

THE RELATIONSHIP BETWEEN ECONOMIC DEVELOPMENT AND CYANOBACTERIA
AND CYANOHAB MONITORING EFFORT IN BRAZIL,
AND ITS IMPLICATIONS FOR PUBLIC HEALTH

A Thesis

by

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ABSTRACT

Cyanobacterial harmful algal blooms (cyanoHABs) are a public health risk because cyanobacteria produce cyanotoxins that affect humans. CyanoHABs have increased globally, causing human intoxications and fatalities. Since cyanoHABs are recurrent, people are constantly exposed to cyanotoxins, through drinking water, food ingestion, aquatic activities, or hemodialysis.

Despite increased cyanoHAB global awareness, little is known about cyanobacteria diversity and distribution in South America. In addition, non-biological variables inducing differential exposure to cyanotoxins have not been addressed. The objective of this study is to assess the relation between economic development and cyanoHAB monitoring effort and the impact of cyanoHABs human exposure to cyanotoxins in Brazil.

I reviewed the occurrence of cyanoHABs in South America with an emphasis on Brazil and analyzed the relationship between gross domestic product per capita (GDPPC) and monitoring effort.

The most common cyanobacterial genera in South America were *Microcystis*, *Anabaena*, *Dolichospermum* and *Planktothrix*, all cyanoHAB and cyanotoxin producers. The most common cyanotoxins for the region were microcystins (MCs) and saxitoxins (STXs). Both have severe adverse health effects in humans and have caused fatalities.

Microcystis aeruginosa and *Cylindrospermopsis raciborskii* were the most commonly identified species, and both produce several cyanotoxins. Therefore, it is likely that their toxins are present in water reservoirs across the region, severely compromising the quality of drinking water. Furthermore, most publications reported mixtures of two or more cyanotoxins, thus

resulting in greater additive or synergistic toxicity than what would be expected from a single cyanotoxin.

GDPPC and monitoring effort were strongly correlated, when monitoring effort was defined as number of municipalities sampled, national percentage of municipalities sampled, and number of monitoring events. Brazilian regions with the lowest GDPPC (i.e. Central-west, North, and Northeast), were monitored significantly less than regions with the highest GDPPC (i.e. South and Southeast). This coincided with Brazil's historic North-Northeast/South-Southeast income gradient. Although Brazil has a cyanoHAB monitoring program, only 2.8% of Brazil's municipalities were monitored at least once a month, and merely 0.1% were monitored monthly. Unfortunately, the data gathered are not biologically informative because cyanobacterial densities, cyanotoxin types, and cyanotoxin concentrations are not recorded. Significant enhancements in the frequency and quality of monitoring efforts need to be implemented to improve public health outcomes.

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NOMENCLATURE

AC	Acre
AER	Aeruginosin
AEPA	AraripeEnvironmental Protection Area
AF	Allocation Factor
AL	Alagoas
AM	Amazonas
Am	Amazonia
AMS	Argentina Ministério de Salud
ANA	Agência National de Águas
ANTX-a	Anatoxin-a
ANTX-a (s)	Anatoxin-a (s)
AP	Amapá
AP-B	anabaenopeptinB
AP-B	anabaenopeptinB
AP-F	anabaenopeptinF
Ar	Argentina
AWWA	AmericanWaterWorksAssociation
BA	Bahía
BCCUSP	Brazilian Cyanobacteria Collection of University of São Paulo
Br	Brazil
C	Central

Carib	Caribbean
CB	Córdoba
CE	Ceara
Co	Colombia
CGSM	Cienaga Grande de Santa Marta
CMEA/UFF	Coleção de Microalgas Elizabeth Aidar/Universidade Federal Fluminense
CONAMA	Conselho Nacional do Meio Ambiente
COSAA	Código do Sistema de Abastecimento de Água
CS	Central-south
CT	Chubut
CW	Central-west
Cy	Cuyo
CyanoHAB	Cyanobacterial Harmful Algal Bloom
CyanoHABs	Cyanobacterial Harmful Algal Blooms
CYN	Cylindrospermopsin
CYNs	Cylindrospermopsins
CyPep	Cyanopeptolin
dc-NEO	Decarbamoylneosaxitoxin
dc-STX	Decarbamoylsaxitoxin
DGGE	Denaturing Gradient Gel Electrophoresis
DF	Brasília
E	East
ELISA	Enzyme-Linked Immunosorbent Assay

ER	Entre Ríos
ES	Espírito Santo
FURG	Federal University of Rio Grande
GDPPC	Gross Domestic Product per Capita
GO	Goiás
GTX	Gonyautoxin
GTXs	Gonyautoxins
GV	Guideline
HPLC	High-Performance Liquid Chromatography
HUCFF	Hospital Universitário Clementino Fraga Filho
IBGE	Instituto Brasileiro de Geografia e Estatística
L	Litoral
LC-MS	Liquid Chromatography-Mass Spectrometry
LC-ESITOF-MS	Liquid chromatography-electrospray ionization-time-of-flight–mass spectrometry
LOAEL	Lowest-Observed-Adverse-Effect-Levels
LYNGTXs	Lyngbytoxins
M	Metropolitan
MA	Maranhão
MALDI-TOF	Matrix Assisted Laser Desorption/Ionization
MC	Microcystin
MCs	Microcystins
MC-LC	Microcystin-LC

MC-LC	Microcystin-LC
MC-RR	Microcystin-RR
MG	Minas Gerais
MI	Misiones
MBFGs	Morphologically Based Functional Groups
MS	Mato Grosso do Sul
MT	Mato Grosso
N	North
NE	Northeast
Neo-STX	Neosaxitoxin
NGA	Norte Grande Argentino
NOAEL	No Observed-Adverse-Effect Levels
NOD	Nodularin
NODs	Nodularins
NQ	Neuquén
P	Patagonia
PA	Pará
PB	Paraíba
PBA	Buenos Aires
PE	Pernambuco
PI	Piauí
PCR	Polymerase Chain Reaction
PQA-VS	Programa de Qualificação das Ações de Vigilância em Saúde

PSP	Paralytic Shellfish Poisoning
PSTs	Paralytic Shellfish Toxins
PR	Paraná
RJ	Rio de Janeiro
RN	Rio Grande do Norte
RN	Río Negro
RO	Rondônia
RR	Roraima
RS	Rio Grande do Sul
S	South
SA	Salta
SAA	Sistema de Abastecimento de Água
SC	Santa Catarina
SE	Santiago del Estero
SE	Sergipe
SE	Southeast
SF	Santa Fe
SISAGUA	Sistema de Informação de Vigilância da Qualidade da Água para Consumo Humano
SL	San Luis
SP	SãoPaulo
STAR	Sistema de Tratamiento de Aguas Residuales Salguero
STX	Saxitoxin

STXs	Saxitoxins
SUFRAMA	Superintendência da Zona Franca de Manaus
TDI	Tolerable Daily Intake
TO	Tocantins
TU	Tucumán
UF	Uncertainty Factor
ULBRA	Universidade Luterana do Brasil
UFSCar	Universidade Federal de São Carlos
Ur	Uruguay
VIGIAGUA	Programa Nacional de Vigilância da Qualidade da Água para Consumo Humano
WHO	World Health Organization

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CHAPTER I
INTRODUCTION

Cyanobacterial Harmful Algal Blooms (cyanoHABs)

Cyanobacterial harmful algal blooms (cyanoHABs) are environmental events that take place when cyanobacteria grow in such high densities that they cause adverse environmental impacts (Clark et al., 2017; Paerl & Otten, 2016). CyanoHABs have been reported worldwide, and mainly occur in in eutrophic and hypereutrophic environments (Azevedo et al., 1994; Carmichael, 1994; Campos et al., 2005; Scarafia et al., 1995).

The occurrence of cyanoHABs is determined by multiple interacting environmental factors. These include water temperature, anthropogenic nutrient enrichment, changes in land-cover and land-use, rainfall patterns, water column stratification, residence times, water column irradiance and clarity, and salinity regimes (Berg & Sutula, 2015; Bista et al., 2014; Chonudomkul et al., 2004; Clark et al. 2017; Davis et al., 2009; Facey et al., 2019; Havens et al., 1998; Lunetta et al., 2015; Moisander et al., 2002; Michalak et al., 2013; Paerl & Huisman, 2008; Paerl et al., 2011; Paerl & Paul, 2012; Paerl & Otten, 2013; Reichwaldt & Ghadouani, 2012; Teferi et al. 2014; Tonk et al., 2008; Waditee et al., 2002).

CyanoHABs incidence is magnified by climate change, particularly warming and extreme hydrologic events (Huisman et al., 2005; Paerl & Huisman, 2008, 2009; O'Neil et al., 2012; Paerl & Paul, 2012; Reichwaldt & Ghadouani, 2012). Nonetheless, the various processes involved in cyanoHAB formation are complex and not yet fully understood (Reichwaldt & Ghadouani, 2012).

CyanoHABs in fresh water reservoirs decrease the quality of drinking water predominantly by the potential release of cyanotoxins. Cyanotoxin production is common to many species; however, not all species or strains produce them. Furthermore, cyanotoxin production is not constant among species and strains that produce cyanotoxins and several cyanotoxin types can be produced by a single species (Farrer et al., 2015; Hitzfeld et al., 2000; Ouahid et al., 2011; Sivonen & Jones, 1999). Nearly 19 genera and 46 species of cyanobacteria have been reported to have cyanotoxins producing capabilities (Codd et al., 2005). Though cyanotoxin production is still under study, some of the factors appearing to regulate it are light intensity, nutrient supply rates, temperature, oxidative stressors, interactions with other biota, growth phase dependent interactions, and environmental chemical cues (Facey et al., 2019; Kearns & Hunter, 2000).

CyanoHABs rarely if ever produce a single cyanotoxin type (Hudnell & Dortch, 2008), during a bloom several cyanotoxins are produced and they interact. Therefore, additive or synergistic toxicity of multiple cyanotoxins could result in greater toxicity than what would have been expected from each individual cyanotoxin thus magnifying the risk to public health (Best et al., 2002; Dietrich et al., 2008; Freitas et al., 2014; Majsterek et al., 2004; Molica et al., 2005; Pinheiro et al., 2016).

CyanoHABs and Cyanotoxins

Due to the presence of cyanotoxins, cyanoHABs represent a technical challenge to water management (AWWA, 2016; Hitzfeld et al., 2000). Worldwide, challenges related to cyanoHABs are expected to intensify, particularly in areas where population growth has spiked, where agro-industrial practices promote the leakage of nutrients to water bodies, and where

sewage treatment is limited or non-existent (Chorus et al., 2000; Paerl, 2008; Cheung et al., 2013).

Because cyanoHABs will become more recurrent, people might be constantly exposed to low levels of cyanotoxins through surface drinking water and recreational water sources (Cheung et al., 2013). Additionally, standard water treatments such as chlorination, boiling, disinfection, filtration, and flocculation (process by which fine particulates are caused to clump together) reduce but do not always eliminate cyanobacteria and cyanotoxins (Hilborn & Beasley, 2015). For example, microcystin are thermostable at temperatures between 10 °C and 150 °C (Yu et al., 2009), therefore resistant to water boiling, a common in-home practice used improve the quality of drinking water (Rosado et al., 2006; de Queiroz et al., 2013; Berry et al., 2018).

Cyanotoxins classification is based on their biological effects on the systems and organs that they affect. They are broadly classified as dermatotoxins, hepatotoxins, neurotoxins, and endotoxins (Table 1). Dermatotoxins cause blistering and irritation, and can be potent carcinogens. Hepatotoxins promote liver tumors, and neurotoxins cause paralytic shellfish poisoning (PSP). Endotoxins are potent immunomodulatory (capable of modifying the immune response or the functioning of the immune system) and immunotoxic products (Bláha et al., 2009; Boopathi & Ki, 2014; Codd et al., 2005; Stewart et al., 2006).

The routes of human exposure to cyanotoxins are dermal absorption, inhalation, oral ingestion, and intravenous injection (Buratti et al., 2017; Codd et al., 2016; Funari, & Testai, 2008; Genitsaris et al., 2011; Metcalf & Codd, 2012; Stewart et al., 2006). Dermal absorption occurs through skin and mucosal contact with cyanotoxins during recreational or working practices, or by showering with raw or inadequately treated water (Giannuzzi et al., 2011; Pilotto et al., 2004).

Inhalation of sprays containing cyanobacteria, their extracts, or toxins can happen during recreational or work activities, or during showering (Annadotter et al., 2005; Falconer, 1996). Inhalation of dust particles from air-dried and lyophilized cyanobacterial biomass, or biogenic desert crusts has been reported during military deployment (Cox et al., 2009).

Oral ingestion occurs via drinking raw or inadequately treated water, and even due to incidental drinking water during recreational activities or showering (Funari & Testai, 2008). It can also happen through the consumption of contaminated seafood (i.e. finfish and shellfish from aquatic marine or fresh water environments) (Ibelings & Chorus, 2007; Funari & Testai, 2008) and vegetables if they were irrigated with cyanotoxin contaminated water (Codd et al., 1999b; Cordeiro-Araújo et al., 2015, 2017; Crush et al., 2008; do Carmo Bittencourt-Oliveira et al., 2016; Freitas et al., 2015; Hereman & Bittencourt-Oliveira, 2012; Wang et al., 2011).

There are reports of cyanotoxin exposure through the consumption of dietary supplements (Drobac et al., 2013; Roy-Lachapelle et al., 2017), or as part of traditional indigenous diets in the highlands of Peru (Johnson et al., 2008). One case of intravenous injection, at a hemodialysis facility in 1996 in Caruaru, Brazil has been reported (Azevedo et al., 2002; Jochimsen et al., 1998; Pouria et al. 1998).

Drinking water is the main route of human exposure to cyanotoxins (Carmichael, 1992; Carmichael et al., 2001; Dolah et al., 2001; Funari & Testai, 2008; Ransom et al. 1994; Van Dolah, 2000). In response to the potential hazards to human health, many countries have followed the provisional guideline proposed by the World Health Organization (WHO) (1998, 2003), and have set maximum acceptable levels of microcystin-LR concentration in drinking water to $1 \mu\text{g l}^{-1}$, a similar value has been proposed for cylindrospermopsin (Burch, 2008; Sivonen & Jones, 1999). Only a few countries, including Australia, Canada, Japan, the United

Kingdom, and Brazil have developed national guidelines for cyanobacteria and cyanotoxins in drinking and recreational waters (AWWA, 2016; Chorus et al., 2000).

CyanoHABs in South America

Although extensive work has been conducted on the acute effects of high-level exposure to cyanotoxins, little research has been done in exposure to low levels of cyanotoxins, even though chronic low-level exposure to microcystins has been linked to major human health threats (Kuiper-Goodman & Fitzgerald, 1999; Lun et al., 2002; Ueno et al., 1996; Yuan et al. 2006; Yu, 1989, 1995; Yu et al., 2001). Hernández et al. (2009) postulated that chronic consumption of tap water containing low doses of microcystins might be a risk factor for liver and colorectal cancer. Hence, the importance of understanding the connection between drinking water containing low levels of cyanotoxins and human health, and the need for more representative regional studies (Kuiper-Goodman et al., 1999).

Regardless of the increased global awareness on the importance of cyanobacteria and cyanotoxins in human and ecosystem health, in South America, little is known about their diversity and distribution (Sant'Anna et al., 2008). In Brazil, the infamous Caruaru syndrome, where 60 patients of a hemodialysis facility died because of accidental intravenous exposure to cyanotoxins (Azevedo et al., 2002; Dörr et al., 2010; Jochimsen et al., 1998), brought cyanoHABs to the attention of the water authorities and the public. This increased awareness lead to increased number of cyanobacteria and cyanotoxin studies in the country, in addition to changes in water policy, sanitary standards, monitoring of water bodies, and institutionalized prevention efforts (Moschini-Carlos et al., 2009; Carvalho et al., 2007; Chellappa et al., 2008a, 2008b; Costa et al., 2006; Hirooka et al., 1999; Molica et al., 2002; Molica et al., 2005; Oliveira et al., 2005; Sant'Anna et al., 2008; Soares et al., 2006; Sotero-Santos et al., 2006; Vieira et al.,

2005; Piccin-Santos & do Carmo Bittencourt-Oliveira, 2012; Yunes et al., 2003). Nonetheless, non-biological variables such as differential policy reinforcement, specifically monitoring effort, have not yet been addressed.

Systematic nationwide monitoring of cyanoHABs in South America is limited and regional studies are scarce (Dörr et al., 2010; Pérez et al., 2013; Sant'Anna et al., 2008). However, scientific publications across the region have documented the occurrence of the phenomenon and show that it has become more frequent. Unfortunately, due to limited analytical capabilities of most countries in the region, the risks imposed by cyanoHABs to public health are understudied (Pérez et al., 2013).

In recent years, the international demand for commodities has prompted massive expansion of agricultural activities in most of South America (Grau & Aide, 2008; Pérez et al., 2013). This added to the rising population and quick economic development of the region, and the subsequent increase in extensive agro-industrial practices, placing the region at risk of increased cyanoHAB incidence. Furthermore, numerous water impoundments and hydropower dams are being built in the region, which in combination with climate change can increase eutrophication and the subsequent occurrence of cyanoHABs (Zarfl et al., 2015).

Since only three countries in South America have specific legislation aiming to monitor, control, and reduce cyanoHABs, the entire region faces monitoring limitations (Pérez et al., 2013). Brazil and Uruguay follow the guidelines established by the WHO in terms of water management and monitoring of cyanobacteria and cyanotoxins in drinking and recreational waters (WHO, 1998, 2003). Both countries have adopted the WHO values for microcystin-LR in drinking and recreational waters. Argentina developed a guideline for cyanobacteria in water and their health effects in 2016 this regulation followed the WHO standards (AMS, 2016).

CyanoHABs in Brazil

Brazil is the focus of this study for five reasons. It is one of the five newly emerging economies in the world (Ahmed, 2017; Cheng et al., 2007), and has a history of regional inequality with wealth concentrating in the Southeast region of the country (Azzoni, 2001; Azzoni & Servo, 2002; Leff, 1972; Savedoff, 1990). Additionally, access to environmental resources and services has been differential (Pedlowski et al., 2002; Perry, 2009; Porto, 2012). Brazil is also Latin America's most populous country, and finally is one of the three countries in the South America with cyanobacteria and cyanotoxins legislation.

Brazil, Russia, India, China, and South Africa have been designated with the acronym BRICS. These countries have experienced increased levels of industrialization and urbanization that generate strong environmental demands (Cowan et al., 2014; Nassani et al., 2017; Santana et al., 2014; Shen et al., 2017; Zaman et al., 2016). Due to increased urbanization, differential access to resources and environmental services have gained greater importance. Given Brazil's history of economic, political and social inequality, this issue is of special concern (Pedlowski et al., 2002) particularly when it comes to access to fresh water (Campos & Studart, 2000).

Brazil's regional income inequality is about three to four times that of the United States (Shankar & Shah, 2003). These differences in income distribution are explained by differences in interregional levels of per capita income (Leff, 1972). In the last seven decades, wealth has concentrated in the Southeast, particularly in the large urban centers of Rio de Janeiro and São Paulo (Azzoni, 2001; Azzoni & Servo, 2002; Leff, 1972; Matos & Baeninger, 2001; Savedoff, 1990).

With 209.3 million inhabitants in 2017 (World Bank, 2019), Brazil is Latin America's most populous country. It also has a history of internal migration towards urban centers in the

Central-West, South, and Southeast regions which triggered the growth of these urban centers (Matos & Baeninger, 2001). After an economic boom in the 1970s, for the first time, the urban population outnumbered the rural population. This increased urbanization became the engine for economic development (Matos & Baeninger, 2001; Shen et al., 2017).

As a result of urbanization, more people in Brazil receive their drinking water from municipal reservoirs. Since water quality varies depending on the ecological characteristic of the reservoirs as well as on the treatments used for potability (Hernández et al., 2009), water quality in different regions of Brazil will be expected to be different. Furthermore, differential water monitoring related to disparate economic development between regions, could contribute to the disparities in the quality of drinking water.

Brazil and Uruguay follow the guidelines and regulations established by the WHO in terms of water management and monitoring of cyanobacteria and cyanotoxins in drinking and recreational waters (WHO, 1998, 2003). Both countries have adopted the WHO guideline values for microcystin-LR in drinking and recreational waters. In 2016, Argentina developed a guideline for cyanobacteria in water and their health effects following the WHO standards (AMS, 2016).

The connection between environmental quality and economic development has long been studied from an economic perspective (Carson & Mitchell, 1993; Machdar et al., 2013; McConnell, 1997; Mohapatra & Giri, 2009; Shafik, 1994; Torras & Boyce, 1998). Building on this notion, I propose to evaluate water quality from a public health perspective, within the frame of economic development. I used cyanobacteria data from Brazil, while exploring regional income as a potential predictor of regional monitoring effort. This approach will allow for better-

informed management strategies, and might bring monitoring disparities to the attention of local water authorities.

In Brazil, potable water is considered a public good, and government institutions are in charge of its regulation and provision (ANA, 2018). In the last seven decades, water demand in Brazil has increased due to economic development and urbanization (ANA, 2018). In order to fulfill these needs many mixed-use reservoirs were built during the 1960s and 1970s. The Ten Year Energy Plan for the country called for the construction of 71 new hydroelectric plants by 2017, the reservoirs built for the hydroelectric projects also to be used for water supply, aquaculture, and recreation (von Sperling, 2012). Due to global warming, industrial and agricultural runoff, and limited or inexistent sewage treatment, many of these reservoirs are at risk of becoming eutrophic and developing cyanoHABs more frequently, which will in turn result in greater cyanotoxin exposure for humans (Paerl & Paul, 2012).

CyanoHABs are a public health risk because numerous cyanobacteria produce cyanotoxins that affect human health. The risk magnifies when municipal systems become contaminated, as entire communities can be exposed, and numerous people can fall sick (Hilborn & Beasley, 2015). Assessing human health risk associated with cyanoHABs is fundamental to develop adequate regulatory measurements to prevent and minimize related risks and impacts (Van Dolah, 2000). Because cyanoHABs are recurrent, people might be constantly exposed to cyanotoxins through drinking water (Hernández et al., 2009; Ueno et al., 1996; Dolah et al., 2001; Van Dolah, 2000; Yu, 1989, 1995; Yu et al., 2001).

The objective of this study is to assess cyanobacteria monitoring effort in Brazil, and evaluate regional differences resulting from affluence. I hypothesized that regions with higher gross domestic product per capita (GDPPC) will have a greater monitoring effort. While

monitoring effort by itself does not guarantee the quality of water that reaches the final consumer, it provides information about how water reservoirs are managed, and might provide the bases for improve those management strategies, which can in turn improve water quality and protect human health.

Assessing human health risks associated with cyanoHABs is fundamental to developing adequate regulatory measurements to prevent and minimize public health risks. Because cyanoHABs are recurrent, people might be constantly exposed to cyanotoxins through drinking water. While most of the reported health incidents associated to cyanotoxins have resulted from acute exposure, the effects of low-level chronic exposure to cyanotoxins are likely underreported and poorly understood.

CHAPTER II

TOXIC CYANOBACTERIAL ALGAL BLOOMS IN SOUTH AMERICA: STATE OF RESEARCH IN THE REGION, KNOWLEDGE GAPS, AND FUTURE DIRECTIONS

Introduction

Cyanobacterial harmful algal blooms (cyanoHABs) are environmental events that take place when one or a few species of cyanobacteria reproduce in high densities resulting in adverse environmental and health impacts (Clark et al., 2017; Paerl & Otten, 2016). CyanoHABs can be visually identified because they change the color of the water, and form mats or scums, Cyanobacteria are considered to have bloomed when the number of cyanobacterial cells is greater than a million per liter (Sulis et al., 2014). CyanoHABs happen in fresh, brackish, and marine waters, in eutrophic and hypereutrophic environments exposed to direct sunlight. CyanoHABs have been reported in the Americas, Europe, Oceania, and southern Africa (Azevedo et al., 1994; Campos et al., 2005; Carmichael, 1994; Scarafia et al., 1995). The presence of cyanobacteria can decrease the quality of drinking water by altering its organoleptic properties (i.e. color, odor, and taste), by interfering with water treatment, and more importantly by potentially releasing cyanotoxins. CyanoHABs are one of the main hazards to public health in fresh water environments, particularly when cyanotoxins are ingested via drinking water or food consumption (Codd et al., 2005; Carmichael, 2008; Fernández et al. 2015; He et al., 2016).

To protect health outcomes a conservative guideline of 20,000 cyanobacterial cells ml⁻¹, corresponding to 10 mg chlorophyll-a l⁻¹, under conditions of cyanobacterial dominance was established by the WHO (2003). This safety threshold was derived from an epidemiological study by Pilotto et al. (1997) and was set based on the irritating or allergic effects of compounds

produced by cyanobacteria. According to the WHO (2003), in a cyanoHAB with a density of 20,000 cells ml⁻¹, a toxin concentration of 2-4 mg microcystin (MC) l⁻¹ could be expected if microcystin-producing cyanobacteria are dominant. Concentrations as high as 10 mg l⁻¹ are possible in highly toxic cyanoHABs.

The WHO provisional guideline for the concentration of microcystins (MCs) in drinking-water is 1 mg l⁻¹ for microcystin-LR (WHO, 1998), which is assumed to be safe for a lifelong consumption. However, a study by Piccin-Santos and do Carmo Bittencourt-Oliveira (2012) conducted in four Brazilian reservoirs, showed that water bodies with the highest cyanobacterial densities did not have the highest microcystin concentrations, suggesting that cyanoHAB toxicity might not be directly related to cyanobacterial cell density. Further studies are needed to determine the precise relation between cyanobacterial density and cyanotoxin concentration during cyanoHABs.

Causes for cyanoHABs Formation

Multiple environmental factors, and their interactions, promote cyanobacterial growth and cyanoHABs occurrence (Bista et al., 2014; Clark et al. 2017). These factors include water temperature (Chonudomkul et al., 2004; Davis et al., 2009; Paerl et al., 2011), water column stratification and residence times (Berg & Sutula, 2015; Paerl & Huisman, 2008), anthropogenic nutrient enrichment (Facey et al., 2019; Michalak et al., 2013; Paerl et al., 2011), changes in land-cover and land-use (Lunetta et al., 2015; Michalak et al., 2013), rainfall patterns (Reichwaldt & Ghadouani, 2012), and to a lesser extend water column irradiance and water clarity (Havens et al., 1998; Teferi et al. 2014), and salinity regimes (Berg & Sutula, 2015; Moisander et al., 2002; Paerl & Otten, 2013; Tonk et al., 2008; Waditee et al., 2002).

Although scientific consensus on the importance of N:P ratios as drivers for cyanoHABs has not been reached (Berg & Sutula, 2015), it is widely accepted that anthropogenic nutrient enrichment (Paerl et al., 2011) leads to eutrophication which in turn increases the frequency and magnitude of cyanoHABs (Paerl & Otten, 2016). Changes in land-cover and land-use increase sediment loading and nutrient delivery in watersheds which also promote cyanobacteria growth (Lunetta et al., 2015; Michalak et al., 2013). Likewise, climate change (Huisman et al., 2005; O'Neil et al., 2012; Paerl & Huisman, 2008, 2009; Paerl & Paul, 2012), particularly warming and extreme hydrologic events have a substantial impact on cyanoHABs. Higher frequency of high intensity rainfall events and longer drought periods influence the occurrence and severity of toxic cyanoHABs (Reichwaldt & Ghadouani, 2012). Nonetheless, the various processes involved in cyanoHAB formation, with or without toxin production, are complex and not yet fully understood (Reichwaldt & Ghadouani, 2012).

Causes for Cyanobacterial Toxicity

Not all species or strains of cyanobacteria produce cyanotoxins, furthermore, cyanotoxin production is not constant even among species and strains carrying the genes necessary to produce toxins (Farrer et al., 2015). It is currently understood that light intensity, nutrient (i.e. nitrogen, phosphorus, and trace metals) supply rates, temperature, oxidative stressors, and interactions with other biota (i.e. bacteria, viruses, and grazers) and their combined effects are some of the reasons for cyanotoxin production (Facey et al., 2019). Cyanotoxin production might also be regulated by complex growth phase dependent interaction and by environmental chemical cues. Suggesting that substances secreted by algae or other cyanobacteria, and even cell-to-cell communication, might influence cyanotoxin production (Kearns & Hunter, 2000). Differential toxicity may depend on several factors, including biomass and cyanotoxin

concentration within the strains present in the cyanoHAB, the amount of contaminated water or food consumed by the human or animal, variations in individual sensibility, the individual's age and sex, as well as the amount of food present in the individual's digestive tract at the time of cyanotoxin ingestion (Carmichael, 2001).

While most of the reported health adverse incidents associated to cyanotoxins have resulted from acute exposure, the effects of low-level chronic exposure to cyanotoxins are likely underreported and poorly understood. Risk assessment for cyanotoxins is normally hindered because cyanotoxins are not easily identifiable, and because the indicators for exposure and their effects in humans and animals remain unclear (Van Dolah, 2000). Human exposure to cyanotoxins occurs via different routes; however, drinking water is often seen as the main source of exposure for humans (Carmichael, 1992; Carmichael, 2001; Dolah et al., 2001; Ransom et al. 1994).

In the past two decades, due to increased evidence of the presence of cyanotoxins in fish and shellfish, the importance of food ingestion as a route of cyanotoxin exposure has become better known (Magalhães et al., 2003; Funari & Testai, 2008; Hereman & Bittencourt-Oliveira, 2012; Gutiérrez-Praena et al., 2013). While most of the literature on cyanotoxin contaminated food focusses in freshwater seafood (for a review on this subject see Ibelings & Chorus, 2007), there has been a recent increase on studies examining the presence of cyanotoxins in vegetables irrigated with cyanotoxin-containing water (Codd et al., 1999b; Cordeiro-Araújo et al., 2015, 2017; Crush et al., 2008; do Carmo Bittencourt-Oliveira et al., 2016; Freitas et al., 2015; Hereman & Bittencourt-Oliveira, 2012; Wang et al., 2011). Though cyanotoxin ingestion via food is of great importance in public health, this topic is outside the scope of this review.

Safety Guidelines for Cyanotoxins

Conservative guidelines have been developed by international, national, and local authorities. The most commonly used metric for safety assessment are tolerable daily intake (TDI), no observed-adverse-effect levels (NOAEL), and lowest-observed-adverse-effect-levels (LOAEL). TDI is the amount of a substance that has been assessed safe for humans to ingest over a lifetime. NOAEL is the level of exposure of an organism at which there is no biologically or statistically significant increase in the frequency or severity of adverse effects. And LOAEL is the lowest concentration or amount of a substance that causes an adverse alteration of morphology, function, capacity, growth, development, or lifespan of an organism. These values have only been established for some cyanotoxins (Codd et al., 2005; Duy et al., 2000; Falconer et al., 1996; Falconer & Humpage, 2005).

TDI for cyanotoxins in drinking water can be calculated using the equation cited by Codd et al. (2005, p. 267) (Eq. 1).

$$\text{TDI} = ((\text{NOAEL or LOAEL}))/\text{UF} \quad (\text{Eq. 1})$$

TDI is given in milligrams per kilogram of body weight per day ($\text{mg kg}^{-1} \text{bw d}^{-1}$), or micrograms per kilogram of body weight per day ($\mu\text{g kg}^{-1} \text{bw d}^{-1}$). UF is a product of uncertainty factor, and it is considered to be equal to three for tumor promotion, five for a LOAEL, and ten for interspecies variation or for a less-than-lifetime study (Codd et al., 2005).

Likewise, guide line (GV) values can be calculated, using the equation (Codd et al. (2005, p. 267) (Eq. 2):

$$GV = (TDI * \text{body weight} * AF) / C \quad (\text{Eq. 2})$$

GV are useful in the setting of risk management strategies for drinking water (Codd et al., 2005; Falconer et al., 1999; Falconer & Humpage, 2005). AF is the allocation factor (i.e. the proportion of the TDI ingested via drinking water), and C is the water consumption per day. To estimate GV body weight for the average adult human is assumed to be 60 kg, and UF is considered to be 80% or 0.8, since people might be exposed to cyanotoxins through food. Two liters was assumed to be the daily water consumption of an adult (Codd et al., 1999a; Codd et al., 2005).

The WHO determined that there is not enough experimental data to determine TDI or GV for anatoxin-a (Codd et al., 2005; Falconer et al., 1999; Falconer & Humpage, 2005).

Nonetheless, Fawell et al. (1999) determined a GV of $1 \mu\text{g l}^{-1}$ for anatoxin-a. This value is the result of a 28-day repeat sublethal oral dosing study, and sets a safety margin of nearly three orders or magnitude. Humpage and Falconer (2003) determined a TDI of $0.03 \text{ mg kg}^{-1} \text{ bw d}^{-1}$ for cylindrospermopsin, based on the NOAEL determined with mice in a 13-week period, and using the adult body weight of 60 kg and an AF of 0.9. A GV of $0.81 \mu\text{g l}^{-1}$ was obtained.

The conservative recommended GV for cylindrospermopsin is $1 \mu\text{g l}^{-1}$. The TDI for microcystin comes from test with mice using pure toxin and from tests with pigs using freeze-thawed *Microcystis* cells containing quantified levels of microcystins. Values were set to and $0.040 \text{ mg kg}^{-1} \text{ bw d}^{-1}$ and $0.067 \text{ mg kg}^{-1} \text{ bw d}^{-1}$ respectively, which produces GV vales of $0.96 \mu\text{g l}^{-1}$ and $1.61 \mu\text{g l}^{-1}$.

Using a conservative approach, the WHO adopted a provisional GV for microcystin in drinking water for adults of 1 µg l⁻¹ (Falconer et al., 1999). When the tumor-promoting action of microcystin is considered, and a UF of 3 is used, GV is equal to 0.3 µg l⁻¹.

NOAEL and LOAEL values are estimated based on quantitative animal oral dosing data, with follow-up over extended periods, and have only been established for anatoxin-a, cylindrospermopsin, and microcystins (Codd et al., 2005).

Cyanotoxin Bioaccumulation and Bioconcentration

Bioaccumulation is the process by which chemical concentrations in the tissues of an organism exceed those in the water, due to uptake by all exposure routes (e.g. absorption from water or food consumption) while bioconcentration specifically refers to absorption directly from water (i.e. dissolved toxins). These processes result in the chemical concentration of a substance being greater in the organism than in the water (Gray, 2002).

Though this subject is beyond the scope of this review, it is worth mentioning that cyanotoxins bioaccumulate in food webs (Al-Sammak et al., 2014; Drobac et al., 2016; Ferrão-Filho et al., 2002; Ferrão-Filho & Kozlowsky-Suzuki, 2011; Hardy et al., 2015; Ibelings et al., 2005; Lehman et al., 2010; Oberhaus, et al., 2007), and that different organism, as well as organs and tissues within them, bioaccumulate cyanotoxins at different rates (Ferrão-Filho et al., 2002; Ferrão-Filho & Kozlowsky-Suzuki, 2011; Ibelings et al., 2005; Ibelings & Chorus, 2007; Lehman et al., 2010; Oberhaus, et al., 2007). Therefore, existing guidelines for acceptable concentration of cyanotoxins in seafood might have limited effectiveness (Farrer et al., 2015).

A study conducted by Paulino et al. (2017) illustrates the necessity to further explore bioaccumulation in aquatic organisms consumed as food, they found evidence of dissimilar organ bioaccumulation of cyanotoxins in *Hoplias malabaricus*. *H. malabaricus* is a Neotropical

fish species that is widespread species in South America, and is well appreciated for human consumption. The specimens were purchased alive from a fish farm in the state of São Paulo in Brazil and were exposed to two microcystin (MC) variants (i.e. MC-RR and MC-YR). Fish were exposed to a single dose of 120.60 MC-RR+MC-LR kg-fish⁻¹ (=100 µg MC-LR equivalents kg-fish⁻¹) for 12 h and 96 h to recreate acute exposure, and for 30 days to a similar dose every 72 h for 30 days to recreate subchronic exposure. Fish did not die during experimental exposure, and no color change was observed in muscle tissue.

Microcystin-YR (MC-YR) accumulated after acute and subchronic exposure while microcystin-RR (MC-RR) only accumulated after subchronic exposure. There was no accumulation of microcystins (MC) in the edible portions of fish (i.e. white muscle), but only in their liver. Consumption of fish immediately after a cyanoHAB might not affect human health, however if the cyanoHAB lasts for an extended period, decreased detoxification capacity of the liver along with increased microcystin bioaccumulation might result in the accumulation of microcystin in muscles with potential health risks implications for humans (Al-Sammak et al., 2014; Drobac et al., 2016; Hardy et al., 2015).

Public Health Risks Associated to Cyanotoxins

Cyanotoxins represent a hazard for drinking water safety, and are a technical challenge to water managers (AWWA, 2016; Hitzfeld et al., 2000). Most cyanotoxins are produced by fresh and brackish water planktonic cyanobacteria. Toxic strains have also been isolated from benthic, and riverine environments, and rarely from terrestrial habitats (Sivonen & Jones, 1999). Cyanotoxins are diverse (Hitzfeld et al., 2000; Ouahid et al., 2011; Sivonen & Jones, 1999), and can be produced by more than one cyanobacterial species. Likewise, several toxins can be produced by one species.

Toxic cyanoHABs are a considerable public health risk because numerous cyanotoxins can affect humans and livestock (Hitzfeld et al., 2000; Sivonene & Jones, 1999). CyanoHABs have caused human poisoning in municipal and recreational water supplies around the world, and have even caused human fatalities as confirmed in Brazil in 1996 (Carmichael et al., 1996; Hirooka et al., 1999; Jochimsen et al., 1998; Pouria et al., 1998). While their occurrence is not new, it has increased in the past few decades with 50% of cyanoHABs linked to toxicity (Carmichael, 2001, 2008; Carmichael et al., 2001; Campos et al., 2005; Cheung et al., 2013; Hudnell, 2010; Sivonen & Jones, 1999). Problems related to cyanoHABs are expected to intensify, particularly in areas where population growth has spiked, with limited or non-existent sewage treatment, and where agricultural practices promote the leakage of nutrients to water bodies (Chorus, 2001; Cheung et al., 2013; Paerl, 2008).

Because cyanoHABs tend to be recurrent, people might be constantly exposed to low levels of cyanotoxins through surface drinking and recreational water sources (Chen et al., 2009). Additionally, standard water treatments such as disinfection, filtration, and flocculation (process by which fine particulates are caused to clump together) reduce but do not always eliminate cyanobacteria and cyanotoxins (Hilborn & Beasley, 2015). For example, microcystin are thermostable at temperatures between 10 °C and 150 °C (Yu et al., 2009), therefore resistant to water boiling a common in-home practice used improve the quality of drinking water in Brazil (Berry et al., 2018; de Queiroz et al., 2013; Rosado et al., 2006).

Assessing human health risks associated with cyanoHABs is fundamental to developing adequate regulatory measurements to prevent and minimize public health risks. Because cyanoHABs are recurrent, people might be constantly exposed to cyanotoxins through drinking water, ingestion of contaminated food, inhalation, contact with recreational waters, or

intravenous injection via hemodialysis (Hirooka et al., 1999; Jochimsen et al., 1998; Pouria et al., 1998; Turner et al., 1990).

Cyanotoxin Classification and Production

There are nearly 19 genera and 46 species of cyanobacteria capable of producing cyanotoxins. These genera include *Anabaena*, *Anabaenopsis*, *Aphanizomenon*, *Cylindrospermopsis*, *Planktothrix*, *Raphidiopsis*, *Microcystis*, and *Nodularia*. Scum formation is common with *Anabaena*, *Anabaenopsis*, *Aphanizomenon* *Microcystis*, and *Planktothrix*, and less common with the remaining genera. Biofilm-forming and mat-forming genera with toxigenic members include *Lyngbya*, *Oscillatoria*, and *Phormidium* (Codd et al., 2005).

Cyanotoxins classification is based on their biological effects on the systems and organs that they affect. They are broadly classified into dermatotoxins, hepatotoxins, neurotoxins, and endotoxins. Dermatotoxins cause blistering and irritation, and can be potent carcinogens. Hepatotoxins promote liver tumors, and neurotoxins cause paralytic shellfish poisoning (PSP). Endotoxins are potent immunomodulatory (capable of modifying the immune response or the functioning of the immune system) and immunotoxic products (Bláha et al., 2009; Boopathi & Ki, 2014; Codd et al., 2005; Pearson et al., 2010; Stewart et al., 2006) (Table 1).

Dermatotoxins

Cyanobacterial dermatotoxins are alkaloids produced by benthic marine cyanobacteria they can damage skin, mucous membranes, or both. The better known cyanobacterial dermatotoxins are lyngbyatoxins, aplysiatoxins, and debromoaplysiatoxins. Exposure to them results in contact dermatitis commonly known as swimmer's itch (Tidgewell et al., 2010). Limited information is available about the environmental factors that regulate dermatotoxin production and degradation.

Lyngbyatoxins and Aplysiatoxin

Lyngbyatoxins (LYNGTXs) and aplysiatoxins (ATXs) are produced by the marine cyanobacterium *Lyngbya majuscula* (Moore et al., 1984; Yadav et al., 2011). Aplysiatoxins have been isolated from *Lyngbya majuscula*, *Schizothrix calcicola*, and *Oscillatoria nigro-viridis* (Chlipala et al., 2010; Moore et al., 1984). Both are inflammatory agents known to promote tumor formation (Fujiki & Suganuma, 1996, 2009; Ito et al., 2002; Suganuma et al., 1984; Yadav et al., 2011). Intraperitoneal injection of these toxins caused intestinal bleeding, stomach ulcers, and eventually death due to hemorrhagic shock in mice (Ito et al., 2002; Yadav et al., 2011), while topical application in mammals resulted in dermatitis, blistering, and necrosis (Yadav et al., 2011)

Debromoaplysiatoxins

Debromoaplysiatoxins (DATs) are compounds related to aplysiatoxin (ATX) and oscillatoxin-a produced by the genera *Lyngbya*, *Oscillatoria*, and *Schizothrix* (Sivonen & Jones, 1999; Solomon & Stoughton, 1978). These toxins produce irritant pustular folliculitis in humans, and severe cutaneous inflammatory reaction in the rabbit and in hairless mice (Solomon & Stoughton, 1978; Tidgewell et al., 2010). Debromoaplysiatoxins (DATs) are potent tumor promoters and protein kinase C activators (Sivonen & Jones, 1999; Tidgewell et al., 2010) (Table 1).

Hepatotoxins

Are hepatotoxic to animals and humans, signs of poisoning including cold extremities, diarrhea, labored breathing, pallor (pale appearance), recumbency (inability to get up from a lying down or reclining position), vomiting, and weakness. Disruption of liver structure and function and hemorrhage into this organ, followed by respiratory arrest, can result in death (Bell

& Codd, 1994; Carmichael, 1992; Codd et al., 1999a). Some of the most common hepatotoxins are cylindrospermopsins, microcystins and nodularins.

Cylindrospermopsins

Cylindrospermopsin (CYN) was first isolated from a culture of *Cylindrospermopsis raciborskii* (Ohtani et al., 1992). It is produced by at least five genera of freshwater cyanobacteria *Anabaenabergii*, *Aphanizomenon*, *Cylindrospermopsis*, *Raphidiopsis* and *Umezakia* (Falconer & Humpage, 2006). They have hepatotoxic, nephrotoxic, and cytotoxic effects. They are also potential carcinogens (Froschio et al., 2003; Humpage et al., 2000; Pearson et al., 2010; Runnegar et al., 2002).

Cylindrospermopsin (CYN) can damage adrenal glands, kidneys, liver, lungs, heart, stomach, as well as the vascular and the lymphatic systems (Hawkins et al., 1985; Falconer & Humpage, 2006). Some of the environmental conditions that seem to regulate cylindrospermopsin production are light intensity, and sulfate and phosphate availability (Saker & Neilan, 2001).

Cylindrospermopsin (CYN) breaks down slowly in temperatures ranging from 4°C to 50°C at pH 7. At 50°C it degrades to 57% of the original concentration after 10 weeks (Chiswell et al., 1999). Cylindrospermopsin (CYN) might break down slowly under artificial light ranging from 42, 29, and 9 $\mu\text{E m}^{-1}\text{s}^{-1}$ and in the dark (Chiswell et al., 1999). Activated carbon and ozone treatment effectively remove cylindrospermopsin (Falconer & Humpage, 2005, 2006; Svrcek & Smith, 2004), and chlorination transforms cylindrospermopsin into nontoxic products (Falconer & Humpage, 2006; Senogles-Derham et al., 2003). Cylindrospermopsin (CYN) does not appear to be affected by pH changes, and boiling does not cause significant cylindrospermopsin break down within 15 minutes (Chiswell et al., 1999).

Microcystins

Microcystins (MCs) are named after *Microcystis aeruginosa*, the cyanobacterium from which the toxin was first isolated and described (Sivonen & Jones, 1999). *M. aeruginosa* is one of the most common cyanobacterial species that produces cyanoHABs. Other cyanobacterial genera that biosynthesized microcystins are *Anabaena*, *Planktothrix*, *Nostoc*, and some species of benthic *Oscillatoria* (Omidi et al., 2018). They are the largest, most study and most structurally diverse group of cyanobacterial toxins, they are also the most commonly produced (Merel et al., 2013). Nearly 90 isoforms have been identified (Hooser et al., 1989; Ito et al., 1997a, 1997b; Neilan et al., 2013; Welker & Von Döhren, 2006; Wickstrom et al., 1996).

Some of the most common variants are microcystin-LR (MC-LR), microcystin-RR (MC-RR), and microcystin-YR (MC-YR). In a comparative study of toxicity conducted in mice, microcystin-LR (MC-LR) was found to be the most potent toxin followed by microcystin-YR (MC-YR) and microcystin-RR (MC-RR) (Harada, 1996; Gupta et al., 2003; Kotak et al., 1995). Microcystins (MCs) are also the most widespread cyanotoxins, having been reported in every continent (Fristachi & Sinclair, 2008; Merel et al., 2013; Pearson et al., 2010).

Microcystins (MCs) mainly target the liver, and inhibit type 1 and 2A phosphatases. Acute microcystins exposure causes liver injury, hemorrhage and necrosis, and promote the formation of tumors, posing a considerable health risk to human health (Hitzfeld et al., 2000; Hooser et al., 1989; Ito et al., 1997a, 1997b; MacKintosh et al., 1990, Nishiwaki-Matsushima, 1991, 1992; Sivonen & Jones, 1999; Ueno et al., 1996, Wickstrom et al., 1996). Doses of as small as 50-70 mg kg⁻¹ of body weight have been reported to produce rapid death in laboratory animals (Sivonen & Jones, 1999). Human fatalities have also been reported following acute

microcystin poisoning (Jochimsen et al., 1998). Chronic, sublethal doses of microcystin may be carcinogenic (Nishiwaki-Matsushima et al., 1991, 1992; Yu, 1989, 1995; Yu et al., 2001).

Production of microcystins (MCs) appears to be associated to physical and environmental parameters, including nitrogen, phosphorous (Sivonen & Jones, 1990; Vezie et al., 2002), iron concentrations (Lukac & Aegerter, 1993), growth temperature, light, and pH (Lukac & Aegerter, 1993; Neilan et al., 2013; Sivonen & Jones, 1990; van der Westhuizen & Eloff, 1985).

Microcystins (MCs) are particularly stable and resist common chemical treatments such as hydrolysis or oxidation, under conditions typically found in most water bodies. They break down slowly at high temperatures (40 °C) at very low or high pH (values <1 to >9). Microcystins (MCs) is thermostable at temperatures between 10 °C and 150 °C, but degrade at 200°C (Yu et al., 2009), indicating that they are very thermostable and regular boiling will not degrade them. Microcystins' half-life, which is the time it takes for half of the toxin to be degraded by biological processes, at pH 1 and 40 °C is 3 weeks. Microcystins (MCs) break down slowly in full sunlight particularly when water-soluble pigments are present, under these environmental conditions its half-life is 10 weeks (Tsuji et al., 1994, 1995). The half-life of microcystin LR by 147 μWcm^{-2} UV irradiation was 10 minutes, and the toxin was completely decomposed by 2550 μWcm^{-2} UV after 10 minutes. Suggesting that a water treatment including UV irradiation could be effective in removing microcystins (MCs) (Tsuji et al., 1995). Chlorination at an appropriate dose could remove microcystins (MCs) from raw water, preoxidation of the cell with chlorine should be avoided, because it causes toxin release from cyanobacterial cells (Tsuji et al., 1997).

Nodularins

Nodularins (NODs) are predominantly produced by *Nodularia spumigena*. They have been found worldwide and have been implicated in human poisoning and animal deaths. At least

10 isoforms have been identified, with nodularin-R (NOD-R) been the most abundant (Chen et al., 2013). Ingestion of nodularins causes liver necrosis and hemorrhage, similar symptoms to those caused by microcystins (Ressom et al., 1994; Runnegar et al., 1988). Nodularins (NODs) bind to and inhibit protein phosphatases and promote tumor formation like microcystins (MCs) do (Aráoz et al. 2010; Gullledge et al., 2002; Ohta et al., 1994; Pearson et al., 2010; Yoshizawa et al., 1990).

Nodularins (NODs) appear to be produced in response to light intensity and macronutrient levels, particularly nitrogen and phosphorus. Most studies report that the nodularin concentration increases under conditions that promote optimal growth, as nodularin production correlates with the cell division rates (Repka et al., 2001).

Nodularin (NOD) persistence in aquatic environments appears to be influenced by other components of the cyanobacterial cells and sun light. Nodularin levels drop at full sun light than under complete darkness (Twist & Codd, 1997). Microorganism diversity might influence microcystins and nodularin biodegradation in freshwaters (Edwards et al., 2008) (Table 1).

Neurotoxins

Cyanobacterial neurotoxins target cholinergic synapses and sodium channels (Aráoz et al., 2010), the most common are anatoxins (ANTXs) and saxitoxins (STXs). Cyanobacterial neurotoxins are responsible for paralytic shellfish poisoning (PSP), when contaminated marine shellfish are consumed (Codd, 2000).

Anatoxins

Anatoxins (ANTXs) are low molecular weight bicyclic secondary amines (organic compounds derived from ammonia) mainly produced by cyanobacteria (Aráoz et al. 2010; Devlin et al., 1977; Skulberg et al., 1992). The most common are anatoxin-a (ANTX-a),

homoanatoxin-a (HTX-a), and anatoxin a(s) (ANTX-a (s)) (Harris, 2017). They are synthesized by members of the genera *Anabaena*, *Aphanizomenon*, *Cylindrospermum*, *Microcystis*, *Oscillatoria*, *Planktothrix* and *Raphidiopsis* (Aráoz et al., 2005; Harada et al., 1989; Namikoshi et al., 2003; Park et al., 1993; Selwood et al., 2007; Sivonen et al., 1989; Viaggiu et al., 2004).

