

DOCTORAL (PH.D.) DISSERTATION

**SPATIAL AND TEMPORAL PATTERNS OF PHYTOPLANKTON IN
TROPICAL AND TEMPERATE LAKES**

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„ The only heritage that I will leave for is your education”

By this, she gave me more than any money could buy.

Cristiane Fernandes, as known as my mom.

„ A única herança que te deixarei será a sua educação”

E dessa forma, ela me deu muito mais

do que qualquer dinheiro pudesse comprar.

Cristiane Fernandes, também conhecida como minha mãe.

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ABBREVIATIONS

Chl-a	Chlorophyll-a
DCM	Deep Chlorophyll Maximum
FSS	Fixed (Inorganic) Suspended Solids
GALD	Greatest Axial Linear Dimension
MBFG	Morphology-Based Functional Groups
SRP	Reactive Soluble Phosphorous
S/V	Surface to Volume ratio
TC	Total Carbon
TDC	Total Dissolved Carbon
Temp	Water Surface Temperature
TN	Total Nitrogen
TDN	Total Dissolved Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids
VSS	Volatile (Organic) Suspended Solids
Z_{eu}	Euphotic depth
Z_{max}	Maximum depth

SUMMARY

Spatial and temporal variability of the environment plays an important role in resource availability and therefore in shaping phytoplankton assemblages and dynamics. In semi-arid tropical regions, seasons are delimited by the extent of rainfall leading to seasonal differences in water-related environmental parameters, thus shaping the phytoplankton community dynamics. Dry periods can intensify eutrophication and often result in seasonal or even perennial cyanobacterial dominance. Yet, the drivers of perennial blooms are less understood when light and temperature do not limit cyanobacterial growth year-round in contrast to lakes in the temperate region. Therefore, this dissertation's first and second chapters focus on phytoplankton response to identify the main drivers of cyanobacteria blooms in the tropics. We evaluated the effects of trophic status and seasonality (dry and rainy periods) on phytoplankton dynamics using the morphology-based functional groups (MBFG) approach, along with the identification of environmental thresholds to the dominance of different MBFGs (**Chapter I**). The dissertation also explores the influence of water levels in promoting cyanobacteria dominance and assess the abiotic conditions underlying the dominance of two bloom-forming genera *Raphidiopsis* and *Microcystis* during perennial blooms (**Chapter II**).

To achieve those aims, phytoplankton, and environmental variables were monthly monitored in lakes with different trophic conditions during the dry and rainy seasons. Our results showed a clear MBFG association with lakes' trophic states. The dominant groups in mesotrophic conditions were members of groups V (unicellular flagellates) and VI (non-flagellated with siliceous exoskeleta), and in meso-eutrophic lakes MBFG IV (medium size without specialized traits) dominated. Conversely, MBFG VII (with mucilage and aerotopes) and VIII (nitrogen-fixing cyanobacteria) dominated mostly under eutrophic conditions, though the latter trait was linked to shallower euphotic zones. Light availability and phosphorous were the most important environmental variables associated with MBFGs' dominance. Furthermore, the majority of the lakes displayed seasonal differences in environmental parameters, still, the effects of seasonality varied across lakes. In contrast to the expectations, the rainy season was characterized by higher concentrations of nutrients and suspended solids, along with reduced euphotic depth compared to the dry season. Overall, our results highlight the effects of seasonality on environmental parameters and eutrophication as the main driver of phytoplankton community composition along with the MBFG functional classification (**Chapter I**).

We also observed that low water levels corresponded to increased phosphorous and nitrogen concentration and consequently, phytoplankton biomass. Cyanobacterial biomass was also proportional to phosphorus concentrations during perennial blooms. Yet, the two dominant cyanobacteria, *Raphidiopsis* and *Microcystis*, seldom co-occurred temporally and the switch between them was driven by water transparency. Furthermore, our results illustrate the effects of drought-induced water level reductions on the biomass and composition of cyanobacterial blooms in tropical shallow lakes. Given

the ideal year-round conditions droughts associated with low water levels may be expected to intensify the risk and multitude of problems linked to eutrophication (**Chapter II**).

In deep oligo-mesotrophic stratified lakes, cyanobacteria can develop a subsurface layer enriched in chlorophyll, the so-called deep chlorophyll maximum (DCM). A wide range of phytoplankton groups has been observed composing DCM; of which cyanobacteria are the most frequently found. The establishment of different layers regulates the input of nutrients and the interface provides a narrow stratum rich in nutrients, typically cold, but with enough light availability for adapted phytoplankton species to grow. Hence, we also aimed to explore the temporal dynamics of cyanobacteria and characterize DCM formation by focusing on the spatial distribution of *Planktothrix* and *Aphanizomenon* in Lake Stechlin, Germany (**Chapter III**).

Our study evidenced the increase of cyanobacteria dominance in Lake Stechlin in the past few years, mostly characterized by potentially toxic filamentous cyanobacteria. Since 2020, *Planktothrix rubescens* (De Candolle ex. Gomont) Anagnostidis & Komárek has been the predominant species in the phytoplankton community, responsible for more than 70% of the total biomass in 2021. Besides, *Planktothrix rubescens* attained extremely high biomass in the upper part of the hypolimnion, while *Aphanizomenon* biomass seldom exceeded *Planktothrix rubescens*' biomass and was mostly found in the epilimnion. The sudden increase in cyanobacteria reflects the ongoing eutrophication in Lake Stechlin and raises concern regarding water quality and further cyanobacterial blooms under the global warming *scenario* (**Chapter III**).