Anatoxins (ANTXs) cause human poisonings, some of which have been fatal (Harris, 2017), they block neuromuscular activity causing gasping, hypersalivation, muscle fasciculations (brief contraction of a group of muscle fibers), and staggering (uncoordinated walking). In birds, they cause opisthotonos (head and neck stretched backwards along the back) and death by respiratory arrest (Codd, 2000).

Some studies have shown that light and temperature affect growth and toxicity in *Anabaena flos-aquae*. Although optimal conditions for growth and toxin production are similar, they are not identical indicating that toxin production is not a direct function of cellular growth (Neilan et al., 2013). Likewise, anatoxin (ANTX) synthesis seems to be regulated by growth phase, light intensity, nitrogen source, and temperature (Gallon et al., 1994; Gupta et al., 2003; Neilan et al. 2013; Rapala et al., 1993). High temperatures reduced anatoxin-a (ANTX-a) levels in *Anabaena* and *Aphanizomenon* regardless of their growth rate, while nitrogen limitations elevate anatoxin-a (ANTX-a) levels (Neilan et al., 2013; Rapala et al., 1993, 1994). High anatoxins (ANTXs) levels were reported under at light intensity and temperatures a slightly suboptimal for growth. Under low light intensity growth conditions, high concentrations of extracellular anatoxin-a (ANTX-a) were detected (Neilan et al., 2013; Rapala & Sivonen, 1998).

Although little is known about the stability of anatoxins, under acidic pH, high photosynthetically active radiation, low temperatures ($\leq 20^{\circ}$ C), and the presence of phycocyanin-C, a blue photosynthetic pigment present in cyanobacteria, anatoxin-a (ANTX-a) is

very stable. Similarly, anatoxin-a (ANTX-a) is stable at low pH values, while high pH values accelerate its degradation. UV-B irradiation lowered the anatoxin-a concentration by 10% at pH 3.5 and over 80% at pH 7.0 and pH 9.5 within 1 hour. Similar values have been observed after 1 hour of radiation at 100°C (Kaminski et al., 2013).

Saxitoxins

Saxitoxins (STXs) include at least 27 isoforms capable of inhibiting neurotransmission by blocking sodium channels in nerve axons, causing loss of sensation, and paralysis (Falconer et al., 2008). The most common are saxitoxin (STX), neosaxitoxin (Neo-STX), gonyautoxins (GTXs), and C-toxins, (Falconer, 2008; Hallegraeff, 1993; Kayal et al., 2008). Saxitoxins are the only known type of cyanotoxins not exclusively produced by cyanobacteria (Aráoz et al., 2010; Codd et al., 1999a; Llewellyn, 2006; Pearson et al., 2010). The cyanobacterial genera that produce them are *Anabaena*, *Aphanizomenon*, *Planktothrix*, *Cylindrospermopsis*, *Lyngbya*, and *Scytonema* (Smith et al., 2012; Wiese, 2012).

Saxitoxins (STXs) cause convulsions, muscle weakness, salivation, and death by respiratory paralysis if ingested in sufficient amounts (Kayal et al., 2008). They are causing paralytic shellfish poisoning (PSP) after the consumption of contaminated filter-feeding marine shellfish (Codd, 2000; Falconer et al., 2008).

Little is known about the environmental factors regulating saxitoxin (STX) production. However, a study on *Cylindrospermopsis raciborskii* T3 toxin production demonstrates a strong correlation between variations in cellular Na⁺ levels and saxitoxin (STX) production suggesting that either saxitoxin metabolism or the toxin itself might be linked to the maintenance of cell homeostasis under high pH values or Na⁺ limited conditions (Pomati et al., 2004).

Saxitoxins (STXs) are very stable, lasting up to 18 months without loss of potency at temperatures of -80°C, -20°C, 4°C, and 37°C (Alfonso et al., 1994). Kayal et al. (2008) examined the biotransformation of saxitoxins in biological filters in freshwater systems, finding increased toxicity in water filtered through biologically active filters containing anthracite (hard coal) sourced from the filter beds of two water treatment plants. Decreased concentration of C-toxins and gonyautoxins (GTXs), the less toxic variants of saxitoxins (STXs), coincided with increased concentrations of the more variants. These results suggest that microorganisms within the biofilm of the filters have the ability to biotransform the saxitoxins (STXs) variants (Table 1).

Endotoxins

Endotoxins are lipopolysaccharides (LPS) characteristic of the outer membrane of cyanobacteria and Gram-negative bacteria (Durai et al., 2015). They are potent immunomodulatory and immunotoxic cyanobacterial products that stimulate a wide variety of responses in humans (Stewart et al., 2006), including inflammation, respiratory and gastrointestinal diseases, allergic reactions, as well as cutaneous and ocular irritation (Durai et al., 2015; Zanchett & Oliveira-Filho, 2013). Endotoxin participation in toxic shock syndrome may increase the hepatic damage triggered by hepatotoxins (Choi & Kim, 1998). They have been characterized from the genera *Anabaena*, *Microcystis*, *Oscillatoria*, *Phormidium*, *Schizothrix*, *Synechococcus*, and *Synechocystis*. Three of this genera *Anabaena*, *Microcystis*, and *Oscillatoria* often occur in eutrophic water bodies many of which are used for water supply and recreation (Sivonen & Jones, 1999). Cyanobacteria endotoxins appear to cause skin rashes, as well as gastrointestinal, respiratory and allergic reactions (See Stewart et al., 2006 for a review on cyanobacterial lipopolysaccharides and human health).

Most significant reduction of endotoxins at water treatment facilities took place at early stages of treatment, during coagulation, settling, and sand filtration (Rapala et al., 2002). Activated carbon filtration either increased or had no effect on endotoxin concentration while ozonation and chlorination had little effect on the endotoxin concentrations (Rapala et al., 2002) (Table 1).

Table 1. Cyanotoxin classification, health effects, production and break down regulation, and treatment.

	Types	Common isoforms	Health effects	Producing genera	Production regulation	Break down regulation	Effective treatments
Dermatotoxins	LYNGTX, APLYTX		Inflammatory agents, tumor promotes	<i>Lyngbya, Schizotrix, Oscillatoria</i>	Limited information available	Limited information available	
	DAT		Cause irritant pustular folliculitis, tumor promoters	<i>Lyngbya, Oscillatoria, Schizothrix</i>	Limited information available	Limited information available	
Hepatotoxins	CYN	CYN, 7-deoxy-CYN, 7-dCYN	Damage adrenal glands, kidneys, liver, lungs, heart, stomach, vascular and the lymphatic systems	<i>Anabaena, Aphanizomenon, Cyndrospermopsis, Raphidiopsis, Umezakia</i>	Light intensity, and sulfate and phosphate availability	Slowly 4°C - 50°C, pH 7. At 50°C, 57% degrades to original concentration after 10 wks	Activated carbon, ozon, chlorination
	MC	> 90 isoforms	Liver injury, hemorrhage, necrosis, promotes tumor formation	<i>Anabaena, Planktothrix, Nostoc, Oscillatoria</i>	Nitrogen, phosphorous and iron availability, temperature, light, and pH	Slowly at 40 °C, pH <1 - >9. Half-life 40 °C, pH 1 is 3 wks. In full sunlight slowly, half-life 10 wks	UV irradiation
	NOD	10 isoforms	Liver necrosis, hemorrhage, promote tumor formation	<i>Nodularia, Nostoc</i>	Light intensity and nitrogen and phosphorus levels	Levels drop at full sunlight	

Note: **AER:** Aeruginosin, **ANTX-a(s):** Anatoxin-a(s), **AP-B:** Anabaenopeptin B, **AP-F:** Anabaenopeptin F, **CyPep:** Cyanopeptolin, **CYN:** Cyndrospermopsis, **dc-NEO:** Decarbamoylneosaxitoxin, **dc-STX:** Decarbamoylsaxitoxin, **GTX:** Gonyautoxin, **MC:** Microcystin, **Neo-STX:** Neosaxitoxin, **NOD:** Nodularins, **STX:** Saxitoxin.

Table 1. Continued.

	Types	Common isoforms	Health effects	Producing genera	Production regulation	Break down regulation	Effective treatments
Neurotoxins	ANTX	ANTX-a, HomoAnTx, ANTX-a(s)	Block neuromuscular activity. Paralytic shellfish poisoning (PSP)	<i>Anabaena</i> , <i>Aphanizomenon</i> , <i>Cylindrospermum</i> , <i>Microcystis</i> , <i>Oscillatoria</i> , <i>Planktothrix</i> , <i>Raphidiopsis</i>	Growth phase, light intensity, nitrogen source, and temperature	UV-B irradiation at high pH 7-9.5, 100°C 1 h	UV-B irradiation
	SXT	~27 isoforms, most common are SXT, neo-SXT, GTX, C-TX	Cause convulsions, muscle weakness, salivation, and death by respiratory paralysis if ingested in sufficient amounts. PSP	<i>Anabaena</i> , <i>Aphanizomenon</i> , <i>Planktothrix</i> , <i>Cylindrospermopsis</i> , <i>Lyngbya</i> , <i>Scytonema</i>	Limited information available. Possible correlation between variations in cellular Na ⁺ and saxitoxin production	Saxitoxin are very stable, lasting up to 18 months without loss of potency at temperatures of -80°C, -20°C, 4°C, and 37°C	
Endotoxins			Potent immunomodulatory and immunotoxic products	<i>Anabaena</i> , <i>Microcystis</i> , <i>Oscillatoria</i> , <i>Phormidium</i> , <i>Schizothrix</i> , <i>Synechococcus</i> , <i>Synechocystis</i>			Coagulation, settling, sand filtration

Note: **AER:** Aeruginosin, **ANTX-a(s):** Anatoxin-a(s), **AP-B:** Anabaenopeptin B, **AP-F:** Anabaenopeptin F, **CyPep:** Cyanopeptolin, **CYN:** Cylindrospermopsin, **dc-NEO:** Decarbamoylneosaxitoxin, **dc-STX:** Decarbamoylsaxitoxin, **GTX:** Gonyautoxin, **MC:** Microcystin, **Neo-STX:** Neosaxitoxin, **NOD:** Nodularins, **STX:** Saxitoxin.

Cyanotoxin Regulation and Control

In response to the potential hazards to human health, many countries have followed the provisional guideline proposed by the World Health Organization (WHO) in 1998, and have set maximum acceptable levels of microcystin-LR (MC-LR) concentration in drinking water of $1 \mu\text{g l}^{-1}$, a similar value has been proposed for cylindrospermopsin (CYN) (Burch, 2008; Sivonen & Jones, 1999). Only a few countries, including Australia, Canada, Japan, the United Kingdom, and Brazil have developed national guidelines for cyanobacteria and their toxins in drinking and recreational waters (AWWA, 2016; Chorus et al., 2000).

Although most studies are designed to evaluate the effects of cyanotoxin exposure using a single cyanotoxin, cyanoHABs rarely if ever produce single analog or a single cyanotoxin type (Hudnell & Dortch, 2008). In freshwater environments, organisms are repeatedly exposed to mixtures of cyanotoxins. In Brazil for example, it is common to find *Microcystis aeruginosa* and *Anabaena spiroides* coexisting in freshwater reservoirs, with the potential to produce hepatotoxins and neurotoxins (Dellamano-Oliveira et al., 2007; Freitas et al., 2014; Matsumura-Tundisi & Tundisi, 2005; Moschini-Carlos et al., 2009). Additive or synergistic toxicity of multiple cyanotoxins could result in greater toxicity than what would have been expected from each individual cyanotoxin thus magnifying the risk to public health (Molica et al., 2005).

Since cyanoHABs generally produce multiple analogues of a cyanotoxin type, and analogues tend to differ in toxicity, toxicity equivalents for microcystin-LR (MC-LR) have been developed. The toxicity equivalent (TEQ) scheme weights the toxicity of the less toxic compounds as fractions of the toxicity of the most toxic compound. The reported value provides toxicity information about the mixture of cyanotoxins. To obtain TEQ values the mass of each cyanotoxin in a mixture is multiplied by its toxic equivalency factor (TEF) and is then summed

with all other cyanotoxins to report the total toxicity-weighted mass. TEQ values are useful for risk assessment and management (Safe, 1998). Australia for example, produced a $1.3 \mu\text{g l}^{-1}$ guideline for the total sum of microcystins in a cyanoHAB based on equivalent toxicity of a number of microcystin-LR analogues (Hudnell & Dortch, 2008).

An additional factor influencing the public health risks associated to cyanoHABs are the additive, antagonistic, or synergistic effects between simultaneously occurring cyanotoxins (Dietrich et al., 2008). There have been observations of synergistic and additive interactions, (Best et al., 2002; Freitas et al., 2014; Majsterek et al., 2004; Pinheiro et al., 2016). Mixtures of cyanotoxins have shown greater toxicity than what would be expected from the amount of toxins contained in single cyanotoxin samples (Dietrich et al., 2008). Although antagonistic effects between cyanotoxins are also likely to occur, they have not been reported (Dietrich et al., 2008). Since most cyanobacterial species are capable of synthesizing a wide range of toxins (Sivonen & Jones, 1999), and the co-occurrence of multiple cyanotoxins in freshwater environments is common, studies on the effects of cyanotoxins would be useful to accurately assess the risk of cyanotoxin exposure, however this is beyond the scope of this study.

Cyanobacteria around the World

Initial cyanoHABs reports were anecdotal including an account by General Zhu Ge-Ling, from the Han dynasty of China, who reported losing some of his troops after drinking the green waters of a river that he and his army crossed (Bartram et al., 1999). Other accounts talk about algal blooms in the Souleseat Loch in southwest Scotland (Codd, 1999a). Indigenous people in Africa, the Americas, Australia and Asia, used to dig filtering holes near the edge of the water to filter the green scums present in it, using a technique known as bankside filtration (Bartram et al., 1999).

The first documented cyanobacterial poisoning of livestock in the scientific literature was reported in 1878 and published in the journal *Nature*; this paper described the deaths of cattle and sheep after drinking water from a lake in Australia (Francis, 1878). A report on human intoxication from mussel consumption in the San Francisco area in 1928 led to the description of saxitoxins by Edward Schantz in the 1950's (Carmichael, 2008). The first international conference on toxic dinoflagellate blooms took place in 1974 after the occurrence of red tides in New England (USA) in 1972 (Carmichael, 2008). In 1981, the proceedings from the first international conference on toxic cyanobacteria were published. In 1998, the Harmful Algal Bloom and Hypoxia Research and Control Act was written by the U.S. Senate-Subcommittee on Oceans and Fisheries. In 2003, this act was revised by the House of Representatives to include freshwater algae and harmful cyanobacteria. These accounts reveal human awareness of the HABs and CyanoHABs (Carmichael, 2008).

Some of the countries where cyanoHABs were first reported include Argentina (Odriozola et al., 1984), Australia (Bourke et al., 1983; Byth, 1980; Griffiths & Saker, 2003; Jackson et al., 1984; Negri et al., 1995), Bangladesh, Belgium, Bermuda, Brazil, Canada (Carmichael & Gorham, 1978; Pybus & Hobson, 1986), Chile, China, Czech Republic, Denmark, Egypt, Finland (Eriksson et al., 1989; Sivonen et al., 1989), France, Germany, Greece, Hungary, Italy, Israel, Japan, Jordan, Mexico, Morocco, Netherlands, New Zealand, Norway (Berg et al., 1987), Pakistan, Poland, Portugal, Russia, Saudi Arabia, South Africa, Sweden, Switzerland, Thailand, Ukraine, the United States of America (Jacoby & Kann, 2007; Mahmood et al., 1988; Tisdale, 1931), and the United Kingdom (Bury et al., 1995; Gunn et al., 1992; Pearson et al., 2001). Surveys of cyanobacterial diversity in mangrove wetlands have been conducted in Brazil, Egypt, India, Mexico, Mozambique, Saudi Arabia, South Africa, and Tanzania reporting large

numbers of cyanobacterial species in mangroves (See Alvarenga et al., 2015 for a list of cyanobacterial genera in mangroves per country).

Kosten et al. (2012) collected worldwide data to evaluate the effect of warm temperature and excessive nutrient loads in lakes and reservoirs, on the dominance of cyanobacteria in phytoplankton communities. They analyzed data along a longitudinal gradient from subarctic Europe to southern South America. The team collected samples from 143 coastal lakes, at altitudes between 200 m and 800 m and an average depth of 1.9 m. They included data from 60 lakes in Europe and 83 in South America. According to their findings, the proportion of cyanobacteria in phytoplankton communities increased as temperature increased.

Bonilla et al. (2012) studied what drives the distribution of cyanohABs of *Planktothrix agardhii* and *Cylindrospermopsis raciborskii* in 29 mesotrophic and hypereutrophic lakes, from the temperate zone in Argentina, Hungary, the Netherlands, Uruguay, and the tropical region in Brazil. *C. raciborskii* was always more abundant and tolerate of a wide range of climates while *P. agardhii* was only observed in subtropical and temperate lakes. The authors also found that global warming might favor *C. raciborskii*, in comparison to *P. agardhii*, because *C. raciborskii* grows faster when temperatures become warmer.

Alvarenga et al. (2015) surveyed cyanobacterial diversity in mangrove wetlands worldwide. In these ecosystems, cyanobacterial colonies were detected on aerial and underground roots, decaying wood, leaves, rocks, sediments and trunks, many in association with algae and seagrass. In Brazil, Egypt, India, Mexico, Mozambique, Saudi Arabia, South Africa, and Tanzania, the countries with the largest mangrove areas in the world, evaluations of cyanobacteria nitrogen fixing abilities and surveys of cyanobacterial diversity in mangrove ecosystems have been conducted. Brazil is the country with the most research on mangrove

cyanobacteria (Alvarenga et al., 2015; Genuario et al., 2015; Rigonato et al., 2012, 2013). In Australia, Indonesia, Malaysia, Mexico, and Nigeria the functionality and the diversity of mangrove cyanobacteria is still understudied (Alvarenga et al., 2015).

Svirčev et al. (2017) reviewed world data to evaluate human health incidents associated with exposure to cyanoHABs, and to identify the occurrence and role of microcystins in these events. The authors reviewed forty-two papers, published between 1960 and 2016. These studies described thirty-three cases of poisonings by cyanotoxins in eleven countries, Australia, Brazil, Canada, China, Namibia, Portugal, Serbia, Sri Lanka, Sweden, the United Kingdom, and the United States of America. At least thirty-six publications showed a direct connection between cyanobacteria and cyanotoxins, and negative human health effects.

Cyanobacteria in South America

Though cyanoHABs are a common phenomenon around the world, there is still limited regional knowledge for South America (Dörr et al., 2010; Pérez et al., 2013; Sant'Anna et al., 2008). Although systematic nationwide monitoring of cyanoHABs in the region is scarce, multiple scientific publications have documented the occurrence of the phenomenon and show that it has become recurrent. Unfortunately, due to limited analytical capabilities of most countries in the region, the risks imposed by cyanoHABs to public health are understudied (Pérez et al., 2013).

In recent years, the international demand for commodities has prompted massive expansion of agricultural activities in most of South America (Grau & Aide, 2008; Pérez et al., 2013). Given the rising population and quick economic development of the region, as well as the subsequent increase in extensive agricultural and industrial activities, specific environmental studies for the region are needed.

Furthermore, in South America numerous water impoundments and hydropower dams are being built which in combination with climate change can increase eutrophication and the subsequent occurrence of cyanoHABs (Zarfl et al., 2015). Since few countries in the region have legislation and other mechanisms to manage and control cyanoHABs, the entire region faces monitoring and research limitations (Pérez et al., 2013).

Dörr et al. (2010) reviewed the occurrence of cyanoHABs and microcystins in Argentina, Brazil, Chile, and Uruguay using published literature from the previous fifteen years. Their goal was to summarize regional understanding of toxic cyanobacteria and microcystin production, and their consequences for the environment and human health. They found that microcystin variants are produced by multiple species, and identified ten variants for the region: microcystin-LR, microcystin-RR, microcystin-FR, microcystin-YR, microcystin-AR, microcystin-LF, microcystin-hRhR, [Asp3]-microcystin-LR, [Asp3]-microcystin-YR and [Leu1]-microcystin-LR. This high level of microcystin diversity is possible, because for each cyanotoxin type, there are multiple analogues, and analogues differ in toxic potency. While chemical synthesis of specific toxins could be important to study chronic exposure, the economic cost of this approach makes it unattainable for South America.

Werner et al. (2012) studied the morphological and molecular characteristics of *Sphaerospermopsis torques-reginae* of the order *Nostocales* in eight water bodies in Argentina, Brazil, and Colombia. *Sphaerospermopsis torques-reginae* was first described by Komárek in 1984 as *Anabaena torques-reginae*. The species was observed in Argentina and Brazil, and in a Colombian tropical swamp. These findings reveal a wide distribution of *A. torques-reginae* in the region, from temperate Argentina to tropical Colombia, in diverse environmental conditions.

Nostocales are important microcystin producers, because they reach high biomasses in relatively short times, and produce high levels of neurotoxins their presence compromises freshwater quality.

Cyanohab Guidelines, Policies, and Regulations in South America

In spite of the substantial hydrologic potential of South America which is home to half of the world's water supply (Bonilla, 2009), high population growth rates, and accelerated impoundment construction, only a few countries of the region have developed legislation regarding cyanobacteria, cyanoHABs, and cyanotoxins in water destined for human use.

Brazil and Uruguay follow the guidelines and regulations established by the WHO in terms of water management and monitoring of cyanobacteria and cyanotoxins in drinking and recreational waters (WHO, 2003). Both countries have adopted the WHO guideline values for microcystin-LR in drinking and recreational waters.

In Brazil, the Lei Portaria do Ministério da Saúde 1469, from 2000 and the Resolução 274 from 2000 from the Conselho Nacional de Meio Ambiente (CONAMA, 2000a) and the Ministério da Saude set the maximum acceptable values for cyanobacterial density and cyanotoxin concentration. If the number of cyanobacterial cells in a reservoir is greater than 20,000 cells ml⁻¹, cyanotoxin analysis and toxicity tests in mice are required. Standards for recreational waters in Brazil are still under development (Azevedo Lopes et al., 2016).

Uruguay uses organoleptic characteristics (i.e. smell, color, and taste) to estimate potability, but this practice does not guarantee the water is safe to drink. No specific toxicity values have been set. The guidelines for microcystin-LR in recreational waters are pending approval by the Uruguayan legislation (Vidal & Britos, 2012; Ibelings et al., 2014). The Comisión Administradora del Río Uruguay (Managing Commission of the Uruguay River)

(Zanoniani et al., 1988), a transboundary agreement between Argentina and Uruguay, does not mention cyanoHABs, cyanobacteria, or cyanotoxins. Uruguay's Decree 253 of 1979 (IMPO, 2019), which aims to prevent environmental pollution by preventing water pollution does not address cyanoHABs.

In Argentina, a book called “Cianobacterias como Determinantes Ambientales de la Salud” was published by the Departamento de Salud Ambiental (Environmental Health Department), a branch of the Dirección Nacional de Determinantes de la Salud (National Health Determinants Office), an updated edition was published digitally in 2017. The book summarized the available knowledge on cyanobacteria, and cyanoHABs around the world and in Argentina, and proposed the development of local and national norms to monitor cyanobacteria in drinking and recreational waters (Giannuzzi et al., 2011). In 2016, the Argentinian ministry of health approved the Resolución 1949, developing a guideline for cyanobacterial exposure, cyanobacteria in water and their health effects. This regulation follows the standards proposed in 1998 and 2003 by the WHO, and includes cyanobacteria genera identified in the country, cyanotoxin impacts in human health, treatment when exposure is suspected, risk factors to exposure, and guideline values to handle bath water containing cyanobacterial cells (AMS, 2016). Before this regulation was in place, treatment plants in the country are required to routinely monitor water reservoirs, however, few plants actually complied and enforcement was limited (Otaño et al., 2012).

In Colombia, regulating the quality of drinking for human consumption is a shared duty of the Ministerio de Protección Social (Ministry of Social Protection) and the Ministerio de Ambiente, Vivienda, and Desarrollo Territorial (Ministry of Environment, Dwelling, and Territorial Development). In 2007, two decrees focused on drinking water were issued, the

Decree 1575 and the Decree 2115. Decreto 1575 established the Sistema de Información de la Vigilancia de la Calidad del Agua para Consumo Humano - SIVICAP (Information System for the Monitoring of Quality of Water for Human Consumption) (MPS, 2007a, 2007b) and Decreto 2115 which established the characteristics, the control mechanisms, and frequencies to monitor drinking water. Neither regulation included cyanobacteria, cyanotoxins or cyanoHABs (Minambiente, 2019; MPS, 2007a, 2007b).

Methods

This review examined the literature available on cyanoHABs, cyanobacteria, and cyanotoxins for South America. Eleven countries (i.e. Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Guyana, Paraguay, Peru, Uruguay, and Venezuela) and one French overseas territory (i.e. French Guiana) were included. Suriname were not considered due to the lack of peer reviewed contributions on the subject available digitally. Primarily, contributions in English were used, but articles in Spanish, Portuguese, and French were also considered.

Different keyword combinations in English, Spanish, and Portuguese were used to find scientific publications. The words cyanobacteria, cyanotoxins, and microcystins (the most common cyanotoxin) plus the name of the country were used in the search. Publications were retrieved from the Web of Science database and the Google Scholar search engine. The search results included peer review articles, theses, dissertations, white papers, and scientific reports. Search limits were set from 1900 until 2018.

Mainly publications examining fresh water bodies used for drinking water or recreation were used, although publications in other environments were considered for the overview, they were not included in results, discussion, or conclusions. Detailed results focused on the countries with the most research regarding cyanobacteria, cyanotoxins, and cyanoHABs in the region,

those countries were Brazil, Argentina, Uruguay, and Colombia. Detailed literature reviews and summaries of the information found for these countries are presented.

Results

Publication Output in South America

The first scientific reference to cyanobacteria in aquatic environments in South America dates back to 1940s, when thousands of farmed ducks died in Argentina (Kühnemann, 1966). Cyanobacteria were reported in 1971 in the Ciénaga Grande de Santa Marta (CGSM), Colombia (Squires & Riveros, 1971). During the 1980s, the occurrence of cyanoHABs in the oyster farming operations and lakes in the Magdalena department continued (Von Cosel, 1986). But the first peer-reviewed article digitally accessible was published in 1990 (Hernández & Gocke, 1990).

A total of 167 publications citing the terms cyanobacteria, cyanotoxins, or microcystins were published in South America between 1990 and 2018. In the 1990s, 16 articles related to cyanobacteria were published. The following decade, this number increased by more than three times, with 51 papers published. The 2010s was the most prolific decade for cyanobacteria related research with 100 publications. Brazil and Argentina were always the greatest contributors (Fig. 1).

The countries with the most research and documentation on cyanobacteria, cyanotoxins, and cyanoHABs were Brazil (286 publications), Argentina (68 publications), Uruguay (39 publications), and Colombia (35 publications). Articles from these four countries accounting for 87.5% of the publications on the subject written in the region (Fig. 1).

Chile had 20 publications, Ecuador 15, Bolivia and Venezuela had eight publications each, Paraguay and Peru had seven, and French Guiana had six. The country with the lowest number of publications citing cyanobacteria was Guyana with only two papers.

After selecting for publications exclusively examining fresh water bodies used for drinking water or recreation the number of publications declined. The countries with the most publications were Brazil with 72 subject specific articles, Argentina with 30, Uruguay with 25, and Colombia with 19. Six publications were available from Chile, five from Ecuador, Bolivia and Paraguay had three publications each, Venezuela had two, and both Peru and French Guiana had one contained useful information. No publications from Guyana addressed cyanobacteria or their toxins in fresh water environments.

The earliest available report of a toxic *Microcystis sp.* cyanoHAB in Chile is from the 1990s in the Biobío region (Campos et al., 1999). Several studies conducted in fresh water lagoons found that the most common cyanobacteria genera in the country were *Anabaena sp.*, *Microcystis sp.*, *Oscillatoria sp.*, and *Spirulina sp.* CyanoHABs were frequently dominated by *Anabaena* and *Microcystis* (Almanza et al., 2016a; Almanza et al. 2016b; Campos et al., 2005; Neumann et al., 2000). The most frequent cyanotoxins were microcystins (MCs) (Campos et al., 1999; Campos et al., 2005; Neumann et al., 2000) and one variant, microcystin-RR (MC-RR), was identified (Almanza et al., 2016a; Almanza et al. 2016b). Nimptsch et al. (2016) reported the occurrence of *Microcystis sp.* and *Dolichospermum sp.* cyanoHABs in oligotrophic lakes in northern Patagonia, as well as the presence of *C. raciborskii* for the first time in Chile.

The presence of cyanobacteria in Ecuador was first reported in the Guayas River Estuary (de Arcos, 1982; Twilley et al., 2001). The cyanobacterial species *Aphanocapsa sp.*, *Merismopedia sp.* (Van Colen et al., 2017a), and *Cylindrospermopsis sp.* (Van Colen et al.

2017b) were reported in glacial lakes, while *Microcystis aeruginosa*, *Lyngbya sp.*, and *Oscillatoria phormidium* were identified in eutrophic tropical lakes (Gómez Rosero, 2017). The first peer-review report of *C. raciborskii* in the country comes from the Limoncocha Lagoon in the Amazonas River basin (Venegas et al., 2018).

In Bolivia cyanobacteria were identified in in alpine streams (McClintic et al., 2003), in a hypereutrophic urban lake (Morales et al., 2017), in Lake Titicaca (Komarkova et al., 2016). The most common species in the country were *M. aeruginosa*, *Oscillatoria subbrevis*, and *P. agardhii* all known cyanotoxin producers. The earliest reference of cyanobacteria in Venezuela is from a study in the Orinoco River, though the study mentioned the presence of nitrogen-fixing cyanobacteria (Hamilton & Lewis, 1992). Cyanobacteria dominated the phytoplankton community of the Pao-Cachinche Reservoir, the dominant species were *C. raciborskii*, *Synechocystis aquatilis*, *Lyngbya limnetica*, *Limnothrix sp.*, *Microcystis spp.*, *Dactylococcopsis acicularis* and *Raphidiopsis curvata* in the central-north region of Venezuela. *Microcystis sp.* and *S. aquatilis* cyanohab were reported (González et al., 2004).

Scientific literature for cyanobacteria in Paraguay started in the 2010s, de Zaburlín et al. (2013) identified the genera *Anabaena sp.*, *Aphanocapsa sp.*, *Chroococcus sp.*, *Lyngbya sp.*, *Merismopedia sp.*, *Microcystis sp.*, *Oscillatoria sp.*, *Planktothrix sp.*, *Pseudanabaena sp.*, and *Raphidiopsis sp.* in the Yacyretá Reservoir. Cyanohab also occurred in the Ypacaraí Lake, where thirteen cyanotoxin producing species were identified, been *C. raciborskii* and *M. aeruginosa* the most common (Benítez Rodas et al., 2017). Cyanobacteria dominated the phytoplankton community of Verde River a tributary of the Paraguay River (Facetti-Masulli et al., 2012).

A single publication for French Guiana and Peru contained information pertaining to this review. The study for French Guiana was conducted in Petit Saut Reservoir, in the Amazon rain forest, where cyanobacteria were abundant in the surface layer and *Oscillatoria neglecta* was the dominant species (Dumestre et al., 2002). While the Peruvian study was conducted in a high-altitude wetland and *Anabaena sp.*, *Lyngbya sp.*, *Nostoc sp.*, and *Oscillatoria sp.*, were identified as the dominant genera (Salazar-Torres & Huszar, 2012).

Only 146 publications from Argentina, Brazil, Colombia, and Uruguay, the countries with the most per review contributions were considered for the analysis, discussion, and conclusions. Seventy-two publications were available for Brazil, they included all five regions of the country. Most publications were written for the Northeast and Southeast regions. In Argentina, 30 publications were available, most of them for the Central region, four of the eight regions of the country were covered. Twenty-five publications were written for Uruguay, most of them for the Metropolitan region, no papers were available for the Central-south region. In Colombia 19 publications were available, mainly for the Andean and Caribbean regions.

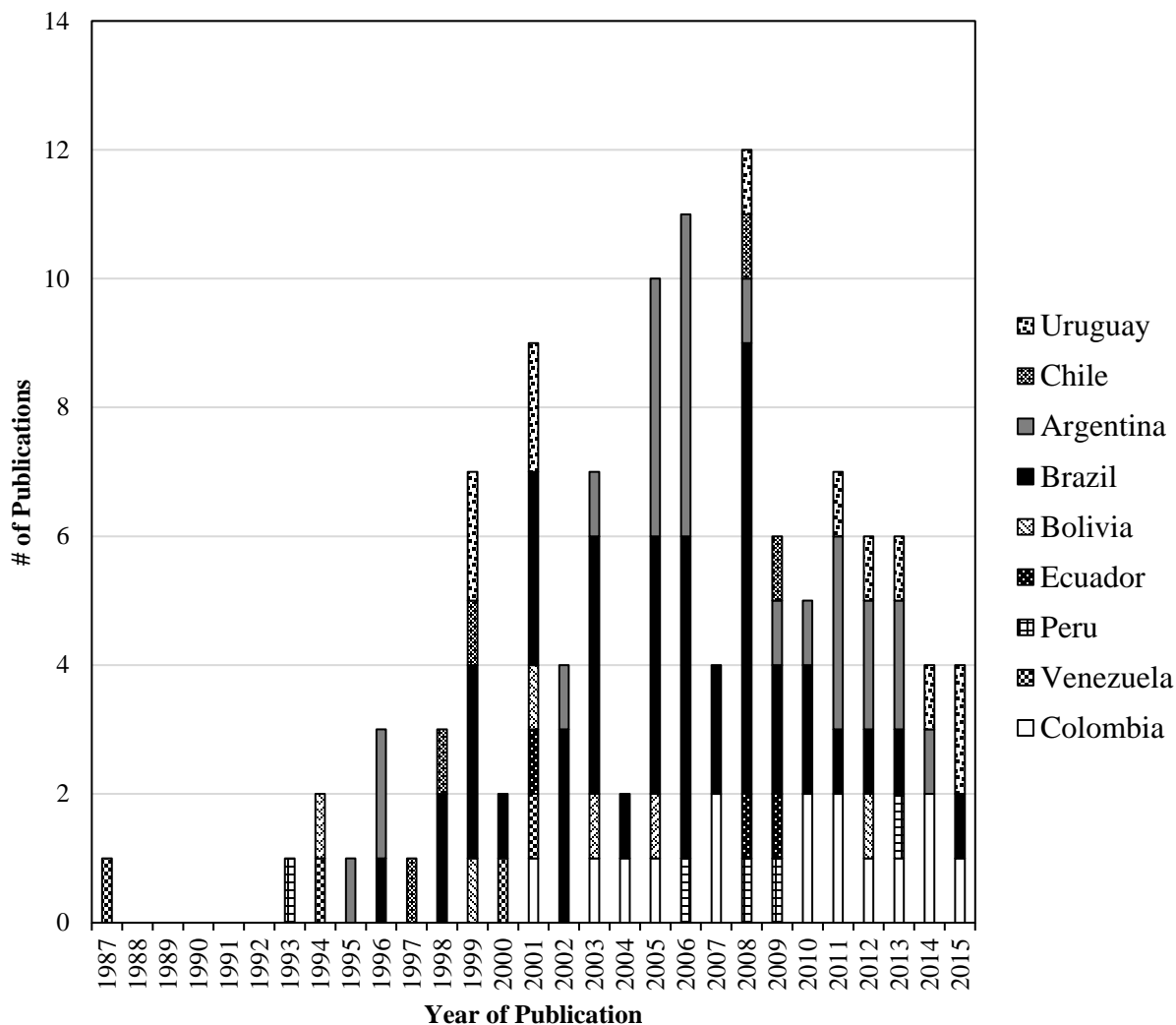


Figure 1. Number of cyanobacteria related publications per country since 1980.

Cyanobacteria Genera and Species Reported in South America

A total of 50 cyanobacterial genera were reported in South America. The genus *Microcystis* was reported by 61% of the publications (89 out of 146), making it is most common genus in the region, with 12 species identified. *Anabaena* was the second most common genus, it was mentioned in 18.5% of the publications (27 out of 146), and 9 species were identified. The third most common genera were *Dolichospermum* and *Planktothrix*, each of them reported by

16.4% (24 out of 146) of the articles available. For *Dolichospermum* 11 species were identified and only two for *Planktothrix sp.*

Other frequently reported genera were *Pseudanabaena*, *Aphanizomenon*, *Oscillatoria*, and *Anabaenopsis* reported by 13% (19 out of 146), 11.6% (17 out of 146), 9% (13 out of 146) and 7% (10 out of 146) of publications respectively. The genera *Alkalinema*, *Amazoninema*, *Brasilonema*, *Cephalothrix*, *Cyanobium*, *Gloeocapsa*, and *Limnococcus* and the species *Limnothrix planctonica*, *Anacystis cyanea*, *Asterocapsa submersa*, and *Dolichospermum uruguayense* were only reported by a one publication making them the rarest genera and species identified in South America.

Microcystis aeruginosa (29.5%, 43 out of 146) and *Cylindrospermopsis raciborskii* (26%, 38 out of 146) were the most commonly reported species in the region. Seven species of *Microcystis* were identified, and the genus was present in in Argentina (Fernández et al., 2015; Giannuzzi et al., 2011), Brazil (Carvalho et al., 2008; dos Anjos et al., 2006; Fonseca & Bicudo, 2008; Hirooka et al., 1999; Moschini-Carlos et al., 2009; Piccin-Santos & do Carmo Bittencourt-Oliveira, 2012; von Sperling et al., 2008; Yunes et al., 1996, 1998), Colombia (Herrera et al., 2014, 2015; Tapia Larios, 2014), and Uruguay (Kruk et al., 2002).

Ten species of *Anabaena* were identified in the region, the genus was present in Argentina (Fernández et al., 2015; O'Farrell et al., 1996, 2015; Pizzolon et al., 1999; Ringuelet et al., 1955), Brazil (Becker et al., 2010; Carvalho et al., 2008; Elias et al., 2015; Molica et al. 2005, Moschini-Carlos et al. 2009; Soares et al., 2006; Walter et al., 2018), Colombia (Mogollón et al., 2014; Tapia Larios, 2014; Palacio et al., 2015a; González & Gómez, 2010; Silva et al., 2018) and Uruguay (Kruk et al., 2002) (Table 6).

Nine species were reported for the genus *Dolichospermum* in the region, it was identified in Argentina (O'Farrell et al., 2015), Brazil (Lins et al., 2016; Piccin-Santos & do Carmo Bittencourt-Oliveira, 2012), Colombia (Herrera et al., 2015; Palacio et al., 2015) and Uruguay (Bordet et al., 2017, González-Madina et al., 2017, 2019; González-Piana et al., 2017; Ferrari et al., 2011; O'Farrell, 2012; Kozlíková-Zapomělová et al., 2016; O'Farrell & Izaguirre, 2014).

Four species of *Planktothrix* were identified, the genus was documented in Argentina (Fernández et al., 2015; O'Farrell et al., 2015), Brazil (Aragão-Tavares et al., 2017; Assis et al., 2018, Barros et al., 2017; Bittencourt-Oliveira et al., 2012; da Silva et al., 2017; de Oliveira et al., 2015; dos Anjos et al., 2006; Lins et al., 2016; Moschini-Carlos et al., 2009; Moura et al., 2011; Schlüter et al., 2018; Vieira et al., 2015), Colombia (González & Gómez, 2010), and Uruguay (Aguilera et al., 2017; Kruk et al., 2002; Scasso et al., 2001; Sommaruga, 1995).

Cyanotoxins Reported in South America

Less than 40% (55 out of 146) of all the publications available reported the presence of cyanotoxins. Eight cyanotoxins types were reported: microcystin (MC), saxitoxin (STX), anatoxin (ANTX), cylindrospermopsin (CYN), nodularin (NOD), aeruginosin (AER), anabaenopeptin (AP), and cyanopeptolin (CyPep) (Table 6).

Microcystin (MC) was the most commonly identified cyanotoxin, it was reported by 34.2% (50 out of 146) of the publications. Four microcystin variants were identified: microcystin-LC (MC-LC), microcystin-LR (MC-LR), microcystin-RR (MC-RR), and microcystin-YR (MC-YR).

Reported by 7.5% (11 out of 146) of the articles, saxitoxins (STXs) were the second most reported cyanotoxins. For variants were identified: decarbamoylneosaxitoxin (dc-NEO), decarbamoylsaxitoxin (dc-STX), and neosaxitoxin (Neo-STX), and gonyautoxins (GTX). And

five gonyautoxin variants were documented: GTX 1, GTX 2, GTX 3, GTX 4, and GTX 6. STXs were mentioned by 15.1% of the papers reviewed (22 out of 146). Two articles reported the presence of microcystins and saxitoxin equivalents, but no details were provided. Three rare and understudied cyanotoxins: aeruginosin (AER), anabaenopeptin (AP), and cyanopeptolin (CyPep), were reported by a single publication (Table 6).

Only 28.1% (41 out of 146) of the regional publications reported testing and identifying cyanotoxins. Out the publications that reported the presence of cyanotoxins, 17.1% (25 out of 146) identified a single cyanobacterial species, 11% (16 out of 146) identified two or more species, and 6.2% (9 out of 146) reported three or more species. Since most cyanobacterial species are capable of synthesizing a wide range of toxins, the co-occurrence of multiple cyanotoxins in reservoirs affected by cyanoHABs is to be expected (Tables 2-6).

In Argentina, only microcystin (MC) (50%, 15 out of 30) and nodularin (NOD) (3.3%, 1 out of 30) were reported. In Colombia, only microcystin was reported (10.5%, 2 out of 19), while in Uruguay microcystin (MC) (16%, 4 out of 25), cylindrospermopsin (CYN) (4%, 1 out of 25), and saxitoxins (STX) (4%, 1 out of 25) were identified.

In Brazil, 44.4% (32 out of 72) of the publications tested for cyanotoxins, and 43.1% (31 out of 72) confirmed their presence. Eight cyanotoxins types (i.e. aeruginosin (AER), anabaenopeptin (AP), anatoxin (ANTX), cylindrospermopsin (CYN), cyanopeptolin (CyPep), nodularin (NOD), microcystin (MC), and saxitoxin (STX)), and 14 variants were identified. Microcystin (MC) (25%, 18 out of 72) and saxitoxin (STX) (11.1%, 8 out of 72), were the most commonly reported cyanotoxins (Table 2).

In Argentina, 30% of the publications (9 out of 30) reported the presence of just one cyanotoxin, and 16.7% (5 out of 30) reported two cyanotoxin types. Mixtures of more than three

cyanotoxin types were not reported for the country. A single cyanotoxin was reported by 23.6% (17 out of 72) of the Brazilian publications, while 19.4% (14 out of 72) reported two or more cyanotoxin types, and 15.3% (11 out of 72) reported mixtures of three or more cyanotoxins. Merely 5.3% (1 out of 19) of the Colombian publications reported the presence of a single cyanotoxin. In Uruguay, 28% (7 out of 25) of the publications reported testing for cyanotoxins, and 20% (5 out of 25) detected them. Only 16% (4 out of 25) identified a single cyanotoxin, and 4% (1 out of 25) reported a cylindrospermopsin and microcystin mixture.

Brazil

In Brazil, research on cyanobacteria and their toxins started in the mid-1980s, when several cyanoHABs of *Microcystis aeruginosa* were observed and documented in Patos Lake in the state of Rio Grande do Sul. This is the second largest coastal lagoon in South America and the biggest in Brazil (Torgan, 1989; Yunes et al., 1996, 1998). By 1992, *M. aeruginosa* was reported to be the most common cyanobacteria in eutrophic lakes and reservoirs across the country (Hirooka et al., 1999). The first peer reviewed work about cyanobacteria was published by Teixeira et al. (1993) about a cyanoHAB that took place in the Itaparica Dam in Pernambuco state and affected the Paulo Afonso municipality in Bahia state in the Northeast region.

Given the inherent difficulty of detecting microcystin-producing cyanobacteria in the field, and how prevalent cyanoHABs have become in Brazil, do Carmo Bittencourt-Oliveira (2003) examined molecular methods for detecting cyanobacteria in Brazilian reservoirs. This study was conducted in 15 reservoirs in the Northeastern, South and Southeastern regions and examined 60 *Microcystis* strains. Eight reservoirs contained toxin producing strains, Duas Unas in Pernambuco state in the Northeast and Cantareira in São Paulo state in the Southeast are mainly used for water supply. While Barra Bonita and Salto Grande in São Paulo state, and Três

Marias and Central in Minas Gerais state, and Jacarepaguá in Rio de Janeiro state in the Southeast are used for recreation and energy generation. Iraí supplies water to the city of Curitiba in Paraná state in the South.

Sant'Anna et al. (2008) reviewed the available literature for Brazil including scientific papers, technical reports, thesis, communications, as well as unpublished data by water treatment companies, and confirmed the presence of cyanotoxins using enzyme-linked immunosorbent assay (ELISA), high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and matrix Assisted laser desorption/ionization (MALDI-TOF). They identified thirty-two species of toxic cyanobacteria. The subtropical areas of the country had a higher diversity of cyanobacteria than the tropical areas. *M. aeruginosa* and *C. raciborskii* were the most widespread species. The genera *Anabaena* and *Microcystis* had the highest numbers of species. *Anabaena* cyanoHABs were mainly restricted to subtropical areas, while *Microcystis* cyanoHABs occurred in tropical and subtropical areas. *Radiocystis fernandoi* only formed cyanoHABs in the tropical areas of Brazil.

Bicudo and Menezes (2010) published the Catálogo de Plantas e Fungos do Brasil which included cyanobacteria, and Menezes et al. (2015) updated of the list of algae and cyanobacteria for the country. In the updated catalog, cyanobacteria had the highest increase in number of species in the five-year period. The genus *Scytonema* was the most species-rich genus of cyanobacteria. The Southeast and South regions had the most species diversity (Table 2).

Regional Studies

Central-west Region

Mato Grosso do Sul (MS) State

Santos and Sant'anna (2010) identified 21 species of cyanobacteria from three types of lagoons representative of the Pantanal da Nhecolândia. The order *Oscillatoriales* had the highest species richness, and a few *Anabaenopsis elenkinii* cyanoHABs were only observed during the dry season. Andreote et al. (2014) identified twenty-eight nonheterocytous cyanobacterial strains organized within the orders Chroococcales, Synechococcales, Pseudanabaenales, and Oscillatoriales in the Pantanal de Nhecolândia. Genuario et al. (2017a) examined cultured nitrogen-fixing heterocytous cyanobacteria from the Pantanal wetland, and found 14 heterocytous cyanobacterial strains belonging to the order Nostocales, specifically the genera *Nostoc*, *Anabaenopsis*, and *Tolypothrix*.

Mato Grosso (MT) State

Assis et al. (2018) created the inventory of cyanobacteria and microalgae for the Chapada dos Guimarães National Park (CGNP). Their review included taxonomic and ecological inventories from peer review, course-completion papers, dissertations, and theses with species lists of cyanobacteria and microalgae from CGNP. They also conducted their own inventory in ten stations in the region, and analyzed 44 plankton samples from the Claro, Coxipó, Coxipozinho, Paciência, and Mutuca Rivers.

North Region

Amazonas (AM) State

Genuario et al. (2017b) collected water samples from the Amazon and Solimões Rivers, and isolated eleven homocytous cyanobacterial strains, identifying a new cyanobacterial genus

Amazoninema. The first genus erected from morphotypes isolated exclusively from Amazonian rivers in Brazil. In a different collaboration, using samples from the Solimões River Genuario et al. (2018b) concluded that the *Cronbergia amazonensis*-cluster is part of the typical *Cronbergia* lineage.

Pará (PA) State

Radiocystis fernandoi strain SPC714, a microcystin producing cyanobacterium, was isolated from water samples collected in the Utinga Reservoir, which is the main drinking water supply for the city of Belém (Vieira et al., 2003). Vieira et al. (2005) also studied cyanobacteria and microcystins in the Utinga Reservoir. Thirty-nine strains of cyanobacteria were isolated, 43.6% of them are capable of producing hepatotoxic microcystins, the genera *Aphanizomenon*, *Microcystis*, *Nostoc*, *Oscillatoria*, *Planktothrix*, and *Radiocystis* were some of the most common. Microcystins were only detected during the dry season.

Northeast Region

Moura et al. (2018) reviewed the occurrence of cyanoHABs in the Northeast. Using Google Scholar, they obtained data from published articles from 1930 to 2016. They produced a list of dominant species recorded per state, including the distribution and occurrence of cyanoHABs involving one or more species. One hundred two publications were chosen, all including information regarding distribution, species richness, biomass, and density. Forty-nine species dominated the region. The state of Pernambuco had the greatest representation (30 species). The most common genera were *Microcystis*, *Cylindrospermopsis*, *Planktothrix*, *Dolichospermum* (= *Anabaena*), and *Geitlerinema*. The most common species were *C. raciborskii*, *M. aeruginosa*, and *P. agardhii*.

Bahía (BA) State

In the city of Paulo Afonso, nearly 2,000 gastroenteritis cases were reported and 88 resulted in death. All cases were registered over a 42-day period. Clinical data and water testing revealed that the cause of the outbreak was a toxin produced by a cyanoHAB in the Itaparica Dam (Teixeira et al., 1993). Affe et al. (2018) conducted metagenomic analysis of the cyanobacteria present in Camamu Bay. Samples were collected during spring tides during two rainfall periods. A total of 219 cyanobacteria taxa were identified. The genera *Synechococcus* and *Prochlorococcus* had the greatest relative abundance. Because of its large mouth, the opening of Camamu Bay has a strong marine influence. However, the freshwater contribution during the rainy season increased the species richness of the cyanobacterial community.

Ceará (CE) State

Borges et al. (2015) isolated cyanobacteria and cyanotoxins from samples from the Mundaú River basin. Monitoring included the Araripe Environmental Protection Area (AEPA). Forty-five strains were isolated, 24 from the Mundaú basin and 21 from AEPA. Benthic cyanobacteria were present in nine of the monitoring sites. This was the first report of saxitoxins production for *Geitlerinema amphibium*, *G. lemmermannii*, and *Cylindrospermum stagnale* and the first report of *Phormidium uncinatum* for Brazil. Barros et al. (2017) studied the impact of filamentous cyanobacteria in the Sítios Novos and Acarape do Meio Reservoirs in Bahía state. ELISA analysis revealed the presence of saxitoxins in both reservoirs, which was confirmed by bioassays in Swiss mice.

Paraíba (PB) State

Barbosa and Mendes (2005) studied the phytoplankton community of the Acauã Reservoir officially known as the Argemiro de Figueiredo Reservoir. The reservoir supplies

drinking water to Campina Grande City and the surrounding municipalities. It is also used for caged fish farming. It also receives domestic, industrial, and agricultural effluents. Cyanobacteria contributed the least to the phytoplankton density in the monitoring period, and the most abundant species was *C. raciborskii*. Lins et al. (2016) conducted 22 samplings in Argemiro de Figueiredo Reservoir. Since it was dammed in 2001, it has had multiple cyanoHABs, most of them dominated by *C. raciborskii* and *M. aeruginosa*. Walter et al. (2018) investigated water quality in five reservoirs close to Campina Grande, three of these ponds are routinely used for human consumption. The other two, are located in hard to reach areas and have low water levels.

Pernambuco (PE) State

After routine treatment, 100 patients showed symptoms of acute neurotoxicity and sub-acute hepatotoxicity and 76 died. Lethal exposure to cyanotoxins was confirmed in 52 of these patients (Hirooka et al., 1999; Yuan et al., 2006). Upon further investigation, microgram to milligram levels of microcystins were found in the filters of the facility, and in the sera and hepatic tissues of the patients (Carmichael et al., 2001; Jochimsen et al., 1998). Water used in the facility normally came from the Tabocas Reservoir, and at the time of the incident, this source water showed signs of cyanoHAB. This was the first confirmed case of human fatalities linked to cyanotoxins, and the first case of fatal microcystins toxicity in a hemodialysis facility in the world (Azevedo et al., 2002; Carmichael et al., 2001; Hirooka et al., 1999, Jochimsen et al., 1998; Pouria et al., 1998; Yuan et al., 2006). After this fatal event, national attention was placed in the regulation and control of the presence of cyanobacteria in the drinking water supplies and in hemodialysis facilities.

Molica et al. (2002) sampled the Tabocas Reservoir from 1997 to 1998. *C. raciborskii*, dominant cyanobacterial species, caused a massive cyanoHAB between July and October 1998.

C. raciborskii strain ITEP-018 was isolated in the laboratory, and produced five saxitoxin (STX) analogues, saxitoxin (SXT), gonyautoxin (GTX), decarbamoylsaxitoxin (dcSTX), neosaxitoxin (neo-STX) and a previously unidentified analog. Yuan et al. (2006) used ELISA, liquid chromatography, and liquid chromatography-mass spectrometry (LC/MS), GC/MS and MS/MS to examine human sera and liver samples from diseased patients of the Caruaru Syndrome.

Molica et al. (2005) studied the presence of neurotoxins during a cyanoHAB in Tapacurá Reservoir. During the cyanoHAB species dominance changed, including the genus *Pseudanabaena*, and the species *A. spiroides*, *C. raciborskii*, and *M. aeruginosa*. Mouse bioassays indicated the presence of neurotoxins when *C. raciborskii* and *A. spiroides* dominated. Three types of cyanobacterial neurotoxins, likely produced by *C. raciborskii*, were identified: saxitoxin (STX), neosaxitoxin (neo-STX) and dc-saxitoxin (dc-STX). *C. raciborskii* also produces microcystin, it is likely that saxitoxins and microcystin co-occur in the Tapacurá Reservoir. Therefore, additive or synergistic toxicity of these cyanotoxins could result in greater toxicity than what would have been expected from each individual cyanotoxin.

Moura et al. (2011) measured the vertical and temporal dynamics of cyanobacteria in the Capina Reservoir that provides drinking and irrigation water and fishing resources, and receives sewage and agricultural drainage from the city of Lagoa do Carro. Fourteen species of cyanobacteria were identified. The genera *Chroococcus* and *Aphanizomenon* occurred in low biomasses, but were present during the entirety of the study. *Oscillatoria* and *Pseudanabaena* were abundant in June below the photic zone. The species *C. raciborskii* and *P. agardhii* were abundant throughout the study at all depths, while *C. minutus*, and *Merismopedia punctata* only occurred in June 2008.

Bittencourt-Oliveira et al. (2012) studied the seasonal dynamics of cyanobacteria in the Arcoverde Reservoir. Cyanobacteria contributed the most to phytoplankton biomass in both seasons, at all times of the day and at both sampling depths. Large biomasses of *P. agardhii*, *G. amphibium*, *M. punctata* competed with *C. raciborskii*. Lorenzi et al. (2016) sequenced the genome of *C. raciborskii* strain ITEP-A1 isolated from the Arcoverde Reservoir. Their analysis showed that *C. raciborskii* is capable of evolving diverse genomic organization and adaptive mechanisms. In a study of the Carpina and Jucazinho Reservoirs, de Oliveira et al. (2015) found higher values of cyanobacteria biomass in the dry season in Jucazinho Reservoir and in the rainy season in Carpina Reservoir. *P. agardhii*, *C. raciborskii* and *Geiterinema amphibium* occurred in all samples. A study of the factors affecting water quality in the Jucazinho Reservoir from 2003 until 2013, showed that cyanobacterial growth was proportional to the concentration of dissolved solids. Likewise, cyanobacteria photosynthetic activity related to variations in the reservoir's volume (de Melo et al., 2017).

Baydum and Oliveira (2017) studied the cyanobacterial community in the Carpina Dam that supplies water to the Lagoa do Carro, Lagoa de Itaenga, Limoeiro and Feira Nova municipalities. Cyanobacteria were observed in high densities in all samples, 13 taxa were identified belonging to the families Phormidiaceae, Nostocaceae, Synechocaceae, Pseudoanabaenaceae, Merismopediaceae, Oscillatoriaceae, and Microcystaceae. During the drier year average cyanobacterial concentration increased. Indicating that rain strongly influences total cyanobacterial biomass, diluting and disturbing aquatic communities.

Piccin-Santos and do Carmo Bittencourt-Oliveira (2012) sampled four reservoirs, two in the northeast Carpina and Mundaú, Pernambuco state, and two in the Southeast Billings and Rio Grande, São Paulo state. They identified fourteen cyanobacterial taxa, eleven of which produce

cyanotoxins. Potentially toxic cyanobacteria were recorded at concentrations $>20,000$ cells ml^{-1} in all samples from all reservoirs indicating the occurrence of a cyanoHAB. Billings showed high concentration of cyanobacteria as well as the highest richness with nine taxa. Rio Grande had a high density of *Dolichospermum flos-aquae*. Mundaú showed the lowest richness, with five taxa having the ability to produce toxins. Carpina had a *Microcystis panniformis* cyanoHAB that lasted four weeks. *Microcystis sp.* was present in all reservoirs, while *Planktothrix sp.* was present in all reservoirs except Mundaú. Considering all samples, the potential toxin-producing genera were *Microcystis*, *Dolichospermum*, *Cylindrospermopsis*, and *Planktothrix* were the most common.

Rio Grande do Norte (RN) State

Costa et al. (2006a) reported on the occurrence of cyanoHABs in the Armando Ribeiro Gonçalves Reservoir, the largest reservoir for irrigation in Latin America. Cyanobacterial cells represented 90% to 100% of the phytoplankton community in raw water from the reservoir. The most abundant genera in most cyanoHABs were *Microcystis*, *Cylindrospermopsis* and *Aphanizomenon*. The presence of microcystins (MCs) and saxitoxins (STXs) was confirmed. Chellappa et al. (2008b) studied the Marechal Dutra Reservoir. Water from the reservoir is destined to human consumption, irrigation, cage aquaculture, and fishing. For fifteen days during December 2003, there was a *C. raciborskii* and *M. aeruginosa* cyanoHAB, during which the presence of microcystin (MC) was detected in seston and fish liver samples, always higher than the acceptable levels for drinking water according to Brazilian regulation (Azevedo et al., 1994).

Vieira et al. (2015) studied the composition and biomass of the phytoplankton community, in the Armando Ribeiro Gonçalves Dam. Cyanobacteria dominated the phytoplankton community in the reservoir, likely due to high nutrient concentrations. The

dominant species throughout the sampling period were *P. agardhii*, *M. aeruginosa*, and *Sphaerocavum brasiliense*. Brasil et al. (2016) analyzed physico-chemical variables and plankton communities from 40 man-made shallow lakes in six hydrographic basins in the state of Rio Grande do Norte. These reservoirs are impoundments of temporary streams, built to store water for drinking, irrigation, ranching, fishing, aquaculture, and recreation during the prolonged dry season. Cyanobacteria contributed the most to total phytoplankton biovolume, during the dry and the wet seasons. Their findings confirm that drought conditions intensify the symptoms of eutrophication and suggest that drought influences the hydrological and physico-chemical characteristics of artificial lakes in tropical semiarid regions favoring cyanoHABs.

Lorenzi et al. (2015) collected samples from Mundaú Reservoir in Ceará state, Arcoverde Reservoirs in Paraíba state, and from Alagoinha, Carpina, Duas Unas, Ingazeira, Ipojuca, Jucazinho, Tapacurá and Venturosa Reservoirs in Pernambuco state, to study the molecular markers of cylindrospermopsins (CYNs) and microcystins (MCs) for the detection of toxic cyanobacteria. Potential toxin-producing cyanobacteria occurred widely in these reservoirs. This is supported by the distribution of genes involved in the synthesis of both cyanotoxins.

South Region

Paraná (PR) State

Hirooka et al. (1999) summarized the occurrence of cyanobacteria and cyanotoxins in water bodies used for recreation in Paraná between 1995 and 1996. The first report of paralytic shellfish toxins (PSTs) freshwaters in southern Brazil occurred upstream in the Salto Hydroelectric Power System during the summer of 1999. A *C. raciborskii* cyanoHAB occurred close to the town of São Francisco de Paula affecting the basins of the Cai and Sinos rivers (Yunes et al. 2003). Wojciechowski et al. (2016) examined the responses of *C. raciborskii* to

light intensity at the Alagados Reservoir. This reservoir is used as a drinking water supply and for hydroelectric power generation. Seasonal *C. raciborskii* cyanoHABs have been reported at the reservoir since 2001. High light intensities increased the growth of *C. raciborskii*, increasing the threat to public health, especially in tropical regions.

Rio Grande do Sul (RS) State

The first *M. aeruginosa* cyanoHAB in southern Brazil and in RS was documented in Patos Lake in the mid-1980s (Torgan, 1989; Yunes et al., 1996, 1998). In the mid-1990s, regular monitoring showed three distinct cyanoHABs of *M. aeruginosa*, from March to May 1994, from July to September 1994, and from December 1994 to March 1995. Variable levels of toxicity were detected, but no animal deaths or human intoxications were registered (Yunes et al., 1996). Matthiensen et al. (2000) reported microcystins containing [D-Leu¹] in the cyclic heptapeptide-structure for the first time in samples from the Patos Lake. [D-Leu¹] microcystin-LR ([D-Leu¹]-MC-LR) is the most abundant microcystin produced by *Microcystis* sp.

Montagnolli et al. (2004) studied the effect of acute toxicity of an aqueous extract of *M. aeruginosa* strain RST9501 on the microcrustacean *Kalliapseudes schubartii*, consumed in large amounts by fish in the Los Patos Lake, and a key species in the trophic web of the reservoir. Mendes et al. (2017) used pigments-based chemotaxonomy to study the distribution and composition of phytoplankton at two sites in the Patos Lake estuary and at the Cassino Beach surf zone. Cyanobacteria was an important group at the lake's mouth, with an average, 37% of the total chlorophyll a.

Becker et al. (2010) reported an *Anabaena crassa* cyanoHAB in the Faxinal Reservoir. This was the first report of anatoxin-a (s) (ANTX-a (s)) in the water for *A. crassa* cyanoHAB in the country. This cyanotoxin had not been included in Brazilian legislation for drinking water

monitoring due to lack of information about toxicity levels and risk calculation for oral doses (Becker et al., 2010). Carvalho et al. (2008) reported a cyanoHAB in Lake Violão, analysis of water samples from the lake revealed that the phytoplankton biota was dominated by *M. protocystis* and *Sphaerocavum cf. brasiliense*. Intraperitoneal tests in mice confirmed the toxicity of the cyanoHAB, and chemical analyses of cyanoHAB extracts showed that the predominant cyanotoxins were microcystin-LR (MC-LR), microcystin-RR (MC-RR), anabaenopeptin B (AP-F), and anabaenopeptin F (AP-F).

Werner et al. (2015) studied the cyanobacterial diversity in the Central pond and Chimarrão marsh of the Universidade Luterana do Brasil - ULBRA (Lutheran University of Brazil), in the city of Canoas. Twenty-four cyanobacterial species were identified, belonging to the orders Oscillatoriales (25%), Nostocales (17%) and Pseudanabaenales (8%). Six species *Cuspidothrix issatschenkoi*, *C. raciborskii*, *M. aeruginosa*, *M. wesenbergi*, *Oscillatoria curviceps* and *O. tenuis* were present in both the pond and the marsh. During the study period, several *C. raciborskii* cyanoHABs occurred in the pond, while *M. aeruginosa* cyanoHABs happened in the marsh during spring and summer.

Pacheco et al. (2016) reported on the occurrence of nodularin in a in the Marine Aquaculture Station (EMA) from the Federal University of Rio Grande (FURG), where repeated cyanoHABs have been reported. The cyanoHAB was caused by high light intensities, and excess fertilizers. The nodularin (NOD) produced by *Nodularia sp.* was extremely toxic to the shrimp growing in the tanks.

Monitoring of the Alagados in Paraná state, Salto and Duro Dams in Rio Grande do Sul state, revealed the occurrence of *C. raciborskii* cyanoHABs and confirmed the presence of six highly toxic structures: saxitoxin (STX), neo-saxitoxin (Neo-STX), and gonyautoxins (GTXs) 1,

3, 2 and 4. The strains of *C. raciborskii* present in the samples all produced PSTs variants, and neo-saxitoxin (Neo-STX), and gonyautoxins (GTXs) 2, 3 and 4 had the highest toxicities. The Alagados Dam, originally constructed to generate power, now supplies water to the city of Ponta Grossa, receives a heavy load of organic and inorganic material released from intensive swine and poultry farms that cause eutrophication (Yunes et al., 2003).

Hirooka et al. (1999) tested samples collected to determine the concentrations of microcystins in water supplies in the states of Paraná and Santa Catarina in the South. Their findings revealed a high incidence of *M. aeruginosa* in all samples and *M. flosaquae*. The situation was particularly critical in the water samples from the Itaipu Dam in Paraná state and treated at a water plant located in Santa Terezinha do Iguaçu. Likewise, samples of freshwater used for animal pasture from these reservoirs showed high levels of *M. aeruginosa*.

Santa Catarina (SC) State

Hennemann and Petrucio (2016) studied the temporal dynamics of trophic water quality parameters in different time scales and their correlation and influence in phytoplankton biomass in the Peri Lake. *C. raciborskii* ability to compete for phosphorus and light seem to be important factors to determine its success and dominance in this low phosphorous coastal lagoon. Twenty years of studies in the lagoon have shown that most of the year *C. raciborskii* dominates the phytoplankton community, and that its density and dominance are increasing (Tonetta et al., 2013).

Southeast Region

Mina Gerais (MG) State

C. raciborskii strain ITEP-018 was isolated from water samples collected in the Tabocas Reservoir in Minas Gerais state. ITEP-018 is one of the most toxic strains of PSP producing

cyanobacteria. Numerous saxitoxin analogues were identified including saxitoxin (STX), gonyautoxin 6 (GTX -6), decarbamoylsaxitoxin (dcSTX), neosaxitoxin (Neo-STX), and a new analogue (Molica et al., 2002).

Von Sperling and Souza (2007) monitored the Vargem das Flores Reservoir for 30 years. This tropical reservoir supplies water to Belo Horizonte City and receives discharges of untreated sewage and agricultural drainage. Cyanobacteria dominated the phytoplankton community and cyanoHABs are frequent due to high nutrient concentrations. Tropical eutrophic lakes tend to be N-limited while oligotrophic temperate lakes are P-limited which might contribute to the dominance of cyanobacteria in tropical eutrophic lakes.

Laux et al. (2018) characterized the picocyanobacteria community of the Volta Grande Reservoir, located between Minas Gerais and São Paulo states. Twenty-two cyanobacteria taxa were identified to the species level, the highest species richness was found in the order Synechococcales and Chroococcales. The genera with the highest species richness were *Microcystis* and *Aphanocapsa*, with five species each, followed by *Anathece* and *Merismopedia* each with three species.

Rio de Janeiro (RJ) State

The Jacarepaguá Lagoon has been extensively studied; de Magalhães et al. (2001) found microcystin concentration close or above the recommended limit for human consumption in muscle of *Tilapia rendalli* collected from the lagoon. Water samples confirmed that the phytoplankton community was dominated by the genus *Microcystis*. While Ferrão-Filho et al. (2002) found that in spite of high levels of microcystins in seston, toxins did not correlate with the density of Cladocera in Jacarepaguá Lagoon. Laboratory experiments showed strong evidence of the toxicity of seston to Cladocera, and that under natural circumstances, *M.*

aeruginosa cyanoHABs are potentially harmful to Cladocera populations with broad ecological implications in food webs.

de Magalhães et al. (2003) evaluated the presence of microcystins (MCs) in shrimp and crab muscle from the Sepetiba Bay. All samples were collected from organisms destined for human consumption and purchased from local fishers. The highest microcystins (MCs) concentration was observed in crab samples, they were above the recommended TDI in 25% of the samples.

In 2005, Oliveira et al. (2005) assessed the effect of the chemical composition of drinking water on the biological activity of microcystins using drinking water samples from the city of Rio de Janeiro. They used ELISA, HPLC, and protein phosphatase-1 inhibition assays, and established that the chemical composition of tap water interfered with the biological activity of microcystins. Chlorine concentrations of 2.5 mg l⁻¹ in deionized water prevented the detection of 10 mg l⁻¹ of added microcystins (MCs). Iron (Fe) and aluminum (Al) ions were also effective in reducing microcystin (MC) detection.

Soares et al. (2006) reported a cyanoHAB dominated by *Microcystis* and *Anabaena* in the Guandu River and Funil Reservoir, which supply drinking water to Rio de Janeiro. Microcystins (MCs) were detected in water as well as in the activated carbon column-treated water used at the renal dialysis center of Hospital Universitário Clementino Fraga Filho - HUCFF (Clementino Fraga Filho Hospital) at the Federal University of Rio de Janeiro. Forty-four patients were exposed to sublethal levels of microcystin via intravenous injection. Twelve of these patients were tested for a two-month period after which microcystins concentrations in their sera were still detectable.

Rezende et al. (2015) characterized the phytoplankton community in Guanabara Bay. Cyanobacteria represented 99% of the abundance in some samples. Two cyanobacterial orders were identified, Oscillatoriales and Nostocales, both with known cyanotoxin producing capabilities. Oscillatoriales were the more abundant than Nostocales, with an average abundance of 7.1×10^5 cells l^{-1} and 1.0×10^8 cells l^{-1} respectively through the entire sampling period. High cyanobacterial densities were associated to rainy periods and high levels of eutrophication.

Hauser-Davis et al. (2015) examined the accumulation of microcystin in tilapia *Oreochromis niloticus*, from specimens captured in the Jacarepaguá Lagoon. Ten specimens were purchased from local fishers, when there was no visual indication of a cyanoHAB. Seven additional specimens, also destined for human consumption, were procured from a commercial aquaculture facility for reference. Microcystin (MC) analysis was conducted from bile, liver, gonads and muscle. For the naturally exposed fish, microcystin (MC) accumulation occurred in higher concentrations in muscle, followed by gonads, liver and bile. Values for tilapia muscle and liver were above the limits suggested for human consumption. de Magalhães et al. (2017) collected samples from the Jacarepaguá Lagoon to test the effectiveness of using coagulants and ballast compounds to remove cyanobacteria from water bodies. During the sampling period, the dominant species was *M. aeruginosa* with some undergrowth of *P. agardhii*. Combined coagulant and ballast are an efficient, and cost-effective way to lessen cyanoHABs.

São Paulo (SP) State

dos Anjos et al. (2006) reported the occurrence of cyanoHABs in the Billings Reservoir, which provides water to the city of São Paulo. Cyanobacterial biota was mainly composed by *M. aeruginosa*, *M. novacekii*, *M. panniformis*, *M. protocystis*, *M. cf. botrys*, *P. agardhii*, *R. fernandoi*, *P. mucicola*, and *C. raciborskii*. Taiaçupeba Reservoir provides drinking water to the

east part of the city of São Paulo. Before 1998, the reservoir was only affected by *Microcystis* cyanoHABs. After 1998, *C. raciborskii* cyanoHABs became common in seven of the main water reservoirs supplying water to the city of São Paulo including the Taiacupeba. Toxin profiles indicated the presence of saxitoxin (STX) and gonyautoxins (GTXs) 2 and 3 (Yunes et al., 2003).

Barra Bonita Reservoir, is located in one of the most populated and industrialized areas in South America, and has a heavy input from agricultural, domestic, and industrial effluents. Sotero-Santos et al. (2006) collected scum samples from the reservoir, prepared crude cyanobacterial extracts and conducted an acute toxicity tests using *Daphnia similis* and *Ceriodaphnia silvestrii*. They also evaluated cyanoHAB toxicity using mouse bioassays. Acute toxicity tests with Cladocerans showed that cyanotoxins from the cyanoHAB are potentially capable of killing Cladocerans.

Tundisi et al. (2008) studied the ecological dynamics of Barra Bonita Reservoir, located in the middle portion of the Tietê River basin. The reservoir is the transition between the tropical and subtropical climates. Sampling was carried out during the rainy (October to March) and the dry (March to October) season, in the reservoir and in the tributaries. Based on their findings, the authors state that long-term eutrophication has resulted in the continuous increase in cyanoHAB frequency, particularly those dominated by *M. aeruginosa*, which will lead to increased toxicity in the ecosystem.

The Guarapiranga and Billings Reservoirs are part of the high Tietê River basin. Water from both reservoirs is intended for human consumption, so samples were taken from where water is captured for supplying to the metropolitan area of city of São Paulo. Cyanobacteria were the second-best represented phytoplankton division in both reservoirs, in Billings 35 taxa were identified (26%), and 34 in Guarapiranga (20%). Cyanobacteria presented the greatest annual

density in both reservoirs and *C. raciborskii* was one of the most abundant species in the Billings Reservoir (Gemelgo et al., 2008).

Moschini-Carlos et al. (2009) evaluate the water quality of the Taquacetuba Channel used to transfer crude water from Billings to Guarapiranga. Thirteen cyanobacterial taxa were identified, and higher densities of cyanobacterial were recorded during winter and summer. During winter, the most abundant species were *M. panniformis* and *A. spiroides* and during summer *C. raciborskii* and *P. agardhii*.

Santana and Carvalho (2011) isolated for the first time *M. aeruginosa* strain SPC777 from samples collected in cyanoHAB that took place in the Racho Grande Arm of the Billings Reservoir. This was the first report of *M. aeruginosa* strain SPC777. In Garças Pond, a reservoir located in state park Fontes do Ipiranga Biological, twenty-nine taxa of cyanobacteria were identified. The most frequent taxa were *Merismopedia glauca*, *M. aeruginosa*, *Sphaerocavum brasiliense* and *Aphanocapsa elachista* cyanobacterial dominance shifted during the year (Fonseca & Bicudo, 2008).

Sotero-Santos et al. (2008) studied the phytoplankton community of a small shallow reservoir located in the Federal University of São Carlos. The main affluent of the reservoir is the Monjolinho River that receives organic effluents from agricultural fields and poultry industry. Samples were collected three times per week, at a single point close to the dam, during October 2004. Higher quantities of cyanobacterial scum were recorded in October 8, 15, and 25. The main species in the CyanoHAB were *Anabaena circinalis* and *A. spiroides*.

Elias et al. (2015) used chemical and molecular analyses to examine cyanoHABs in four lagoons used for recreational purposes: ESALQ1, ESALQ2, Taquaral, and Limeira. Nine cyanobacterial genera were identified. Taquaral lagoon had low diversity and richness values,

with low cyanobacteria diversity. Limeira and ESALQ2 the predominantly observed genus was *Microcystis*. Lagoons ESALQ2 had 89%, Limeira had 82%, and Taquaral 81%, of their sequences related to the genus *Microcystis*. ESALQ1 lagoon had the highest richness and diversity for cyanobacteria. Limeira had a high number of uncultured cyanobacteria and no sequences matching *Microcystis* was observed in this lagoon.

The UHE Carlos Botelho Reservoir, also known as Lobo-Broa, has been part of a research program since 1971. In the 2014 winter a cyanoHAB was observed in this reservoir for the first time, it was dominated by *C. raciborskii*. The cyanoHAB was attributed to a 2°C temperature increase in the water during winter added to lower rainfall during the previous summer. Microcystins (MCs) and saxitoxins (STXs) were present in low concentrations. Fish mortalities occurred during this intense cyanoHAB affecting recreational activities (Tundisi et al., 2015).

do Carmo Bittencourt-Oliveira et al. (2015) examined the interaction between unbroken cells of toxic *M. aeruginosa* and the non-toxic *M. panniformis* with those of green algae *Monoraphidium convolutum* and *Scenedesmus acuminatus* and examined the effects of cyanobacteria extracts on green algae growth as well as the impact of completion in cyanotoxin allelopathy. Cyanobacterial strains were obtained from the Brazilian Cyanobacteria Collection of University of São Paulo (BCCUSP), and the strains of green algae from the Coleção de Microalgas Elizabeth Aidar/Universidade Federal Fluminense - CMEA/UFF (Elizabeth Aidar Collection of Microalgae/Fluminense Federal University) and from the Universidade Federal de São Carlos - UFSCar (Federal University of São Carlos). The growth of cyanobacteria and green algae was impacted by the presence of competing species. Green algae inhibited the growth of

M. aeruginosa, but *M. aeruginosa* increased microcystin (MC) production when green algae were present.

Schlüter et al. (2018) examined the influence of light and nutrient starvation on chlorophyll a ratios from cyanobacteria samples collected in six fish farms in the state and then cultured in the laboratory. Cyanobacteria generally dominated in all reservoirs (44% to 66% of the average phytoplankton biomass). Fourteen strains of cyanobacteria including the genera *Microcystis*, *Dolichospermum*, *Planktothrix*, *Oscillatoria*, *Pseudanabaena*, *Cylindrospermopsis*, *Pseudana*, and *Radiocystis* were selected for a culture experiment. Five of the cultured strains, *P. cf. agardhii* K-0546, *P. rubescens* K-0569, *M. aeruginosa* NIES-107, *Microcystis* PCC-7820, and *Microcystis* sp. produced microcystin (MC) at concentrations above the detection limit. Eight microcystins (MCs) variants were detected, microcystin-LR (MC-LR), microcystin-YR (MC-YR), microcystin-RR (MC-RR), demethylated microcystin-LR (DM MC-LR), demethylated microcystin-RR (DM MC-RR), microcystin-LY (MC-LR), microcystin-LF (MC-FL), and microcystin-LW (MC-LW). Microcystin (MC) concentrations significantly correlated with chlorophyll-a concentrations of cyanobacteria.

Piccin-Santos and do Carmo Bittencourt-Oliveira (2012) sampled four public water reservoirs, the Billings and Rio Grande Reservoirs in São Paulo state in the Southeast, and Carpina and the Mundaú Reservoirs in Pernambuco state in the Northeast. They identified fourteen cyanobacterial taxa, eleven of which are potential cyanotoxin producers. All these taxa were recorded at concentrations greater than 20,000 cells ml⁻¹ in all reservoirs. According to Brazilian legislation, at these concentrations, microcystin monitoring for drinking water is required. Billings Reservoir showed high concentration of cyanobacteria and the highest richness (9 taxa). Rio Grande Reservoir had a high density of *D. flos-aquae*. Mundaú Reservoir showed

the lowest richness, with five taxa having the ability to produce toxins. Carpina Reservoir had a *M. panniformis* cyanoHAB that lasted the four weeks of sampling. *Microcystis* was present in all reservoirs, while *Planktothrix* was present in all reservoirs except Mundaú. Among all samples, *Microcystis*, *Dolichospermum*, *Cylindrospermopsis*, and *Planktothrix* were the most common toxin-producing genera.

Table 2. List of cyanobacterial genera and species reported for Brazil in peer-review contributions from 1990 to 2018.

	State	Reference	Water body	Highlighted species	Cyanotoxin
Central-west	MS	Santos and Sant'anna (2010)	Pantanal da Nhecolândia	<i>Anabaenopsis elenkinii</i>	
		Andreote et al. (2014)	Pantanal da Nhecolândia		
		Genuario et al. (2017)	4 lakes in Centenário farm	<i>Nostoc sp.</i> , <i>Anabaenopsis sp.</i> , <i>Tolypothrix sp.</i>	
Central-west	MT	Assis et al. (2018)	Claro, Coxipó, Coxipozinho, Paciência and Mutuca Rivers	<i>Cylindrospermopsis raciborskii</i> , <i>Asterocapsa submersa</i> , <i>Chroococcus dispersus</i> , <i>C. minor</i> , <i>Snowella lacustris</i> , <i>Komvophoron crassum</i> , <i>K. schmidlei</i> , <i>Merismopedia tenuissima</i> , <i>Planktothrix agardhii</i> , <i>Microcystis aeruginosa</i> , <i>Sphaerocavum brasiliense</i> , <i>Lyngbya major</i> , <i>Pseudanabaena galeata</i>	
North	AM	Genuario et al. (2017b)	Amazon and Solimões Rivers	<i>Planktothrix sp.</i> , <i>Pseudanabaena sp.</i> , <i>Cephalothrix sp.</i> , <i>Pantanalinema sp.</i> , <i>Alkalinema sp.</i>	
		Genuario et al. (2018a)	Amazon and Solimões Rivers	<i>Amazoninema gen. nov.</i>	
		Genuario et al. (2018b)	Solimões River	<i>Cronbergia siamensis</i> , <i>Cronbergia amazonensis</i>	
North	PA	Vieira et al. (2003)	Utinga Reservoir	<i>R. fernandoi</i>	MC
		Vieira et al. (2005)	Água Preta and Bolonha Lakes, Bolonha and São Braz water treatment plants	<i>Aphanizomenon sp.</i> , <i>Microcystis sp.</i> , <i>Nostoc sp.</i> , <i>Oscillatoria sp.</i> , <i>Planktothrix sp.</i> , <i>Radiocystis sp.</i>	MC
NE	BA	Teixeira et al. (1993)	Itaparica Dam (PE)		

Note: **AER:** Aeruginosin, **ANTX-a:** Anatoxin-a(s), **AP-B:** Anabaenopeptin B, **AP-F:** Anabaenopeptin F, **CyPep:** Cyanopeptolin, **CYN:** Cylindrospermopsin, **dc-NEO:** Decarbamoylneosaxitoxin, **dc-STX:** Decarbamoylsaxitoxin, **GTX:** Gonyautoxin, **MC:** Microcystin, **Neo-STX:** Neosaxitoxin, **NOD:** Nodularins, **STX:** Saxitoxin, **NE:** Northeast.

Table 2. Continued.

	State	Reference	Water body	Highlighted species	Cyanotoxin
Northeast	BA	Affe et al. (2018)	Camamu Bay	<i>Synechococcus sp.</i> , <i>Prochlorococcus sp.</i>	
	CE	Von Sperling and Souza (2008)	Gavião Reservoir	<i>Planktothrix sp.</i> , <i>Cylindrospermopsis sp.</i>	Not detected
		Barros et al. (2017)	Sítios Novos and Acarape do Meio Reservoirs	<i>P. agardhii</i> , <i>C. raciborskii</i> , <i>Merismopedia sp.</i> , <i>Pseudanabaena sp.</i>	STX
		Borges et al. (2015)	Mundaú River Basin	<i>Geitlerinema amphibium</i> , <i>G. lemmermannii</i> , <i>C. stagnale</i> , <i>Phormidium uncinatum</i>	Neo-STX, dc-STX, STX, GTX, GTX4
	PB	Barbosa & Mendes (2005)	Acauã or Argemiro de Figueiredo Dam	<i>C. raciborskii</i>	
		Lins et al. (2016)	Acauã or Argemiro de Figueiredo Dam	<i>P. agardhii</i> , <i>Aphanocapsa incerta</i> , <i>C. raciborskii</i> , <i>Dolichospermum circinalis</i> , <i>Microcystis sp.</i> , <i>M. aeruginosa</i> , <i>Pseudanabaena limnetica</i> , <i>Trichodesmium lacustre</i>	
		Walter et al. (2018)	Araçagi, Boqueirão or Epitácio Pessoa, Saulo Maia, Galante and Mazagão Ponds	<i>Microcystis sp.</i> , <i>Synechococcus sp.</i> , <i>Anabaena sp.</i> , <i>Cyanobium sp.</i> and <i>Cylindrospermopsis sp.</i>	MC, NOD, CYN
	PE	Carmichael et al. (1996)	Tabocas Reservoir		

Note: **AER:** Aeruginosin, **ANTX-a:** Anatoxin-a(s), **AP-B:** Anabaenopeptin B, **AP-F:** Anabaenopeptin F, **CyPep:** Cyanopeptolin, **CYN:** Cylindrospermopsin, **dc-NEO:** Decarbamoylneosaxitoxin, **dc-STX:** Decarbamoylsaxitoxin, **GTX:** Gonyautoxin, **MC:** Microcystin, **Neo-STX:** Neosaxitoxin, **NOD:** Nodularins, **STX:** Saxitoxin.

Table 2. Continued.

	State	Reference	Water body	Highlighted species	Cyanotoxin
Northeast	PE	Molica et al. (2002)	Tabocas Reservoir	<i>C. raciborskii</i>	STX, GTX, dc-STX, dc-NEO, Neo-STX, a new STX analogue.
		Molica et al. (2005)	Tapacurá Reservoir	<i>A. spiroides</i> , <i>Pseudanabaena</i> sp., <i>C. raciborskii</i> and <i>M. aeruginosa</i>	STX, Neo-STX, dc-STX
		Moura et al. (2011)	Capina Reservoir	<i>Chroococcus</i> sp., <i>Aphanizomenon</i> sp., <i>C. raciborskii</i> sp., <i>P. agardhii</i> , <i>Oscillatoria</i> sp., <i>C. minutus</i> , <i>Pseudanabaena</i> sp., <i>Merismopedia punctata</i>	
		Piccin-Santos and do Carmo Bittencourt-Oliveira (2012)	Carpina and Mundaú Reservoirs	<i>Microcystis</i> sp., <i>Dolichospermum</i> sp., <i>Cylindrospermopsis</i> sp., <i>Planktothrix</i> sp.	
		Bittencourt-Oliveira et al. (2012)	Arcoverde Reservoir	<i>Planktothrix agardhii</i> , <i>G. amphibium</i> , <i>Merismopedia punctata</i> , <i>C. raciborskii</i> .	
		Aquino et al. (2015)	Capibaribe River estuary		
		Ferreira et al. (2015)	Reef ecosystem of São José da Coroa Grande	<i>Oscillatoria</i> sp., <i>Spirulina</i> sp., <i>Pseudoanabaenopsis</i> sp.	
		Portella et al. (2015)	Jucazinho and Carpina Reservoirs	<i>P. agardhii</i> , <i>C. raciborskii</i> and <i>G. amphibium</i>	
		Lorenzi et al. (2016)	Arcoverde reservoir	<i>C. raciborskii</i>	
		da Silva et al. (2017)	Jaboatão River estuary	<i>M. aeruginosa</i> , <i>P. agardhii</i>	

Note: **AER:** Aeruginosin, **ANTX-a:** Anatoxin-a(s), **AP-B:** Anabaenopeptin B, **AP-F:** Anabaenopeptin F, **CyPep:** Cyanopeptolin, **CYN:** Cylindrospermopsis, **dc-NEO:** Decarbamoylneosaxitoxin, **dc-STX:** Decarbamoylsaxitoxin, **GTX:** Gonyautoxin, **MC:** Microcystin, **Neo-STX:** Neosaxitoxin, **NOD:** Nodularins, **STX:** Saxitoxin.

Table 2. Continued.

	State	Reference	Water body	Highlighted species	Cyanotoxin
Northeast	PE	Baydum and Oliveira (2017)	Carpina Reservoir on the Capibaribe River	<i>Phormidiaceae, Nostocaceae, Synechococcaceae, Pseudoanabaenaceae, Merismopediaceae, Oscillatoriaceae, Microcystaceae</i>	
		Aragão-Tavares et al. (2017)	Ingazeira, Ipojuca and Pedra Reservoirs		
		de Melo et al. (2017)	Jucazinho Reservoir		
	RN	Costa et al. (2006a)	Armando Ribeiro Gonçalves Reservoir	<i>Microcystis spp, C. raciborskii, Aphanizomenon spp.</i>	MCs, STX. CYN not confirmed
		Chellappa et al. (2008 a,b)	Marechal Dutra Reservoir		MC
		Brasil et al. (2016)	40 man-made lakes in 6 hydrographic basins		
		Vieira et al. (2015)	Armando Ribeiro Gonçalves Dam	<i>P. agardhii, M. aeruginosa, S. brasiliense</i>	
CE, PB, PE	Lorenzi et al. (2015)	Mundaú (CE), Arcoverde (PB), Alagoinha, Carpina, Duas Unas, Ingazeira, Ipojuca, Jucazinho, Tapacurá and Venturosa (PE)			
South	PR	Wojciechowski et al. (2016)	Alagados Reservoir		
		Hirooka et al. 1999	Itaipu Dam	<i>M. aeruginosa, M. flosaquae</i>	MC

Note: **AER:** Aeruginosin, **ANTX-a:** Anatoxin-a(s), **AP-B:** Anabaenopeptin B, **AP-F:** Anabaenopeptin F, **CyPep:** Cyanopeptolin, **CYN:** Cylindrospermopsin, **dc-NEO:** Decarbamoylneosaxitoxin, **dc-STX:** Decarbamoylsaxitoxin, **GTX:** Gonyautoxin, **MC:** Microcystin, **Neo-STX:** Neosaxitoxin, **NOD:** Nodularins, **STX:** Saxitoxin.

Table 2. Continued.

	State	Reference	Water body	Highlighted species	Cyanotoxin
South	PR, RS	Yunes et al. (2003)	Alagados (PR), Salto (RS) and Duro (RS) Dams	<i>C. raciborskii</i> , <i>P. limnetica</i> , <i>Pseudoanabaena</i> sp.	Neurotoxins, STX, Neo-STX, GTX 1, 3, 2 and 4. STX and MC equivalents
	RS	Yunes et al. (1996, 1998)	Los Patos Lake	<i>M. aeruginosa</i> , <i>M. flosaquae</i>	
		Montagnolli et al. (2004)	Los Patos Lake	<i>M. aeruginosa</i> RST9501	MC
		Matthiensen et al. (2000)	Los Patos Lake	<i>Microcystis</i> sp.	MC
		Becker et al. (2010)	Faxinal Reservoir	<i>Anabaena crassa</i>	ANTX-a
		Carvalho et al. (2008)	Lake Violão	<i>M. protocystis</i> , <i>Sphaerocavum</i> cf. <i>brasiliense</i> , <i>M. panniformis</i> , <i>A. oumiana</i> , <i>C. raciborskii</i> , <i>Anabaenopsis elenkinii</i> f. <i>circularis</i>	MC-LR, MC-RR, AP-F, AP-B
		Werner et al. (2015)	Central Pond and Chimarrão Marsh (Lutheran University of Brazil)	<i>C. raciborskii</i> , <i>M. aeruginosa</i> , <i>M. wesenbergi</i> , <i>Oscillatoria curviceps</i> , <i>O. tenuis</i>	
		Martins et al. (2016)	Triunfo Reservoir		
		Mendes et al. (2017)	Patos Lake		
		Costa et al. (2016)	Marine Aquaculture Station	<i>Nodularia</i> sp.	NOD
SC	Tonetta et al. (2015)	Peri Lake	<i>C. raciborskii</i>		

Note: **AER:** Aeruginosin, **ANTX-a:** Anatoxin-a(s), **AP-B:** Anabaenopeptin B, **AP-F:** Anabaenopeptin F, **CyPep:** Cyanopeptolin, **CYN:** Cylindrospermopsin, **dc-NEO:** Decarbamoylneosaxitoxin, **dc-STX:** Decarbamoylsaxitoxin, **GTX:** Gonyautoxin, **MC:** Microcystin, **Neo-STX:** Neosaxitoxin, **NOD:** Nodularins, **STX:** Saxitoxin.

Table 2. Continued.

	State	Reference	Water body	Highlighted species	Cyanotoxin
Southeast	MG	Molica et al. (2002)	Tabocas Reservoir	<i>C. raciborskii</i> strain ITEP-018	STX, GTX 6, dcSTX, Neo-STX, new saxitoxin analogue
		Von Sperling and Souza (2008)	Vargem das Flores Reservoir		
		Paulino et al. (2017)	Furnas Reservoir	<i>R. fernandoi</i> R28 strain	MC-YR, MC-RR
	MG, SP	Laux et al. (2018)	Volta Grande and Rio Grande Reservoirs	<i>Microcystis</i> sp., <i>Aphanocapsa</i> sp., <i>Anathece</i> sp., <i>Merismopedia</i> sp.	
	RJ	Ferrão-Filho et al. (2002)	Jacarepaguá Lagoon	<i>M. aeruginosa</i> , <i>Aphanizomenon</i> sp.	MC
		de Magalhães et al. (2001)	Jacarepaguá Lagoon	<i>M. aeruginosa</i>	MCs
		Magalhães et al. (2003)	Sepetiba Bay		MCs
		Soares et al. 2006	Funil Reservoir and the Guandu River	<i>Microcystis</i> sp., <i>Anabaena</i> sp.	MC
		Rezende et al. (2015)	Guanabara Bay		
	SP	Hauser-Davis et al. (2015)	Jacarepaguá lagoon		MC
		Tundisi et al. (2008)	Barra Bonita Reservoir	<i>M. aeruginosa</i>	
Fonseca and Bicudo (2008)		Garças Pond	<i>Merismopedia glauca</i> , <i>M. aeruginosa</i> , <i>Sphaerocavum brasiliense</i> , <i>Aphanocapsa elachista</i> , <i>Raphidiopsis/Cylindrospermopsis</i> sp.		

Note: **AER:** Aeruginosin, **ANTX-a:** Anatoxin-a(s), **AP-B:** Anabaenopeptin B, **AP-F:** Anabaenopeptin F, **CyPep:** Cyanopeptolin, **CYN:** Cylindrospermopsin, **dc-NEO:** Decarbamoylneosaxitoxin, **dc-STX:** Decarbamoylsaxitoxin, **GTX:** Gonyautoxin, **MC:** Microcystin, **Neo-STX:** Neosaxitoxin, **NOD:** Nodularins, **STX:** Saxitoxin.

Table 2. Continued.

	State	Reference	Water body	Highlighted species	Cyanotoxin
Southeast	SP	Sant'Anna et al. (2011)	Racho Grande arm of the Billings Reservoir	<i>M. aeruginosa</i> SPC777	
		Sotero-Santos et al. (2006)	Barra Bonita Reservoir		MC
		Gemelgo et al. (2008)	Billings and Guarapiranga Reservoirs		
		dos Anjos et al. (2006)	Billings Reservoir	<i>M. aeruginosa</i> , <i>M. novacekii</i> , <i>M. panniformis</i> , <i>M. protocystis</i> , <i>M. cf. botrys</i> , <i>Planktothrix agardhii</i> , <i>Radiocystis fernandoi</i> , <i>P. mucicola</i> , <i>C. raciborskii</i> .	MC, MC-LR, MC-RR, MC - YR, STX, Neo-STX, GTX, GTX 3
		Sotero-Santos et al. (2008)	Monjolinho Reservoir	<i>Anabaena circinalis</i> , <i>Anabaena spiroides</i>	MC. SXT and GTX measured but not detected
		Moschini-Carlos et al. (2009)	Taquacetuba branch of the Billings complex	<i>Cylindrospermopsis raciborskii</i> , <i>Planktothrix agardhii</i> , <i>Microcrocis sp.</i> , and <i>Microcystis aeruginosa</i> , <i>Microcystis panniformis</i> found, <i>Anabaena spiroides</i> , <i>Woronichinia naegeliana</i> , <i>Microcystis aeruginosa</i>	MC-RR, MC-LR, MC-YR

Note: **AER:** Aeruginosin, **ANTX-a:** Anatoxin-a(s), **AP-B:** Anabaenopeptin B, **AP-F:** Anabaenopeptin F, **CyPep:** Cyanopeptolin, **CYN:** Cylindrospermopsin, **dc-NEO:** Decarbamoylneosaxitoxin, **dc-STX:** Decarbamoylsaxitoxin, **GTX:** Gonyautoxin, **MC:** Microcystin, **Neo-STX:** Neosaxitoxin, **NOD:** Nodularins, **STX:** Saxitoxin.

Table 2. Continued.

	State	Reference	Water body	Highlighted species	Cyanotoxin
Southeast	SP	Piccin-Santos and do Carmo Bittencourt-Oliveira (2012)	Billings and Rio Grande Reservoirs	<i>Microcystis sp.</i> , <i>Dolichospermum sp.</i> , <i>Cylindrospermopsis sp.</i> , <i>Dolichospermum flos-aquae</i> , <i>Planktothrix sp.</i>	MCs
		Elias et al. (2015)	ESALQ1 and ESALQ2 in the University of São Paulo, Limeira and Taquaral	<i>Anabaena sp.</i> , <i>Brasilonema sp.</i> , <i>Cylindrospermopsis sp.</i> , <i>Limnococcus sp.</i> , <i>Microcystis sp.</i> , <i>Nostoc sp.</i> , <i>Pseudanabaena sp.</i> , <i>Synechococcus sp.</i> , <i>Woronichinia sp.</i>	MC, AER, MC-LR
		Martins et al. (2016)	Barra Funda and Jacaré stream		
		Tundisi et al. (2015)	Carlos Botelho or Lobo-Broa Reservoir	<i>C. raciborskii</i>	MC, STX
		Schlüter et al. (2018)	Chavantes, Ilha Solteira and Nova Avanhanda	<i>P. cf. agardhii</i> K-0546, <i>P. rubescens</i> K-0569, <i>M. aeruginosa</i> NIES-107, <i>Microcystis sp.</i> PCC-7820, and <i>Microcystis sp.</i>	MCs

Note: **AER:** Aeruginosin, **ANTX-a:** Anatoxin-a(s), **AP-B:** Anabaenopeptin B, **AP-F:** Anabaenopeptin F, **CyPep:** Cyanopeptolin, **CYN:** Cylindrospermopsin, **dc-NEO:** Decarbamoylneosaxitoxin, **dc-STX:** Decarbamoylsaxitoxin, **GTX:** Gonyautoxin, **MC:** Microcystin, **Neo-STX:** Neosaxitoxin, **NOD:** Nodularins, **STX:** Saxitoxin.

Summary

In Brazil, research on cyanobacteria and their toxins started in the mid-1980s, when several cyanoHABs of *Microcystis aeruginosa* were observed and documented in the state of Rio Grande do Sul, in the South region (Torgan, 1989; Yunes et al., 1996, 1998). By 1992, *M. aeruginosa* was the most common cyanobacteria in eutrophic lakes and reservoirs in the country (Hirooka et al., 1999). The first peer-reviewed work about cyanobacteria was published by Teixeira et al. (1993) after a cyanoHAB that took place in the state of Pernambuco.

In 1996, a fatal cyanotoxin intoxication took place in the city of Caruaru in northeastern Brazil, as result a phytoplankton-monitoring program was established at the city's main water supply, and then it extended to the rest of the country. Routine monitoring included cyanobacterial density, and cyanotoxin monitoring, but only presence/absence were reported.

Seventy-two publications were digitally available, they included all five regions of the country. The greatest number of publications were written for the Northeast (37.5%, 27 out of 72), followed by the Southeast (31.9%, 23 out of 72). Most papers were written for the state of Pernambuco (18.1%, 13 out of 72). No cyanobacteria related studies were available for the states of Goiás in the Central-west and Espírito Santo in the Southeast. Seventeen of the 26 states of the country were covered by scientific publications related to the cyanobacteria (Table 2).

Forty genera and 55 species were reported for Brazil. The most reported genera for the country were *Microcystis* (54.2%, 39 out of 72), *Cylindrospermopsis* (36.1%, 26 out of 72), *Planktothrix* (23.6%, 17 out of 72), and *Pseudanabaena* (13.9%, 10 out of 72).

Seven species were reported for *Micromixturecystis* (i.e. *aeruginosa*, cf. *botrys*, *flos-aquae*, *novacekii*, *panniformis*, *protocystis* and *wesenbergi*) (Table 6). And three strains of *M. aeruginosa* (i.e. NIES-107, RST9501, and SPC777) were identified. Only one species was

identified for *Cylindrospermopsis*, and one publication identified *C. raciborskii* strain ITEP-018 and determined that it produces at least five saxitoxin analogues, including the most toxic ones as assessed by mouse bioassay to the time of publication (Molica et al., 2002).

The most common species were *Microcystis aeruginosa* (5.6%, 4 out of 72) and *Cylindrospermopsis raciborskii* (2.8%, 2 out of 72). Nearly 13% of the publications (12.5%, 9 out of 72) reported the occurrence of a single cyanobacterial species. Nearly 88% (63 out of 72) of the articles, identified the presence of two or more species, and 82% (59 out of 72) reported three or more species (Table 6).

A new genus of cyanobacteria *Ancyllothrix* with two species *rivularis* and *terrestris* was established. The new genus was isolated from seven strains in the South and Southeast regions of Brazil (Martins et al., 2016). *Cuspidothrix issatschenkoi* was recorded in Brazil for the first time in May 2010 (Werner et al., 2015). *Spirulina princeps* and *Oscillatoria tenuis* were reported for the first time in Rio Grande do Sul state, in the South region, in February and April and 2010 respectively (Werner et al., 2015). This were the first reports for both species in temperate latitudes in Brazil (Table 2).

Less than a third of the publications available (30.6%, 22 out of 72) reported cyanobacterial densities, 44.4% (32 out of 72) of them tested for the presence of cyanotoxins, and 43% (31 out of 72) confirmed it.

Eight types of cyanotoxins, and fourteen variants were identified. A single cyanotoxin type was rarely reported, nearly 41% (29 out of 72) of the publications reporting cyanotoxins identified two or more types, and 31% (22 out of 72) reported mixtures of three or more cyanotoxins.

Microcystins (MCs) and saxitoxins (SXs) were the most commonly identified cyanotoxins. Three microcystin variants were referenced, microcystin-LR (MC-LR), microcystin-RR (MC-RR), and microcystin-YR (MC-YR). Yunes et al. (2003), reported the presence of microcystin equivalents, but no details were provided.

Saxitoxins (STXs) were saxitoxin (STX) and gonyautoxins (GTX) were commonly reported. Three saxitoxin variants: decarbamoylneosaxitoxin (dc-NEO), decarbamoylsaxitoxin (dc-STX), and neosaxitoxin (Neo-STX), and five gonyautoxins variants: gonyautoxin-1 (GTX-1), gonyautoxin-2 (GTX-2), gonyautoxin-3 (GTX-3), gonyautoxin-4 (GTX-4), and gonyautoxin-6 (GTX-6) were identified (Table 2).

Molica et al. (2002) recounted the presence of a previously unidentified saxitoxin analog after a massive cyanoHAB that took place between July and October 1998 at Tabocas Reservoir. The cyanoHAB was dominated by *C. raciborskii* strain ITEP-018. The unidentified analog, was considered to be the most toxic cyanotoxin reported at the moment of publication.

Cylindrospermopsin (CYN), was reported by only one publication (Walter et al., 2018), this was an unexpected finding given that the *Cylindrospermopsis* was reported by 36% (26 out of 72) of the publications, and *C. raciborskii* specifically by 30% (21 out of 72) of them. This can be explained by the fact that Brazilian strains of *C. raciborskii* have not shown to produce cylindrospermopsins (Molica et al., 2002) (Table 2).

Argentina

In Argentina, cyanoHABs have been known since 1944, when nearly thousand ducks died after an *Anabaena flos-aquae* cyanoHAB at Benedetti Lake (Kühnemann, 1966). The first account of a massive fish mortality caused by cyanobacteria was recorded in 1954 in Miguel del Monte Lagoon in La Pampa region (Ringuelet et al., 1955). Kühnemann (1966) wrote the first

encompassing review for the country, and it was revised a decade later by Emiliani and Rodriguez (1974). Emiliani and Rodriguez (1974) reviewed the negative impacts of harmful algal blooms (HABs) and cyanoHABs in natural, urban, and industrial environments. Odriozola et al. (1984) reported the death of seventy-two cows after drinking from a pond with a *M. aeruginosa* cyanoHAB.

A review of the phytoplankton ecology of the middle Paraná River revealed that Cyanophyceae are typically subdominant or dominant in the upper Paraná, but decrease in importance downstream and were rarely abundant in the middle Paraná (García de Emiliani, 1990). Pizzolon et al. (1999) examined water bodies in fourteen localities across Argentina to determine the state of cyanoHABs in coastal and continental environments from 1963 until 1997. *Microcystis* and *Anabaena* were the dominant genera. CyanoHABs happened during summer and fall, in the Central and North regions, and in spring in the Patagonia region. O'Farrell et al. (1996) sampled the phytoplankton community of the lower Paraná River. The greatest abundance and diversity were recorded during spring. The most common species were *Anabena oscillaroides*, *Aphanizomenon flos-aquae* and *Raphidiopsis mediterranea* (Table 3).

Regional Studies

Central Region

Buenos Aires (PBA) Province

The San Miguel del Monte Lagoon was sampled after massive fish deaths occurred in March 1954. The mortality was caused by a cyanoHAB dominated by *Anabaena inaequalis*, *A. circinalis* and *Polycystis flos-aquae* (Ringuelet et al., 1955).

In 1999, Planetario Lake, a recreational pond in the city of Buenos Aires had a *M. aeruginosa* cyanoHAB that resulted in massive bird and fish mortality, likely caused by

microcystins. Monthly sampling by the local water authority showed that cyanobacteria dominated the phytoplankton community in the lake at the time of the cyanoHAB (Ehrenhaus & Vigna, 2006).

In Río de La Plata Estuary, cyanobacteria were less abundant than green algae. The dominant cyanobacteria genera were *Microcystis*, *Oscillatoria*, and *Merismopedia*, all potential cyanotoxin producers (Conti et al., 2005b). A *M. aeruginosa* cyanoHAB occurred at Puerto Madero docks, in the Río de La Plata Estuary. *M. aeruginosa* was the dominant species representing more than 90% of the phytoplankton biomass. CyanoHAB extracts were hepatotoxic. A microcystin (MC) variant likely microcystins-XR (MC-XR), where X represents an unidentified amino acid, was present in all samples. This was the first report of a microcystin (MC) in an urban site in the estuary (Ouahid et al., 2011). In samples from two locations at the Río de La Plata Estuary *M. aeruginosa* accounted for 97% of the total phytoplankton biomass. Both areas were used for recreational activities and water supply. Microcystin-LR (MC-LR) was present in water from the estuary as well as in domestic water samples (Giannuzzi et al., 2012).

As part of an encompassing study, Echenique and Aguilera (2014) had one case in a popular tourist destination near La Plata City in the Río de La Plata Estuary. *M. aeruginosa* was typically the dominant species. Microcystins (MCs) were detected in 10 out of 13 samples, at higher concentrations than those suggested by the WHO for drinking-water (1 mg l⁻¹ of microcystin-LR) (WHO, 1998).

Increased water turbidity and a foul odor, resembling that of organochloride pesticides, added to skin and respiratory problems reported by residents of Punta Alta and Bahía Blanca Cities. These issues prompted an investigation on the water quality of the Grünbein and Planta Patagonia water treatment plants as well as several houses supplied by the Paso de las Piedras

Reservoir. High concentrations of *M. aeruginosa* and *A. circinalis* were reported for domestic water samples. This was the first report of cyanobacteria in drinking water for Argentina. In a different publication, Echenique et al. (2006) reported the presence of *M. aeruginosa* and microcystins (MCs) in the water supply of the cities of La Plata and Ensenada.

Water samples collected at input and output points of two water treatment facilities from the Paso de las Piedras Reservoir, and at specific households in Bahía Blanca City showed high concentrations *M. aeruginosa* and *D. circinalis*. These findings indicate the inefficiency of standard water treatments to remove cyanobacteria (Echenique & Aguilera, 2014).

Paso de las Piedras Reservoir has had recurrent cyanoHABs since 1982 (Echenique et al., 2001; Fernández et al., 2009; Pizzolon et al., 1999). Throughout the study period, two cyanoHABs were recorded where *Microcystis natans* and *Anabaena circinalis* were the most common species. Four potentially toxic species were also recorded *Snowella fennica*, *M. aeruginosa*, *P. agardhii*, and *A. circinalis*. Nutrient enrichment, phosphorus levels, temperature, and solar radiation were the mechanisms that induced cyanoHAB formation in Paso de las Piedras Reservoir (Fernández et al., 2015).

A field experiment in the Grande Lagoon in the Otamendi Natural Reserve showed that algal biomass was highest during warm periods. The phytoplankton community was dominated by flagellates, small coccoid algae, and cyanobacteria, while nitrogen-fixing heterocystous cyanobacteria were rare (Unrein et al., 2010). Environmental conditions in Grande Lagoon were marked by drought and flooding. The phytoplankton community was dominated by eight cyanoHAB forming species *Anabaenopsis cf. elenkinii*, *Cuspidothrix issatschenkoi*, *Dolichospermum cf. bituri*, *Raphidiopsis mediterranea*, *Sphaerospermopsis cf. aphanizonemoides*, *Sphaerospermopsis torques-reginae*, *Planktothrix agardhii*, and *M.*

aeruginosa. CyanoHABs intensity and frequency in Grande Lagoon has increased in the last two decades (O'Farrell et al., 2015).

Five *Anabaenopsis* morphospecies were identified in Los Patos Lake, a shallow lake used for fishing and recreational activities in Ensenada City. The phytoplankton community is dominated by cyanobacteria, and almost all species contributed to cyanoHABs during warm periods. Some species like *Anabaenopsis milleri* and *A. elenkinii* were also present in low temperatures and others like *A. cf. cunningtonii* formed cyanoHABs during winter (Aguilera et al., 2016).

Córdoba (CB) Province

Quality monitoring from the Tercero River water treatment plant, revealed the presence of cyanobacteria, predominantly *M. aeruginosa*. The increase in cyanobacterial density coincided with bad taste and odor in tap water reported by members of the community (Lerda & Prospero, 1996).

The San Roque Reservoir created by the damming the Suquía and Cosquín Rivers, provides water to the city of Córdoba and its adjacent communities, and it is used for recreation and power generation. Several field and laboratory experiments have been conducted at this reservoir where cyanoHABs have been a common occurrence. In 1989, HPLC revealed the presence of microcystin traces in water samples collected during a *M. aeruginosa* cyanoHAB (Scarafia et al., 1995). Systematic monitoring of this reservoir conducted between 1998 and 2002 confirmed the presence of high concentrations of microcystin-RR (MC-RR) and microcystin-LR (MC-LR) in 97% of the observed cyanoHABs (Amé et al., 2003).

The uptake, accumulation, and distribution of microcystin-RR (MC-RR) in the tissue of wild fish, under field and laboratory conditions, during a *Microcystis* cyanoHAB were studied by

Cazenave et al. (2005). In the laboratory, two species of fish *Jenynsia multidentata* and *Corydoras paleatus* were exposed to microcystins (MCs). In the field, *Odontesthes bonariensis* captured from the San Roque Reservoir and water samples were examined for microcystins (MCs). Liquid chromatography-electrospray ionization-time-of-flight-mass spectrometry (LC-ESITOF-MS) and HPLC showed the presence of microcystin-RR (MC-RR) in liver, gills, brain and muscle of *J. multidentata*, and in liver, gills, intestine, and muscle of *C. paleatus*. The concentration of microcystin-RR (MC-RR) in muscle of *O. bonariensis* exceeded the values suggested by the WHO. The uptake and accumulation of microcystin-LR (MC-LR) by the freshwater shrimp *Palaemonetes argentinus* was studied in laboratory and field assays.

In the laboratory, shrimp were obtained alive from microcystin-LR (MC-LR) free water from the San Antonio River, and exposed to the toxin at different concentrations. Field exposures were conducted in the San Roque Reservoir after a cyanoHAB. This was the first-time nodularin (NOD) was reported for this reservoir and in South America (Galanti et al., 2013). The bacterium *Sphingomonas sp.* CBA4, isolated from the San Roque Reservoir, was capable of biodegrading microcystin-RR (MC-RR) (Valeria et al., 2006).

Anatoxin-a (ANTX-a) and microcystin (MC) were reported co-occurring in the San Roque Reservoir. This was the first report of anatoxin-a (ANTX-a) for South America. Microcystin (MC) concentrations were higher than the guidelines suggested by WHO for drinking and recreational waters (WHO, 2003). *Microcystis*, *Oscillatoria*, *Anabaena*, and *Pseudoanabaena* were likely the main producers of microcystin (MC) in the reservoir. The highest cyanotoxin concentrations were registered during spring and summer (Ruiz et al., 2013).

Entre Ríos (ER) Province

The Salto Grande Reservoir is a transnational hydroelectric; it is part of the Uruguay River and it is located in the Entre Ríos province of Argentina and the Salto department in Uruguay. Though it is mainly used for power generation, it is also used for recreational purposes particularly during summer. In 2007, a 19-year-old man, accidentally fell into the reservoir, and remained in the water for two hours. At the time, there was a *M. aeruginosa* and *M. wesenbergii* cyanoHAB. The young man was exposed to cyanotoxins via immersion, oral ingestion, and inhalation. Four hours after exposure he suffered from abdominal pain, nausea, vomiting, muscle weakness, and fever. Within three days, he exhibited respiratory distress, and dyspnea. A week after, he developed hepatotoxicosis. Twenty days after exposure, he recovered completely with no signs of permanent damage. Microcystin-LR (MC-LR) was present in all water samples collected during the incident (Giannuzzi et al., 2011).

The introduced bivalve *Limnoperna fortunei* was first reported for Argentina in the Rio de la Plata Estuary in the 1990s. Its presence in the Salto Grande Reservoir changes the structure of the *Microcystis spp.* population boosting its growth. Bivalves consume algae and modify nutrient supply for *Microcystis spp.*, favoring their aggregation into colonies, which might have enhanced the formation of *Microcystis* cyanoHABs (Cataldo et al., 2012).

Santa Fe (SF) Province

García de Emiliani and Emiliani (1997) reported the death of cattle and wild birds linked to an *Anabaena spiroides* cyanoHAB in the Portmann Lagoon in the Salado River. This was the first report of an *A. spiroides* cyanoHAB in the province. *Merismopedia minima*, *Raphidiopsis mediterranea*, and *R. curvata* were also present. Although no toxicity tests were conducted, *A. spiroides* is a known neurotoxin producer. The cyanoHAB took place under typical

environmental conditions for this phenomenon, drought, strong winds, high temperatures, and high pH values.

Norte Grande Argentino/North Region

Corrientes (CR) Province

Zalocar de Domitrovic et al. (1998) studied the spatial and temporal variations of phytoplankton in an urban pond, the Aeroclub Lagoon. With 25% to 83% of the total biomass, cyanobacteria dominated the phytoplankton community. Five species were responsible for this elevated biomass (i.e. *C. raciborskii*, *Aphanizomenon sp.*, *Phormidium mucicola*, *M. aeruginosa* and *Merismopedia tenuissima*). The dominant species were *C. raciborskii*, *Aphanizomenon sp.*, and *M. aeruginosa*. Over a decade later, Vallejos et al. (2015) studied periphytic algae in the same lagoon. They collected three plants of *Nymphoides indica* and *Potamogeton illinoensis* and scraped periphyton from selected areas on each plant. *P. illinoensis* samples showed the highest cyanobacterial density and frequency, with *Stigonema hormoides* as the dominant species. In *N. indica* cyanobacteria were the second group in terms of abundance and frequency.

Coelosphaerium kuetzingianum and *Aphanocapsa elachista* were the dominant species. Both species are known cyanotoxins producers.

Misiones (MI) Province

Otaño (2009) examined the presence of potentially toxic cyanobacteria in the Uruguay River that supplies drinking water to the Corrientes and Misiones provinces. This was the first report of *Aphanizomenon schindleri* in Uruguay River and it accounted for 93% of the phytoplankton biomass from March to April. *A. spiroides* accounted for 91% of the phytoplankton from January to March. *A. spiroides*, *M. aeruginosa*, *C. raciborskii*,

Aphanizomenon sp., and *Anabaena sp.* were also present. Neither microcystins (MCs) nor saxitoxins (STXs) were found in water samples.

Patagonia Region

Echenique and Aguilera (2014) presented the results from four case studies involving cyanoHABs in Argentina. One case included areas of the Limay River, downstream from the Arroyito Reservoir in Neuquén province. During the study, *Doclichospernum* cyanoHABs were recorded, especially during the austral summer (November to January) with densities ranging from 1,100 and 3,500 cells m⁻¹. Toxicity of the cyanoHAB was assessed in mice from samples taken in the Exequiel Ramos Mexía reservoir indicating the presence of cyanobacterial neurotoxins.

Another study was conducted when fragments of cyanobacteria were detected in the tap water of Tolhuin City in Tierra del Fuego province. Water samples were taken from Fandando Lake, the water supply for Tolhuin, to estimate phytoplankton density. *Snowella lacustris* and *Woronichinia naegeliana*, both cyanotoxin producers, were the dominant species. *S. lacustris* highest densities were >1,000 cells m⁻¹ in December 2002 and January 2004. Similar values were recorded from November 2003 until the fall of 2004, coinciding with high nutrient availability.

Table 3. List of cyanobacterial genera and species reported for Argentina in peer-review contributions from 1990 to 2018.

	State	Reference	Water body	Highlighted species	Cyanotoxin
All	C: CB, SF, ER, PBA. Cy: SL. NGA: MI, SA, TU, SE. P: RN, NQ, CT	Pizzolon et al. (1999)	C: San Roque, Río Tercero, Río Cuarto. SFe: Portmann. ER: Salto Grande. PBA: Paso de Piedras. SL: Cruz de Piedra. MI: Uruguáí. SA: Cabra Corral. Tucumán: El Cadillal, Río Hondo. SE: Río Hondo. Río Negro: Pellegrini, Ramos Mexía, Limay-Negro. Neuquén: Ramos Mexía, Limay-Negro. CT: Willimanco, F. Ameghino, Chubut inf.	<i>Microcystis sp.</i> , <i>Anabaena sp.</i> , <i>Aphanizomenon sp.</i> , <i>Gomphosphaeria lacustris</i> and <i>Oscillatoria sp.</i>	MC-RR
Central	PBA	Ringuelet et al. (1955)	San Miguel del Monte Lagoon	<i>Anabaena inaequalis</i> , <i>A. circinalis</i> , <i>Polycystis flos-aquae</i>	
		Odriozola et al. (1984)	Artificial pond	<i>M. aeruginosa</i>	
		Ehrenhaus & Vigna (2006)	Lake Planetario	<i>M. aeruginosa</i>	
		Unrein et al. (2010)	Laguna Grande		
		Echenique et al. (2006)	Paso de las Piedras Reservoir	<i>M. aeruginosa</i> , <i>A. circinalis</i>	
		Ouahid et al. (2011)	Puerto Madero docks, in the Río de La Plata Estuary	<i>M. aeruginosa</i>	MC-LC
		Echenique (2001)	Paso de las Piedras Reservoir	<i>M. aeruginosa</i> , <i>A. circinalis</i>	
		Echenique et al. (2006)	Río de la Plata River	<i>M. aeruginosa</i>	MC-LR, other MCs

Note: **C:** Central, **CB:** Córdoba, **ER:** Entre Ríos, **SF:** Santa Fe, **PBA:** Buenos Aires, **Cy:** Cuyo, **SL:** San Luis, **NGA:** Norte Grande Argentino, **MI:** Misiones, **SA:** Salta, **TU:** Tucumán, **SE:** Santiago del Estero, **P:** Patagonia, **RN:** Río Negro, **NQ:** Neuquén, **CT:** Chubut. **MCs:** Microcystins, **MC-LC:** Microcystin-LC, **MC-RR:** Microcystin-RR, **NOD:** Nodularin.

Table 3. Continued.

	State	Reference	Water body	Highlighted species	Cyanotoxin
		Echenique & Aguilera (2014)	Paso de las Piedras Reservoir and Río de La Plata Estuary	<i>M. aeruginosa</i> , <i>D. circinalis</i>	MCs, MC-LR, MC variant with a molecular ion
		Fernández et al. (2015)	Paso de las Piedras Reservoir	<i>Microcystis natans</i> , <i>Anabaena circinalis</i> , <i>Snowella fennica</i> , <i>M. aeruginosa</i> , <i>Planktothrix agardhii</i> , <i>A. circinalis</i> .	MC-LR
		O'Farrell et al. (2015)	Laguna Grande	<i>A. cf. elenkinii</i> , <i>C. issatschenkoi</i> , <i>D. cf. bituri</i> , <i>R. mediterranea</i> , <i>S. cf. aphanizonemoides</i> , <i>S. torques-reginae</i> , <i>P. agardhii</i> , <i>M. aeruginosa</i>	
		Aguilera et al. (2016)	Los Patos Lake	<i>Anabaenopsis milleri</i> , <i>A. elenkinii</i> , <i>A. cf. cunningtonii</i>	
Central	ER, PBA	Giannuzzi et al. (2012)	Río de La Plata Estuary	<i>M. aeruginosa</i>	MC-LR
	CB	Scarafia et al. (1995)	San Roque Reservoir	<i>M. aeruginosa</i>	MC-LR, MC-RR
		Amé et al. (2003)	San Roque Reservoir		MC-LR, MC-RR
		Lerda & Prospero (1996)	Tercero River	<i>M. aeruginosa</i> , <i>Lyngbya sp.</i> , <i>Phormidium sp.</i>	
		Cazenave et al. (2005)	San Roque Reservoir	N/A	MC-RR
		Valeria et al. (2006)	San Roque Reservoir	<i>Microcystis sp.</i>	

Note: **C:** Central, **CB:** Córdoba, **ER:** Entre Ríos, **SF:** Santa Fe, **PBA:** Buenos Aires, **Cy:** Cuyo, **SL:** San Luis, **NGA:** Norte Grande Argentino, **MI:** Misiones, **SA:** Salta, **TU:** Tucumán, **SE:** Santiago del Estero, **P:** Patagonia, **RN:** Río Negro, **NQ:** Neuquén, **CT:** Chubut. **MCs:** Microcystins, **MC-LC:** Microcystin-LC, **MC-RR:** Microcystin-RR, **NOD:** Nodularin.

Table 3. Continued.

	State	Reference	Water body	Highlighted species	Cyanotoxin
Central		Galanti et al. (2013)	San Roque Reservoir	N/A	NOD
		Ruiz et al. (2013)	San Roque Reservoir	<i>Microcystis sp.</i> , <i>Oscillatoria sp.</i> , <i>Anabaena sp.</i> , and <i>Pseudoanabaena sp.</i>	MC
	ER	Conti et al. (2005b)	Río de La Plata Estuary	<i>Microcystis sp.</i> , <i>Oscillatoria sp.</i> and <i>Merismopedia sp.</i>	MC, MC-LR
		Giannuzzi et al. (2011)	Salto Grande Reservoir	<i>M. aeruginosa</i> , <i>M. wesenbergii</i>	MC-LR
		Cataldo et al. (2012)	Salto Grande Reservoir	<i>Microcystis spp.</i>	
	SF	O'Farrell et al. (1996)	Lower Paraná River	<i>A. oscillaroides</i> , <i>A. flos-aquae</i> and <i>R. mediterranea</i>	
Cuy	SF	Kühnemann (1965)	Bedetti Lake	<i>A. flosaquae</i>	
NGA	CR	Zalocar de Domitrovic et al. (1998)	Aeroclub Lagoon	<i>C. raciborskii</i> , <i>Aphanizomenon sp.</i> , <i>P. mucicola</i> , <i>M. aeruginosa</i> and <i>M. tenuissima</i>	
		Vallejos et al. (2015)	Aeroclub Lagoon	<i>Stigonema hormoides</i> , <i>Coelosphaerium kuetzingianum</i> Nägeli and <i>Aphanocapsa elachista</i> West & G.S.	
	CR, MI	Otaño (2009)	Uruguay River	<i>Aphanizomenon schindleri</i> , <i>A. spiroides</i> , <i>M. aeruginosa</i> , <i>Cylindrospermopsis raciborskii</i> , <i>Aphanizomenon sp.</i> , <i>Anabaena sp.</i>	Not detected

Note: **C:** Central, **CB:** Córdoba, **ER:** Entre Ríos, **SF:** Santa Fe, **PBA:** Buenos Aires, **Cy:** Cuyo, **SL:** San Luis, **NGA:** Norte Grande Argentino, **MI:** Misiones, **SA:** Salta, **TU:** Tucumán, **SE:** Santiago del Estero, **P:** Patagonia, **RN:** Río Negro, **NQ:** Neuquén, **CT:** Chubut. **MCs:** Microcystins, **MC-LC:** Microcystin-LC, **MC-RR:** Microcystin-RR, **NOD:** Nodularin.

Summary

The first reference to cyanoHABs in Argentina dates back to 1944, when a thousand ducks died after an *A. flosaquae* bloom in the Santa Fe providence in the Central region (Kühnemann, 1966). However, the first peer-review article digitally available for the country was published by Ringuelet et al. (1955) after a massive fish death attributed to a cyanoHAB in the Buenos Aires province. Only one paper was available for the 1960s and 1970s. No cyanobacteria related articles were published in the 1980s and only four papers were published in the 1990s.

Thirty publications regarding cyanobacteria were digitally available for Argentina. Most publications were written for the Central region (53.3%, 16 out of 30), specifically for the Buenos Aires province (46.7%, 14 out of 30) (Table 3). Only 6.7% (2 out of 30) of the studies were conducted in the Norte Grande Argentino or North, the largest region in the country. In spite of been the site of the first documented cyanoHAB in the country (Kühnemann, 1966), only one publication was available for the Cuyo region (3.3%). For Patagonia, the southernmost region, one case study and one article were available. Four of the eight regions of the country and nine of the 23 provinces in the country were covered by the cyanobacteria literature (Table 3).

Twenty-two genera and 30 species were reported for Argentina, the most common genera across the country were *Microcystis* (76.7%, 23 out of 30), *Anabaena* (36.7%, 11 out of 30), *Aphanizomenon* (20%, 6 out of 30), and *Dolichospermum* (16.7%, 11 out of 30). Three species were identified for *Microcystis* (i.e. *aeruginosa*, *natans*, and *wesenbergii*) and four for *Anabaena* (*inaequalis*, *circinalis*, *cf. elenkinii* and *oscillaroides*) (Table 3). *Microcystis aeruginosa* (53.3%,

16 out of 30) and *Anabaena circinalis* (16.7%, 5 out of 30) were the most commonly reported species (Table 3).

Nearly 60% of the Argentinian publications (57%, 17 out of 30) reported the presence of at least two species of cyanobacteria, while 43.3% (13 out of 30) reported three or more species. Cyanobacterial density was reported by 46.7% (14 out of 30) of the publications available, and 50% (15 out of 30) reported the presence of cyanotoxins.

Microcystins (MCs) were the most common cyanotoxins (40%, 12 out of 30). Four variants were identified: microcystin-LC (MC-LC), microcystin-LR (MC-LR), microcystin-RR (MC-RR) and variant with a molecular ion. Microcystin-LR (MC-LR) was the most commonly reported microcystin in Argentina (26.7%, 8 out of 30). Only one publication reported the presence of nodularins (NOD) (Galanti et al., 2013), and one mentioned the presence of cyanobacterial neurotoxins without providing specifics (Echenique & Aguilera, 2014) (Tables 3, 6). A single cyanotoxin was reported by 30% (9 out of 30) of the articles, and only 16.7% (5 out of 30) reported mixtures of two or more cyanotoxins types. Mixtures of three or more cyanotoxins were not reported (Table 3).

Uruguay

CyanoHAB reports for Uruguay date back to the 1960s, when dense scums were observed in Sauce Lagoon in the East region. The occurrence of this cyanoHABs was likely due to intensification in anthropogenic pressures (González-Madina et al., 2019; Pacheco et al., 2010).

By 1982, cyanoHABs were observed in most water bodies in the country, particularly during the austral summer and in eutrophic ecosystems (Vidal & Britos, 2012). During the

1998/1999 summer a *M. aeruginosa* cyanoHAB was registered in the coastal city of Colonia, and the first cyanoHAB in Montevideo beaches was observed in 2001 (Sienra & Ferrari, 2006).

By 1997, cyanoHAB occurrences had become common and cyanotoxins analysis, particularly for microcystins (MCs) was a standard component of cyanoHAB monitoring in the country. By 2008, Uruguay adopted routine saxitoxin (STX) and cylindrospermopsin (CYN) analysis after a cyanoHAB (Bonilla et al., 2015).

Several nationwide studies on cyanobacteria were in the 2000s and 2010s. Vidal and Kruk (2008) investigated the spatial occurrence and relative frequency of *C. raciborskii*, in forty-seven freshwater lakes in southern Uruguay. This was the southernmost observation of the species in the Americas and the first report from the country. At the time of publication *C. raciborskii* had only been seen in four of the studied lakes (i.e. Blanca, Chica, Javier, and Sauce). All lakes had high water temperatures, high nutrient concentrations, low light availability, and well-mixed waters.

Bonilla (2009) edited a national guide on identification and management of planktonic cyanobacteria including case studies in the Salto Grande Reservoir in the North and in the Castillos and de Rocha coastal lagoons in the East, as well as the distribution of the invasive species *C. raciborskii* in the country.

Pacheco et al. (2010) used morphologically based functional groups (MBFGs) to study the phytoplankton community of five reservoirs in the southern coast of Uruguay. One hundred fifty-three species were identified, mainly green microalgae and cyanobacteria. Dominant groups were different between lakes and between seasons. Cyanobacteria were identified in two lakes, in Lake Blanca Chroococcales, *M. aeruginosa*, and *Aphanocapsa spp.* dominated during winter,

while Oscillatoriales, *C. raciborskii* and *Anabaena spp.* dominated during summer. In Lake Escondida *Microcystis spp.* were abundant during summer and winter.

Phylogenetic analysis of *C. raciborskii* obtained from lakes in the East and Metropolitan regions, showed that the Uruguayan strains were closely affiliated to other *C. raciborskii* strains isolated from the Americas, particularly from Brazil (Piccini et al., 2011). As part of a world distribution study for *P. agardhii* and *C. raciborskii*, Bonilla et al. (2012) examined data from seven lakes in the subtropical zone of Uruguay. In most lakes, the distribution of *P. agardhii* and *C. raciborskii* overlapped, and it was affected by temperature, light, and trophic status.

O'Farrell (2012) studied phytoplankton communities along the Uruguay River. CyanoHABs commonly occur in the river during the summer due to high temperatures and lack of water mixing. The most common cyanobacteria taxa were *M. aeruginosa*, *Dolichospermum spiroides* and *D. circinale*. The highest cyanobacteria abundance was recorded during summer constituted mainly by *M. aeruginosa* and *D. cf. pseudocompactum*. Toxicity analyses did not indicate the presence of microcystin-LR (MC-LR).

In 2015, a national cyanobacteria data base was published, including 3,061 records from 1980 until 2014. The data base examined the distribution of cyanobacteria and three common cyanotoxins: microcystin (MC), saxitoxin (STX), and cylindrospermopsin (CYN), in sixty-four water bodies in Uruguay (Bonilla et al., 2015).

Kruk et al. (2015) studied cyanoHABs along six sites along the Uruguay River and Río de La Plata Estuary. MBFGs and PCR were used as indicators of the presence of encoding genes for cyanotoxins production. The most abundant populations of toxic cyanobacteria were found in Salto in the North region, belonging to the *M. aeruginosa* complex. Microcystin-LR (MC-LR) were detected in five samples. Kozlíková-Zapomělová et al. (2016) also studied sites along the

Uruguay River to clarify the taxonomic status of the genus *Dolichospermum*. They named and classified a new species *Dolichospermum uruguayense*. CyanoHABs in the lower Uruguay River normally include numerous *Dolichospermum* morphospecies.

González-Piana et al. (2017) analyzed the temporal dynamics of the concentration of total microcystins (MCs) in three reservoirs destined to power generation Bonete in the North, Baygorria in the Central-south, and Palmar in the Littoral region. Microcystin (MC) concentrations were the lowest in Bonete, followed by Baygorria, and Palmar. Microcystin-LR (MC-LR) concentration appeared to be related to water temperature and cyanobacterial biomass. Nearly 27% of the samples were in the slight to moderate risk to health category according to the WHO guidelines (Table 4).

Regional Studies

East Region

Maldonado (MA) Department

Sauce Lagoon is main source of drinking water for Maldonado City and the second supplier of drinking water in the country. Recurrent cyanoHABs dominated by *Cuspidothrix issatschenkoi*, *Dolichospermum crassum*, *Microcystis aeruginosa*, *M. panniformis* and *Sphaerocavum brasiliense* were observed in the lagoon. The genera *Dolichospermum* and *Microcystis* are known cyanotoxin producers. Total nitrogen and orthophosphate (PO₄) concentrations determined the differential presence of potentially toxic cyanobacteria (González-Madina et al., 2017). Sauce Lagoon also provides recreational services during summer. Weekly samples were collected during the 2015/2016 and 2016/2017 summers. Both periods had distinctive phytoplankton biomass and composition. In the 2015/2016 summer cyanobacteria biomass surpassed the alert level 3. This cyanoHABs was promoted by low N:P ratios and

dominated by *Aphanizomenon gracile*, *Dolichospermum crassum*, and *Cuspidothrix issatschenkoi*. *Aphanizomenon* and *Dolichospermum* are known cyanotoxin producers (González-Madina et al., 2019).

Rocha (RO) Department

Castillos Lagoon is part of the Bañados del Este Biosphere Reserve and an Important Bird and Biodiversity Area (IBA). A *Nodularia baltica-spumigena* cyanoHAB was reported in January 1990 (Pérez et al., 1999). In the late 1990's, the lagoon had limited human activity and cyanoHABs appeared to be determined by meteorological and limnological conditions exclusively.

Littoral Region

Río Negro (RN) Department

Ferrari et al. (2011) studied the lower Uruguay River from 2006 to 2009. Twenty-four cyanobacteria taxa were recorded. Among the cyanoHAB forming genera *Dolichospermum* and *Microcystis* had the highest number of species. CyanoHABs occurred during summer, and were dominated by *M. aeruginosa* and *D. cf. pseudocompactum*. Although both species are known to produce microcystins, microcystin-LR (MC-LR) was not detected after toxicity analysis.

Cyanobacterial densities were associated to the presence of nitrogen (N) and phosphorus (P), and to flow changes. Densities increasing during summer when the temperatures were high and flow levels were low. This was the first time *R. fernandoi* was recorded in Uruguay and the first time *Dolichospermum cf. pseudocompactum* was recorded in South America.

North Region

Salto (SA) Department

The Salto Grande Reservoir is a polymictic and eutrophic reservoir with recurrent summer cyanoHABs dominated by *Microcystis* and *Dolichospermum* (Chalar, 2009). Bonilla (2009) proposed the use of environmental variables to develop predictive models for cyanoHAB occurrence based on phytoplankton abundance and diversity. They used some of the environmental conditions that promote *Microcystis* cyanoHABs as well as the factors that can make the system resilient to them. The co-occurrence of *Dolichospermum* and *Microcystis* in the reservoir is determined by hydrological conditions and reservoir morphology. In general, *Microcystis* had higher densities than the *Dolichospermum*, the reasons for *Microcystis* dominance is unclear, but does not appear to be explained by nitrogen-fixation behavior.

The regular occurrence of *Microcystis* cyanoHABs in Salto Grande Reservoir inhibited the recruitment of the invasive bivalve *Limnoperna fortunei*. This reduced recruitment might make the reservoir more susceptible to cyanoHABs (Boltovskoy et al., 2013). Other variables that might have contributed to the formation of cyanoHABs in the reservoir were inflow discharge, water level, wind velocity, and temperature. These factors produced water mixing, which in combination with light regimes influenced the vertical distribution of *Microcystis* and *Dolichospermum* (Bordet et al., 2017).

Metropolitan Region

Montevideo (MO) Department

De Leon and Yunes (2001) reported for the first time the occurrence of a toxic *Microcystis aeruginosa* cyanoHAB in the Uruguayan side of the Río de la Plata Estuary. Prior to that, a non-toxic *M. aeruginosa* cyanoHAB was registered in the Río de la Plata Estuary in 1981

(De Leon & Yunes, 2001). The first cyanoHAB in Montevideo beaches was observed in 2001 (Sienra & Ferrari, 2006).

Rodó Lake is an urban lake in the city of Montevideo, with high phytoplankton biomasses always dominated by the filamentous cyanobacterium *P. agardhii* (Sommaruga, 1995). In 1996, a restoration effort was conducted in the lake. This was the first restoration attempt for Uruguay. The lake was drained, sediments were removed, and stream inputs diverted. Since restoration, the phytoplankton community became more diverse, favoring grazing by mesozooplankton, decreasing phytoplankton biomass, and increasing water transparency (Scasso et al., 2001). In terms of MBFGs, the phytoplankton community of the Rodó Lake has high phytoplankton abundance. While chlorophytes, cyanobacteria, and diatoms were numerically dominant, cyanobacteria and chlorophytes were the groups with the greatest biovolume (Kruk et al., 2002).

Canelones (CA) Department

Seven man-made lakes (i.e. Botavara, Jardín, Javier, Leandro, Pomacea, Prohibido, and Ton-Ton) formed by the removal of sand for construction in an urban area in the department of Canelones were studied to examine the connection between nitrogen (N), the euphotic/mixing zone, and the presence of cyanobacteria. The growth rate of nitrogen-fixing cyanobacteria increased with increased nitrogen (N) concentrations. However, there was no correlation between cyanobacterial growth and lower diversity in the systems (Fabre et al., 2010).

The effect of light and temperature in cyanobacteria biomass and cyanotoxin concentration were examined in Javier Lake. *C. raciborskii* dominated the phytoplankton community, and maintained a high biomass in a broad range of radiation and temperature. This was the first report of a *C. raciborskii* in low temperatures (10.5°C). In spite of *C. raciborskii*

high biomass, saxitoxin (STX) levels were always low, below the values suggested by the WHO (Somma, 2014). Samples from Javier Lake were used in laboratory bioassays to test the synergistic effect of light and nutrients in the growth of cyanobacteria from the order Nostocales in comparison to non-heterocystous cyanobacteria. The combination on nutrients and light promoted changes in the dominance of Nostocales and non-heterocystous cyanobacteria. By the end of the experiment, Nostocales either replaced or co-dominated in all treatments. The highest saxitoxin (SXT) concentrations were observed in treatments with no addition of nitrogen (N) or phosphorus (P), indicating a potential link between toxicity and nutrient limitations (Aguilera et al., 2017).

Summary

The first accounts of cyanobacteria and their toxins for Uruguay date back to the 1960s (González-Madina et al., 2019; Pacheco et al., 2010). In the 1980s, cyanoHABs were reported in most water bodies in the country particularly in eutrophic ecosystems during the austral summer (Bonilla et al., 2015; Vidal & Britos, 2012). However, the first article digitally available was published in 1999 by Pérez et al. (1999) about a *Nodularia baltica-spumigena* cyanoHAB in the East region.

Twenty-five articles were available for Uruguay, most of them for the Metropolitan region (32%, 8 out of 25), followed but the East (20%, 5 out of 25), then the North (16%, 4 out of 25) and Littoral regions (8%, 2 out of 25). No papers were available for the Central-south region. Most studies covered several regions and departments. Seven of the nineteen departments in the country were covered in the literature. The most studied region, Metropolitan region, is the most urbanized and industrialized (Table 4).

Seventeen genera and 29 species were reported for the country. The most commonly reported genera were *Microcystis* (72%, 18 out of 25), *Dolichospermum* (44%, 11 out of 25), *Cylindrospermopsis* (28%, 7 out of 25) and *Aphanizomenon* (24%, 6 out of 25). Four species were identified for *Microcystis* (i.e. *aeruginosa*, *flos-aquae*, *panniformis*, and *wesenbergii*) and five for *Dolichospermum* (i.e. *circinale*, *crassum*, *cf. pseudocompactum*, *spiroides*, and *uruguayense*) (Table 6).

Dolichospermum uruguayense, was a newly described and named cyanobacterial species, it was isolated from samples from the lower Uruguay River. *M. aeruginosa* (36%, 9 out of 25) and *C. raciborskii* (28%, 7 out of 25) were the most commonly reported species for the country, (Table 1-6). The first report of *C. raciborskii* for Uruguay occurred in 2008, at the time that was that southernmost record of the species in the Americas (Vidal & Kruk, 2008).

Close to 50% (12 out of 25) of the available publications reported cyanobacterial density, 28% (7 out of 25) of them reported testing for cyanotoxins, and 20% (5 out of 25) detected them. Three cyanotoxin types were identified (i.e. cylindrospermopsin (CYN), microcystin (MC), and saxitoxin (STX)). Microcystin (MC) was the most commonly reported (16%, 4 out of 25). Saxitoxin was only reported once (Aguilera et al., 2017) and only one publication reported a mixture of cylindrospermopsin and microcystin (Bonilla et al., 2015) (Table 6).

Table 4. List of cyanobacterial genera and species reported for Uruguay in peer-review contributions from 1990 to 2018.

	State	Reference	Water body	Highlighted species	Cyanotoxin
All	Multiple	O'Farrell & Izaguirre (2014)	Uruguay River	<i>Microcystis aeruginosa</i> , <i>Dolichospermum spiroides</i> , <i>D. circinale</i> , <i>Dolichospermum spiroides</i> , <i>Pandorina morum</i> , <i>Pediastrum duplex</i> , <i>Spermatozopsis exsultans</i> , <i>Dinobryon eurystoma</i> , <i>Aulacoseira granulata</i> , <i>A. granulata</i> var. <i>angustissima</i> , <i>A. granulata</i> fo. <i>curvata</i> , <i>Aulacoseira islandica</i> , <i>Melosira varians</i> , <i>Nitzschia palea</i> , <i>Surirella guatemalensis</i> , <i>S. tenera</i> , <i>Synedra ulna</i> , <i>Cryptomonas marsonii</i> , <i>Plagioselmis lacustris</i> , <i>P. nannoplanctica</i>	Not detected
		Bonilla et al. (2015)	Multiple	N/A	MC, CYN
CS, N, L	Tacuarembó, Rio Negro, Durazno, Soriano	González-Piana et al. (2017)	Bonete, Baygorria and Palmar Reservoirs	<i>Microcystis</i> spp., <i>Dolichospermum</i> spp.	MC
E, L, M, N	Salto, Río Negro, Colonia, Montevideo, Maldonado	Kruk et al. (2015)	Uruguay and the Rio de la Plata Rivers	<i>Microcystis aeruginosa</i>	MC
E, M, N	Canelones, Maldonado	Vidal & Kurk (2008)	Laguna Chica and Lago Javier (Canelones), Laguna Blanca and Laguna del Sauce complex (Maldonado)	<i>Cylindrospermopsis raciborskii</i> , <i>Planktolyngbya</i> spp., <i>Ceratium hirudinella</i> , <i>Planktolyngbya limnetica</i> , <i>Aphanizomenon issatschenkoi</i>	

Note: CS: Central-south, E: East, L: Litoral, M: Metropolitan, N: North, MC: Microcystin, CYN: Cylindrospermopsin, STX: Saxitoxin.

Table 4. Continued.

	State	Reference	Water body	Highlighted species	Cyanotoxin
E, M	Canelones, Lavalleja, Maldonado	Pacheco et al. (2010)	Cisne, Blanca, Escondida, Clotilde, García	<i>Microcystis aeruginosa</i> , <i>Aphanocapsa</i> spp., <i>Cylindrospermopsis raciborskii</i> , <i>Anabaena</i> spp.	
	East	Maldonado	Piccini et al. (2011)	Laguna Blanca, Lago Javier	<i>Cylindrospermopsis raciborskii</i>
González-Madina et al. (2017)			Sauce Lagoon, Cisne and Potrero Lakes	<i>Dolichospermum crassum</i> , <i>Cuspidothrix issatschenkoi</i> , <i>Aphanizomenon</i> sp., <i>Microcystis aeruginosa</i> , <i>Microcystis panniformis</i> , <i>Sphaerocavum brasiliense</i>	
González-Madina et al. (2019)			Sauce Lagoon	<i>Aphanizomenon gracile</i> , <i>Cuspidothrix issatschenkoi</i> , <i>Dolichospermum crassum</i>	
Rocha		Pérez et al. (1999)	Laguna de Castillos	<i>Nodularia baltica-spumigena</i>	
Rocha, Salto		Bonilla (2009)	Salto Grande, Castillos and de Rocha	<i>Microcystis</i> sp., <i>C. raciborskii</i> , <i>Pseudanabaena</i> cf. <i>moniliformis</i>	
Littoral	Río Negro	Ferrari et al. (2011)	Lower Uruguay River	<i>Microcystis aeruginosa</i> , <i>M. wessenbergii</i> , <i>Dolichospermum circinale</i> and <i>Dolichospermum</i> cf. <i>pseudocompactum</i>	Not detected
		Kozlíková-Zapomělová et al. (2016)	Uruguay River	<i>Dolichospermum uruguayense</i>	
M	Canelones	Fabre et al. (2010)	Botavara, Jardín, Javier, Pomacea, Prohibido, Leandro and Ton-Ton	<i>M. aeruginosa</i> , <i>Cylindrospermopsis raciborskii</i>	
		Somma (2014)	Javier Lake	<i>C. raciborskii</i>	

Note: CS: Central-south, **E:** East, **L:** Littoral, **M:** Metropolitan, **N:** North, **MC:** Microcystin, **CYN:** Cylindrospermopsis, **STX:** Saxitoxin.

Table 4. Continued.

	State	Reference	Water body	Highlighted species	Cyanotoxin
Metropolitan	Montevideo	Aguilera et al. (2017)	Javier Lake	<i>Planktothrix agardhii</i> , <i>Pseudanabaena raphidioides</i> , <i>Pseudanabaena aff. limnetica</i> , <i>Planktolyngbya limnetica</i> , <i>Cylindrospermopsis raciborskii</i> , <i>Aphanizomenon aff. gracile</i>	SXT
		Amaral (2014)	Lago Javier	<i>C. raciborskii</i> MVCC19	
		Sommaruga (1995)	Lake Rodó	<i>P. agardhii</i>	
		De Leon & Yunes (2001)	Río de La Plata Estuary	<i>M. aeruginosa</i>	MC
		Scasso et al. (2001)	Lake Rodó	<i>P. agardhii</i>	
		Kruk et al. (2002)	Lake Rodó	<i>Anabaena planctonica</i> , <i>A. spiroides</i> , <i>Aphanizomenin flos-aquaw</i> , <i>A. gracile</i> , <i>Anabaenopsis sp.</i> , <i>Microcystis flos-aquae</i> , <i>M. wesenbergii</i> , <i>M. aeruginosa</i> , <i>P. agardhii</i> , <i>Pseudoanabena galeata</i> , <i>Oscillatoria spp.</i> , <i>Limnothrix planctonica</i> , <i>Raphidiopsis mediterranea</i>	
North	Salto	Charlar (2009)	Salto Grande Reservoir	<i>M. aeruginosa</i>	
		O'Farrell (2012)	Salto Grande Reservoir	<i>Dolichospermum sp.</i> , <i>Microcystis sp.</i>	
		Boltovskoy et al. (2013)	Salto Grande Reservoir	<i>Microcystis spp.</i>	
		Bordet et al. (2017)	Salto Grande Reservoir	<i>Microcystis sp.</i> , <i>Dolichospermum sp.</i>	

Note: CS: Central-south, E: East, L: Litoral, M: Metropolitan, N: North, MC: microcystin, CYN: Cylindrospermopsin, STX: Saxitoxin.

Colombia

The first references to cyanobacteria in Colombia was date back to the 1970s in the Ciénaga Grande de Santa Marta (CGSM) (Hernandez & Gocke, 1990). In the 1980s cyanobacteria were referenced in a catalog of the mollusks of the CGSM (Von Cosel, 1986). The earliest peer review report of cyanobacteria in Colombia is from the 1990s, in the Ciénaga Grande de Santa Marta (CGSM) (Hernandez & Gocke 1990). In 1994, *Anabaeopsis sp.* was associated to massive fish deaths in the CGSM during July and August (Mancera et al., 1994).

Most accounts on cyanoHAB, cyanobacteria and cyanotoxins in the country occurred during the 2010s.

Botero and Mancera-Pineda (1996) summarized the negative anthropomorphic impacts in the CGSM over five decades, from 1946 until 1994. They referenced the occurrence of fish mortalities associated to cyanoHABs after strong rains. Rainwaters flush of organic matter from mangroves into the CGSM, promoting nutrient accumulation, a determining factor for cyanoHABs.

Regional Studies

Amazon Region

Caquetá (CQ) Department

Pinilla (2006) studied the relations between the physico-chemical variables and the vertical distribution of phytoplankton community in Boa Lake. Cyanobacteria dominated during high waters, they accumulated in the water surface in areas with high sun exposure, but they were also abundant in the hypolimnion, the lower layer of water, which was dark and hypoxic. During the flow stage conditions were variable, and significant associations between the vertical arrangement of green algae and cyanobacteria were detected. The cyanobacterium *Oscillatoria*

splendida was identified as well as small cyanobacteria of the genera *Synechococcus* and *Dactylococcopsis*.

Andean Region

Rios-Pulgarin et al. (2016) examined seasonal and interannual variabilities in the taxonomic composition, richness, and density of periphytic algae in Guarinó River in the Los Nevados National Natural Park. They identified 17 cyanobacteria taxa. Cyanobacterial cells developed abundant mucilaginous matrix that allowed them to attach to each other, improving stress tolerance during high flow periods. Temperature was not decisive for tropical periphytic algae, but some genera (i.e. *Anabaena*, *Chroococcus*, *Oscillatoria*, and *Microcystis*) tended to have higher densities at high temperature because low temperature reduce their metabolic rate.

Antioquía (AN) Department

The Porce II and Rio Grande II Reservoirs are two of the biggest reservoirs in the country, both located in the Antioquía department. Both reservoirs are used for power generation. Rio Grande II also supplies drinking water to the nearby urban areas, and Porce II supports small-scale commercial fishing (Herrera et al., 2015; Palacio et al., 2015a). Riogrande II has two main tributaries, the Grande and Chico Rivers, both affected by anthropogenic disturbances (i.e. swine production, cattle ranching, and extensive agriculture). The tributaries received discharges from tanning industries, dairy farming, and sewage from urban settlements (Palacio et al., 2015a).

Ferrão-Filho et al. (2014) collected cyanoHAB samples from the Porce II and Rio Grande, to test the accumulation of dissolved microcystins from aqueous extracts in three Cladoceran species (i.e. *Moina micrura*, *Daphnia laevis* and *Daphnia similis*) in the laboratory.

Cladoceran uptake of microcystins increased with increased exposure to the toxin, indicating that Cladocerans bioaccumulate microcystins which corroborates previous findings.

Herrera et al. (2014) took samples of a cyanoHAB in Rio Grande II, and used them to test the effect of microcystin-LR (MC-LR) on the ecophysiology of *Daphnia similis* in the laboratory. *M. wesenberguii* was the most abundant species in the cyanoHAB, but *Sphaerospermopsis torques-reginae* was also present in high densities. Microcystin-LR (MC-LR) concentrations increased second antennae movements, but significantly reduced mandibular and thoracic movements, and heart rate. These physiological alterations could indicate intoxication by microcystins (MCs) or a behavioral response to their presence in the water.

Hurtado-Alarcón and Polanía-Vorenberg (2014), used polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) to detect cyanobacteria in the Riogrande II and La Fe Reservoirs. They found 35 DGGE bands in Riogrande II and 30 bands in La Fe. This reflects the molecular diversity of cyanobacteria in the reservoirs. PCR and DGGE are useful techniques to detect genes associated with toxicity and molecular diversity of cyanobacteria.

Herrera et al. (2015) tested the effects of six microcystis (MCs) extracts containing microcystin-LR (MC-LR) on the survival and reproduction of *Cladocerans* using water samples from Porce II and Rio Grande II. The most abundant species was *M. wesenberguii*, which was present in all samples. *Sphaerospermopsis torques-reginae*, *Dolichospermum sp.* and *Synechocystis sp.* were also identified.

Palacio et al. (2015a) monitored the *Dolichospermum lemmermannii*, a species typically found in high latitudes and temperate environments, in Rio Grande II. This was the first record of this species in Neotropical eutrophic environment. Its presence likely correlated with precipitation and reservoir level. Palacio et al. (2015b) also studied cyanobacterial species

richness of Riogrande II, and identified eleven species belonging to the order *Chroococcales*.

Microcystis sp. and *Dolichospermum sp.*, both toxic cyanoHAB producers, were the genera with the highest number of taxa.

Cundinamarca (CD) Department

Pinilla (2010) reported the presence of cyanobacteria in the phytoplankton community in five wetlands of the Bogota River basin: Guaymaral, Jaboque, Tibanica, Santa Maria del Lago, and Juan Amarillo, and one rural wetland in the Tenjo municipality for comparison. In the Tibanica, Juan Amarillo, and Guaymaral wetlands, cyanobacteria indicated high levels of organic matter and nutrients. In Guaymaral, cyanobacteria were the most abundant group within the phytoplankton community.

Huila (HU) Department

Silva et al. (2018) studied the composition of the phytoplanktonic community in the Quimbo Dam. Twelve species of cyanobacteria were recorded: *Anabaena circinalis*, *A. sphaerica*, *Nostoc sp.*, *Aphanizomenon gracile*, *Aphanocapsa delicatissima*, *Chroococcus turgidus*, *Gloeocapsa sp.*, *Lyngbya gracile*, *Merismopedia convolute*, *Microcystis aeruginosa*, *Oscillatoria princeps*, and *Spirulina platensis*. The most abundant species were *M. aeruginosa* and *Aphanizomenon gracile*, both cyanotoxin producers.

Caribbean Region

Atlántico (AT) Department

Tapia Larios (2014) characterized the cyanobacteria of the El Guájaro, a big fishing area in the Atlántico department. Eight species were identified: *M. aeruginosa*, *M. flos-aquae*, *Chroococcus minutus*, *Aphanocapsa grevillea*, *Coelosphaerium kuetzingianum*, *Anabaena sp.*, *A. phanizomenoides*, and *C. raciborskii*.

Córdoba (CR) Department

Jaramillo-Londoño and Aguirre-Ramírez (2012) assessed the spatio-temporal changes in the plankton community in the Ciénaga de Ayapel. Cyanobacteria represented 70% of the phytoplankton density. *C. raciborskii* and *Planktolyngbya limnetica* were the dominant species.

Magdalena (MA) Department

CyanoHABs in the CGSM, the biggest coastal lagoon in the country, were reported since the 1900s, likely due to high concentrations of phosphorous (P). The frequency of the cyanoHABs varied in time and space, but they were always dominated by *Anacystis cyanea* and *Nostoc commune* (Hernández & Gocke, 1990). In the CGSM, cyanoHABs have been presumed to cause multiple fish and mollusks mortalities either by toxicity or by reducing oxygen concentrations. CyanoHABs occurrence was only confirmed via visual assessment (Botero & Mancera-Pineda, 1996; Mancera et al., 1994; Polanía et al., 2001; Von Cosel, 1986).

Using HPLC-pigment analysis Gocke et al. (2003) determined that cyanobacteria were the predominant group in coastal lagoons (i.e. Nenguange Bay, CGSM, Ciénaga Pajarales lagoon complex and Occidental Salamanca Island complex) in the Colombian Caribbean. Cyanobacteria percentages increased from fresh waters to transient to the brackish lagoons.

In a study about the tilapia *Oreochromis niloticus* fishery in northern Colombia, Blanco et al. (2007), noticed that some cyanobacteria genera such as *Anabaeopsis* showed increased growth under salinity values greater than 10, indicating the occurrence of a cyanoHAB between June and July 2000. Cyanotoxins probably altered the tilapia food supply.

González and Gómez (2010) sampled water quality in the Sistema de Tratamiento de Aguas Residuales Salguero (STAR). A water treatment plant in the Cesar River. They identified

eight genera of toxin producing cyanobacteria *Phormidium*, *Planktothrix*, *Oscillatoria*, *Pseudanabaena*, *Woronichinia*, *Microcystis*, *Lyngbya*, and *Synechocystis*.

Plata-Díaz and Pimienta-Rueda (2011) studied plankton variability in twenty-seven swamps of the Momposina depression, part of the hydrographic network of the Magdalena River. They found high cyanobacterial densities in three of the thirty-four stations sampled in 2006. In 2006 and 2007 samples cyanobacteria was the most abundant group. In 2008, cyanobacterial seasonal variability was more evident. There was a strong correlation between filamentous cyanobacteria of the order Oscillatoriales and nitrates (NO_3), likely linked to high nutrient inputs during the high-water season.

Mogollón et al. (2014) studied spatio-temporal variation of cyanobacteria in seven areas of the Sinú River in the south end of the Morrosquillo Gulf, they identified fourteen genera. *Oscillatoria* had the greatest richness in all hydrological periods, except during low waters. The genera *Anabaenopsis*, *Aphanocapsa*, *Dolichospermum*, *Lyngbya*, *Merimopedia*, *Microcystis*, and *Pseudoanabaena* showed high species richness. The spatio-temporal variation of cyanobacterial abundance correlated with salinity, and nutrient concentrations (i.e. nitrates (NO_3^-) and ammoniacal nitrogen ($\text{NH}_3\text{-N}$)), from agriculture and aquaculture runoff.

Rosales Loaiza et al. (2016) compared the biochemical composition and growth of *Anabaena* and *Nostoc* in relation to concentrations of sodium nitrate (NaNO_3), using samples isolated from a humid environment in Bogotá, and a rice field in Valencia, Spain. *Anabaena sp.* 1 and *Anabaena sp.* 2, needed high nitrogen (N) concentrations to grow and increased their biomass, whereas, *Nostoc sp.* strain LAUN0015 grew without external sources of nitrogen (N).

Table 5. List of cyanobacterial genera and species reported for Colombia in peer-review contributions from 1990 to 2018.

	Department	Reference	Water body	Highlighted species	Cyanotoxin
Am.	Caquetá	Pinilla (2006)	Lake Boa	<i>Oscillatoria splendida</i> , <i>Synechococcus sp.</i> , <i>Dactylococcopsis sp.</i>	
	Caldas and Tolima	Ríos-Pulgarín et al. (2016)	Guarínó River in the Los Nevados Natural Park	N/A	
Andean	Antioquia	Ferrão-Filho et al. (2014)	Porce II and Rio Grande II Reservoirs	N/A	
		Herrera et al. (2014)	Riogrande II Reservoir	<i>Microcystis wesenbergui</i> , <i>Sphaerospermopsis torques-reginae</i>	
		Herrera et al. (2015)	Porce II and Riogrande II	<i>M. wesenberguii</i> , <i>S. torques-reginae</i> , <i>Dolichospermum sp.</i> , <i>Synechocystis sp.</i>	MCs, MC-LR
		Palacio et al. (2015a)	Riogrande II Reservoir	<i>Dolichospermum lemmermannii</i>	
		Palacio et al. (2015b)	Riogrande II Reservoir	<i>Microcystis sp.</i> , <i>Dolichospermum sp.</i>	
		Hurtado-Alarcón & Polanía-Vorenberg (2014)	Riogrande II and La Fe Reservoirs	N/A	

Note: **Am.:** Amazonia, **Carib.:** Caribbean, **MCs:** Microcystins, **MC-LC:** Microcystin-LC.

Table 5. Continued.

	Department	Reference	Water body	Highlighted species	Cyanotoxin
Andean	Cundinamarca	Pinilla (2010)	Guaimaral, Jaboque, Tibanica, Santa María del Lago, Juan Amarillo	N/A	
	Huila	Silva et al. (2018)	Quimbo Dam	<i>Anabaena circinalis</i> , <i>A. sphaerica</i> , <i>Nostoc sp.</i> , <i>Aphanizomenon gracile</i> , <i>Aphanocapsa delicatissima</i> , <i>Chroococcus turgidus</i> , <i>Gloeocapsa sp.</i> , <i>Lyngbya gracile</i> , <i>Merismopedia convolute</i> , <i>Microcystis aeruginosa</i> , <i>Oscillatoria princeps</i> , and <i>Spirulina platensis</i>	
Caribbean	Bolívar, Cesar, Córdoba, Magdalena, Sucre	Plata-Díaz & Pimienta-Rueda (2011)	27 swamps of the Momposina depression	order <i>Oscillatoriales</i>	

Note: **Am.:** Amazonia, **Carib.:** Caribbean, **MCs:** Microcystins, **MC-LC:** Microcystin-LC.

Table 5. Continued.

	Department	Reference	Water body	Highlighted species	Cyanotoxin
Caribbean	Córdoba, Sucre	Mogollón et al. (2014)	Sinú River, south end of the Morrosquillo Gulf	<i>Oscillatoria sp.</i> , <i>Anabaenopsis sp.</i> , <i>Aphanocapsa sp.</i> , <i>Dolichospermum sp.</i> , <i>Lyngbya sp.</i> , <i>Merimopedia sp.</i> , <i>Microcystis sp.</i> , and <i>Pseudanabaena sp.</i>	
	Atlántico	Tapia Larios (2014)	El Guájaro Reservoir	<i>M. aeruginosa</i> , <i>M.</i> <i>flos-aquae</i> , <i>C.</i> <i>minutus</i> , <i>A. grevillei</i> , <i>C. kuetzingianum</i> , <i>Anabaena sp.</i> , <i>A.</i> <i>phanizomenoides</i> , <i>C.</i> <i>raciborskii</i> .	
	Cesar	Rivera-González & Gómez-Gómez (2010)	El Salguero Water Treatment Plant (STAR)	<i>Phormidium sp.</i> , <i>Planktothrix sp.</i> , <i>Oscillatoria sp.</i> , <i>Pseudanabaena sp.</i> , <i>Woronichinia sp.</i> , <i>Microcystis sp.</i> , <i>Lyngbya sp.</i> , <i>Synechocystis sp.</i>	
	Córdoba	Jaramillo-Londoño & Aguirre-Ramírez (2012)	Ciénaga de Ayapel	<i>C. raciborskii</i> , <i>P.</i> <i>limnetica</i>	

Note: **Am.:** Amazonia, **Carib.:** Caribbean, **MCs:** Microcystins, **MC-LC:** Microcystin-LC.

Table 5. Continued.

	Department	Reference	Water body	Highlighted species	Cyanotoxin
Caribbean	Magdalena	Hernández & Gocke (1990)	Ciénaga Grande de Santa Marta (CGSM)	<i>Anacystis cyanea</i> , <i>Nostoc commune</i>	
		Gocke et al. (2003)	Nenguange Bay, CGSM, Pajarales lagoon complex, Salamanca Island complex	<i>Oscillatoria sp.</i>	
	Magdalena	Mancera et al. (1994)	Pajarales lagoon complex, Caño Grande, CGSM	<i>Anabaenopsis sp.</i>	
		Blanco et al. (2007)	Pajarales lagoon complex	<i>Anabaeopsis sp.</i>	

Note: **Am.:** Amazonia, **Carib.:** Caribbean, **MCs:** Microcystins, **MC-LC:** Microcystin-LC.

Summary

The first references to cyanobacteria in Colombia is from the 1970s in the Ciénaga Grande de Santa Marta (CGSM) (Hernández & Gocke, 1990). In the 1980s cyanobacteria were referenced in a catalog of the mollusks of the CGSM (Von Cosel, 1986). Most accounts on cyanoHAB, cyanobacteria and cyanotoxins in the country occurred during the 2010s.

Nineteen publications were digitally available for Colombia, they included three of the five regions of the country. The Andean (47.4%, 9 out of 19) and Caribbean (47.4%, 9 out of 19) regions account for 94.7% of the publications in the country. Only one publication was available for Amazonia, and there were no publications for the Orinoquía and Pacific regions. Most articles were written for the departments of Antioquia (31.6%, 6 out of 19) and Magdalena (21.1%, 4 out of 19). Seven of the 32 departments of the country were included in the publications available for the country (Table 5).

Twenty-four genera and 22 species of cyanobacteria were reported in Colombia. The most commonly reported genera were *Microcystis* (42.1%, 8 out of 19), and *Anabaena*, *Dolichospermum* (21.1%, 4 out of 19 each), and *Oscillatoria* (21.1%, 4 out of 19) (Table 6).

Three species were identified for *Microcystis* (i.e. *aeruginosa*, *flos-aquae*, and *wesenbergii*), three for *Anabaena* (i.e. *circinalis*, *phanizomenoides*, and *sphaerica*), one for *Dolichospermum* (i.e. *lemmermannii*) and one for *Oscillatoria* (i.e. *princeps*). Four species were reported by two publications (i.e. *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa*, *M. wesenbergii*, and *Sphaerospermopsis torques-reginae*), all other species were reported only once. Nearly 53% of the publications (10 out of 19) reported the presence of two or more cyanobacterial species, while 31.6% (6 out of 19) reported the presence of three or more species.

Merely 15.8% of the publications (3 out of 19) reported cyanobacterial density and only 5.3% (1 out of 19) reported the presence of cyanotoxins. Herrera et al. (2015) reported the presence of microcystis in all samples from the Porce II and Riogrande II reservoirs in the Andean region (Tables 5, 6).

Discussion

The first report of a cyanoHABs in South America comes from Argentina, when thousands of ducks died after an *Anabaena flos-aquae* cyanoHAB at Benedetti Lake in the early 1940s (Kühnemann, 1966). CyanoHAB reports continued to increase in the 1950s, and 1960s in Argentina and Uruguay particularly in aquaculture facilities (Ringuelet et al., 1955; Pacheco et al., 2010; González-Madina et al., 2019). In Colombia, the first reports of cyanobacteria date back to the 1970s in the Ciénaga Grande de Santa Marta (CGSM), in oyster farming operations (Hernández, 1983; Hernández & Gocke, 1990; Squires & Riveros, 1971). The occurrence of cyanoHABs in Colombia continue to be reported during the 1980s in aquaculture facilities (Von Cosel, 1986), but the first peer-reviewed publication digitally accessible was published in 1990 (Hernández & Gocke, 1990). The first peer review publications in Brazil, regarding the subject started in the mid-1980s, when several *M. aeruginosa* cyanoHABs were documented in the state of Rio Grande do Sul, in the South region (Torgan, 1989; Yunes et al., 1996, 1998).

A total of 167 publications related to cyanobacteria in fresh water reservoirs were published in South America between 1990 and 2018. In the 1990s, 9.6% (16 out of 167) of the articles related to cyanobacteria were published. The following decade, this percentage increased to 30.5% (51 out of 167). The 2010s was the most prolific decade for cyanobacteria related research when 60% of the publications were written (100 out of 167). The countries with the most publications were Brazil (43.1%, 72 out of 167), Argentina (18%, 30 out of 167), Uruguay

(15%, 25 out of 167), and Colombia (11.4%, 19 out of 167). Articles from these four countries accounted for 87.4% (146 out of 167) of the publications on the subject in South America.

Most publications for Brazil were written for the Northeast (37.5%, 27 out of 72), and Southeast (31.9%, 23 out of 72) regions, including 17 of the 26 states of the country. Most papers were written for the state of Pernambuco (18.1%, 13 out of 72). In Argentina, most publications were available for the Central region (70%, 21 out of 30). Only nine of the 23 provinces in the country were covered by the cyanobacteria literature. Most of the publications for Uruguay covered the Metropolitan region (32%, 8 out of 25). Seven of the 19 departments in the country were included. In Colombia, most publications for were written for the Andean and Caribbean regions (47.4%, 9 out of 19 each). Seven of the 32 departments of the country were included (Tables 2-5).

Fifty-eight genera and 98 species reported for Argentina, Brazil, Colombia, and Uruguay. The four most common genera were *Microcystis* (60.69%, 89 out of 146), *Anabaena* (18.48%, 27 out of 146), *Dolichospermum* (16.44%, 24 out of 146), and *Planktothrix* (16.44 %, 24 out of 146), all known cyanoHAB and cyanotoxin producers (Table 6).

Forty genera and 55 species were reported for Brazil. The most reported genera for the country were *Microcystis* (54.2%, 39 out of 72), *Cylindrospermopsis* (36.1%, 26 out of 72), *Planktothrix* (23.6%, 17 out of 72), and *Pseudanabaena* (13.9%, 10 out of 72). Twenty-two genera and 30 species were reported for Argentina, the most common were *Microcystis* (76.7%, 23 out of 30), *Anabaena* (36.7%, 11 out of 30), *Aphanizomenon* (20%, 6 out of 30), and *Dolichospermum* (16.7%, 5 out of 30). Seventeen genera and 29 species were reported for Uruguay. The most commonly reported species were *Microcystis* (72%, 18 out of 25), *Dolichospermum* (44%, 11 out of 25), *Cylindrospermopsis* (28%, 7 out of 25) and

Aphanizomenon (24%, 6 out of 25). Twenty-four genera and 22 species of cyanobacteria were reported in Colombia. The most commonly reported genera were *Microcystis* (42.1%, 8 out of 19), followed by *Anabaena*, *Dolichospermum*, and *Oscillatoria* each with four publications (21.1%) (Tables 2-5).

Microcystis aeruginosa (29.5%, 43 out of 146) and *Cylindrospermopsis raciborskii* (26%, 38 out of 146) were the most commonly reported species in the region. *M. aeruginosa* is the most common toxic cyanoHAB producer in eutrophic fresh water bodies (Qu et al., 2018). The species has been identified in multiple South American countries and has caused frequent cyanoHABs. *M. aeruginosa* produces several microcystins (MCs) variants, including the highly toxic microcystin-LR (MC-LR) (Harada, 1996; Gupta et al., 2003; Kotak et al., 1995; Oh et al., 2000). Given how wide spread *M. aeruginosa* is in south America and how recurrent its cyanoHABs are, the regions fresh water bodies may always contain elevated concentrations of potent cyanotoxins to which humans are constantly exposed.

In the past decade *C. raciborskii* has acquired the status of a cosmopolitan species (Chapman & Schelske, 1997; Padisak, 1997; Saker et al., 1999; Saker & Neilan, 2001). *C. raciborskii* was found several countries in South America in environments as diverse as oligotrophic lakes in northern Patagonia in Chile (Nimptsch et al., 2016) and eutrophic lagoons in the Amazonas River basin in Ecuador (Venegas et al., 2018), attesting to the species adaptability. Moreover, *C. raciborskii* produces several cyanotoxins including anatoxin-a (ANTX-a), cylindrospermopsins (CYNs), and saxitoxin (STXs) (Smith et al., 2012; Wiese, 2012). These cyanotoxins have severe adverse health effects in humans, and have caused human fatalities (Table 1).

The presence of *M. aeruginosa* and *C. raciborskii* and their toxins in fresh water bodies across the region compromises the quality of drinking water, potentially compromising human health and thus constituting a serious public health risk.

Though it has been assumed that cyanobacterial density correlates to cyanoHAB toxicity that might not be the case, indicating that water reservoirs destined to human consumption might contain high cyanotoxin concentrations even when cyanoHABs are not visible (Piccin-Santos & do Carmo Bittencourt-Oliveira, 2012).

CyanoHABs were rarely quantified, for instance, cyanobacterial densities were reported by less than half of the Argentinian (46.7%, 14 out of 30) and Uruguayan (48%, 12 out of 25) publications. Only by a third of the papers available for Brazil (30.6%, 22 out of 72) and by merely 15.8% of those available for Colombia (3 out of 19) reported this variable. Though cyanoHAB toxicity does not always correlate to its density (Piccin-Santos & do Carmo Bittencourt-Oliveira, 2012), cyanotoxin density provides useful information to understand the risks imposed by cyanoHABs and to better design management strategies.

Another useful cyanoHAB quantifier is chlorophyll a concentration, Schlüter et al. (2018) found that microcystin (MC) concentration significantly correlated with chlorophyll-a concentrations of cyanobacteria. Indicating that measuring pigment concentration could be used to detect microcystin-producing cyanobacteria.

For South America, 37.7% (55 out of 146) of the publications reported the presence of cyanotoxins. Eight cyanotoxin types were reported, the most common were microcystins (MCs) (28.1%, 41 out of 146), and saxitoxins (STXs) (7.53%, 11 out of 146). Microcystins (MCs) were reported in Argentina (Amé et al., 2003; Cazenave et al., 2005; Conti et al., 2005a; Echenique et al., 2006; Echenique & Aguilera, 2014; Fernández et al., 2015; Giannuzzi et al., 2011, 2012;

Ouahid et al., 2011; Pizzolon et al., 1999; Ruiz et al., 2013; Scarafia et al., 1995), Brazil (Borges et al., 2015; Chellappa et al., 2008a, 2008b; Costa et al., 2006; Carvalho et al., 2008; de Magalhães et al., 2001; dos Anjos et al., 2006; Elias et al., 2015; Ferrão-Filho et al., 2002; Hauser-Davis et al., 2015; Hirooka et al., 1999; Lorenzi et al., 2015; Magalhães et al., 2003; Matthiensen et al., 2000; Montagnolli et al., 2004; Moschini-Carlos et al., 2009; Paulino et al., 2017; Piccin-Santos & do Carmo Bittencourt-Oliveira, 2012; Schlüter et al., 2018; Soares et al., 2006; Sotero-Santos et al., 2006; Vieira et al., 2003, 2005; Yunes et al., 2003; Walter et al., 2018), Colombia (Herrera et al., 2015), and Uruguay (Bonilla et al., 2015; Ferrari et al., 2011; González-Piana et al., 2017; O'Farrell & Izaguirre, 2014). While saxitoxins (STXs) were only identified in Brazil (Barros et al., 2017; Borges et al., 2015; Costa et al., 2006; Tundisi et al., 2015) and Uruguay (Aguilera et al., 2017). Nodularin (NOD) was only reported by 2.05% (3 out of 146) of the publications and were only identified in Argentina (Galanti et al., 2013) and Brazil (Costa et al., 2016; Walter et al., 2018). Cylindrospermopsin (CYN) was reported by 1.4% (2 out of 146) of the publications, were present in Brazil (Costa et al., 2006; Lorenzi et al., 2015; Walter et al., 2018) and Uruguay (Bonilla et al., 2015) (Tables 2-6).

Anatoxin (ANTX), aeruginosa (AER), anabaenopeptin (AP), and cyanopeptolin (CyPep) were only reported in Brazil (Becker et al., 2010; Carvalho et al., 2008; Elias et al., 2015; Lins et al., 2016) (Table 6). In a worldwide study, Svirčev et al. (2019) reported the presence of microcystins (MCs), and saxitoxins (STXs) as the most abundant cyanotoxins types in the region, however, they did not report the presence of nodularins (NODs).

Microcystins (MCs), saxitoxins (STXs), anatoxins (ANTXs), and cylindrospermopsins (CYNs), have severe adverse health effects in humans and have caused human fatalities. Microcystins (MCs) and cylindrospermopsins (CYNs) capable of promoting tumor formation

and liver and colorectal cancer after prolonged low-level exposure, and responsible for liver injury, hemorrhage, and necrosis after acute exposure. Saxitoxins (STXs) and anatoxins (ANTXs), are both neurotoxins, capable of causing death by respiratory paralysis.

The most commonly reported saxitoxins (STXs) types were saxitoxin (STX) and gonyautoxins (GTX). Eight variants were reported (i.e. decarbamoylneosaxitoxin (dc-NEO), decarbamoylsaxitoxin (dc-STX), neosaxitoxin (Neo-STX), gonyautoxin-1 (GTX-1), gonyautoxin-2 (GTX-2), gonyautoxin-3 (GTX-3), gonyautoxin-4 (GTX-4), and gonyautoxin-6 (GTX-6)) (Table 6).

Although aeruginosin (AER), anabaenopeptin (AP), and cyanopeptolin (CyPep) are understudied and poorly understood cyanotoxins. The toxicity of anabaenopeptin (AP) appears to be greater than that of microcystin-RR (MC-RR), while the toxicity of cyanopeptolin (CyPep) toxicity might be comparable to that of microcystin-RR (MC-RR) (Lenz et al., 2019).

In Brazil, 44.4% (32 out of 72) of the publications tested for cyanotoxins, and 43.1% (31 out of 72) confirmed their presence. A single cyanotoxin was reported by 23.61% (17 out of 72) of the articles, 19.4% (14 out of 72) reported two or more types, and 15.3% (11 out of 72) reported mixtures of three or more cyanotoxins (Table 2).

Half of the Argentinian publications (15 out of 30) reported the presence of cyanotoxins. A single cyanotoxin was reported by 30% (9 out of 30) of the articles, and only 16.7% (5 out of 30) reported mixtures of two or more cyanotoxins types. Mixtures of three or more cyanotoxin types were not reported in Argentina. Microcystin (MC) was the most commonly reported cyanotoxin (40%, 12 out of 30) in the country. Four variants were identified: microcystin-LC (MC-LC), microcystin-LR (MC-LR), microcystin-RR (MC-RR) and variant with a molecular ion. Microcystin-LR (MC-LR), the most toxic microcystin variant, was the most reported

cyanotoxin in Argentina (26.7%, 8 out of 30). Only one publication reported the presence of nodularins (NOD) (Galanti et al., 2013), and one mentioned the presence of cyanobacterial neurotoxins without providing specifics (Echenique & Aguilera, 2014). A single cyanotoxin was reported by 30% (9 out of 30) of the articles, and only 16.67% (5 out of 30) reported mixtures of two or more 2 cyanotoxins types (Table 3).

In Uruguay, 28% (7 out of 25) of them reported testing for cyanotoxins, and 20% (5 out of 25) detected them. Three cyanotoxin types were identified: cylindrospermopsin (CYN), microcystin (MC), and saxitoxin (STX). Microcystin was the most commonly reported (16%, 4 out of 25). Saxitoxin (STX) was only reported by one publication (Aguilera et al., 2017) and just one article reported a mixture of cylindrospermopsin and microcystin (Bonilla et al., 2015) (Table 4). Merely 15.7% of the Colombian publications (3 out of 19) reported cyanobacterial density and only one article, reported the presence of microcystins (Herrera et al., 2015) (Table 5).

Table 6. List of cyanobacteria genera and species reported in the literature for Argentina, Brazil, Colombia and Uruguay, including the cyanotoxins reported for the genera as well as those specifically identified per country.

Genus	Species	Country	Cyanotoxin
<i>Alkalinema</i>	<i>sp.</i>	Br (Genuario et al., 2017a)	
<i>Amazoninema</i>	<i>gen. nov.</i>	Br (Genuario et al., 2018a)	
<i>Anabaena</i>	<i>sp.</i>	Ar (Pizzolon et al., 1999). Br (Elias et al., 2015; Soares et al., 2006; Walter et al., 2018). Co (Mogollón et al., 2014; Tapia Larios, 2014; Palacio et al., 2015b; Rivera-González & Gómez-Gómez, 2010)	Br MCs, MC-LR, AER, CyPep (Elias et al. 2015), MCs, NOD, CYN (Walter et al. 2018)
	<i>spp.</i>	Ur (Aguilera et al., 2017; Bonilla, 2009; Kruk et al., 2002; Pacheco et al., 2010)	Ur STX (Aguilera et al. 2017)
	<i>cf. elenkinii</i>	Ar (O'Farrell et al., 2015)	
	<i>circinalis</i>	Ar (Fernández et al., 2015). Br (Sotero-Santos et al., 2008). Co (Silva et al., 2018)	Ar MC-LR (Fernández et al., 2015), Br MC (Sotero-Santos et al., 2008)
	<i>crassa</i>	Br (Becker et al., 2010)	Br ANTX-a (Becker et al., 2010)
	<i>inaequalis</i>	Ar (Ringuelet et al., 1955)	
	<i>oscillaroides</i>	Ar (O'Farrell et al., 1996)	
	<i>oumiana</i>	Br (Carvalho et al., 2008)	Br MC-LR, MC-RR, AP-F, AP-B (Carvalho et al., 2008)
	<i>phanizomenoides</i>	Co (Tapia Larios, 2014)	

Note: **Ar:** Argentina, **Br:** Brazil, **Co:** Colombia, **Ur:** Uruguay, **AER:** Aeruginosin, **ANTX-a:** Anatoxin-a(s), **AP-B:** Anabaenopeptin B, **AP-F:** Anabaenopeptin F, **CyPep:** Cyanopeptolin, **CYN:** Cylindrospermopsin, **dc-NEO:** Decarbamoylneosaxitoxin, **dc-STX:** Decarbamoylsaxitoxin, **GTX:** Gonyautoxin, **MC:** Microcystin, **Neo-STX:** Neosaxitoxin, **NOD:** Nodularins, **STX:** Saxitoxin.

Table 6. Continued.

Genus	Species	Country	Cyanotoxin
<i>Anabaena</i>	<i>planctonica</i>	Ur (Kruk et al., 2002)	
	<i>sphaerica</i>	Co (Silva et al., 2018)	
	<i>spiroides</i>	Br (Molica et al., 2005, Moschini-Carlos et al., 2009, Sotero-Santos et al., 2008). Ur (Kruk et al., 2002)	Br SXT, Neo-SXT, dc-SXT (Molica et al., 2005), MC (Sotero-Santos et al., 2008), MC-RR, MC-LR, MC-YR (Moschini-Carlos et al., 2009)
<i>Anabaenopsis</i>	<i>sp.</i>	Br (Genuario et al., 2017a). Co (Blanco et al., 2007; Mancera et al., 1994; Mogollón et al., 2014). Ur (Kruk et al., 2002)	
	<i>cf. cunningtonii</i>	Ar (Aguilera et al., 2016)	
	<i>elenkinii</i>	Ar (Aguilera et al., 2016, O'Farrell et al., 2015). Br (Carvalho et al., 2008; Genuario et al. 2017a; Santos & Sant'Anna, 2010)	
	<i>milleri</i>	Ar (Aguilera et al., 2016)	
<i>Anacystis</i>	<i>cyanea</i>	Co (Hernández & Gocke, 1990)	
<i>Anathece</i>	<i>sp.</i>	Br (Laux et al., 2018)	
<i>Aphanizomenon</i>	<i>sp.</i>	Ar (Pizzolon et al., 1999; Zalocar de Domitrovic et al., 1998). Br (Costa et al., 2006; Ferrão-Filho et al., 2002, Moura et al., 2011, Vieira et al., 2005). Ur (González-Madina et al., 2017)	Br MC, STX (Costa et al., 2006), MC (Ferrão-Filho et al., 2002), MC (Vieira et al., 2005)
	<i>spp.</i>	Br (Costa et al., 2006)	Br MCs, STXs (Costa et al. 2006)
	<i>aff. gracile</i>	Ur (González-Madina et al., 2019)	

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Table 6. Continued.

Genus	Species	Country	Cyanotoxin
<i>Aphanizomenon</i>	<i>flos-aquae</i>	Ar (O'Farrell et al., 1996), Ur (Kruk et al., 2002)	
	<i>gracile</i>	Co (Silva et al., 2018). Ur (González-Madina et al., 2019)	
	<i>issatschenkoi</i>	Ur (González-Madina et al., 2017, 2019; Vidal & Kurk, 2008)	
	<i>schindleri</i>	Ar (Otaño, 2009)	
	<i>spiroides</i>	Ar (Otaño, 2009)	
<i>Aphanocapsa</i>	<i>sp.</i>	Br (Laux et al., 2018). Co (Mogollón et al., 2014)	
	<i>spp.</i>	Ur (Pacheco et al., 2010)	
	<i>delicatisima</i>	Co (Silva et al., 2018)	
	<i>elachista</i>	Ar (Vallejos et al., 2015). Br (Fonseca & Bicudo, 2008)	
	<i>grevillei</i>	Co (Tapia Larios, 2014)	
	<i>incerta</i>	Br (Lins et al., 2016)	
<i>Asterocapsa</i>	<i>submersa</i>	Br (Assis et al., 2018)	
<i>Brasilonema</i>	<i>sp.</i>	Br (Elias et al., 2015)	Br MCs, MC-LR, AER, CyPep (Elias et al., 2015)
<i>Cephalothrix</i>	<i>sp.</i>	Br (Genuario et al., 2017b)	
<i>Cryptomonas</i>	<i>brasiliensis</i>	Br Schlüter et al., (2018)	
<i>Chroococcus</i>	<i>sp.</i>	Br (Moura et al., 2011)	
<i>Coelosphaerium</i>	<i>dispersus</i>	Br (Assis et al., 2018)	
	<i>minor</i>	Br (Assis et al., 2018)	

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Table 6. Continued.

Genus	Species	Country	Cyanotoxin
<i>Coelosphaerium</i>	<i>minutus</i>	Br (Moura et al., 2011). Co (Tapia Larios, 2014)	
	<i>turgidus</i>	Co (Silva et al., 2018)	
	<i>kuetzingianum</i>	Ar (Vallejos et al., 2015). Co (Tapia Larios, 2014)	
<i>Cronbergia</i>	<i>amazonensis</i>	Br (Genuario et al., 2018)	
	<i>siamensis</i>	Br (Genuario et al., 2018)	
<i>Cuspidothrix</i>	<i>issatschenkoi</i>	Ar (O'Farrell et al., 2015). Ur (González-Madina et al., 2017, 2019)	
<i>Cyanobium</i>	<i>sp.</i>	Br (Walter et al., 2018)	
<i>Cylindrospermopsis</i>	<i>sp.</i>	Br (Barros et al., 2017; Elias et al., 2015; Fonseca & Bicudo, 2008; Piccin-Santos & do Carmo Bittencourt-Oliveira, 2012; von Sperling et al., 2008; Walter et al., 2018)	Br STX (Barros et al., 2017), MCs, MC-LR, AER, CyPep (Elias et al., 2015), MC (Piccin-Santos & do Carmo Bittencourt-Oliveira, 2012), MCs, NOD, CYN (Walter et al., 2018)
<i>Cylindrospermum</i>	<i>stagnale</i>	Br (Borges et al. 2015)	Br Neo-STX, dc-STX, STX, GTX 1 (Borges et al., 2015)

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Table 6. Continued.

Genus	Species	Country	Cyanotoxin
<i>Cylindrospermopsis</i>	<i>raciborskii</i>	<p>Ar (Otaño, 2009, Zalocar de Domitrovic et al., 1998). Br (Barros et al., 2017, Costa et al., 2006, dos Anjos et al., 2006, Lins et al., 2016, Fonseca & Bicudo, 2008, Moschini-Carlos et al., 2009, Molica et al., 2002, 2005, Piccin-Santos & do Carmo Bittencourt-Oliveira, 2012, Tundisi et al., 2015, Yunes et al., 2003, Schlüter et al., 2018). Co (Jaramillo-Londoño & Aguirre-Ramírez, 2012, Tapia Larios, 2014). Ur (Aguilera et al., 2017, Amaral, 2014, Pacheco et al., 2010, Piccini et al., 2011, Vidal & Kurk, 2008)</p>	<p>Br STX (Aguilera et al., 2017; Barros et al., 2017), MCs, STXs (Costa et al., 2006); MC-LR, MC-RR, MC-YR, STX, Neo-STX, GTXs, GTX 3 (dos Anjos et al., 2006), MC-LR, MC-RR, AP-B, AP-F (Lins et al., 2016), MC-RR, MC-LR, MC-YR (Moschini-Carlos et al., 2009), STX, GTX, dcSTX, dcNEO, Neo-STX, new STX analog (Molica et al., 2002), STX, Neo-STX, dc-STX (Molica et al., 2005), MC (Piccin-Santos & do Carmo Bittencourt-Oliveira, 2012), STX, Neo-STX, GTX 1, 2,3,4 (Yunes et al., 2003), MC, STX (Tundisi et al., 2015), MC (Schlüter et al., 2018)</p>

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Table 6. Continued.

Genus	Species	Country	Cyanotoxin
<i>Dolichospermum</i>	<i>sp.</i>	Br (Lins et al., 2016; Piccin-Santos & do Carmo Bittencourt-Oliveira, 2012). Co (Herrera et al., 2015; Palacio et al., 2015b). Ur (Bordet et al., 2017; Ferrari et al., 2011, O'Farrell, 2012)	
	<i>spp.</i>	Ur (González-Piana et al., 2017)	
	<i>cf. bituri</i>	Ar (O'Farrell et al., 2015)	
	<i>cf. pseudocompactum</i>	Ur (Ferrari et al., 2011)	
	<i>circinale</i>	Ur (Ferrari et al., 2011)	
	<i>circinalis</i>	Br (Lins et al., 2016)	
	<i>crassum</i>	Ur (González-Madina et al., 2017, 2019)	
	<i>flos-aquae</i>	Br (Piccin-Santos & do Carmo Bittencourt-Oliveira, 2012)	Br MC (Piccin-Santos & do Carmo Bittencourt-Oliveira, 2012)
	<i>lemmermannii</i>	Co (Palacio et al., 2015a)	
	<i>spiroides</i>	Ur (O'Farrell & Izaguirre, 2014)	
	<i>uruguayense</i>	Ur (Kozlíková-Zapomělová et al., 2016)	
<i>Geitlerinema</i>	<i>amphibium</i>	Br (Borges et al., 2015)	Br GTX 4 (Borges et al., 2015)
	<i>lemmermannii</i>	Br (Borges et al., 2015)	Br GTX 1 (Borges et al., 2015)
<i>Gloeocapsa</i>	<i>sp.</i>	Co (Silva et al., 2018)	
<i>Gomphosphaeria</i>	<i>lacustris</i>	Ar (Pizzolon et al., 1999)	Ar MC-RR (Pizzolon et al., 1999)
<i>Komvophoron</i>	<i>crassum</i>	Br (Assis et al., 2018)	

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Table 6. Continued.

Genus	Species	Country	Cyanotoxin
<i>Komvophoron</i>	<i>schmidlei</i>	Br (Assis et al., 2018)	
<i>Limnococcus</i>	<i>sp.</i>	Br (Elias et al., 2015)	
<i>Limnothrix</i>	<i>planctonica</i>	Ur (Kruk et al., 2002)	
<i>Lyngbya</i>	<i>sp.</i>	Ar (Lerda and Prosperi 1996), Co (Mogollón et al. 2014, Rivera-González & Gómez-Gómez 2010)	
	<i>gracile</i>	Co (Silva et al. 2018)	
	<i>major</i>	Br (Assis et al. 2018)	
<i>Merismopedia</i>	<i>sp.</i>	Ar (Conti et al. 2005a), Br (Barros et al. 2017), Co (Mogollón et al. 2014)	Ar MCs and MC-LR (Conti et al. 2005a)
	<i>convolute</i>	Co (Silva et al. 2018)	
	<i>glauca</i>	Br (Fonseca & Bicudo 2008)	
	<i>punctata</i>	Br (Bittencourt-Oliveira et al. 2012, Moura et al. 2011)	
	<i>tenuissima</i>	Ar (Zalocar de Domitrovic et al. 1998), Br (Assis et al. 2018)	
<i>Microcystis</i>	<i>spp.</i>	Ar (Cataldo et al., 2012, Giannuzzi et al., 2011), Ur (Boltovskoy et al., 2013, Bordet et al., 2017, O'Farrell, 2012)	Ar MC-LR (Giannuzzi et al., 2011)

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Table 6. Continued.

Genus	Species	Country	Cyanotoxin
<i>Microcystis</i>	<i>sp.</i>	Ar (Conti et al., 2005a; Fernández et al., 2015; Pizzolon et al., 1999; Ruiz et al., 2013; Valeria et al., 2006), Br (Assis et al., 2018; de Magalhães et al., 2001; Elias et al., 2015; Laux et al., 2018; Lins et al., 2016; Martins et al., 2016; Matthiensen et al., 2000; Montagnolli et al., 2004; Moschini-Carlos et al., 2009; Piccin-Santos & do Carmo Bittencourt-Oliveira, 2012; Schlüter et al., 2018; Soares et al., 2006; Tundisi et al., 2008; Vieira et al., 2005, 2015; von Sperling et al., 2008; Walter et al., 2018), Co (Mogollón et al., 2014; Palacio et al. 2015b; Rivera-González & Gómez-Gómez, 2010), Ur (Bonilla, 2009; Bordet et al., 2017; Ferrari et al., 2011; O'Farrell, 2012)	Ar MCs, MC-LR (Conti et al., 2005a), MCs (Ruiz et al., 2013), MC-LR (Fernández et al., 2015). Br MC (de Magalhães et al., 2001), MCs, MC-LR, MC-LR 995, AER, CyPep (Elias et al., 2015), MC (Matthiensen et al., 2000), MC (Montagnolli et al., 2004), MC-RR, MC-LR, MC-YR (Moschini-Carlos et al., 2009), MC (Piccin-Santos & do Carmo Bittencourt-Oliveira, 2012), MC (Schlüter et al., 2018), MC (Soares et al., 2006), MC (Vieira et al., 2005), MC, NOD, CYN (Walter et al., 2018)

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Table 6. Continued.

Genus	Species	Country	Cyanotoxin
<i>Microcystis</i>	<i>aeruginosa</i>	<p>Ar (Echenique, 2001; Echenique et al., 2006; Echenique & Aguilera, 2014; Ehrenhaus & Vigna, 2006; Fernández et al., 2015; Giannuzzi et al., 2011, 2012; Lerda & Prospero, 1996; Odriozola et al., 1984; O’Farrell et al., 2015; Ouahid et al., 2011; Otaño, 2009; Scarafia et al., 1995; Zalocar de Domitrovic et al., 1998). Br (da Silva et al., 2017; de Magalhães et al., 2001; dos Anjos et al., 2006; Ferrão-Filho et al. 2002; Hirooka et al., 1999; Lins et al., 2016; Molica et al., 2005; Montagnolli et al., 2004; Moschini-Carlos et al., 2009; Sant’Anna et al., 2011; Schlüter et al., 2018; Tundisi et al., 2008; Vieira et al., 2015; Werner et al., 2015; Yunes et al., 1996, 1998). Co (Tapia Larios, 2014; Silva et al., 2018). Ur (De Leon & Yunes, 2001; Fabre et al., 2010; Ferrari et al., 2011; González-Madina et al., 2017; Kruk et al., 2015; O’Farrell & Izaguirre, 2014; Pacheco et al., 2010)</p>	<p>Ar MC-LC (Ouahid et al., 2011), MC-LR, MCs (Echenique et al., 2006), MCs, MC-LR, MC with molecular ion (Echenique & Aguilera, 2014), MCs, MC-LR (Giannuzzi et al., 2012), MC-RR, MC-LR, MCs (Scarafia et al., 1995), MC-LR (Giannuzzi et al., 2011). Br MC, STX, Neo-STX, dc-STX (de Magalhães et al., 2001), MC, MC-LR, MC-RR, MC-YR, STX, Neo-STX, GTX, GTX 3 (dos Anjos et al., 2006), MC (Ferrão-Filho et al., 2002), MC (Hirooka et al., 1999), MC (Montagnolli et al., 2004), MC-RR, MC-LR, MC-YR (Moschini-Carlos et al., 2009). Ur MCs (De Leon & Yunes, 2001), MC (Kruk et al., 2015)</p>

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Table 6. Continued.

Genus	Species	Country	Cyanotoxin
<i>Microcystis</i>	<i>cf. botrys</i>	Br (dos Anjos et al. 2006)	Br MC-LR, MC-RR, MC-YR, STX, Neo-STX, GTX, GTX 3 (dos Anjos et al., 2006)
	<i>flos-aquae</i>	Br (Hirooka et al., 1999, Yunes et al., 1996, 1998), Co (Tapia Larios, 2014), Ur (Kruk et al., 2002)	Br MC (Hirooka et al., 1999)
	<i>natans</i>	Ar (Fernández et al., 2015)	Ar MC-LR (Fernández et al., 2015)
	<i>novacekii</i>	Br (dos Anjos et al., 2006; von Sperling et al., 2008)	Br Neo-STX (von Sperling et al., 2008), MC-LR, MC-RR, MC-YR, STX, neo-STX, GTX, GTX 3 (dos Anjos et al., 2006)
	<i>panniformis</i>	Br (Carvalho et al., 2008; Fonseca & Bicudo, 2008; Moschini-Carlos et al., 2009; Piccin-Santos & do Carmo Bittencourt-Oliveira, 2012)	Br MC-LR, MC-RR, AP-B, AP-F (Carvalho et al., 2008), MC-RR (Fonseca & Bicudo, 2008), MC-LR, MC-YR (Moschini-Carlos et al., 2009)
	<i>protocystis</i>	Br (Carvalho et al., 2008; dos Anjos et al., 2006)	Br MC-LR, MC-RR, AP-B, AP-F (Carvalho et al., 2008), MC, MC-LR, MC-RR, MC-YR, STX, Neo-STX, GTX, GTX 3 (dos Anjos et al., 2006)

Note: **Ar:** Argentina, **Br:** Brazil, **Co:** Colombia, **Ur:** Uruguay, **AER:** Aeruginosin, **ANTX-a:** Anatoxin-a(s), **AP-B:** Anabaenopeptin B, **AP-F:** Anabaenopeptin F, **CyPep:** Cyanopeptolin, **CYN:** Cylindrospermopsin, **dc-NEO:** Decarbamoylneosaxitoxin, **dc-STX:** Decarbamoylsaxitoxin, **GTX:** Gonyautoxin, **MC:** Microcystin, **Neo-STX:** Neosaxitoxin, **NOD:** Nodularins, **STX:** Saxitoxin.

Table 6. Continued.

Genus	Species	Country	Cyanotoxin
<i>Microcystis</i>	<i>wesenbergii</i>	Ar (Giannuzzi et al., 2011), Ur (Kruk et al., 2002)	Ar MC-LR (Giannuzzi et al., 2011)
	<i>wesenbergii</i>	Co (Herrera et al., 2014, 2015)	
<i>Nodularia</i>	<i>sp.</i>	Br (Costa et al., 2016)	Br NOD (Costa et al., 2016)
	<i>baltica-spumigena</i>	Ur (Pérez et al., 1999)	
<i>Nostoc</i>	<i>sp.</i>	Br (Baydum & Oliveira, 2017; Genuario et al., 2017; Elias et al., 2015; Lins et al., 2016; Rezende et al., 2015; Vieira et al., 2005; Werner et al., 2015), Co (Silva et al., 2018)	Br MC, MC-LR, AER, CyPep (Elias et al., 2015), MC (Vieira et al., 2005)
	<i>commune</i>	Co (Hernández & Gocke 1990)	
<i>Oscillatoria</i>	<i>sp.</i>	Ar (Conti et al., 2005a; Pizzolon et al., 1999; Ruiz et al., 2013), Br (Baydum & Oliveira, 2017; de Magalhães et al., 2001; Lins et al., 2016; Ferreira et al., 2015; Martins et al., 2016; Moura et al., 2011; Rezende et al., 2015; Santos & Sant'Anna, 2010; Vieira et al., 2005; Werner et al., 2015), Co (Gocke et al., 2003; Mogollón et al., 2014; Rivera-González & Gómez-Gómez, 2010)	Ar MCs, MC-LR (Conti et al., 2005a), MCs (Ruiz et al., 2013)
	<i>spp.</i>	Ur (Kruk et al., 2002)	
	<i>curviceps</i>	Br (Werner et al., 2015)	
	<i>princeps</i>	Co (Silva et al., 2018)	
	<i>tenuis</i>	Br (Assis et al., 2018; Werner et al., 2015)	
<i>Pantanalinema</i>	<i>sp.</i>	Br (Genuario et al., 2017)	

Note: **Ar:** Argentina, **Br:** Brazil, **Co:** Colombia, **Ur:** Uruguay, **AER:** Aeruginosin, **ANTX-a:** Anatoxin-a(s), **AP-B:** Anabaenopeptin B, **AP-F:** Anabaenopeptin F, **CyPep:** Cyanopeptolin, **CYN:** Cylindrospermopsin, **dc-NEO:** Decarbamoylneosaxitoxin, **dc-STX:** Decarbamoylsaxitoxin, **GTX:** Gonyautoxin, **MC:** Microcystin, **Neo-STX:** Neosaxitoxin, **NOD:** Nodularins, **STX:** Saxitoxin.

Table 6. Continued.

Genus	Species	Country	Cyanotoxin
<i>Phormidium</i>	<i>sp.</i>	Ar (Lerda & Prosperi, 1996; Zalocar de Domitrovic et al., 1998), Co (Rivera-González & Gómez-Gómez, 2010)	
	<i>uncinatum</i>	Br (Borges et al., 2015)	Br GTX 1 (Borges et al., 2015)
	<i>mucicola</i>	Ar (Zalocar de Domitrovic et al., 1998)	
<i>Planktolyngbya</i>	<i>spp.</i>	Ur (Vidal & Kurk, 2008)	
	<i>limnetica</i>	Ur (Aguilera et al., 2017; Vidal & Kurk, 2008)	Ur STX (Aguilera et al., 2017)
<i>Planktothrix</i>	<i>sp.</i>	Br (Lins et al., 2016; Schlüter et al., 2018), Co (Rivera-González & Gómez-Gómez, 2010)	
	<i>agardhii</i>	Ar (Fernández et al., 2015; O'Farrell et al., 2015), Br (Aragão-Tavares et al., 2017; Assis et al., 2018; Barros et al., 2017; Bittencourt-Oliveira et al., 2012; da Silva et al., 2017; dos Anjos et al., 2006; Lins et al., 2016; Moschini-Carlos et al., 2009; Moura et al., 2011; Portella et al., 2015; Schlüter et al., 2018; Vieira et al., 2015), Ur (Aguilera et al., 2017; Kruk et al., 2002; Scasso et al., 2001; Sommaruga, 1995)	Br STX (Barros et al. 2017), MC, MC-LR, MC-RR, MC-YR, STX, Neo-STX, GTXs, GTX 3 (dos Anjos et al., 2006), MC-RR, MC-LR, MC-YR (Moschini-Carlos et al., 2009), MC (Schlüter et al., 2018). Ur STX (Aguilera et al., 2017)
	<i>cf. agardhii</i>	Br (Schlüter et al., 2018)	Br MC (Schlüter et al., 2018)
	<i>rubescens</i>	Br (Schlüter et al., 2018)	Br MC (Schlüter et al., 2018)

Note: **Ar:** Argentina, **Br:** Brazil, **Co:** Colombia, **Ur:** Uruguay, **AER:** Aeruginosin, **ANTX-a:** Anatoxin-a(s), **AP-B:** Anabaenopeptin B, **AP-F:** Anabaenopeptin F, **CyPep:** Cyanopeptolin, **CYN:** Cylindrospermopsin, **dc-NEO:** Decarbamoylneosaxitoxin, **dc-STX:** Decarbamoylsaxitoxin, **GTX:** Gonyautoxin, **MC:** Microcystin, **Neo-STX:** Neosaxitoxin, **NOD:** Nodularins, **STX:** Saxitoxin.

Table 6. Continued.

Genus	Species	Country	Cyanotoxin
<i>Polycystis</i>	<i>flos-aquae</i>	Ar (Ringuelet et al., 1955)	
<i>Prochlorococcus</i>	<i>sp.</i>	Br (Affe et al., 2018)	
<i>Pseudanabaena</i>	<i>sp.</i>	Br (Barros et al., 2017; Genuario et al., 2017; Molica et al., 2005; Moura et al., 2011), Co (Mogollón et al., 2014)	Ar STX (Barros et al., 2017), MCs, AER, CyPep, MC-LR (Elias et al., 2015), STX, Neo-STX, dc-STX (Molica et al., 2005)
	<i>aff. limnetica</i>	Ur (Aguilera et al., 2017)	Ur STX (Aguilera et al., 2017)
	<i>cf. moniliformis</i>	Ur (Bonilla, 2009)	
	<i>galeata</i>	Br (Assis et al., 2018), Ur (Kruk et al., 2002)	
	<i>limnetica</i>	Br (Lins et al., 2016; Yunes et al., 2003)	Br STX, Neo-STX, GTX 1, GTX 2, GTX 3, GTX 4, not specified MC, STX equivalents (Yunes et al., 2003)
	<i>mucicola</i>	Br (dos Anjos et al., 2006)	Br MC, MC-LR, MC-RR, MC-YR, STX, Neo-STX, GTXs, GTX 3 (dos Anjos et al., 2006)
	<i>raphidioides</i>	Ur (Aguilera et al., 2017)	Ur STX (Aguilera et al., 2017)
<i>Pseudoanabaenopsis</i>	<i>sp.</i>	Br (Ferreira et al., 2015)	

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Table 6. Continued.

Genus	Species	Country	Cyanotoxin
<i>Radiocystis</i>	<i>sp.</i>	Br (Vieira et al., 2003, 2005)	Br MCs (Vieira et al., 2003 2005)
	<i>fernandoi</i>	Br (Vieira et al., 2003)	Br MC, MS-YR, MS-RR (Vieira et al., 2003)
<i>Raphidiopsis</i>	<i>sp.</i>	Br (Barros et al., 2017)	Br STXs (Barros et al., 2017)
	<i>mediterranea</i>	Ar (O'Farrell et al., 1996, 2015), Br (Fonseca & Bicudo, 2008), Ur (Kruk et al., 2002)	
<i>Rhodomonas</i>	<i>lacustris</i>	Br (Schlüter et al., 2018)	Br MC (Schlüter et al., 2018)
<i>Snowella</i>	<i>fennica</i>	Ar (Fernández et al., 2015)	Ar MC-LR (Fernández et al., 2015)
	<i>lacustris</i>	Ar (Echenique & Aguilera 2014), Br (Assis et al., 2018)	Ar Neurotoxins (Echenique & Aguilera, 2014)
<i>Sphaerocavum</i>	<i>brasiliense</i>	Br (Assis et al., 2018; Fonseca & Bicudo, 2008; Vieira et al., 2015; von Sperling et al., 2008), Ur (González-Madina et al., 2017)	
	<i>cf. brasiliense</i>	Br (Carvalho et al., 2008)	
<i>Sphaerospermopsis</i>	<i>cf. aphanizonemoides</i>	Ar (O'Farrell et al., 2015)	
	<i>torques-reginae</i>	Ar (O'Farrell et al., 2015), Co (Herrera et al., 2014, 2015)	Br MCs, MC-LR (Herrera et al. 2015)
<i>Spirulina</i>	<i>sp.</i>	Br (Ferreira et al., 2015)	
	<i>platensis</i>	Co (Silva et al., 2018)	

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Table 6. Continued.

Genus	Species	Country	Cyanotoxin
<i>Stigonema</i>	<i>hormoides</i>	Ar (Vallejos et al., 2015)	
<i>Synechococcus</i>	<i>sp.</i>	Br (Elias et al., 2015)	Br MC, MC-LR, AER, CyPeP (Elias et al., 2015)
<i>Synechocystis</i>	<i>sp.</i>	Co (Herrera et al., 2015, Rivera-González & Gómez-Gómez, 2010)	Co MC-LR (Herrera et al., 2015)
<i>Tolypothrix</i>	<i>sp.</i>	Br (Genuario et al., 2017)	
<i>Trichodesmium</i>	<i>lacustre</i>	Br (Lins et al., 2016)	
<i>Woronichinia</i>	<i>sp.</i>	Br (Moschini-Carlos et al., 2009), Co (Herrera et al., 2015, Rivera-González & Gómez-Gómez, 2010)	Br MC-RR, MC-LR, MC-YR (Moschini-Carlos et al., 2009). Co MC-LR (Herrera et al., 2015)
	<i>naegeliana</i>	Ar (Echenique & Aguilera, 2014), Br (Moschini-Carlos et al., 2009)	Ar Neurotoxins (Echenique & Aguilera, 2014), Br MC-RR, MC-LR, MC-YR (Moschini-Carlos et al., 2009)

Note: **Ar:** Argentina, **Br:** Brazil, **Co:** Colombia, **Ur:** Uruguay, **AER:** Aeruginosin, **ANTX-a:** anatoxin-a(s), **AP-B:** anabaenopeptin B, **AP-F:** anabaenopeptin F, **CyPep:** Cyanopeptolin, **CYN:** Cylindrospermopsin, **dc-NEO:** decarbamoylneosaxitoxin, **dc-STX:** decarbamoylsaxitoxin, **GTX:** gonyautoxin, **MC:** microcystin, **Neo-STX:** neosaxitoxin, **NOD:** nodularins, **STX:** saxitoxin.

CHAPTER III

THE RELATIONSHIP BETWEEN ECONOMIC DEVELOPMENT AND CYANOBACTERIA MONITORING EFFORT IN FRESH-WATER RESERVOIRS IN BRAZIL

Introduction

Water Quality and Economic Development

The connection between environmental quality and economic development has long been studied from an economic perspective (Carson & Mitchell, 1993; Machdar et al., 2013; McConnell, 1997; Mohapatra & Giri, 2009; Shafik, 1994; Torras & Boyce, 1998). Two conflicting views persist, one states that economic development comes at the expense of the environment, while the other states that pressing environmental problems will be better addressed because of economic development. While both of these notions continued to be examined and reassessed, the connection between economic development and the environment is not contested.

Building on the notion that human and environmental health are interconnected, I propose evaluating water quality from a public health perspective, within the frame of economic development. I used cyanobacteria data from Brazil as a biological indicator of water quality, while exploring regional income as a predictor of monitoring effort. This approach will allow for better-informed management strategies, and might bring monitoring disparities to the attention of local water authorities.

In Brazil, potable water is considered a public good. Therefore, government institutions are in charge of its regulation and provision. Current water management in Brazil is based on the Política Nacional de Recursos Hídricos (National Policy of Hydrologic Resources) developed in

1997, and commonly known as Lei das Águas (Water Law) (ANA, 2018). According to the Agência Nacional de Águas (ANA), the national water agency, in Brazil water is mainly used for irrigation (52%), human consumption (23.8%), and industry (9.1%). Secondary uses include animal husbandry (8%), hydroelectric power generation (3.8%), rural supply (1.7%) and mining (1.6%).

In the last seven decades, water demand in Brazil has increased due to economic development and urbanization (ANA, 2018). In order to fulfill these needs many mixed-use reservoirs were built during the 1960s and 1970s. The Ten-Year Energy Plan for the country called for the construction of 71 new hydroelectric plants by 2017; the reservoirs built for the hydroelectric projects also to be used for water supply, aquaculture, and recreation (von Sperling, 2012).

The governmental policy Política para construção de açudes (Policy for the construction of Reservoirs) promoted the construction of reservoirs, particularly in the Northeast is known as the Polígono das Secas (Drought Polygon) to store water for use during periods of drought (Bouvy et al., 2000). Out of the 710 reservoirs registered in the Sistema de Acompanhamento de Reservatórios – SAR (Reservoir Follow-up System), 77.9% (553) are located in the Northeast. In contrast, only 11% (78) of the reservoirs are located in the Southeast. 4.4% (31) in the South, 4.5% (32) in the Central-west, and 2.3% (16) are in the North.

Due to global warming, industrial and agricultural runoff, and limited or inexistent sewage treatment, many of these reservoirs are at risk of becoming eutrophic and developing cyanobacterial harmful algal blooms (cyanoHABs), which will in turn result in greater cyanotoxin exposure for humans (Paerl & Paul, 2012).

CyanoHABs are a public health risk because numerous cyanobacteria produce cyanotoxins that affect human health. The risk magnifies when municipal systems become contaminated, as entire communities can be exposed, and numerous people can fall sick (Hilborn & Beasley, 2015). In fact, cyanotoxin poisonings related to municipal and recreational water supplies have increased worldwide (Carmichael, 2001; Dolah et al., 2001; Dörr et al., 2010; de Figueiredo et al., 2004; Vieira et al., 2005). In some cases, this had lethal consequences, as happened in Caruaru, Brazil in 1996, the first confirmed case of human fatalities linked to intravenous exposure to cyanotoxins present in treated water (Azevedo et al., 2002; Carmichael et al., 2001; Jochimsen et al., 1998; Pouria et al., 1998; Yuan et al., 2006). Furthermore, conventional water treatments often reduce but do not completely eliminate all cyanobacteria and cyanotoxins (Hilborn & Beasley, 2015; Manali et al., 2017; Yu et al., 2009).

Assessing human health risk associated with cyanoHABs is fundamental to develop adequate regulatory measurements to prevent and minimize related risks and impacts (Dolah et al., 2001). Because cyanoHABs are recurrent, people might be constantly exposed to cyanotoxins, through drinking water (Dolah et al., 2001; Hernández et al., 2009; Ueno et al., 1996; WHO, 1998; Yu, 1989, 1995), ingestion of contaminated food (Amorim & Vasconcelos, 1999; Codd et al. 1999b; de Magalhães et al., 2001; de Magalhães et al., 2003; Schaeffer et al., 1999; Williams et al., 1997), exposure to recreational waters (de Figueiredo et al., 2004; WHO, 2003) or hemodialysis (Azevedo et al., 2002; Carmichael et al., 2001; Jochimsen et al., 1998; Pouria et al., 1998; Soares et al., 2006).

The objective of this study is to assess cyanobacteria monitoring effort in Brazil, and evaluate regional differences resulting from affluence. I hypothesized that regions with higher gross domestic product per capita (GDPPC) will have a greater monitoring effort. While

monitoring effort by itself does not guarantee the quality of water that reaches the final consumer, it provides information about how water reservoirs are managed. Additionally, more information provides bases for better management strategies, which can improve water quality and protect human and ecosystem health.

Importance of Cyanobacteria and Cyanotoxin Monitoring

Although extensive work has been conducted on the acute effects of high-level exposure to cyanotoxins, little research has been done in exposure to low levels of cyanotoxins, even though chronic low-level exposure to microcystins has been linked to major human health threats (Kuiper-Goodman & Fitzgerald, 1999; Lun et al., 2002; Ueno et al., 1996; Yuan et al., 2006; Yu, 1989, 1995; Yu et al., 2001). Hernández et al. (2009) postulated that chronic consumption of tap water containing low doses of microcystins might be a risk factor for liver and colorectal cancer. Hence, the importance of understanding the connection between drinking water containing low levels of cyanotoxins and human health, and the need for more representative regional studies (Kuiper-Goodman et al., 1999).

Regardless of the increased global awareness on the importance of cyanobacteria and cyanotoxins in human and ecosystem health, in South America (SA) little is known about their diversity and distribution (Sant'Anna et al., 2008). In Brazil, the infamous Caruaru syndrome, where 60 patients of a hemodialysis facility died because of accidental intravenous exposure to cyanotoxins (Azevedo et al., 2002; Dörr et al., 2010; Jochimsen et al., 1998), brought cyanoHABs to the attention of the water authorities and the public. This increased awareness lead to increased number of cyanobacteria and cyanotoxin studies in the country, in addition to changes in water policy, sanitary standards, monitoring of water bodies, and institutionalized prevention efforts (Moschini-Carlos et al., 2009; Carvalho et al., 2007; Chellappa et al., 2008a;

Costa et al., 2006; Hirooka et al., 1999; Molica et al., 2002; Molica et al., 2005; Oliveira et al., 2005; Sant'Anna et al., 2008; Soares et al., 2006; Sotero-Santos et al., 2006; Vieira et al., 2005; Piccin-Santos & do Carmo Bittencourt-Oliveira, 2012; Yunes et al., 2003). Nonetheless, non-biological variables such as differential policy reinforcement, specifically monitoring effort, have not yet been addressed.

Income Inequality in Brazil

Brazil's regional income inequality rose in the 1980s, slightly fell in the early 1990s, and rose slightly again between 1995 and 1997 (Shankar & Shah, 2003). These differences in income distribution are explained by ample differences in interregional levels of per capita income (Leff, 1972). Over the past seventy years, Brazil has shown a marked economic concentration in the Southeast, particularly in the large urban centers of São Paulo and Rio de Janeiro, and there is a strong North-Northeast/South-Southeast per capita income gradient dating back to the nineteenth century (Azzoni, 2001; Leff, 1972).

By the 1850s, per capita income between regions grew at considerably different rates, at relatively low rates in the Northeast and at high rates in the Southeast. Many factors contributed to these differences, including poor conditions of human capital formation in the Northeast, and greater joint marginal-value resulting from labor and capital in the Southeast. By the early 20th century, differential growth in export of commodities, and the rates of those exports contributed to the onset of this long-lasting economic gradient (Leff, 1972).

Since 1985, the Southeast region has increased its GDP consistently. By 1998, the region represented 43% of total population and produced 58% of the country's GDP. Meanwhile, the Northeast only produced 13% of the national GDP, although by 2000 it had 28% of the country's population (Azzoni, 2001; Azzoni & Servo, 2002). In the Northeast, where most people with low

income level, wages are significantly lower than those in the South and Southeast regions.

Unskilled laborers from the Northeast could double their earnings if they were to find comparable employment in the Southeast (Savedoff, 1990).

Given the long history of economic inequity and the differential access to environmental resources and services in Brazil (Pedlowski et al., 2002; Perry, 2009; Porto, 2012), it is possible that reinforcement of water policies varies as a function of wealth, which will in turn influence the quality of drinking water available in northern and northeastern states. Differential policy reinforcement, would subsequently determine human exposure to cyanotoxins in less affluent states. Under these circumstances, cyanoHABs, a pressing environmental issue becomes a public health issue and furthermore an environmental justice issue. This study proposes to address this knowledge gap by studying the impact of GDPPC capita in monitoring effort in fresh water reservoirs destined to human consumption and recreation across Brazil.

Brazilian Water Legislation

Since 2000, the Brazilian legislation for drinking water incorporates cyanobacteria and cyanotoxins as parameters to be considered for water quality control (Pírez et al., 2013). The Ministério da Saúde (Ministry of Health), in Ordinance 2914 (Ministério da Saúde, 2017) and the Conselho Nacional de Meio Ambiente – CONAMA (National Environment Council) (CONAMA, 2005; Walter et al., 2018) adopted the threshold for microcystins in drinking water proposed by the World Health Organization (WHO) of less than $1 \mu\text{g l}^{-1}$ (Falconer et al., 1999).

Ordinance 1,469 (Portaria N° 1,469) decreed by the Ministry of Health (CONAMA, 2000b) established mandatory monitoring for cyanotoxins (i.e. microcystins, cylindrospermopsins, and saxitoxins), if the number of cyanobacterial cells at the intake point was greater than $20,000 \text{ cells ml}^{-1}$ ($2 \text{ mm}^3 \text{ l}^{-1}$ of biovolume), as well as weekly cyanotoxin

analysis at the exit-point of treatment plants, and at the intake-point of hemodialysis facilities and injectable pharmaceutical manufacturing industries. Likewise, monthly toxicity analysis in mice is required if the number of cyanobacterial cells does not exceed 10,000 cells ml⁻¹ or 1 mm³ l⁻¹ of biovolume.

Ordinance 1,469 (CONAMA, 2000b) also established procedures to reduce nutrient levels, mainly phosphorous and nitrogen, which can trigger the occurrence of cyanoHABs. In addition, it prohibited the use of algacides to control cyanobacterial growth in water bodies, since this practice lyses the cells and contributes to the release of cyanotoxins.

In 2005, a law passed by the Brazilian National Congress known as Resolução Nº 357 (CONAMA, 2005) defined the standards for cyanobacteria in water intended for animal consumption. According to the law, cyanobacterial density should not exceed 5,000 cells ml⁻¹ or 5 mm³ l⁻¹ of biovolume.

The Ministério da Saúde (Ministry of Health), the Secretaria de Vigilância em Saúde (Health Monitoring Office), and the General Coordenação-Geral de Vigilância em Saúde Ambiental (General Office of Monitoring in Environmental Health), created the Programa Nacional de Vigilância da Qualidade da Água para Consumo Humano – VIGIAGUA (National Program for the Monitoring of Water Quality for Human Consumption). The program was established and organized according to the principle of the Sistema Único de Saúde – SUS (Universal Health System), in charge of guaranteeing water quality and security for human consumption in Brazil.

VIGIAGUA is a series of actions adopted by public health authorities to guarantee access to water to the general population, and to guarantee that the quantity and quality of the water received by the public conforms to the potability standards established by current legislation. The

Sistema de Informação de Vigilância da Qualidade da Água para Consumo Humano – SISAGUA (Information System for the Monitoring of Water Quality) was developed based on VIGIAGUA and Ordinance 2,914 (Ministério da Saúde, 2017). The purpose of the system is to manage the health risks associated to drinking water and its quality, a fundamental action for health promotion set by the SUS.

SISAGUA collects data about water supply systems and water quality for each of the registered municipalities. The system stores data in three modules: cadastro (registry), controle (control), and vigilância (monitoring). The objective of the registry module is to store information about the physical and operational characteristics of the forms of water supply used by the local population. The control module aims to store information about water quality monitoring conducted by the institutions responsible to provide drinking water, and the monitoring module seeks to store data regarding sanitary inspections of water supply and water quality monitoring conducted by public health and sanitary authorities.

SISAGUA is the main tool used to assess the indicators set by VIGIAGUA. It is also used by other Brazilian health initiatives such as the Pacto pela Saúde (Health Pact), the Contrato Organizativo da Ação Pública da Saúde – COAP (Organizational Contract of Health Public Action), and the Programa de Qualificação das Ações de Vigilância em Saúde – PQA-VS (Program for the Assessment of Health Monitoring Actions). Data can be used by health care professionals, employees of the Ministry of Health working with VIGIAGUA, companies supplying water for human consumption, and any other practitioners working in areas related to drinking water.

The Sistema de Abastecimento de Água – SAA (Water Supply System) consists of at least one intake point (superficial or underground), one or more water treatment stations, storage

facilities with one or more reservoirs, and a distribution network, that can supply water to one or more municipalities (Queiroz et al., 2012). The forms used in the registration process include information regarding the state, municipality, name of the water supply system or water provider, the names of the official in charge of the treatment facility, whether water was captured superficially or underground, the types and stages of treatment. The register includes stations, or units, that are part of the water supply system, alternative supply solutions and individual supply solutions. Registry is expected to happen at least annually, and monitoring is expected to include the registry of water supply conditions in rural and urban environments. Nine data collection forms are available on line under Formulário de Cadastro de SAA (registration forms for water supply systems) (SISAGUA, 2016).

The SAA is an assortment of civil works, materials, and equipment destined to the production and distribution of drinking water. Each SAA has a unique national code known as Código do Sistema de Abastecimento de Água – COSAA (Water Supply System Code). Depending in their population size, municipalities have different numbers of COSAAs. Larger municipalities have more COSAAs than smaller municipalities.

Water supply in Brazil occurs in a series of combinations, and there is no single arrangement that characterizes water supply. In general, the complete cycle includes four components: 1) source, 2) capturing, 3) treatment and storage, and 4) distribution. This type of supply is called soluções clássicas (classic arrangement), and includes supply through the network. The main difference with the arrangement known as soluções alternativas coletivas (collective alternative), is that the municipality is responsible for the water supply, even if the service is provided by a public or by a private institution (Queiroz et al., 2012). Solução alternativa coletiva – SAC (collective alternative arrangement) is a type of communal water

supply different from SAA, and includes water fountains, community wells, supply by water tankers, and community installations (i.e. apartment buildings and apartment complexes) (Ministério da Saúde, 2005; Queiroz et al., 2012). These alternative solutions can function as part of the network or not. Alternatives that are not part of the distribution network are normally associated to water fountains, wells, and public tabs. These alternatives tend to rely in water tanker trucks for distribution.

In Brazil, many apartment buildings, apartment complexes, hotels, and clubs install and operate their own water plants, and in many cases are similar to the SAA. There are many arrangements that are likely to be found, and can be group based on the type of source (surface and underground) and on the way water is distributed (public water fountain or tab, water tank, or wagon). Solução alternativa individual – SAI (Alternative Individual Arrangement) is any alternative solution for water supply that provides the service to a single residence. These alternatives include well water, rainwater, river water, and spring water. Isolated systems are those that supply water in isolation to neighborhoods, sectors, or localities. Integrated systems provide water to multiple municipalities simultaneously or when more than one productive unit provides water to a single municipality, neighborhood, sector, or locality (SISAGUA, 2016).

SISAGUA created instructional and operational manuals (i.e. Instrutivo para solicitação de acesso ao SISAGUA, Manual de Procedimentos de Entrada de Dados do SISAGUA – Vigilância, Manual de Procedimentos de Entrada de Dados do SISAGUA – Prestadores), as well as documents to integrate the systems of water suppliers with those of SISAGUA (i.e. Anexo 1 – Web service de controle mensal – descrição da ferramenta, Anexo 2 – descrição dos arquivos relacionados aos cadastros de SAA e SAC, Anexo 3 – descrição dos arquivos relacionados ao controle mensal de SAA, Anexo 4 – descrição dos arquivos relacionados ao controle mensal de

SAC, Anexo 5 – regras de negócio, Anexo 6 – mensagens, Anexo 7 – opções de tags (SISAGUA, 2016).

Methods

I used GDPPC, an indicator of economic development, as a predictor of monitoring effort for cyanobacterial density. I defined monitoring effort in terms of number and percentage of municipalities sampled per state per year, and as the number and percentage of monitoring events per COSAA code per state per year. And examined the relationship between regional wealth and monitoring effort for cyanobacteria.

Data

Cyanobacteria

I used seven years of data collected by SISAGUA from 2007 to 2013. The data was recorded as part of VIGIAGUA, the national water quality monitoring program. These data were available upon request, and after providing evidence of payment to the Ministério da Fazenda (Ministry of Finance) and the Secretaria do Tesouro Nacional (Secretary of the National Treasury). Data included date of sampling, water provider code, cyanobacterial density (cells l⁻¹), cyanotoxin sampling, as well as, the number of samples collected at the entrance and exit points at hemodialysis facilities, treatment plants, and springs.

Some records for cyanobacterial density were blank, and some were reported as zero. For the purpose of the analysis blank records were assumed to be a non-monitoring event. The validity of zero values as actually representing a monitoring event in which no cyanobacterial cells were found was questionable. However, since I did not have data to corroborate the real meaning of the zeros, they were kept in the record and were analyzed accordingly.

Brazil has 5,569 municipalities, organized in twenty-six states and one Federal District (Brasilia D.F.), and grouped in five regions: North, Northeast, Central-west, Southeast, and South. Data included all regions of the country, but not all states or municipalities were included every year. Each municipality has at least one water supply system, and each supplier has a unique national code, known as Código do Sistema de Abastecimento de Água – COSAA. SISAGUA collects data about water supply systems and water quality for each of the registered municipalities.

Gross Domestic Product per Capita (GDPPC)

Nominal GDP measures the value of economic activity, in a country, state, or municipality. It is the sum of market values, or prices, of all the goods and services produced in an economy for a set period. When it is divided by the number of people in the population, it provides the average output of each individual, which is the average amount of money each person makes. GDPPC, is a metric for average wealth. I used this metric to compare the standard of living between Brazilian regions. GDP and population data were downloaded from the Instituto Brasileiro de Geografia e Estatística (IBGE), the Brazilian Institute of Geography and Statistics website. Data were available from 2007 until 2013. Data was obtained by IBGE from the Órgãos Estaduais de Estatística (State Offices of Statistics), the Secretarias Estaduais de Governo (State Secretary of Government) and the Superintendência da Zona Franca de Manaus (SUFRAMA), the Duty-Free Agency of Manaus.

Nominal GDP was adjusted to real GDP using a deflator provided by the World Bank (World Bank, 2018) to account for inflation and the devaluation of the Brazilian Real (R\$). The GDP implicit deflator is the ratio of GDP in current local currency to GDP in constant local currency, the base year varies by country. Inflation as measured by the annual growth rate of the

GDP implicit deflator shows the rate of price change in the economy as a whole. The GDP implicit deflator is the ratio of GDP in current local currency to GDP in constant local currency. Real GDP values in 2000 USA dollars (USA\$) were calculated from Brazilian Real (R\$) from 2007 to 2013 using a modified version of the equation proposed by Chowdhury (2008, p. 267). (Eq. 3).

$$\text{Constant USA\$} = (\text{Current R\$/Deflator}) * 100 \quad \text{Eq. 3}$$

A GDP deflator is a measure of price inflation. Nominal GDP is GDP at current market prices, so it includes all the changes in the market prices that occurred during a year due to deflation or inflation. The difference between nominal and real GDP values is that the real GDP is adjusted to inflation allowing comparison between years.

Monitoring Effort

I assessed monitoring effort five ways: (1) as the number of municipalities sampled in each state each year, (2) as the state percentage of municipalities sampled each year, (3) as the national percentage of municipalities sampled each year, (4) in terms of number of monitoring events per municipality per year, and (5) in terms of the percentage of monitoring events per municipality per year. I considered municipalities and COSAA codes monitored at least one month of the year, three or more months per year, six or more months per year, and 12 months.

For the number of monitoring events per municipality per year I used the supplier codes per municipality, and assumed monthly monitoring as the standard. Since each COSAA code could be sampled 12 months each year, I determined the potential number of monitoring events by multiplying the total number of COSAA codes times 12 (Eq. 4).

$$\text{Potential N}^\circ \text{ monitoring events} = \text{Total N}^\circ \text{ of CO SAA codes} * 12 \quad (\text{Eq. 4})$$

Then, using the actual number of months a particular COSAA code was monitored, I determined the proportion of monitoring events (Eq. 4), and then multiplied that value times 100 to determine the percentage of monitoring events per COSAA code (Eq. 5).

$$\% \text{ monitoring events} = (\text{N}^\circ \text{ actual monitoring events} / \text{N}^\circ \text{ potential monitoring events}) * 100 \quad (\text{Eq. 5})$$

Analysis

Normality Tests

Data were tested for normality and homogeneity of variance using the Shapiro-Wilks and Bartlett's tests respectively. The Shapiro-Wilks test examines whether the samples for the variables studied come from normally distributed populations. Bartlett's test assesses if variances were equal across all the regions of Brazil, and during the seven years of data available.

Correlation Analysis

I performed a Pearson correlation analysis, including all regions, to establish if there was a correlation between GDPPC and monitoring effort. GDPPC was the independent variable. Monitoring effort, defined as the number and percentage (state and national) of municipalities sampled, and as the number and percentage of monitoring events were the dependent variables. I ran these tests per year, including data from municipalities and COSAA codes sampled at least once a year, ≥ 3 months, ≥ 6 months, and 12 months of the year from all regions.

Regression Analysis

I calculated a single linear regression, including all regions, to determine if GDPPC determined monitoring effort. This analysis describes how much of the variation in monitoring effort was driven by regional GDPPC. I ran these tests per year, including data for all regions, for municipalities and SAA codes sampled at least once a year, ≥ 3 months, ≥ 6 months, and 12 months of the year.

Kruskal-Wallis Test and Dunn's Test

Based on the results of the normally tests and unequal sample size and variances, I conducted a Kruskal-Wallis test for GDPPC, and monitoring effort per region and per year. Based on the Kruskal-Wallis results, I carried out a nonparametric pairwise multiple comparisons in independent groups using Dunn's test (Table 7).

Results

Brazil is the one country in SA with a program in place for systematic cyanobacteria monitoring in fresh-water reservoirs. However, most municipalities in the country were under sampled and not all municipalities were part of the seven-year data set. Out of the 5,569 municipalities in the country, 4,559 municipalities (81.9%) were listed and only 1,801 (32.3%) monitored. Merely 155 (2.8%) were monitored at least one month of the year, and only five (0.1%) were sampled every month. The five municipalities sampled monthly (i.e. Balneário Camboriú, Blumenau, Gaspar, Guaramirim, and Timbó) were all located in the state of Santa Catarina in the South region. Moreover, not the same COSAAs were monitored every year. Four COSAAs (i.e. 4078, 4087, 4091, and 4092) in the municipality of Blumenau, were monitored every month for five years. But only two COSAAs were monitored monthly during the seven years, 4338 in the municipality of Guaramirim, and 6622 in Timbó both in Santa Catarina.

From 2007 until 2013, five states were never sampled: Acre, Amapá, and Roraima in the North, and Alagoas and Paraíba in the Northeast. In the South and Southeast, all states were sampled every year (Table 8).

The number of municipalities registered with SISAGUA varied every year, and a small percentage of the municipalities registered were monitored at least once each year. In 2007, the first year digital registry was available, 1,940 municipalities that is 34.84% of the country's total number of municipalities were registered, and only 338 were (6.1%) sampled at least once a year. This year had the lowest number of municipalities sampled. The year with the highest number of registries was 2011, when 3,935 nearly 71% of the country's municipalities were registered. However, only 1,224 (22%) were sampled. The year with the most municipalities sampled was 2012 when 3,859 nearly 70% of the municipalities were monitored. However, the percentage of municipalities sampled remained low 23.77% (Tables 8, 9).

In terms of regional percentages, the South and Southeast regions consistently had the highest number of municipalities registered with VIGIAGUA, as well as the highest number of municipalities sampled. The Central-west region, had low numbers of municipalities registered, but most of them were in fact sampled. The South region had the highest percentage of municipalities sampled every year except for 2007. The North region had the lowest percentage of municipalities sampled every year except for 2013. The Central-west, South, and Southeast regions consistently had the higher percentage of municipalities registered (Table 10, Fig. 2).

The North region always reported the lowest numbers of municipalities registered and sampled. The highest values of registered and sampled municipalities belong the South, Southeast, and Central-west regions. In terms of percentage of municipalities sampled the Northeast and Southeast regions had comparable values every year, with the exception of 2013.

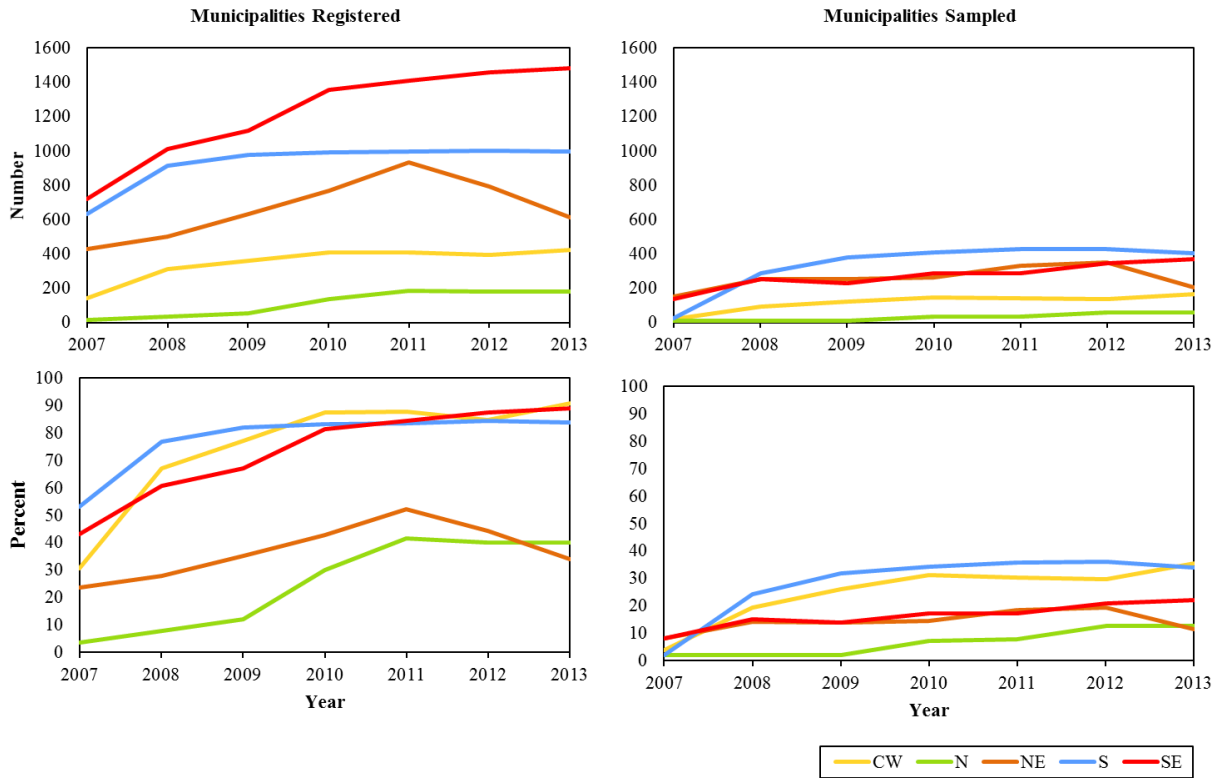


Figure 2. Number and regional percentage of municipalities registered with VIGIAGUA, and sampled at least once from 2007 to 2013.

From 2010 to 2013, all states in the Central-west region, with the exception of Brasília (D.F.), reported more than 75% of the municipalities in the state registered. However, only the state of Goiás had more than 50% of its municipalities sampled in 2013. From 2011 to 2013, more than 80% of the municipalities in the Southeast were registered. However, in this period none of the states in this region reached 75% of municipalities sampled. In 2010, the state of Espírito Santo had the highest percentage (66.7%) of municipalities sampled in the country for the seven-year period (Tables 8-10, Fig. 2).

National percentages are even lower, in the seven years. On average, only 11.8% of Brazil's municipalities were registered and on average, only 3.7% of the municipalities were

sampled at least one month of the year. The percentage of municipalities registered ranged between 34.8% and 70.7%, and the percentage of municipalities sampled between 6.1% and 23.8%. Year by year the number of municipalities registered and sampled increased. Every year, the Southeast had the highest national percentage of municipalities registered. The South region had the highest national percentage of municipalities sampled every year except for 2007. Every year, the North had the lowest regional and national percentages for the number of municipalities registered and sampled (Table 10).

Normality Tests

None of the data sets had a normal distribution, and all regions and years have different sample sizes and variances. Therefore, I conducted a Kruskal-Wallis test for GDPPC and monitoring effort per region and per year.

The Kruskal-Wallis test by region showed that at least one region had a higher mean GDPPC ($\chi^2= 40.423$, $df = 4$, $p\text{-value} = 3.539e-8$). Likewise, when monitoring effort was examined as the number of municipalities sampled ($\chi^2= 42.571$, $df = 4$, $p\text{-value} = 1.27e-8$), and as the percentage of municipalities sampled once ($\chi^2= 21.502$, $df = 4$, $p\text{-value} = 0.0002518$), at least one region experienced higher monitoring effort. The number of monitoring events was higher in at least one region ($\chi^2= 53.077$, $df = 4$, $p\text{-value} = 8.212e-11$). However, all regions have a similar percentage of monitoring events ($\chi^2= 10.963$, $df = 4$, $p\text{-value} = 0.02698$) (Table 7).

The Kruskal-Wallis test by year showed that all variables had p-values greater > 0.05 . Therefore, there were no significant differences between years for any of the variables examined (Table 7). However, data limitations may mean that the standard errors are inflated.

Table 7. Kruskal-Wallis test by region and by year.

	Variable	χ^2	df	p-value
by region	* GDPPC	40.423	4	3.54E-08
	* # Municipalities	42.571	4	1.27E-08
	* State % Municipalities	21.502	4	0.00025
	* National % Municipalities	40.423	4	3.54E-08
	* # Monitoring of events	53.077	4	8.21E-11
	% Monitoring of events	10.963	4	0.269
by year	GDP per capita	4.1903	6	0.651
	# Municipalities	3.707	6	0.800
	State % Municipalities	9.6239	6	0.141
	National % Municipalities	4.8017	6	0.569
	# Monitoring of events	2.8659	6	0.826
	% Monitoring of events	2.3486	6	0.885

Note: Variables with an * were significant

Table 8. Number and percentage of municipalities registered in VIGIAGUA at least once from 2007 to 2013. Bold values show states where $\geq 75\%$ of the municipalities were registered. Grayed states were never sampled.

	State	# Mun.	2007		2008		2009		2010		2011		2012		2013	
			#	%	#	%	#	%	#	%	#	%	#	%	#	%
CW	DF	1									1	100	1	100	1	100
	GO	246	89	36.2	163	66.3	179	72.8	217	88.2	220	89.4	213	86.6	235	95.5
	MS	79	54	68.4	57	72.2	66	83.5	75	94.9	73	92.4	67	84.8	68	86.1
	MT	141			93	66.0	114	80.6	116	82.3	115	81.6	114	80.9	119	84.4
N	AC	22														
	AP	16														
	AM	62	17	27.4	22	35.5	20	32.3	21	33.9	25	40.3	17	27.4	20	32.3
	PA	144					6	4.17	11	7.6	17	11.8	18	12.5	16	11.1
	RO	52							11	21.2	16	30.8	16	30.8	15	28.9
	RR	15														
	TO	139			14	10.1	29	21.0	92	66.2	129	92.8	130	95.5	130	95.5
NE	AL	102														
	BA	417	295	70.7	287	68.8	357	85.6	364	87.3	367	88.0	377	90.4	296	71.0
	CE	184	132	71.7	160	87.0	168	91.3	171	92.9	173	94.0	168	91.3	178	96.7
	MA	217					1	0.5			1	0.5				
	PB	223														
	PE	185							116	62.7	154	83.2	168	90.8		
	PI	224			12	5.4	17	7.6	30	13.4	36	16.1	39	17.4	43	19.2
	RN	167					7	4.2	14	3.8	55	32.9	43	25.8	95	57.0
SE	75			40	53.3	58	77.3	75	100	73	97.3					

Note: Mun: Municipalities, DF: Brasília, GO: Goiás, MS: Mato Grosso do Sul, MT: Mato Grosso, AC: Acre, AP: Amapá, AM: Amazonas, PA: Pará, RO: Rondônia, RR: Roraima, TO: Tocantins, AL: Alagoas, BA: Bahia, CE: Ceara, MA: Maranhão, PB: Paraíba, PE: Pernambuco, PI: Piauí, RN: Rio Grande do Norte, SE: Sergipe, PR: Paraná, RS: Rio Grande do Sul, SC: Santa Catarina, ES: Espírito Santo, MG: Minas Gerais, RJ: Rio de Janeiro, SP: São Paulo.

Table 8. Continued.

	State	# Mun.	2007		2008		2009		2010		2011		2012		2013	
			#	%	#	%	#	%	#	%	#	%	#	%	#	%
S	PR	399	370	92.7	386	96.7	387	97.0	387	97.0	395	99.0	398	100	148	37.1
	RS	497	234	47.1	332	66.8	358	72.0	360	72.4	361	72.6	363	73	346	69.6
	SC	295	30	10.2	196	66.4	231	78.3	244	82.7	240	81.4	246	83.4	253	85.8
SE	ES	78	47	60.3	62	80.0	71	91.0	72	92.3	70	89.7	70	89.7	65	83.3
	MG	853	447	52.4	631	74.0	702	82.3	719	84.3	719	84.3	729	85.5	765	89.7
	RJ	92	9	9.8	27	29.4	41	44.6	65	70.7	74	80.4	84	91.3	82	89.1
	SP	216	216	33.5	293	45.4	306	47.4	500	77.5	545	84.5	577	89.5	573	88.8
Brazil		5,569	1,940	34.8	2,775	50.0	3,118	56.0	3,660	65.7	3,859	22.0	3,838	69.0	3,448	62.0

Note: **Mun:** Municipalities, **DF:** Brasília, **GO:** Goiás, **MS:** Mato Grosso do Sul, **MT:** Mato Grosso, **AC:** Acre, **AP:** Amapá, **AM:** Amazonas, **PA:** Pará, **RO:** Rondônia, **RR:** Roraima, **TO:** Tocantins, **AL:** Alagoas, **BA:** Bahia, **CE:** Ceara, **MA:** Maranhão, **PB:** Paraíba, **PE:** Pernambuco, **PI:** Piauí, **RN:** Rio Grande do Norte, **SE:** Sergipe, **PR:** Paraná, **RS:** Rio Grande do Sul, **SC:** Santa Catarina, **ES:** Espírito Santo, **MG:** Minas Gerais, **RJ:** Rio de Janeiro, **SP:** São Paulo.

Table 9. Number and percentage of municipalities sampled per state at least once from 2007 to 2013. Bold values show states where $\geq 50\%$ of their municipalities were sampled. Grayed states were never sampled.

	State	# Mun.	2007		2008		2009		2010		2011		2012		2013	
			#	%	#	%	#	%	#	%	#	%	#	%	#	%
CW	DF	1									1	100	1	100	1	100
	GO	246	17	6.9	85	34.6	101	41.1	122	50.0	114	46.3	111	45.1	136	55.3
	MS	79	2	2.5	2	2.5	14	17.7	16	20.3	17	21.5	15	19.0	13	16.5
	MT	141			4	2.8	7	5.0	8	5.7	9	6.4	12	8.5	15	10.6
N	AC	22														
	AP	16														
	AM	62	9	14.5	1	1.6	1	1.6	1	1.6	1	1.6	1	1.6	2	3.2
	PA	144					1	0.7	2	1.4	1	0.7	2	1.4	2	1.4
	RO	52							1	1.9	3	5.8	3	5.8	3	5.8
	RR	15														
	TO	139			8	5.8	7	5.0	29	20.9	31	22.3	52	37.4	51	36.7
NE	AL	102								0						
	BA	417	104	25.0	152	37.0	143	34.3	148	36.0	108	26	122	29.3	74	17.8
	CE	184	45	24.5	100	54.4	102	55.4	103	56.0	106	57.6	105	57.1	109	59.2
	MA	217					1	0.5			1	0.5				
	PB	223														
	PE	185							4	2.2	105	56.8	114	61.6		
	PI	224			1	0.5	1	0.5	1	0.5	3	1.3	3	1.3	4	1.8
	RN	167					1	0.6	5	3.0	7	4.2	6	3.6	20	12.0
	SE	75			1	1.3	3	4.0	1	1.3	2	2.7				

Note: Mun: Municipalities, DF: Brasília, GO: Goiás, MS: Mato Grosso do Sul, MT: Mato Grosso, AC: Acre, AP: Amapá, AM: Amazonas, PA: Pará, RO: Rondônia, RR: Roraima, TO: Tocantins, AL: Alagoas, BA: Bahia, CE: Ceara, MA: Maranhão, PB: Paraíba, PE: Pernambuco, PI: Piauí, RN: Rio Grande do Norte, SE: Sergipe, PR: Paraná, RS: Rio Grande do Sul, SC: Santa Catarina, ES: Espírito Santo, MG: Minas Gerais, RJ: Rio de Janeiro, SP: São Paulo.

Table 9. Continued.

	State	# Mun.	2007		2008		2009		2010		2011		2012		2013	
			#	%	#	%	#	%	#	%	#	%	#	%	#	%
S	PR	399	7	1.8	106	26.6	119	29.8	145	36.3	150	37.6	148	37.1	148	37.1
	RS	497	11	2.2	159	32	166	33.4	160	32.2	166	33.4	168	33.8	141	28.4
	SC	295	6	2.0	24	8.1	94	31.9	103	34.9	111	37.6	113	38.3	116	39.3
SE	ES	78	21	26.9	42	53.9	50	64.1	52	66.7	50	64.1	51	65.4	48	61.5
	MG	853	103	12.1	160	18.8	125	14.7	134	15.7	132	15.5	186	21.8	207	24.3
	RJ	92	1	1.1	13	14.1	25	27.2	47	51.1	50	54.4	59	64.1	54	58.7
	SP	216	12	1.9	40	6.2	31	4.8	55	8.5	56	8.7	52	8.1	60	9.3
Brazil		5,569	338	6.1	898	16.1	992	17.8	1,137	20.4	1,224	22.0	1,324	23.8	1,204	21.6

Note: **Mun:** Municipalities, **DF:** Brasília, **GO:** Goiás, **MS:** Mato Grosso do Sul, **MT:** Mato Grosso, **AC:** Acre, **AP:** Amapá, **AM:** Amazonas, **PA:** Pará, **RO:** Rondônia, **RR:** Roraima, **TO:** Tocantins, **AL:** Alagoas, **BA:** Bahia, **CE:** Ceara, **MA:** Maranhão, **PB:** Paraíba, **PE:** Pernambuco, **PI:** Piauí, **RN:** Rio Grande do Norte, **SE:** Sergipe, **PR:** Paraná, **RS:** Rio Grande do Sul, **SC:** Santa Catarina, **ES:** Espírito Santo, **MG:** Minas Gerais, **RJ:** Rio de Janeiro, **SP:** São Paulo.

Table 10. Number, regional percentage (Re. %), and national percentage (Br. %) of municipalities registered and sampled at least once from 2007 to 2013 per region. Highest percentages bolded; lowest percentages grayed.

		Municipalities					
		Registered	Re. %	Br. %	Sampled	Re. %	Br. %
2007	CW	143	30.7	2.6	19	4.1	0.3
	N	17	3.8	0.3	9	2.0	0.2
	NE	427	23.8	7.7	149	8.3	2.7
	S	634	53.3	11.4	24	2.0	0.4
	SE	719	43.1	12.9	137	8.2	2.5
2008	CW	313	67.2	5.6	91	19.5	1.6
	N	36	8.0	0.7	9	2.0	0.2
	NE	499	27.8	9.0	254	14.2	4.6
	S	914	76.7	16.4	289	24.3	5.2
	SE	1,013	60.7	18.2	255	15.3	4.6
2009	CW	359	77.0	6.5	122	26.2	2.0
	N	55	12.2	1.0	9	2.0	0.2
	NE	632	35.2	11.4	251	14.0	4.5
	S	976	82	17.5	379	31.8	6.8
	SE	1,120	67.15	20.1	231	13.9	4.2
2010	CW	408	87.6	7.3	146	31.3	2.6
	N	135	30.0	2.4	33	7.3	0.6
	NE	770	42.9	13.8	262	14.6	4.7
	S	991	83.2	17.8	408	34.3	7.3
	SE	1,356	81.3	24.4	288	17.3	5.2
2011	CW	409	87.8	7.4	141	30.3	2.5
	N	187	41.6	3.6	36	8.0	0.7
	NE	935	52.1	16.8	332	18.5	6.0
	S	996	83.6	17.9	427	35.9	7.7
	SE	1,408	84.4	25.3	288	17.3	5.2
2012	CW	395	84.8	7.1	139	29.8	2.5
	N	181	40.2	3.3	58	12.9	1.0
	NE	795	44.3	14.3	350	19.5	6.3
	S	1,004	84.3	18.0	429	36.0	7.7
	SE	1,460	87.5	26.2	348	20.9	6.3
2013	CW	423	90.8	7.6	165	35.4	3.0
	N	181	40.2	3.3	58	12.9	1.0
	NE	612	34.1	11.0	207	11.5	3.7
	S	997	83.7	17.9	405	34.0	7.3
	SE	1,485	89.0	26.7	369	22.1	6.6

Correlation Analysis

A Pearson correlation between GDPPC and monitoring effort showed similar trends regardless of the number of months sampled. Although more data were available for municipalities and COSAAs sampled at least one month of the year, the trend persisted for municipalities and COSAAs sampled ≥ 3 months, ≥ 6 months, and 12 months of the year. I considered values between 0.76 and 1 to denote a strong correlation, values between 0.5-0.75 a moderate correlation, values between 0.26 and 0.5 a fair correlation and values between 0 and 0.25 a weak correlation.

The correlation was strong between GDPPC and the number of municipalities sampled, the national percentage of municipalities sampled, and number of monitoring events irrespective of the number for months sampled. The correlation between GDPPC and state percentage of municipalities sampled, and percentage of monitoring events was always fair or a weak.

Exceptions to these trends were the correlation between GDPPC and the national percentage of municipalities sampled at least one month in 2007 ($p = 0.2633$), when the correlation was fair. The correlation between GDPPC and the number of municipalities sampled 12 months in 2012 ($p = 0.7050$) was moderate. And the correlation between GDPPC and percentage of monitoring events for COSAAs sampled 12 months in 2010 ($p = 0.5233$), 2011 ($p = 0.6277$), 2012 ($p = 0.6983$), and 2103 ($p = 0.7444$). Other exceptions were the weak negative correlations between percentage of monitoring events for COSAAs sampled at least 6 months in 2007 ($p = -0.2681$), and state percentage of municipalities sampled 12 months in 2013 ($p = -0.0037$) (Table 9).

Table 11. Correlation coefficients (r) for the Pearson correlation analysis between GDPPC and the number and percentage (state and national) of municipalities sampled, and GDPPC and number and percentage of monitoring events that occurred ≥ 1 month, ≥ 3 months, ≥ 6 months, and 12 months of the year from 2007 to 2013. Values between 0.76-1 denote a strong correlation, between 0.5-0.75 a moderate correlation, between 0.26-0.5 a fair correlation, and between 0-0.25 a weak correlation.

			2007	2008	2009	2010	2011	2012	2013
1 mo.	Mun.	#	0.8624	0.8663	0.8736	0.8667	0.8525	0.8655	0.9082
		St. %	0.3673	0.4628	0.5341	0.6136	0.3018	0.2351	0.2529
		Br. %	0.2633	0.8663	0.8736	0.8667	0.8525	0.8655	0.9082
	S.E.	#	0.8717	0.8585	0.8598	0.8907	0.8829	0.8953	0.9007
		%	0.0582	0.2340	0.4321	0.3750	0.4357	0.3679	0.3571
≥ 3 mo.	Mun.	#	0.9718	0.9126	0.8977	0.9352	0.8696	0.8798	0.8909
		St. %	0.4725	0.4795	0.4560	0.6360	0.2652	0.1728	0.2234
		Br. %	0.9718	0.9126	0.8977	0.9352	0.8696	0.8798	0.8626
	S.E.	#	0.9824	0.8935	0.8741	0.9472	0.8952	0.8953	0.8763
		%	0.0793	0.2630	0.3569	0.4941	0.4575	0.3232	0.3897
≥ 6 mo.	Mun.	#	0.7906	0.9091	0.9153	0.9365	0.8829	0.8984	0.8444
		St. %	0.0872	0.5287	0.4423	0.5822	0.0805	0.2141	0.1577
		%	0.7906	0.9091	0.9153	0.9365	0.8613	0.8984	0.8444
	S.E.	#	0.7790	0.8923	0.9197	0.9517	0.8855	0.8985	0.8368
		St. %	-0.2681	0.2854	0.3185	0.5097	0.4496	0.4515	0.4303
		%							
12 mo.	Mun.	#	0.9987	0.9739	0.9782	0.9082	0.8655	0.7050	0.9134
		St. %	0.3898	0.5993	0.8749	0.6203	0.5532	0.6227	-0.0037
		Br. %	0.9987	0.9739	0.9782	0.9082	0.8655	0.705	0.9134
	S.E.	#	0.7776	0.9949	0.9807	0.8693	0.9093	0.8040	0.9889
		%	0.0311	0.3051	0.4524	0.5233	0.6277	0.6983	0.7444

Note: Bold values show negative correlations, and grayed values correlations outside the general trends.

Regression Analysis

The regression analysis was carried out to determine how much of the variance in regional cyanobacteria monitoring effort was predicted by wealth. The number and state and national percentages of municipalities sampled, as well as the number and percentage of

monitoring events were used as indicators of monitoring effort and were the dependent variables. GDPPC was used as an indicator of economic development, and was the independent variable.

For municipalities and COSAAs sampled at least one month, three or more months, and six or more months of the year, GDPPC was a strong ($R^2 = 0.76-1$) or moderate ($R^2 = 0.5-0.75$) predictor of regional monitoring effort when considered in terms of the number of municipalities sampled, national percentage of municipalities sampled, and number of monitoring events. But it was a fair ($R^2 = 0.26-0.5$) or weak ($R^2 = 0-0.25$) predictor of monitoring effort when it defined as state percentage of municipalities sampled or percentage of monitoring events (Tables 10-12).

The pattern slightly changed for municipalities and COSAAs sampled 12 months, when GDPPC was a strong ($R^2 = 0.76-1$) or moderate ($R^2 = 0.5-0.75$) predictor of regional monitoring effort, when defined as number of municipalities, national percentage of municipalities sampled, and number of monitoring events. GDPPC was mostly a fair ($R^2 = 0.26-0.5$) predictor of monitoring when considering percentage of monitoring events, and mostly a weak predictor ($R^2 = 0-0.25$) for state percentage of municipalities sampled (Table 15).

Given the similar trends of the linear regressions, regardless of the number of months sampled, only the results for municipalities and COSAAs sampled at least once will be plotted and reported, but the information for three or more months, six or more months, and twelve months is summarized in tables 2-6, 2-7, and 2-8 respectively. Table 2-9 presents the regression equations for regional monitoring effort (y) as a function of GDPPC (x) for municipalities and COSAAs sampled at least one month, three or more months, six or more months, and twelve months from 2007 to 2013.

Table 12. Results for the single linear regression between GDPPC and monitoring effort metrics for ≥ 1 month from 2007 to 2013. R^2 in dark orange were considered strong (0.76-1), in orange moderate (0.5-0.75), in yellow fair (0.26-0.5) and in green weak (0-0.25).

GDPPC								
	Year	<i>df</i>		<i>F</i>	<i>Significance F</i>	R^2	Coefficients	
		Regression	Residual				GDPPC	Intercept
# Municipalities sampled	2007	1	10	15.7546	0.0003	0.7438	0.3344	6.3325
	2008	1	14	42.1069	1.43e-5	0.7505	0.2858	7.8111
	2009	1	17	54.7802	1.04e-6	0.7632	0.2891	9.0439
	2010	1	18	54.3320	7.71e-7	0.7511	0.2276	8.8059
	2011	1	20	53.2007	4.71e-7	0.7268	0.1989	12.5658
	2012	1	18	53.7353	8.31e-7	0.7491	0.2417	13.4034
	2013	1	17	80.0963	7.67e-8	0.8249	0.2679	9.1226
St. % Municipalities	2007	1	10	1.5592	0.2402	0.1349	0.0392	7.5463
	2008	1	14	3.8162	0.0710	0.2142	0.0464	10.9480
	2009	1	17	6.7863	0.0185	0.2853	0.0610	10.4630
	2010	1	18	10.8721	0.0040	0.3766	0.0583	9.9411
	2011	1	20	2.0047	0.1722	0.0911	0.0332	20.3078
	2012	1	18	1.0536	0.3183	0.0553	0.0290	25.6752
	2013	1	17	1.1613	0.2963	0.0639	0.0319	23.9980
Br. % Municipalities	2007	1	10	0.7448	0.4084	0.0693	0.0014	0.2550
	2008	1	14	42.1069	1.43e-5	0.7505	0.0051	0.1403
	2009	1	17	54.7802	1.04e-6	0.7632	0.0052	0.1624
	2010	1	18	54.3320	7.71e-7	0.7511	0.0041	0.1581
	2011	1	20	53.2007	4.71e-7	0.7268	0.0036	0.2256
	2012	1	18	53.7353	8.31e-7	0.7491	0.0043	0.2407
	2013	1	17	80.0963	7.67e-8	0.8249	0.0048	0.1638
# Monitoring events	2007	1	10	31.6415	0.0002	0.7599	5.1048	151.6446
	2008	1	14	39.2419	2.08e-5	0.7370	4.3134	200.5247
	2009	1	17	48.1829	2.38e-6	0.7392	4.4061	234.0372
	2010	1	18	69.1337	1.41e-7	0.7934	3.7464	176.4055
	2011	1	20	70.7221	5.34e-8	0.7795	3.3284	226.6192
	2012	1	18	72.7385	9.74e-8	0.8016	3.9802	254.5303
	2013	1	17	73.0475	1.47e-7	0.8112	4.3874	233.1729
% Monitoring events	2007	1	10	0.0340	0.8574	0.0034	0.0101	19.0935
	2008	1	14	0.8107	0.3831	0.0547	0.0209	15.9739
	2009	1	17	3.9027	0.0647	0.1867	0.0396	12.2551
	2010	1	18	2.9457	0.1033	0.1406	0.0290	15.0094
	2011	1	20	4.6862	0.0427	0.1898	0.0311	12.5904
	2012	1	18	2.8186	0.1105	0.1354	0.0263	16.6854
	2013	1	17	2.4853	0.1333	0.1275	0.0255	16.8966

Table 13. Results for the single linear regression between GDPPC and monitoring effort metrics for ≥ 3 month from 2007 to 2013. R^2 in dark orange were considered strong (0.76-1), in orange moderate (0.5-0.75), in yellow fair (0.26-0.5) and in green weak (0-0.25).

GDPPC								
	Year	<i>df</i>		<i>F</i>	<i>Significance</i>	R^2	Coefficients	
		Regression	Residual				GDPPC	Intercept
# Municipalities	2007	1	8	29.0281	0.0003	0.7438	0.3344	6.3325
	2008	1	14	42.1069	1.43e-5	0.7505	0.2858	7.8111
	2009	1	14	54.7802	1.04e-6	0.7632	0.2891	9.0439
	2010	1	17	54.3320	7.71e-7	0.7511	0.2276	8.8059
	2011	1	18	53.2007	4.71e-7	0.7268	0.1989	12.5658
	2012	1	17	53.7353	8.31e-7	0.7491	0.2417	13.4034
	2013	1	17	80.0963	7.67e-8	0.8249	0.2679	9.1226
St % Municipalities	2007	1	8	2.2994	0.1679	0.2233	0.0522	2.7928
	2008	1	14	4.1810	0.0602	0.2300	0.0506	7.3283
	2009	1	14	3.6757	0.0758	0.2080	0.0501	11.2645
	2010	1	17	11.5466	0.0034	0.4045	0.0574	7.5521
	2011	1	18	1.3615	0.2585	0.0703	0.0314	18.9972
	2012	1	17	0.5232	0.4793	0.0299	0.0236	24.4105
	2013	1	17	0.8932	0.3578	0.0499	0.0315	20.1528
Br %	2007	1	8	135.9450	2.67e-6	0.9444	0.2925	1.2642
	2008	1	14	69.7837	8.23e-7	0.8329	0.0047	0.0883
	2009	1	14	58.1266	2.34e-6	0.8059	0.0048	0.1503
	2010	1	17	118.5795	4.38e-9	0.8746	0.0039	0.0886
	2011	1	18	55.8522	6.37e-7	0.7563	0.0035	0.1547
	2012	1	17	58.2442	6.89e-7	0.7741	0.0042	0.1996
	2013	1	17	49.4461	2.02e-6	0.7442	0.0053	0.2295
# Monitoring	2007	1	8	221.6754	4.08e-7	0.9652	4.7256	64.4009
	2008	1	14	55.4258	3.13e-6	0.7983	3.8386	137.6578
	2009	1	14	45.3454	9.57e-6	0.7641	3.9200	216.4483
	2010	1	17	148.2659	8.05e-10	0.8971	3.4749	117.6826
	2011	1	18	72.6060	9.87e-8	0.8013	3.2196	161.9925
	2012	1	17	68.6880	2.25e-7	0.8016	3.7744	214.8452
	2013	1	17	56.2621	8.68e-7	0.7680	3.8516	190.4959
% Monitoring	2007	1	8	0.0506	0.8277	0.0063	0.0148	10.1327
	2008	1	14	1.0405	0.3250	0.0692	0.0210	10.6525
	2009	1	14	2.0441	0.1747	0.1274	0.0290	11.4625
	2010	1	17	5.4894	0.0316	0.2441	0.0340	9.0922
	2011	1	18	4.7635	0.0426	0.2093	0.0335	9.4667
	2012	1	17	1.9827	0.1771	0.1044	0.0252	14.2158
	2013	1	17	3.0447	0.0990	0.1519	0.0286	12.1633

Table 14. Results for the single linear regression between GDPPC and monitoring effort metrics for ≥ 6 month from 2007 to 2013. R^2 in dark orange were considered strong (0.76-1), in orange moderate (0.5-0.75), in yellow fair (0.26-0.5) and in green weak (0-0.25).

GDPPC								
	Year	<i>df</i>		<i>F</i>	<i>Significance F</i>	R^2	Coefficients	
		Regression	Residual				GDPPC	Intercept
# Municipalities sampled	2007	1	7	11.66744	0.0112	0.6250	0.240638	2.468387
	2008	1	12	57.14981	6.68e-6	0.8265	0.254374	4.068369
	2009	1	13	67.1748	1.71e-6	0.8379	0.278696	4.857124
	2010	1	14	99.9369	9.38e-8	0.8771	0.21109	6.157842
	2011	1	16	45.97917	4.41e-6	0.7418	3.132028	139.1458
	2012	1	16	66.96462	4.13e-7	0.8071	0.241999	5.348975
	2013	1	16	39.73937	1.05e-5	0.7129	0.224225	7.462667
St % Municipalities sampled	2007	1	7	0.0536	0.8235	0.0076	0.0106	2.2126
	2008	1	12	4.6553	0.0519	0.2795	0.0475	5.5004
	2009	1	13	3.1617	0.0988	0.1956	0.0499	9.0767
	2010	1	14	7.1788	0.0180	0.3390	0.0481	8.9368
	2011	1	16	0.1043	0.7510	0.0065	0.0090	18.9256
	2012	1	16	0.7690	0.3935	0.0459	0.0337	18.7264
	2013	1	16	0.4080	0.5320	0.0249	0.0253	18.9112
Br % Municipalities	2007	1	7	11.66744	0.0112	0.6250	0.004321	0.044324
	2008	1	12	57.14981	6.68e-6	0.8265	0.004568	0.073054
	2009	1	13	67.1748	1.71e-6	0.8379	0.005004	0.087217
	2010	1	14	99.9369	9.38e-8	0.8771	0.00379	0.110574
	2011	1	16	45.97917	4.41e-6	0.7418	0.003409	0.155102
	2012	1	16	66.96462	4.13e-7	0.8071	0.004345	0.096049
	2013	1	16	39.73937	1.05e-5	0.7129	0.004026	0.134004
# Monitoring events	2007	1	7	10.8011	0.0134	0.6068	3.1593	88.5047
	2008	1	12	46.8748	1.78e-5	0.7962	3.5247	133.1978
	2009	1	13	71.3177	1.23e-6	0.8458	4.1068	140.7526
	2010	1	14	134.5384	1.44e-8	0.9057	3.2699	137.6103
	2011	1	16	58.0953	1.03e-6	0.7841	3.0674	171.3977
	2012	1	16	67.0692	4.09e-7	0.8074	3.8785	138.6158
	2013	1	16	37.3691	1.50e-5	0.7002	3.6234	173.4523
% Monitoring events	2007	1	7	0.5422	0.4855	0.0719	-0.0828	8.8525
	2008	1	12	1.1713	0.3004	0.0889	0.0192	7.8773
	2009	1	13	1.4681	0.2472	0.1015	0.0252	9.0098
	2010	1	14	4.9147	0.0437	0.2598	0.0300	7.6754
	2011	1	16	4.0543	0.0612	0.2022	0.0315	8.6449
	2012	1	16	4.0956	0.0600	0.2038	0.0380	8.7714
	2013	1	16	3.6363	0.0747	0.1852	0.0324	9.2915

Table 15. Results for the single linear regression between GDPPC and monitoring effort metrics for 12 months from 2007 to 2013. R² in dark orange were considered strong (0.76-1), in orange moderate (0.5-0.75), in yellow fair (0.26-0.5) and in green weak (0-0.25).

GDPPC								
	Year	df		F	Significance F	R ²	Coefficients	
		Regression	Residual				GDPPC	Intercept
# Municipalities sampled	2007	1	3	1179.6265	5.43e-5	0.9975	0.2728	0.3364
	2008	1	9	57.1498	6.68e-6	0.8265	0.2544	4.0684
	2009	1	11	243.9263	7.44e-9	0.9569	0.2580	-0.0255
	2010	1	12	56.5345	7.05e-6	0.8249	0.1994	1.2199
	2011	1	11	32.8548	0.0001	0.7492	0.1976	2.5638
	2012	1	12	11.8548	0.0049	0.4970	0.1737	4.4358
	2013	1	14	70.4609	7.79e-7	0.8342	0.2104	1.4143
St % Municipalities sampled	2007	1	3	0.5376	0.5165	0.1520	0.0238	0.4389
	2008	1	9	5.0436	0.0514	0.3591	0.0411	2.0543
	2009	1	11	35.8839	9.05e-5	0.7654	0.0465	1.3210
	2010	1	12	7.5057	0.0179	0.3848	0.0464	3.0993
	2011	1	11	4.8514	0.0499	0.3061	0.0385	4.6095
	2012	1	12	7.5982	0.0174	0.3877	0.0770	2.6894
	2013	1	14	0.0002	0.9891	1.38e-5	-0.0015	12.0707
# Municipalities Br %	2007	1	3	1179.6265	0.0001	0.9975	0.0049	0.006
	2008	1	9	165.6493	4.23e-7	0.9485	0.0042	0.0139
	2009	1	11	243.9263	7.44e-9	0.9569	0.0046	-0.0005
	2010	1	12	56.5345	7.05e-6	0.8249	0.0036	0.0219
	2011	1	11	32.8548	0.0001	0.7492	0.0035	0.046
	2012	1	12	11.8548	0.0049	0.4970	0.0031	0.0797
	2013	1	14	70.4609	7.79e-7	0.8342	0.0038	0.0254
# Monitoring events	2007	1	3	4.5871	0.1217	0.6046	5.1222	26.3609
	2008	1	9	876.4561	2.8e-10	0.9898	3.6270	20.2621
	2009	1	11	276.6938	3.82e-9	0.9618	3.5742	24.9998
	2010	1	12	37.1211	5.4e-5	0.7557	6.0362	330.1614
	2011	1	11	52.5327	1.65e-5	0.8269	3.0133	40.2478
	2012	1	12	21.9410	0.0005	0.6464	3.1353	75.4106
	2013	1	14	618.9816	5.48e-13	0.9779	3.7762	15.9622
% Monitoring events	2007	1	3	0.0029	0.9604	0.0010	0.0077	1.9991
	2008	1	9	0.9239	0.3616	0.0931	0.0137	2.8527
	2009	1	11	2.8304	0.1206	0.2047	0.0185	2.2394
	2010	1	12	4.5259	0.0548	0.2739	0.0646	9.0416
	2011	1	11	7.1514	0.0216	0.3940	0.0266	2.8393
	2012	1	12	11.4173	0.0055	0.4876	0.0637	1.5343
	2013	1	14	17.3980	0.0009	0.5541	0.0650	1.3869

Analyses for Municipalities and COSAAs Sampled at Least One Month of the Year

Relationship Analyses for the Year 2007

There was a strong positive correlation between the number of municipalities sampled and GDPPC (Table 11; $r = 0.8624$). The linear regression between the number of municipalities sampled and GDPPC was highly significant ($F(1, 10) = 29.0281, p = 0.0003$). The resulting linear equation $y = 0.3344(\text{GDPPC}) + 6.3325$, indicates that the number of municipalities sampled increased by 0.3344 with each additional USD of regional GDPPC. The coefficient of the determination showed that 74.4% of the variation in the number of municipalities sampled was explained by GDPPC (Table 12, Fig. 3A; $R^2 = 0.7438$).

There was a moderate positive correlation between state percentage of municipalities sampled and GDPPC (Table 11; $r = 0.3673$). The linear regression between the two variables was non-significant ($F(1, 10) = 1.5592, p = 0.2402$). The resulting linear equation $y = 0.0392(\text{GDPPC}) + 7.5463$, indicates that the state percentage of municipalities sampled increased by 0.0392 for each USD. However, only 13.5% of the variation in regional monitoring effort was explained by GDPPC (Table 12, Fig. 3B; $R^2 = 0.1349$).

For the national percentage of municipalities sampled and GDPPC, there was a fair correlation (Table 11; $r = 0.2633$), and the regression was non-significant ($F(1, 10) = 0.7448, p = 0.4084$). Predicted monitoring effort was equal to $0.0014(\text{GDPPC}) + 0.2550$. Monitoring effort only increased by 0.0014 for each USD, and only 7% of the variation in monitoring effort could be explained by GDPPC (Table 12, Fig. 3C; $R^2 = 0.0693$).

When considering the number of monitoring events in relation to GDPPC there was a strong positive correlation (Table 11; $r = 0.8717$). Likewise, the linear regression was highly significant ($F(1, 10) = 31.6415, p = 0.0002$), with nearly 76% of the variation in the number of

monitoring events explained by GDPPC. Predicted monitoring effort was determined by the equation $y = 5.1048(\text{GDPPC}) + 15.6446$. Thus, the number of monitoring events increased by 5.1048 for each real USD (Table 12, Fig. 3D; $R^2 = 0.7599$).

There was a weak correlation between percentage of monitoring events and GDPPC (Table 11; $r = 0.0582$), and no significant relationship was found between these two variables ($F(1, 10) = 0.0340$, $p = 0.8574$). Only 0.3% of the variation in the percentage of monitoring events was explained by GDPPC, and predicted monitoring effort was equal to $0.0101(\text{GDPPC}) + 19.093$, indicating that the percentage of monitoring events only increased by 0.0101 for each USD of GDPPC (Table 12, Fig. 3E; $R^2 = 0.0034$).

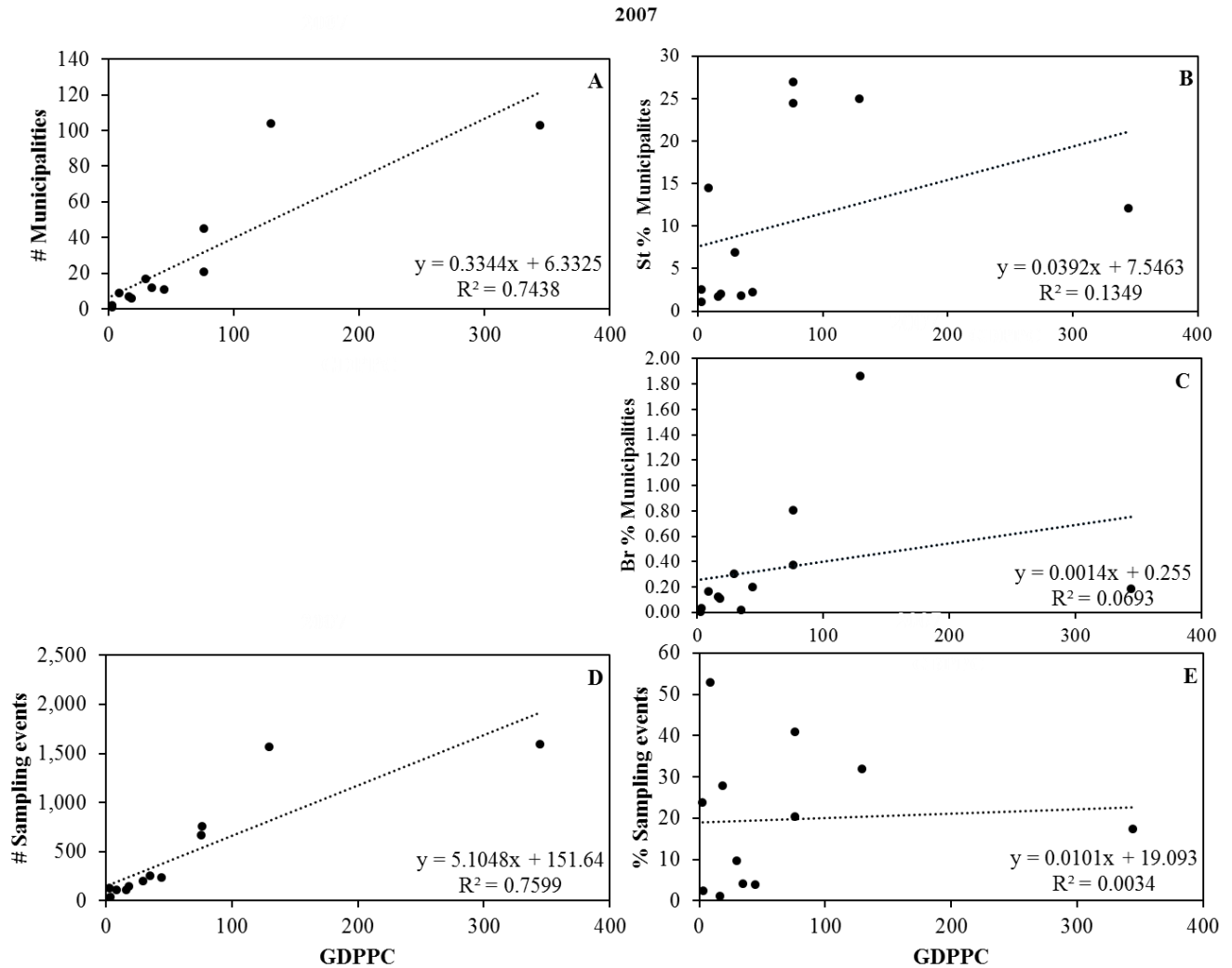


Figure 3. Simple linear regression between GDPPC and monitoring effort. Monitoring effort was defined as (A) number of municipalities sampled, (B) state percentage of municipalities sampled, (C) national percentage of municipalities sampled, (D) number of monitoring events, and (E) percentage of monitoring events in 2007.

Relationship Analyses for the Year 2008

For the year 2008, there was a strong positive correlation between the number of municipalities sampled and GDPPC (Table 11; $r = 0.8663$), with a highly significant linear relationship between the two variables ($F(1, 14) = 42.1069$, $p = 1.43e-5$). More than 75% of the variation in the number of municipalities was explained by regional GDPPC. The resulting linear

equation was $y = 0.2858(\text{GDPPC}) + 7.8111$, thus monitoring effort increased by 0.2858 for each USD of GDPPC (Table 12, Fig. 4A; $R^2 = 0.7505$).

Though there was a fair positive correlation (Table 11; $r = 0.4628$) for the state percentage of municipalities sampled and GDPPC, this linear regression was moderately non-significant ($F(1, 14) = 3.8162$, $p = 0.0710$). Only 21.4 % of the variation in the percentage of sampled municipalities that was explained by GDPPC. Predicted monitoring effort was equal to $0.0464(\text{GDPPC}) + 10.9480$. Therefore, the percentage of municipalities sampled increased by 0.0464 for each USD of GDPPC (Table 12, Fig. 4B; $R^2 = 0.2142$).

There was a strong positive correlation between the national percentage of municipalities and GDPPC (Table 11; $r = 0.8663$), and a highly significant relationship between the two variables ($F(1, 14) = 42.1069$, $p = 1.43e-5$), Predicted monitoring effort was equal to $0.0051(\text{GDPPC}) + 0.1403$. Therefore, for each USD of regional GDPPC, monitoring effort increased by 0.0051. The coefficient of the determination showed that 75.05% of the variation in the number of municipalities sampled was explained by GDPPC. (Table 12, Fig. 4C; $R^2 = 0.7505$).

Similarly, for the number of monitoring events and GDPPC there was a strong positive correlation (Table 11; $r = 0.8585$), as well as a highly significant linear regression ($F(1, 14) = 39.2419$, $p = 2.0761e-5$). Nearly 74% of the variation in the number of monitoring events was explained by GDPPC, and the predicted monitoring effort was determined by the equation $y = 4.3134(\text{GDPC}) + 200.5247$. The mean number of municipalities sampled increased by 4.3134 for each USD (Table 12, Fig. 4D; $R^2 = 0.7370$).

There was a weak positive correlation between percentage of monitoring events and GDPPC (Table 11; $r = 0.2340$), with no statistical significance ($F(1, 14) = 0.8107$, $p = 0.3831$).

Only 5.5% of the variation in the percentage of municipalities sampled explained by GDPPC.

The predicted monitoring effort was determined by the equation $y = 0.0209(\text{GDPPC}) + 15.9739$, revealing that the percentage of municipalities sampled increased by 0.0209 for each USD (Table 12, Fig. 4E; $R^2 = 0.0547$).

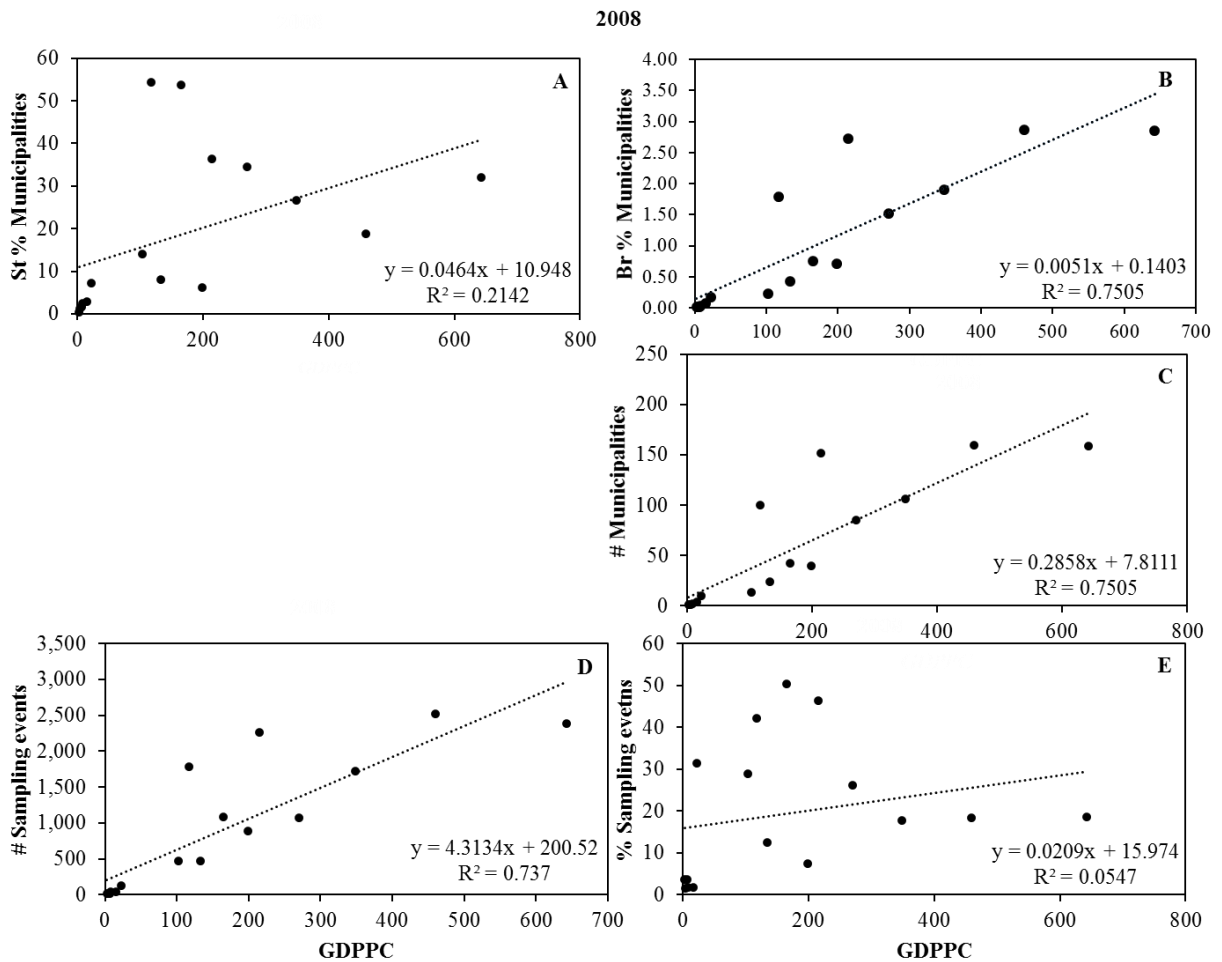


Figure 4. This Simple linear regression between GDPPC and monitoring effort. Monitoring effort was defined as (A) number of municipalities sampled, (B) state percentage of municipalities sampled, (C) national percentage of municipalities sampled, (D) number of monitoring events, and (E) percentage of monitoring events in 2008.

Relationship Analyses for the Year 2009

In the year 2009, there was a strong positive correlation between the number of municipalities sampled and GDPPC (Table 11; $r = 0.8736$), with a highly significant linear regression ($F(1, 17) = 54.7802, p = 1.04e-6$). Predicted monitoring effort equal $0.2891(\text{GDPPC}) + 9.0439$, showing that monitoring effort increased by 0.2891 for each USD, and 76.3% of the variation in the number of municipalities sampled was explained by GDPPC (Table 12, Fig. 5A; $R^2 = 0.7632$).

State percentage of municipalities sampled and GDDPPC moderately correlated (Table 11; $r = 0.5341$), and there was a significant linear regression between these variables ($F(1, 17) = 6.7863, p = 0.0185$). Predicted monitoring effort was equal to $0.0610(\text{GDPPC}) + 10.4630$. The percentage of municipalities sampled increased by 0.0610 for each real USD, 28.5% of the variation is explained by GDPPC (Table 12, Fig. 5B; $R^2 = 0.2853$).

There was a strong positive correlation between the national percentage of municipalities sampled and GDPPC (Table 11; $r = 0.8736$). Their linear regression was highly significant ($F(1, 17) = 54.7802, p = 1.04e-6$). The equation for predicted monitoring effort is $y = 0.0052(\text{GDPPC}) + 0.1624$. Monitoring effort increased by 0.0052 for each USD, and 76.3% of the variation in the number of municipalities sampled was explained by regional GDPPC (Table 12, Fig. 5C; $R^2 = 0.7632$).

For number of monitoring events and GDPPC, the correlation was strong (Table 11; $r = 0.8598$), and the linear relationship was highly significant ($F(1, 17) = 48.12829, p = 2.38e-6$). Predicted number of monitoring events was equal to $4.4061(\text{GDPPC}) + 234.0372$, indicating that the number of monitoring events increased by 4.4061 for each USA\$, and 73.9% of the variation could be explained by GDPPC (Table 12, Fig. 5D; $R^2 = 0.7392$).

For the percentage of monitoring events and GDPPC the correlation was fair (Table 11; $r = 0.4321$), while the linear regression was moderately non-significant ($F(1, 17) = 3.9027$, $p = 0.0647$). Predicted monitoring effort was equal to $0.0396(\text{GDPPC}) + 12.2551$, and the percentage of monitoring events increased by 0.0396 for each USD. However, only 18.7% of the variation in the percentage of monitoring events could be explained by regional GDPPC (Table 12, Fig. 5E; $R^2 = 0.1867$).

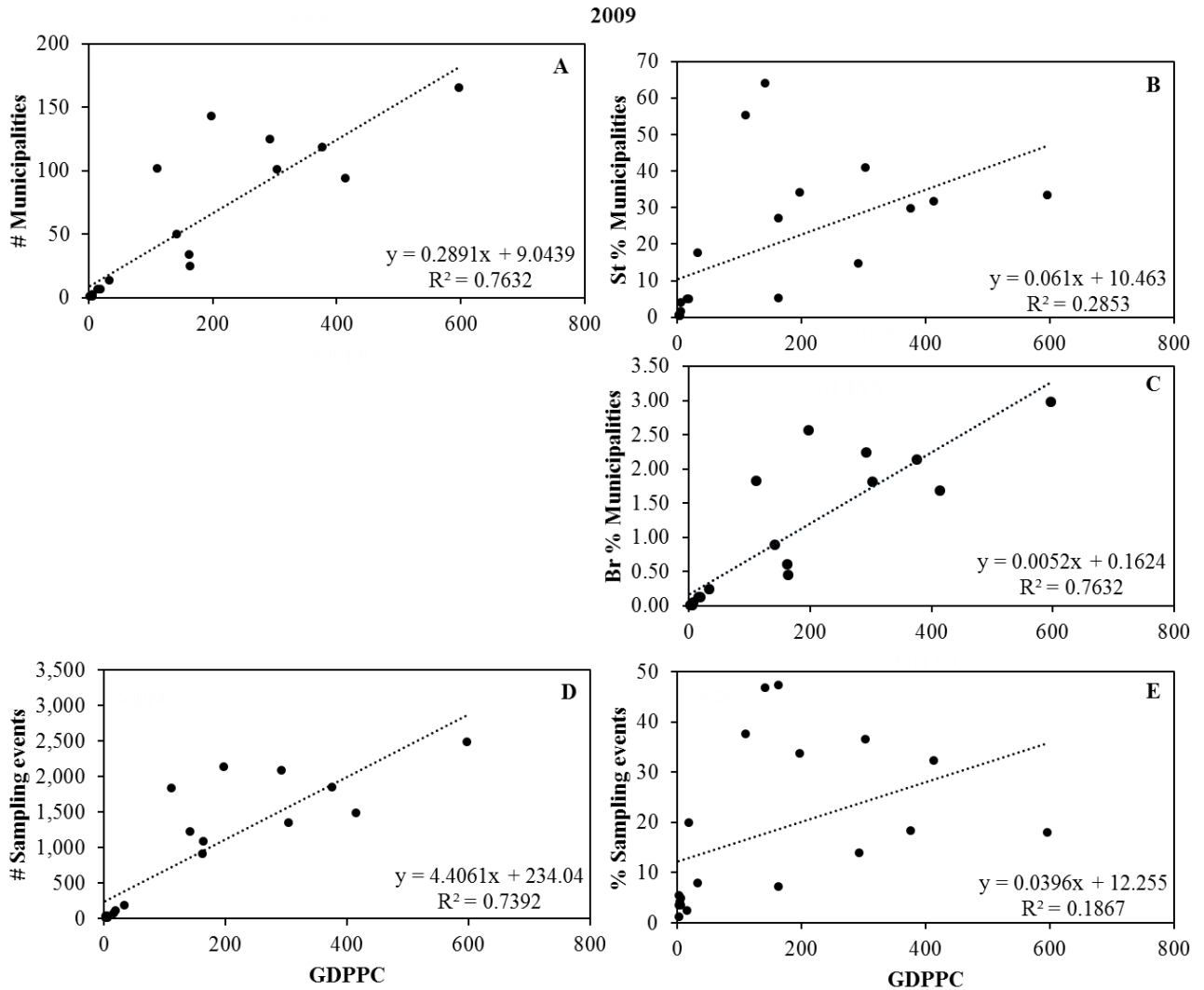


Figure 5. Simple linear regression between GDPPC and monitoring effort. Monitoring effort was defined as (A) number of municipalities sampled, (B) percentage of municipalities sampled, (C) national percentage of municipalities sampled, (D) number of monitoring events, and (E) percentage of monitoring events in 2009.

Relationship Analyses for the Year 2010

In the year 2010, the correlation between the number of municipalities sampled and GDPPC was strong (Table 11, $r = 0.8667$), and the linear regression highly significant ($F(1, 18) = 54.3320$, $p = 7.71e-7$). Predicted monitoring effort was equal to $0.2276(\text{GDPPC}) + 8.8059$.

Monitoring effort increased by 0.2276 for each USD, regional GDPPC explained 75% of the variation in the number of municipalities sampled (Table 12, Fig. 6A; $R^2 = 0.7511$).

There was a moderate correlation between state percentage of sampled municipalities and GDPPC (Table 11; $r = 0.6136$) and a highly significant linear relationship ($F(1, 18) = 10.8721$, $p = 0.0040$). Predicted monitoring effort was determined by the equation $y = 0.0583(\text{GDPPC}) + 9.9411$. Monitoring effort increased by 0.0583 for each USD of GDPPC. Nearly 38% of the variation in the percentage of monitoring events was explained by regional GDPPC (Table 12, Fig. 6B; $R^2 = 0.3766$).

The correlation between national percentage of municipalities sampled and GDPPC was strong (Table 11; $r = 0.8907$), and the regression was highly significant ($F(1, 18) = 54.3320$, $p = 7.71e-7$). Predicted number of monitoring events was equal to $0.0041(\text{GDPPC}) + 0.1581$. The national percentage of municipalities sampled increased by 0.0041 for each USD, with 75.1% of the variation explained by GDPPC (Table 12, Fig. 6C; $R^2 = 0.7511$).

Similarly, between the number of monitoring events and GDPPC the correlation was strong (Table 11; $r = 0.8907$), highly significant regression ($F(1, 18) = 69.1337$, $p = 1.41e-7$). Predicted number of monitoring events was equal to $3.7464(\text{GDPPC}) + 176.41$. The number of monitoring events increased by 4.4061 for each USD, and 80% of the variation was explained by regional GDPPC (Table 12, Fig. 6C; $R^2 = 0.7934$).

The correlation between the percentage of monitoring events and GDPPC was fair (Table 11; $r = 0.3750$). The regression equation for the two variables was significant ($F(1, 18) = 2.9457$, $p = 0.01033$). The predicted percentage of monitoring events was determined by the equation $0.0290(\text{GDPPC}) + 15.0094$. Monitoring effort increased by 0.0290 for each USD, but only 14.1% of the variation was explained by GDPPC (Table 12, Fig. 6D; $R^2 = 0.1406$).

2010

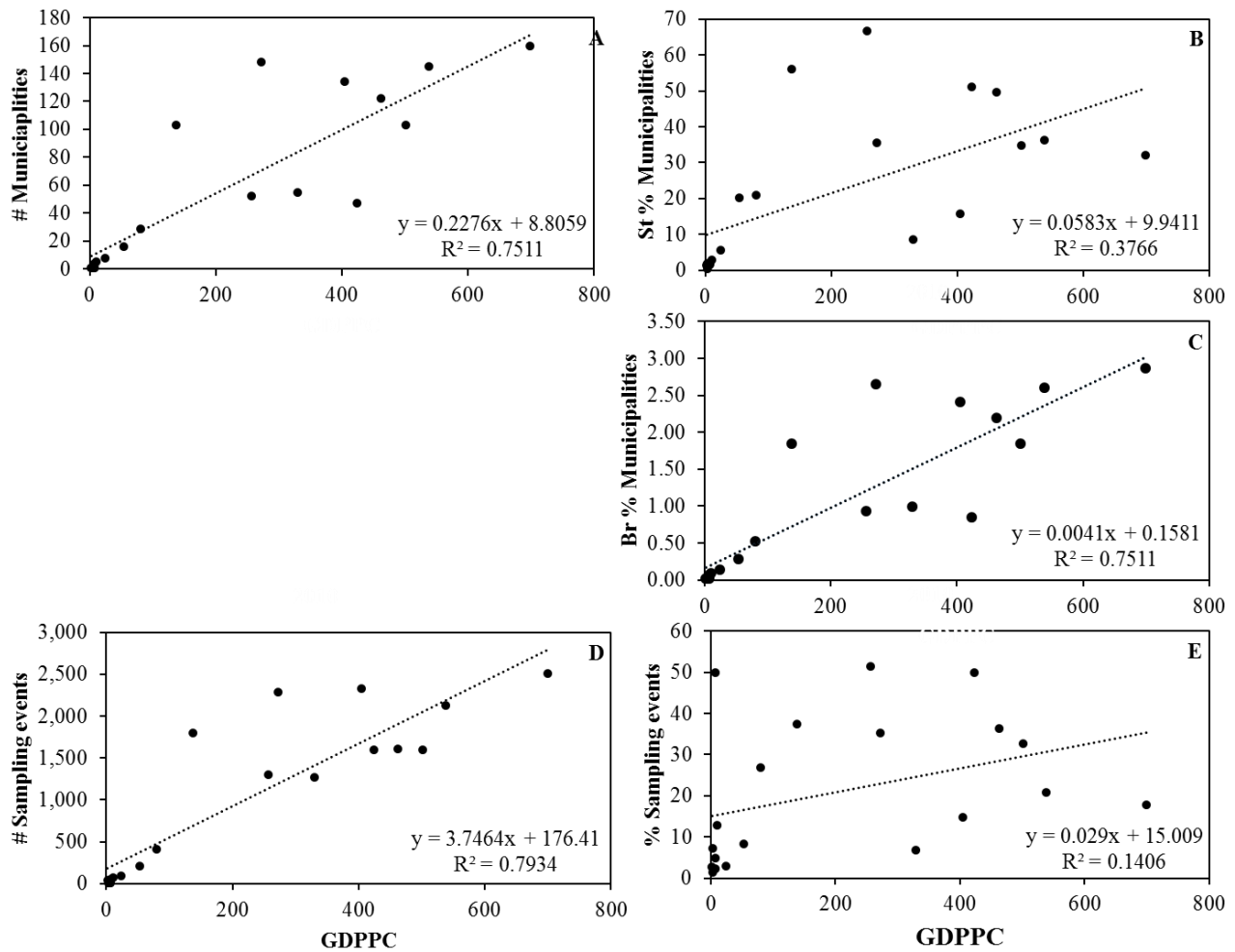


Figure 6. Simple linear regression between GDPPC and monitoring effort. Monitoring effort was defined as (A) number of municipalities sampled, (B) percentage of municipalities sampled, (C) national percentage of municipalities sampled, (D) number of monitoring events, and (E) percentage of monitoring events in 2010.

Relationship Analyses for the Year 2011

For the year 2011, there was a strong positive correlation between number of municipalities sampled and GPPC (Table 11; $r = 0.8525$), with a highly significant linear regression ($F(1, 20) = 53.2007$, $p < 4.71e-7$). Predicted monitoring effort is equal to $0.1989(\text{GDPPC}) + 12.5658$. The number municipalities sampled increased by 0.1989 for each

USD of GDPPC, and close to 73% of the variation could be explained by GDPPC (Table 12, Fig. 7A; $R^2 = 0.7268$).

A moderate positive correlation occurred between state percentage of municipalities sampled and GDPPC (Table 11; $r = 0.3018$). The linear regression between these variables was non-significant ($F(1, 20) = 2.0047, p = 0.1722$). The predicted percentage of municipalities sampled was determined by the equation $y = 0.0332(\text{GDPPC}) + 20.3078$. The percentage of municipalities sampled increased by 0.0392 for each USD. Nonetheless, only 9.1% of the variation in the percentage of municipalities sampled was explained by GDPPC (Table 12, Fig. 7B; $R^2 = 0.0911$).

The correlation between national percentage of municipalities sampled and GDPPC was strong (Table 11, $r = 0.8525$). While the linear regression between the two variables was highly significant ($F(1, 20) = 53.2007, p = 4.71e-7$). The predicted national percentage of municipalities sampled was equal to $0.0036(\text{GDPPC}) + 0.2256$, and it increased by 0.0036 for each USD. Nearly 73% of the variation in the percentage of municipalities sampled was explained by regional GDPPC (Table 12, Fig. 7B; $R^2 = 0.7268$).

The correlation between the number of monitoring events and GDPPC was strong (Table 11; $r = 0.8829$), and the linear regression was highly significant ($F(1, 20) = 70.7221, p = 5.34e-8$). The predicted monitoring effort was equal to $3.3284(\text{GDPPC}) + 226.6192$. Thus, the mean number of monitoring events increased by 5.1048 for each real USD. The coefficient of the determination showed that almost 78% of the variation in the number of monitoring events could be explained by GDPPC (Table 12, Fig. 7C; $R^2 = 0.77795$).

For the percentage of monitoring events in relation to GDPPC, the correlation was moderate (Table 11; $r = 0.4357$) while the regression was moderately non-significant ($F(1, 20) =$

4.6862, $p = 0.0427$). Predicted monitoring effort was equal to $0.0311(\text{GDPPC}) + 12.5904$. The percentage of monitoring events increased by 0.0311 for each USD. However, merely 19% of the variation in the percentage of monitoring events could be explained by regional GDPPC (Table 12, Fig. 7D; $R^2 = 0.1898$).

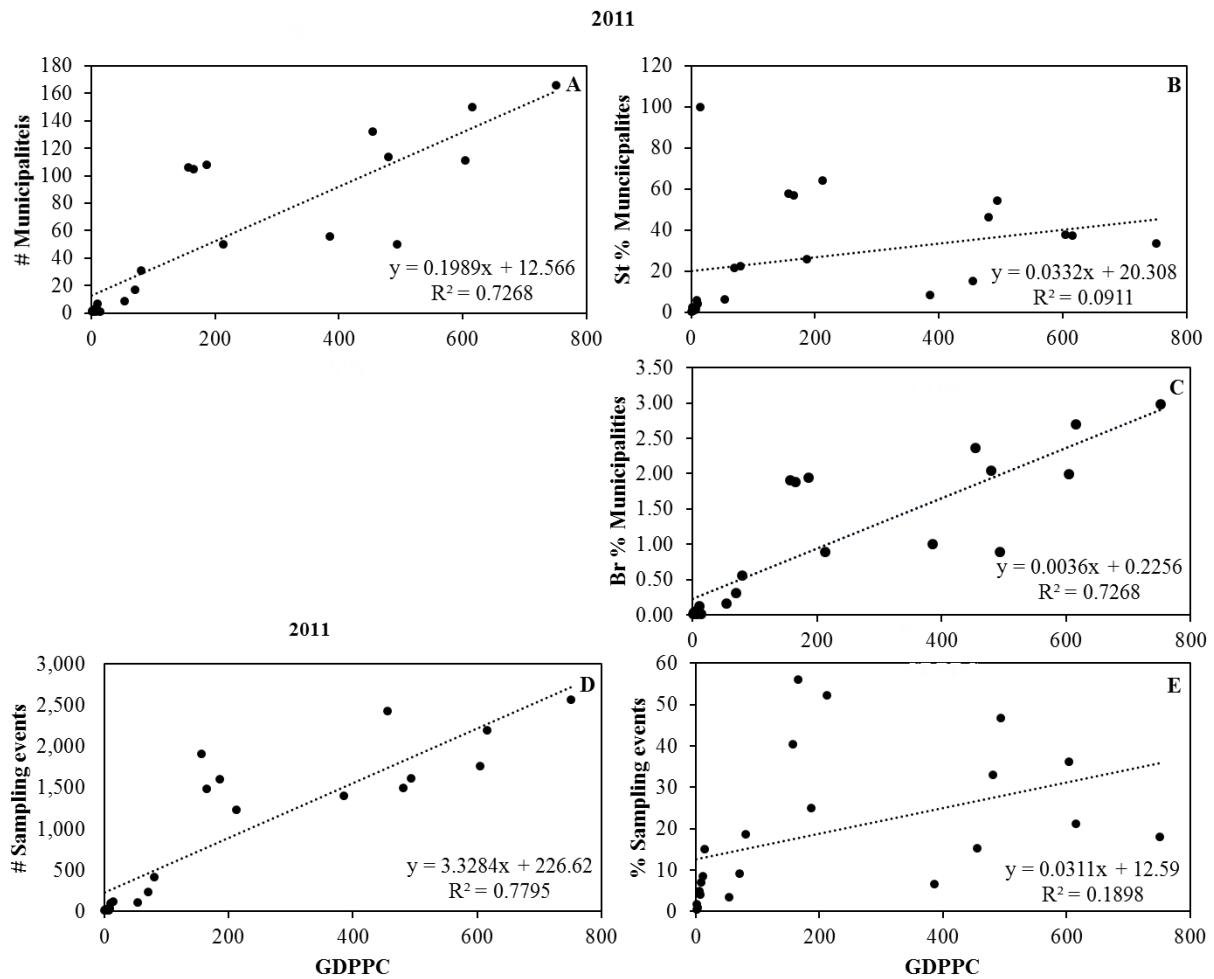


Figure 7. Simple linear regression between GDPPC and monitoring effort. Monitoring effort was defined as (A) number of municipalities sampled, (B) percentage of municipalities sampled, (C) national percentage of municipalities sampled, (D) number of monitoring events, and (E) percentage of monitoring events in 2011.

Relationship Analyses for the Year 2012

For the year 2012, the correlation was strong between the number of municipalities sampled and GDPPC (Table 11; $r = 0.8655$). The regression for these two variables was highly significant ($F(1, 18) = 53.7353, p = 8.31e-7$). The predicted number of municipalities sampled was equal to $0.2417(\text{GDPPC}) + 13.4034$. Monitoring effort, in terms of number of municipalities sampled, increased by 0.2417 for each USD, 75% of the variation could be explained by GDPPC (Table 12, Fig. 8A; $R^2 = 0.7491$).

For the state percentage of municipalities sampled and GDPPC there was a weak positive correlation (Table 11; $r = 0.2351$). The linear regression for the variable was non-significant ($F(1, 18) = 1.0536, p = 0.3183$). Predicted monitoring effort was determined by the equation $y = 0.029(\text{GDPPC}) + 25.675$. Monitoring effort increased by 0.029 for each USD of regional GDPPC, but merely 5.5% of the variation was explained by GDPPC (Table 12, Fig. 8B; $R^2 = 0.0553$).

The correlation between the national percentage of municipalities sampled and GDPPC was strongly positive (Table 11; $r = 0.8655$), and the regression was highly significant ($F(1, 18) = 53.7353, p = 8.3e-7$). Predicted monitoring effort is equal to $0.029(\text{GDPPC}) + 25.675$. The national percentage of municipalities sampled increased by 0.029 for each USD of GDPPC, and almost 75% of the variation in the percentage of sampled municipalities was explained by regional GDPPC (Table 12, Fig. 8C; $R^2 = 0.7491$).

Likewise, the correlation between the number of monitoring events and GDPPC was strong (Table 11; $r = 0.8953$) with a highly significant linear regression ($F(1, 18) = 72.7385, p < 9.74e-8$). Predicted monitoring effort is equal to $3.9802(\text{GDPPC}) + 254.53$. The number of

monitoring events increased by 3.9802 for each USD, and 80.16% of the variation was explained by GDPPC (Table 12, Fig. 8D; $R^2 = 0.8016$).

For percentage of monitoring events and GDPPC, the correlation was fair (Table 11; $r = 0.3679$), and the linear regression between the variables was non-significant ($F(1, 18) = 2.8186$, $p = 0.1105$). The predicted percentage of monitoring events is given by the equation $y = 0.0263(\text{GDPPC}) + 16.685$. The percentage of monitoring events increased by 0.0263 for each USD, and 13.54% of the variation was explained by regional GDPPC (Table 12, Fig. 8E; $R^2 = 0.1354$).

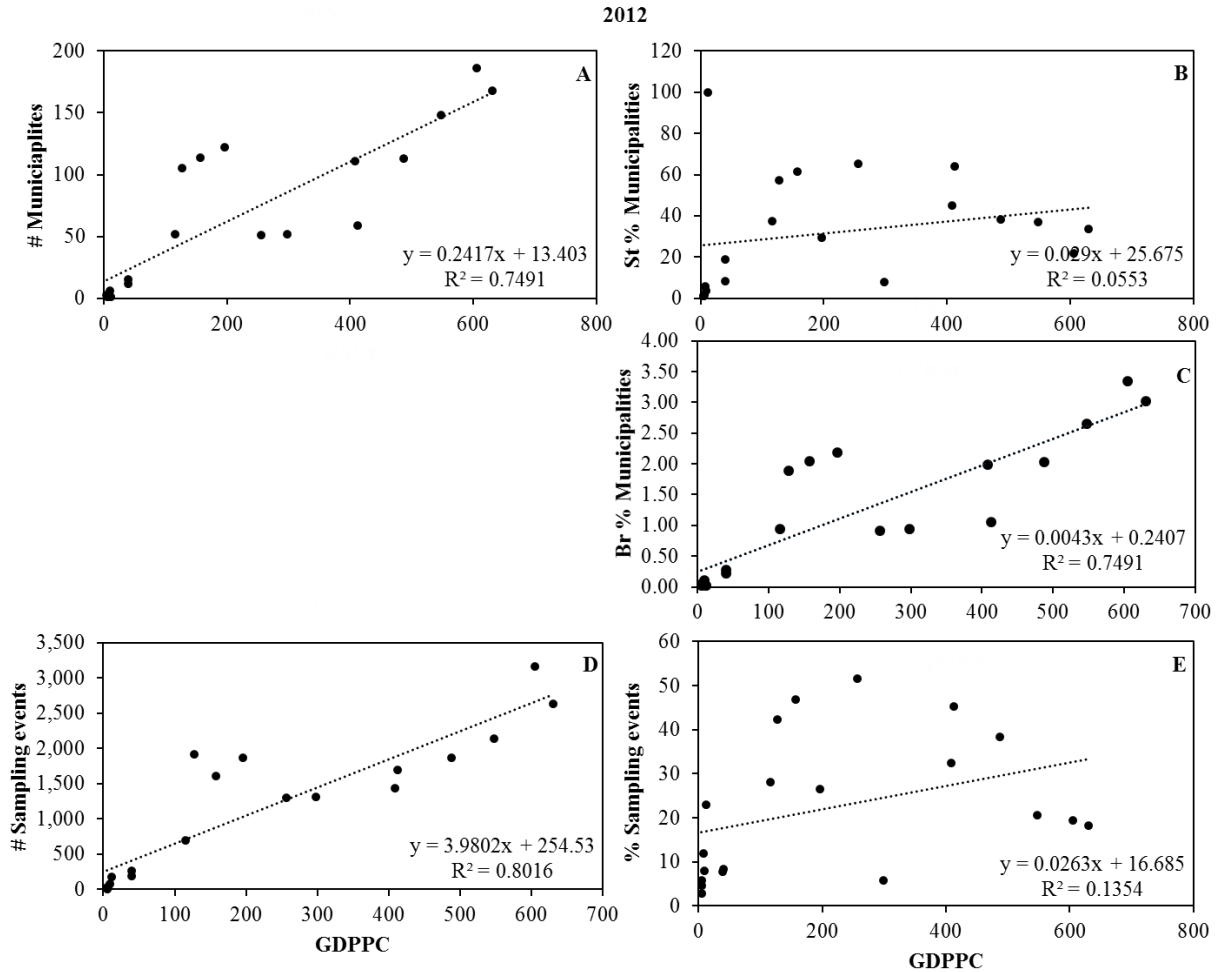


Figure 8. Simple linear regression between GDPPC and monitoring effort. Monitoring effort was defined as (A) number of municipalities sampled, (B) percentage of municipalities sampled, (C) national percentage of municipalities sampled, (D) number of monitoring events, and (E) percentage of monitoring events in 2012.

Relationship Analyses for the Year 2013

In the year 2013, there was a strong positive correlation between the number of municipalities sampled and GPDPPC (Table 11; $r = 0.9082$), with a highly significant regression was ($F(1, 17) = 80.0963$, $p = 7.67e-8$). Predicted monitoring effort was determined by the equation $y = 0.2679(\text{GDPPC}) + 9.1226$. The number of municipalities sampled increased by

0.2679 for each USD, and 82.5% of the variation in monitoring effort was explained by GDPPC (Table 12, Fig. 9A; $R^2 = 0.8249$).

The correlation between the state percentage of sampled municipalities and GDPPC was weak (Table 11; $r = 0.2529$), and the linear regression between them was non-significant ($F(1, 17) = 1.1613$, $p = 0.2963$). Predicted monitoring effort was equal to $0.0319(\text{GDPPC}) + 23.998$, indicating that the percentage of sampled municipalities increased by 0.0319 for each USD. However, only 6.4% of the variation was explained by regional GDPPC (Table 12, Fig. 9B; $R^2 = 0.0639$).

There was a strong positive correlation between the national percentage of municipalities sampled and GDPPC (Table 11; $r = 0.9082$), and the linear regression was moderately significant ($F(1, 17) = 80.0963$, $p = 7.67e-8$). The predicted percentage of monitoring events was determined by the equation $y = 0.0048(\text{GDPPC}) + 0.1638$. The percentage of monitoring events increased by 0.0255 for each USD. And 82.5% of the variation in the percentage of monitoring events was explained by GDPPC (Table 12, Fig. 9C; $R^2 = 0.8249$).

The correlation between the number of monitoring events and GDPPC was strong (Table 11; $r = 0.9007$). Likewise, the regression for these two variables was highly significant ($F(1, 17) = 73.0475$, $p = 1.47e-7$). Predicted monitoring effort, in terms of number of monitoring events, was equal to $4.3874(\text{GDPPC}) + 233.17$, and it increased by 4.3874 for each USD of GDPPC. Slightly more than 80% of the variation in the number of monitoring events was explained by GDPPC (Table 12, Fig. 9D; $R^2 = 0.8112$).

There was a strong positive correlation between the percentage of monitoring events and GDPPC (Table 11; $r = 0.3571$). However, the regression between the two variables was only moderately significant ($F(1, 17) = 2.4853$, $p = 0.1333$). The predicted percentage of monitoring

events was equal to $0.0255(\text{GDPPC}) + 16.897$, indicating that the percentage of monitoring events increased by 0.0255 for each USD, but merely 12.8% of the variation in monitoring effort was explained by regional GDPPC (Table 12-, Fig. 9E; $R^2 = 0.1275$).

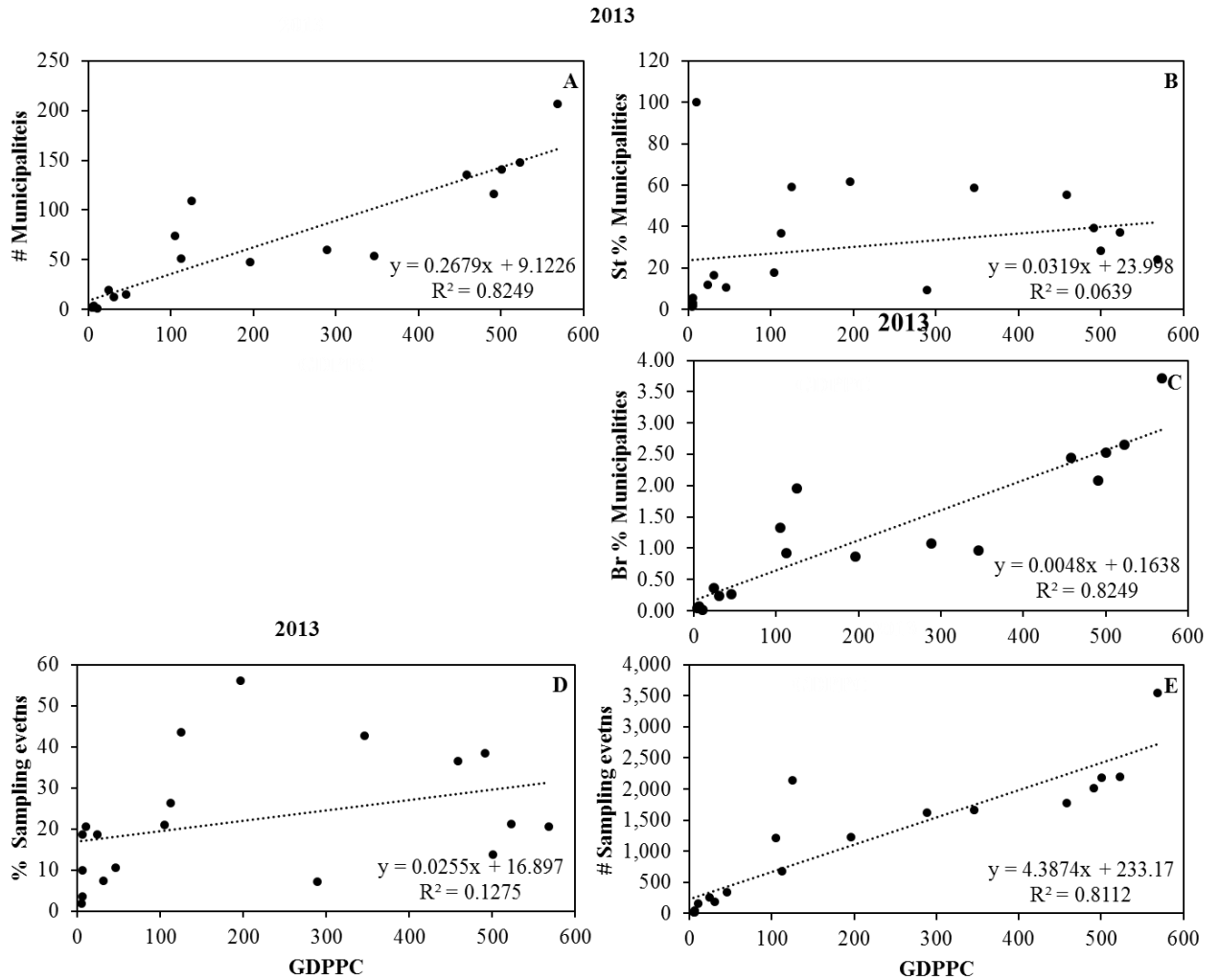


Figure 9. Simple linear regression between GDPPC and monitoring effort. Monitoring effort was defined as (A) number of municipalities sampled, (B) percentage of municipalities sampled, (C) national percentage of municipalities sampled, (D) number of monitoring events, and (E) percentage of monitoring events in 2013

Dunn's Multiple Comparison Test

Because the Kruskal-Wallis test by region showed significant differences between all variables, I used a pairwise Dunn's-test for multiple independent samples, to determine which regions were significantly different from the others for each of the examined variables.

GDPPC Comparison between Regions

There were no statistically significant differences between the GDPPC of the Central-west and North, Central-west and Northeast, and North and Northeast regions, or between the South and Southeast regions ($p < 0.05$). However, there were significant differences in GDPPC of the Central-west, North, and Northeast regions, and the GDPPC of the South and Southeast regions ($p > 0.05$) (Table 16, Fig. 10).

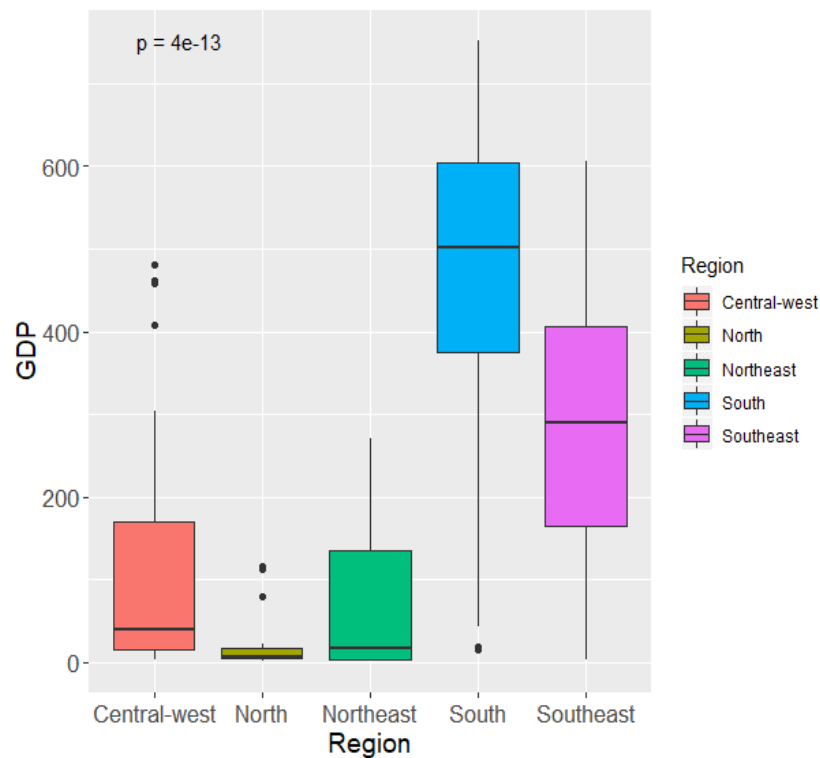


Figure 10. Box plot GDPPC per region

Table 16. Dunn's multiple comparison test for GDPPC for the five regions of Brazil.

	CW	N	Ne	S
N	0.09900	-	-	-
NE	1	1	-	-
S	0.00061	8.70e-10	5.60e-8	-
SE	0.06003	6.20e-7	3.70e-5	1

Monitoring Effort Comparison between Regions

Number of Municipalities Sampled

There were no statistically significant differences in the number of municipalities sampled at least one month of the year between the Central-west and North regions, the Central-west and Northeast regions, the Central-west and Southeast, the North and Northeast, the Northeast and Southeast, or the South and Southeast regions. Nevertheless, there were significant differences between the Central-west and South, the North and South, the North and Southeast, and between the Northeast and South regions. The number of municipalities sampled in the South, the region with the highest GDPPC in the country, was significantly different from all the other regions except the Southeast (Table 17, Fig. 11).

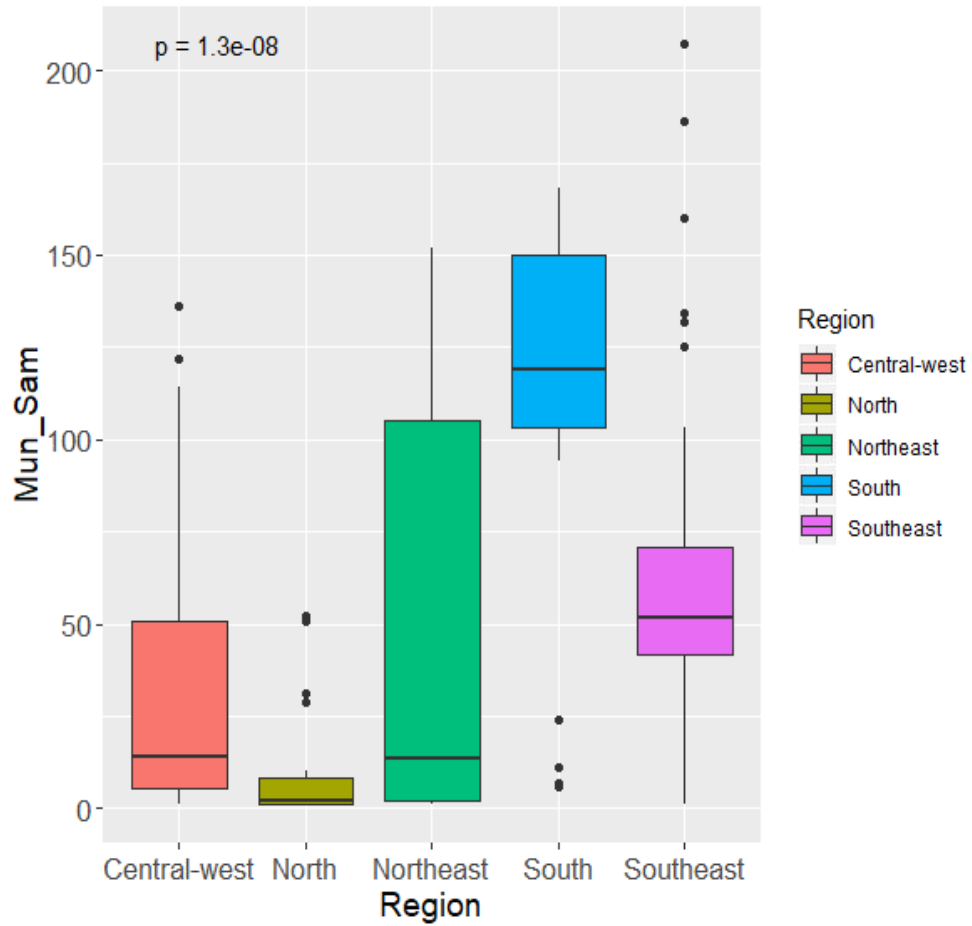


Figure 11. Box plot of the number of municipalities sampled at least one month of the year per region.

Table 17. Dunn's multiple comparison test for the number of municipalities sampled at least one month of the year for the five regions of Brazil.

	CW	N	NE	S
N	0.272	-	-	-
NE	1	0.061	-	-
S	0.001	2.0e-8	0.001	-
SE	0.226	5.0e-5	0.312	0.666

State Percentage of Municipalities Sampled

There were no significant differences between the percentages of municipalities sampled at least once a year between the Central-west and Northeast, the Central-west and South, the Central-west and Southeast, the North and Northeast, the Northeast and South, the Northeast and Southeast, or between the South and the Southeast. However, there were significant differences between the Central-west and North, the North and South, the North and Southeast (Table 18, Fig. 4). This indicates that the state percentage of municipalities sampled in all regions, except the North, were comparable. However, not all regions have the same number of municipalities. The Central-west region has 446 municipalities, accounting for 8.4% of the total number of municipalities in the country, followed the North with 450 municipalities (8.1%). The regions with the largest numbers of municipalities are the Northeast with 1,794 (32.2%), followed by the Southeast with 1,668 municipalities (30%), and the South with 1,191 (21.4%) (Table 18, Fig. 12).

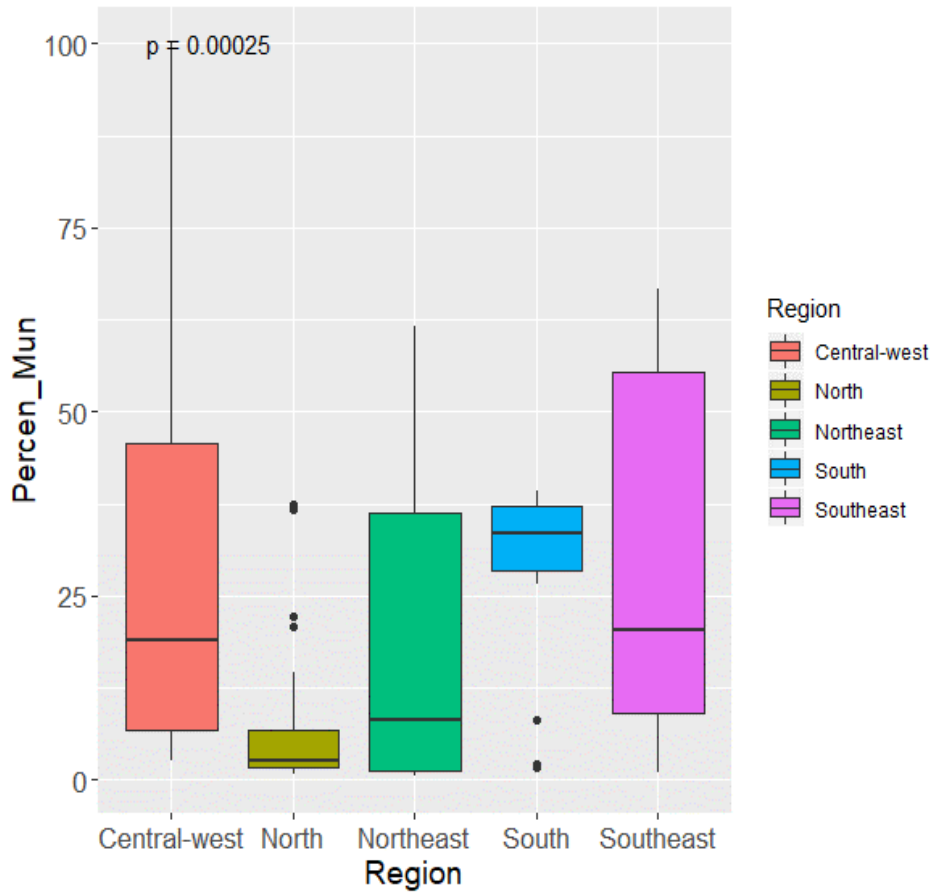


Figure 12. Box plot for the state percentage of municipalities sampled at least one month of the year per region

Table 18. Dunn's multiple comparison test for the state percentage of municipalities sampled at least one month of the year for the five regions of Brazil.

	CW	N	NE	S
N	0.0095	-	-	-
NE	0.7509	0.6506	-	-
S	1	0.0025	0.2696	-
SE	1	0.0016	0.2482	1

National Percentage of Municipalities Sampled

In terms of the national percentage of municipalities sampled, there were no significant differences between the Central-west and North, the Central-west and Northeast, Central-west and Southeast, North and Northeast, Northeast and Southeast, and the South and Southeast regions. There were statistically significant differences between the Central-west and South, the North and the South, and the North and the Southeast and the Northeast and South. The North, the region with the lowest GDPPC, has the lowest percentage of municipalities sampled in the country, but is not significantly different from the Northeast (Table 19, Fig. 13).

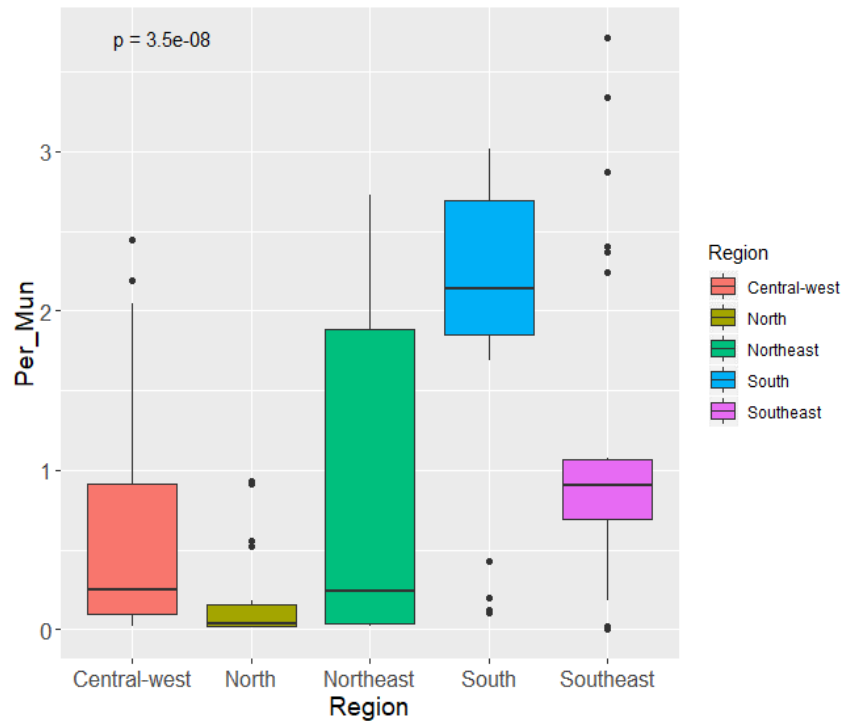


Figure 13. Box plot for the national percentage of municipalities sampled at least one month of the year per region.

Table 19. Dunn's multiple comparison test for the national percentage of municipalities sampled at least one month of the year for the five regions of Brazil.

	CW	N	NE	S
N	0.27428	-	-	-
NE	1	0.06606	-	-
S	0.00124	2.7e-8	0.00111	-
SE	0.45989	0.00019	0.62117	0.38773

Number of Monitoring Events per Region

There were no significant differences between the Central-west and North, Central-west and Northeast, and between the South and Southeast regions. There were significant differences between the number of monitoring events between the Central-west and South, Central-west and Southeast, the North and Northeast, the North and South, the North and-Southeast, Northeast and South and the Northeast and Southeast (Table 20, Fig. 14).

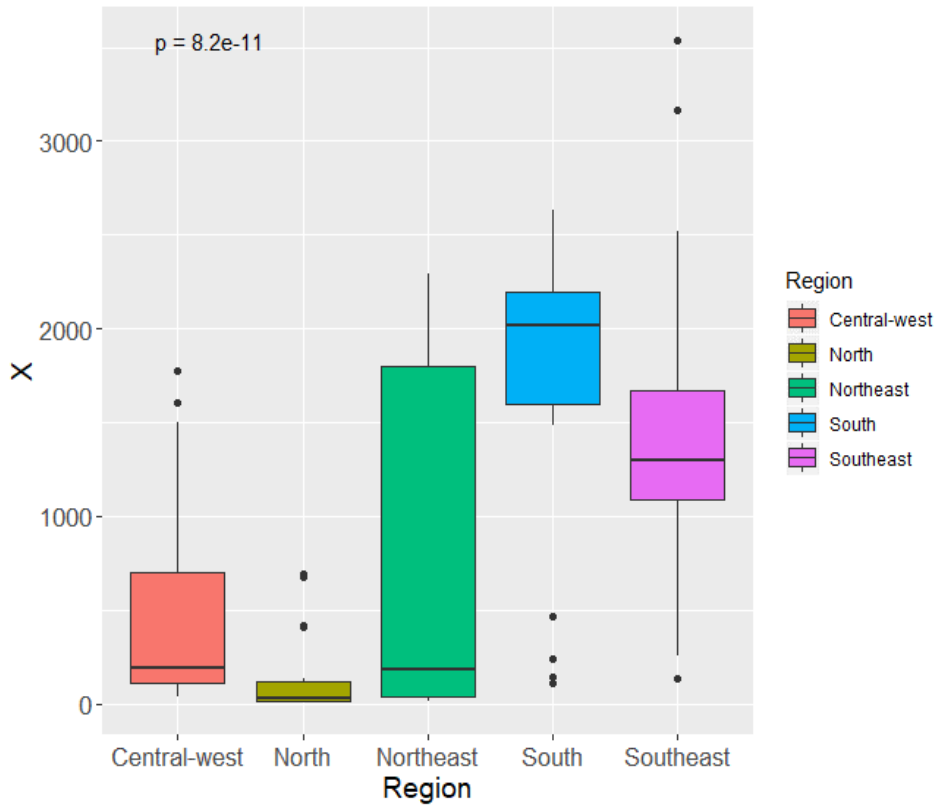


Figure 14. Box plot number of monitoring events, when they happened at least one month of the year (≥ 1).

Table 20. Dunn's multiple comparison test for the number monitoring events for the five regions of Brazil.

	CW	N	NE	S
N	0.10553	-	-	-
NE	1	0.01527	-	-
S	0.00082	1.6e-9	0.00094	-
SE	0.02632	1.6e-7	0.03654	1

Percentage of Monitoring Events per Region

Even though the Kruskal- Wallis test ($p = 0.02698$) indicated that at least one of the regions was different in terms of the percentage of monitoring events, the Dunn's test indicated that in fact there were no statistical differences between the regions. Indicating that in regards to

the national percentage of monitoring events, all regions could be comparable. However, pairwise test are slightly more conservative than experiment wise tests (Table 21, Fig. 15).

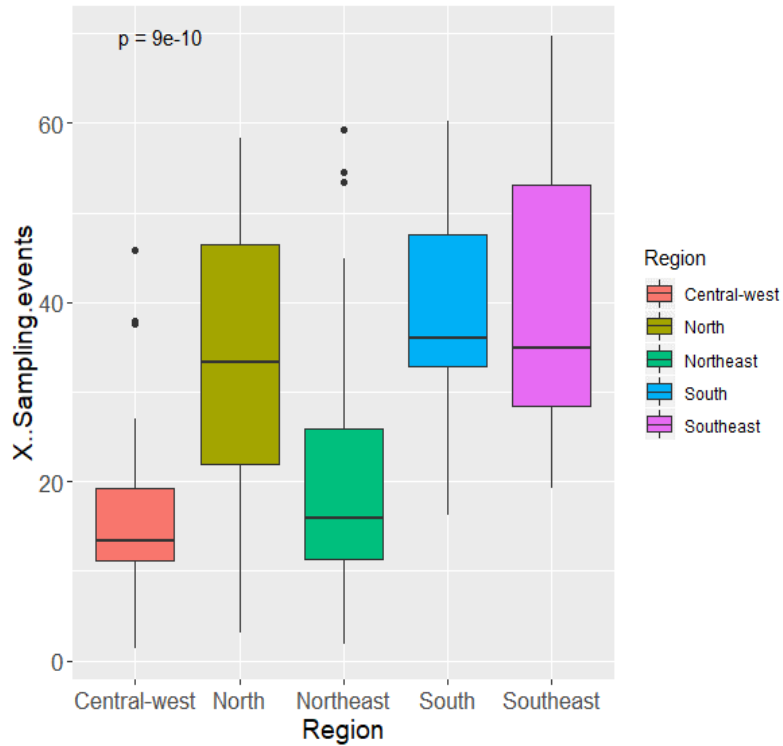


Figure 15. Box plot percentage of monitoring events at least one month of the year per region

Table 21. Dunn's multiple comparison test for the percentage of monitoring events for the five regions of Brazil.

	CW	N	NE	S
N	1	-	-	-
NE	1	1	-	-
S	1	1	1	-
SE	0.075	0.073	0.250	1

Discussion

Nationwide, long-term environmental studies are rare, because of the challenges of conducting systematic monitoring. These challenges are more prominent in developing and emerging countries, and even more so in country as large and diverse as Brazil. In the last two decades, there have been a few studies about the public health implications of cyanoHABs at the national level. Some examples include (Yu et al., 2001) in China, Svirčev et al. (2009) in Serbia and Zhang et al. (2015) in the USA. The current study represents the first attempted to study the relationship between GDPPC and cyanobacteria and cyanotoxins in Brazil and in South America.

Out of the five metrics established to assess monitoring effort; number of municipalities sampled, national percentage of municipalities sampled, and number of monitoring events showed a strong positive correlation to GDPPC (Table 11).

Analysis of the regional relationship between economic development and monitoring effort showed that more municipalities per state were sampled in the South and Southeast regions which have the highest GDPPC in Brazil (Table 18, Fig. 12). The same pattern was observed when the national percentage of municipalizes sampled was considered (Table 19, Fig. 13).

Likewise, more monitoring events per year occurred in these two regions (Table 20, Fig. 14). This was true regardless of the difference in the number of reservoirs present per region, and of the need for monitoring. Therefore, GDPPC is a good predictor of cyanobacteria and cyanoHAB monitoring effort in Brazil. There was a North-Northeast/South-Southeast monitoring effort gradient in Brazil, which reflects the historical and well documented GDPPC gradient in the country (Savedoff, 1990; Azzoni, 2001; Azzoni & Servo, 2002; Shankar & Shah, 2003).

Monitoring effort showed a similar trend to GDPPC (Table 16, Fig. 10). However, the trend was disrupted by the fact that the Northeast region grouped with Southeast for most monitoring effort metrics, except for number and percentage of monitoring events. Furthermore, the state percentage of municipalities sampled in the Northeast was not significantly different from the state percentage sampled in the South. This result is explained by the large number of reservoirs in the Northeast.

Higher cyanobacteria and cyanotoxins awareness in the Northeast, after the Caruaru syndrome (Azevedo et al., 2002; Carlos et al., 2009; Carvalho et al., 2007; Chellappa et al., 2008b; Costa et al., 2006; Dörr et al., 2010; Hirooka et al., 1999; Jochimsen et al., 1998; Molica et al., 2002; Molica et al., 2005; Oliveira et al., 2005; Piccin-Santos & Bittencourt-Oliveira, 2012; Sant'Anna et al., 2008; Soares et al., 2006; Sotero-Santos et al., 2006; Vieira et al., 2005; Yunes et al., 2003), might have contributed to higher monitoring in the Northeast even it is was to a lesser extent.

Number of municipalities as a metric for monitoring effort, is biased toward regions with the highest number of municipalities, which are the Northeast 1,794 municipalities (32.2%), the South 1,191 municipalities (21.4%), and the Southeast 1,668 municipalities (30%). This could

explain monitoring similarities between the Northeast and the South and the Southeast, since these regions have the higher number of municipalities than the North and the Central-west. However, the median number of municipalities sampled for the Northeast was low, with broad variance (Table 17, Fig. 11).

Furthermore, R^2 is seriously biased upward in small samples Cramer (1987), and samples sizes for all metrics were always small. Ranging from 11 to 21 for municipalities and COSAAs sampled at least one month (Table 12), 9 to 19 for municipalities and COSAAs sampled three or more months (Table 13), 8 to 17 for municipalities and COSAAs sampled six or more months (Table 14) and 4 to 15 for municipalities and COSAAs sampled twelve months (Table 15).

Regarding the percentage of monitoring events, the Kruskal- Wallis test ($p = 0.02698$) (Table 7) indicated that the percentage of monitoring events was significantly different in at least one of the regions. However, Dunn's test indicated that in fact there were no statistical differences between the regions (Table 21, Fig. 15). Though the differences are not significant, the North-Northeast/South-Southeast gradient was still observed. Nonetheless, for this metric the North grouped with the South and Southeast and not with the Central-west and Northeast like it did for all other metrics of monitoring effort. Monitoring effort in the North was comparable to monitoring effort that took place in the South and Southeast. This is explained by the fact that not all regions have the same number of reservoirs. Out of the main reservoirs in the country, 11% (78 out of 710) are located in the Southeast, the South 4.4% (31 out of 710), and only 2.3% (16 out of 710) in the North (SAR, 2019).

Since there are so few reservoirs in the Northern region, any amount of monitoring of those reservoirs will represent a high percentage of monitoring events for the region. Lack of significance in regional differences could be explained by the small sample size. Alternatively,

this could mean that in terms of percentage of monitoring events, all regions are making comparable monitoring efforts. Indicating that all regions are monitoring all COSAAs a similar number of months per year.

While absolute predictability of monitoring effort based on GDPPC cannot be expected, it provides information about the extent to which regional wealth impacts the amount of monitoring, and therefore information on cyanobacterial density and cyanotoxins is available per region. And the marked North-Northeast/South-Southeast gradient is still observable.

GDPPC and monitoring effort strongly correlated when monitoring effort was defined as number of municipalities sampled (Table 17, Fig. 11), national percentage of municipalities sampled (Table 19, Fig. 13), and number of monitoring events (Table 20, Fig. 14). Regions with the lowest GDPPC values, the Central-west, the North, and the Northeast, were monitored significantly less than the South and Southeast, the regions with the highest GDPPC values. This coincides with the historic income gradient in Brazil.

The number of municipalities sampled in the Northeast, was not significantly different from the number of municipalities sampled in the Southeast. This can be explained because there are more reservoirs in the Northeast (77.9%, 553 out of 710) than in the Southeast (11%, 78 out of 710). And to a lesser extent to increased cyanotoxin awareness in the Northeast after the Caruaru syndrome.

Though Brazil has a cyanobacteria and cyanotoxin monitoring program, the Brazilian regulation guidelines are not fulfilled as intended by the Ordinance 1,469 from the Ministry of Health (Portaria N° 1,469 do Ministério da Saúde) (CONAMA, 2000b), Resolution 274 from the National Council for the Environment (Resolução 274 do Conselho Nacional de Meio Ambiente) (CONAMA, 2000a), and Ordinance 2,914 from the Ministry of Health and the National Council

for the Environment (Portaria N° 2,914 do Ministério da Saúde e o Conselho Nacional de Meio Ambiente). Furthermore, the data are not biologically informative because cyanobacterial densities, cyanotoxin type, and cyanotoxin concentration are not recorded. Since only 2.8% (155 out of 5,569) of the municipalities in the country were monitored at least once a month, and merely 0.1% (5 out of 5,569) were monitored every month, the entire country and particularly the Northeast region are under monitored. The Northeast has the largest number of reservoirs in the country, and its distinctive environmental conditions (i.e. high temperatures, limited rain, and long retention times in reservoirs) make it prone to cyanoHABs.

Because of this, monitoring in the Northeast should be more systematic and frequent. Particularly considering that even with limited monitoring effort, the Northeast had the highest cyanobacterial densities reported in the country over a seven-year period.

With more than 53 million inhabitants, the Brazilian Northeast it is the world's most densely populated dry land region and Brazil's second most populous region after the Southeast (Bouvy et al., 2000; IBGE, 2010; Marengo et al., 2017; Molica et al., 2005).

In the context of climate change, cyanoHABs are likely to become even more frequent. Standard water treatments such as disinfection, filtration, and flocculation reduce but do not always eliminate cyanobacteria and cyanotoxins. While others such as boiling and biological filters are ineffective or even increase toxicity (Berry et al., 2018; de Queiroz et al., 2013; Hilborn & Beasley, 2015; Manali et al., 2017; Rosado et al., 2006; Yu et al., 2009).

Chronic exposure to low-level cyanotoxins can decrease the detoxification capacity of the liver, and this factor combined with amplified cyanotoxin bioaccumulation increases health risks (Galanti, 2013; Griffiths & Saker, 2003).

Adequate cyanoHABs monitoring is fundamental to prevent human exposure to cyanotoxins and to minimize the public health risks associated to them. Because cyanoHABs are recurrent, people might be constantly exposed to cyanotoxins through drinking water, ingestion of contaminated food, inhalation, or contact with recreational waters (Chen et al., 2009; Hernández et al., 2009; Hirooka et al., 1999; Turner et al., 1990; Ueno et al., 1996; Dolah et al., 2001; Yu, 1989, 1995; Yu et al., 2001).

A study in four Brazilian reservoirs by Piccin-Santos and Bittencourt-Oliveira (2012) showed that water bodies with the highest cyanobacterial densities had the lowest microcystin concentrations. If cyanoHAB toxicity is not directly related to cyanobacterial density, effective and biologically relevant cyanoHAB monitoring is needed to prevent human exposure to cyanotoxins and its potential impacts to public health.

Expanding cyanobacterial monitoring effort in Brazil, to at least quarterly events, that prioritizes the Northeast and North regions is necessary. Monitoring should be conducted proportional to the number of reservoirs and municipalities present in each region. And cyanobacterial species and densities, as well as cyanotoxin type and concentration should be reported.

Because monitoring might not be attainable, due to limited technical and financial resources, monthly and rapid diagnostic tests have not been developed for all known cyanotoxins. Routine analysis of the most common cyanotoxins identified in Brazil should be conducted, that is microcystins (MCs) and saxitoxins (STXs). Since cylindrospermopsins (CYNs) are very common around the world, including them as part of the standard monitoring should be strongly considered.

If testing for specific cyanotoxins is not a viable option, environmental factors such as light intensity, nutrient supply rates, temperature, oxidative stressors, and interactions with other biota should be measured, as they have that have been determined to be associated to cyanotoxin production and degradation as they might provide clues into when cyanotoxin production might occur.

Conclusions

There is a strong correlation between regional wealth and cyanobacteria and cyanoHAB monitoring effort in Brazil. The trend observed for monitoring effort were similar for all metrics used to defined it except percentage of monitoring events were clear. There is there is a strong North-Northeast/South-Southeast monitoring effort gradient. That was strongly or moderately predicted by regional GDPPC. Monitoring effort was greater in the South and Southeast, the regions with the highest GDPPC. Followed by the Central-west and Northeast, while monitoring effort was extremely low in the North, the region with the lowest GDPPC. Percentage of monitoring events might indicate that all regions are monitoring all COSAAs a similar number of months per year, which will imply that all regions are making comparable monitoring efforts.

Thought Brazil is the one country in the region with a program in place for systematic cyanobacterial and cyanotoxin monitoring of fresh-water reservoirs, most municipalities are under sampled. About 82% of the total number of municipalities in the country were registered in the monitoring program, but only 32.3% were actually monitored. Merely 2.8% were monitored at least once a year, and only 0.1% were sampled monthly. The five municipalities sampled every month were all located in the state of Santa Catarina in the South region, and only two COSAA codes were monitored during the seven years of data available. The states of Acre,

Amapá, and Roraima in the North, and Alagoas and Paraíba in the Northeast were never sampled.

The percentage of monitoring events in the North was comparable to percentage of monitoring events South and Southeast regions. Since there are so few reservoirs in the North, any amount of monitoring of those reservoirs will represent a high percentage of monitoring events for the region. Monitoring effort in the Northeast was not significantly different from monitoring effort in the South and Southeast. These were explained by the high number of reservoirs in the NE, and to an extent to higher cyanobacteria and cyanotoxin awareness in this region. The Northeast had low median values, but great variability. While monitoring effort in the Central-west region was low, it was consistent. Most municipalities registered with SISAGUA were sampled at least once, three or more months, six or more months and 12 months.

For the seven years of data available, municipalities and COSAAs in regions with higher GDPPC were monitored more frequently. Therefore, more information about the presence of cyanobacteria and cyanotoxins in drinking water was available in these regions. This differential information availability on the quality of drinking water reflects Brazil's history of differential access to environmental resources and services. Indicating that economically disenfranchised communities might be at a higher risk of exposure to cyanotoxins and their potential risk factors to human health.

CHAPTER IV

CONCLUSIONS

Though Brazil has a nationwide cyanobacteria and cyanotoxin monitoring program, the data are not biologically informative. Based on seven years of available data, from 2007 until 2013, the Brazilian regulation guidelines are not fulfilled as established by Ordinance 1,469 from the Ministry of Health (Portaria N° 1,469 do Ministério da Saúde), Resolution 274 from the National Council for the Environment (Resolução 274 do Conselho Nacional de Meio Ambiente), and Ordinance 2,914 from the Ministry of Health and the National Council for the Environment (Portaria N° 2,914 do Ministério da Saúde e o Conselho Nacional de Meio Ambiente).

These regulations set the maximum acceptable values for cyanobacterial density and microcystin (MC) concentration, and called for toxicity analysis when densities surpassed 20,000 cells ml⁻¹. However, monitoring standards do not require agencies to reference the exact species or toxins. The presence of cyanotoxins was reported as a yes/no variable, which is not informative given that eight cyanotoxins types and fourteen variants were reported in the scientific literature available for the country.

Furthermore, only 2.8% (155 out of 5,569) of the municipalities in the country were monitored at least once a month, and merely 0.1% (5 out of 5,569) were monitored monthly. The entire country is under monitored, and the Northeast even more so, given the large number of reservoirs present in the region and how prone the Northeast is to cyanoHABs due to high yearly temperatures, prolonged drought periods, and long retention times in all reservoirs. Although monitoring effort was significantly lower than in other regions, the Northeast still had the highest

cyanobacterial densities reported for the country, and in the context of climate change, cyanoHABs are likely to become even more frequent.

The Northeast is the second most populous region in Brazil after the Southeast, and it is the world's most densely populated dry land region. The high incidence of cyanoHABs in the region compounded by limited monitoring, means that more than 50 million people might be constantly exposed to cyanotoxins via drinking water, with considerable impacts on the region's public health.

Though it was not possible to establish a linear relation between gross domestic product per capita (GDPPC) and monitoring effort, these two variables were strongly correlated when monitoring effort was defined as number of municipalities sampled, national percentage of municipalities sampled, and number of monitoring events. The regions with the lowest GDPPC, the Central-west, North, and Northeast, had a significantly lower monitoring effort than the South and the Southeast, the regions with the highest GDPPC. This coincides with the historic North-Northeast/South-Southeast per capita income gradient and the historic differential access to environmental resources and services in Brazil.

The number of municipalities sampled in the Southeast was not significantly different from the number of municipalities sampled in the Northeast. This could be explained because there are more reservoirs in the Northeast (77.9%, 553 out of 710) than in the Southeast (11%, 78 out of 710), and to a lesser extent to increased cyanotoxin awareness in the Northeast after the Caruaru syndrome.

The most common cyanobacterial genera reported in Brazil were *Microcystis*, *Cylindrospermopsis*, *Planktothrix*, and *Pseudanabaena*, all known cyanoHAB and cyanotoxin producers. Since the most commonly reported species were *Microcystis aeruginosa* and

Cylindrospermopsis raciborskii, it is likely that their toxins are present across Brazil compromising the quality of drinking water and constituting a serious public health risk.

M. aeruginosa produces MC-LR, MC-YR, and MC-RR. MC-LR was responsible for the death of 50 people during the Caruaru syndrome, exemplifying how inadequate water monitoring and treatment can lead to severe public health outcomes.

Though *C. raciborskii* is a common species reported in Brazil, and around the world it commonly produces cylindrospermopsin (CYN), only one publication out of 72 reported its presence in Brazil. This could imply that environmental conditions in the country are not conducive to the production of this specific toxin by *C. raciborskii*. A better understanding of this phenomenon could provide alternative control strategies to limit or even stop the production of this toxin. *C. raciborskii* is also known to produce anatoxin-a (ANTX-a), and saxitoxins (STXs) two neurotoxins known to cause paralytic shellfish poisoning (PSP).

In Brazil, 44.44% (32 out of 72) of the publications tested for cyanotoxins, and 43.06% (31 out of 72) confirmed their presence. Eight cyanotoxins types and fourteen variants were reported for Brazil; four of these types (i.e. MCs, STXs, ANTXs, and CYNs), have severe adverse health effects in humans and have caused human fatalities. MCs and CYNs are hepatotoxins, acute exposure to them causes liver injury, hemorrhage, and necrosis. While chronic exposure promotes tumor formation and has been associated with liver and colorectal cancer.

MCs were the most commonly identified cyanotoxins in Brazil, 25% of the publications (18 out of 72) reported their presence, and three variants were identified (i.e. MC-LR, MC-YR, and MC-RR). STXs and ANTXs are both neurotoxins, and they cause convulsions, muscle weakness, salivation, and death by respiratory paralysis. STXs were the second most reported

cyanotoxins in Brazil, they were reported in 11.1% of the publications (8 out of 72), and four variants were identified (i.e. decarbamoylneosaxitoxin (dc-NEO), decarbamoylsaxitoxin (dc-STX), neosaxitoxin (Neo-STX), and gonyautoxins (GTXs)).

Anatoxins (ANTX) and three understudied cyanotoxins (i.e. aeruginosin (AER), anabaenopeptin (AP), and cyanopeptolin (CyPep)), were reported by a single publication each. Although these types are poorly studied and their toxicity is not well understood, their toxicity appears to be comparable or greater than that of microcystin-RR (MC-RR). This was the first report of these cyanotoxin types for Brazil and South America. As more specific research continues to be conducted, it is likely that more evidence on cyanobacteria and their toxins will emerge.

In Brazil, only 22.2 % of the publications (16 out of 72) reported a single cyanobacterial species, 47.2% (34 out of 72) identified two or more species, and 37.5% (27 out of 72) reported three or more species. Since most cyanobacterial species are capable of synthesizing a wide range of toxins, the co-occurrence of multiple cyanotoxins in reservoirs affected by cyanoHABs is to be expected. Likewise, single cyanotoxin types were rarely reported. Only 19.4% (14 out of 72) of the publications reported a single cyanotoxin, while 15.3% (11 out of 72) reported mixtures of two or more cyanotoxin types, and 11% (8 out of 72) reported three or more cyanotoxin types. CyanoHABs do not produce a single cyanotoxin type or a single variant, therefore the additive or synergistic toxicity of multiple cyanotoxins could result in greater toxicity than what would be expected from each individual cyanotoxin, magnifying the risks to public health. Globally cyanoHAB occurrences have increased in the past few decades, and most of them have been linked to toxicity. This global trend reflects on the increased number of publications on the subject, however, increased awareness alone does not explain the increased

number of cyanoHAB report worldwide. Additionally, standard water treatments such as disinfection, filtration, and flocculation reduce but do not always eliminate cyanobacteria and cyanotoxins. Other common treatments such as boiling, a common in-home practice used improve the quality of drinking water in Brazil, are ineffective therefore increasing the possibility for human exposure.

Furthermore, chronic exposure to low-level cyanotoxins can decrease the detoxification capacity of the liver, and this factor combined with amplified cyanotoxin bioaccumulation increases health risks. Adequate cyanoHABs monitoring is fundamental to prevent human exposure to cyanotoxins and to minimize the public health risks associated to them. Because cyanoHABs are recurrent, people might be constantly exposed to cyanotoxins through drinking water, ingestion of contaminated food, inhalation, or contact with recreational waters.

Currently, the Brazilian regulations for cyanoHABs use cyanobacterial density as criteria to measure toxicity, as it has been assumed that denser cyanoHABs have higher toxicities. Nevertheless, a study in four reservoirs in northeastern Brazil showed that water bodies with the highest cyanobacterial densities actually had the lowest microcystin concentrations. This could indicate that cyanoHAB toxicity is directly related to cyanobacterial density, and that toxicity might not be an accurately assess without direct measurement, indicating the need for new approaches and standards. This should open alternatives for cyanoHABs monitoring, management, and control.

Effective and biologically relevant cyanoHAB monitoring is needed to prevent human exposure to cyanotoxins and their subsequent impact in public health. Due to climate change and increased androgenic nutrient enrichment and eutrophication, cyanoHABs will increase, and the range of certain cyanobacterial species will expand as it has been seen with *C. raciborskii*.

Monthly monitoring might be logistically unattainable and extremely costly for all 5,569 municipalities. In order to improve Brazil's monitoring standards, I suggest establishing mandatory quarterly monitoring prioritizing the Northeast and North regions. Cyanobacterial monitoring should be conducted proportional to the number of reservoirs present in each region, or proportional to the number of municipalities served by each reservoir. Monitoring should always include species identification and densities counts. Since cyanotoxin analysis is costly, and rapid tests have not yet been developed for all identified cyanotoxins, routine analysis of the three most common cyanotoxins (i.e. microcystins, cylindrospermopsins, and saxitoxins), should be established as has been the case in Uruguay.

In order to take effective actions that minimize the occurrence and impact of cyanoHABs, environmental factors regulating cyanotoxin production and degradation (i.e. light intensity, nutrient supply rates, temperature, oxidative stressors, and interactions with other biota) should be closely monitored, as they might provide clues into when cyanotoxin production might occur.

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