PADRÕES ESPACIAIS E TEMPORAIS DO FITOPLÂNKTON EM LAGOS TROPICAIS E TEMPORAIS

RESUMO

Os padrões espaciais e temporais exercem um importante papel regulando a disponibilidade de recursos e, portanto, na composição e dinâmicas do fitoplâncton. Nas regiões tropicais, sazonalidade é caracterizada pela ocorrência ou ausência de chuva, nas quais resultam em diferenças sazonais nos parâmetros químicos e físicos da água, influenciando assim a dinâmica da comunidade fitoplanctônica. Os períodos de seca associados com redução do volume de água podem diminuir os efeitos da diluição e intensificar a eutrofização em corpos d'água, favorecendo, assim, florações sazonais ou até perenes de cianobactérias. No entanto, os mecanismos regulando as florações perenes de cianobactérias são menos compreendidos quando condições como luz e temperatura são favoráveis ao crescimento de cianobactérias durante todo o ano. Portanto, o primeiro e o segundo capítulos desta dissertação avaliam as dinâmicas do fitoplâncton para identificar o principal mecanismo influenciando as florações de cianobactérias nos trópicos. No mais, avaliamos os efeitos do estado trófico e da sazonalidade (período de seco e chuva) na dinâmica do fitoplâncton usando a abordagem de Grupos Funcionais Baseados na Morfologia (MBFG), juntamente com a identificação de limiares ambientais favorecendo a dominância de diferentes MBFGs (**Capítulo I**). Além disso, também abordamos a influência dos níveis de água na dominância de cianobactérias e identificamos as condições abióticas relacionadas à abundância e dominância de *Raphidiopsis* e *Microcystis* durante uma floração perene (**Capítulo II**).

Para atingir esses objetivos, avaliamos seis reservatórios com diferentes condições tróficas localizados na região semiárida do Nordeste do Brasil durante uma seca regional prolongada, no qual monitoramos mensalmente a comunidade do fitoplâncton e as variáveis ambientais durante as estações seca e chuvosa. Nossos resultados mostraram uma clara associação do MBFG com os estados tróficos dos lagos. Os grupos dominantes em condições mesotróficas foram os membros dos grupos V (flagelados unicelulares) e VI (não flagelados com exoesqueletos silicosos), enquanto, MBFG IV (tamanho médio sem traços especializados) dominou em condições meso-eutróficas. Por outro lado, MBFG VII (com mucilagem e aerótopos) e VIII (cianobactérias fixadoras de nitrogênio) dominaram principalmente sob condições eutróficas, embora o último tenha sido beneficiando em zonas eufóticas mais rasas. A disponibilidade de luz e concentrações de fósforo foram os limiares ambientais mais importantes associados ao domínio dos MBFGs. Além disso, a maioria dos reservatórios apresentaram diferenças sazonais nos parâmetros ambientais, ainda assim, os efeitos da sazonalidade variaram entre eles. Ao contrário do esperado, a estação chuvosa apresentou maiores concentrações de nutrientes e sólidos em suspensão, juntamente com profundidade eufótica reduzida em relação à estação seca. No geral, nossos resultados enfatizaram os efeitos da sazonalidade nos parâmetros ambientais e a eutrofização como o principal fator para a seleção de MBFG (**Capítulo I**).

Também observamos que baixos níveis de água corresponderam ao aumento da concentração de fósforo e nitrogênio e, conseqüentemente, da biomassa fitoplanctônica. A biomassa de cianobactérias também foi proporcional às concentrações de fósforo durante as florações perenes. No entanto, os dois gêneros dominantes de cianobactérias, *Raphidiopsis* e *Microcystis*, raramente co-ocorreram temporalmente e a alternância na dominância foi impulsionada pela transparência da água. No mais, nossos resultados ilustram os efeitos das reduções do nível de água induzidas pela seca na biomassa e na composição das florações de cianobactérias em lagos tropicais rasos. Dadas as condições ideais durante todo o ano, espera-se que os baixos níveis de água decorrentes do período de seca intensifiquem o risco e a multiplicidade de problemas ligados à eutrofização (**Capítulo II**).

Em contraste às típicas florações no epilímnio de lagos eutróficos, em lagos profundos oligomesotróficos estratificados as florações de cianobactérias tipicamente podem se desenvolver em camadas mais profundas. Quando a maior concentração de clorofila encontra-se em uma camada subsuperficial, esse fenômeno é conhecido como profundidade da clorofila máxima (*Deep Chlorophyll Maximum* – DCM). Uma diversidade de grupos fitoplanctônicos tem sido observada compondo o DCM, sendo cianobactéria o mais frequente. O estabelecimento de diferentes camadas por meio da estratificação regula a disponibilidade de nutrientes, além disso, a interface fornece um ambiente rico em nutrientes, tipicamente frio, e com disponibilidade de luz suficiente para o crescimento de espécies de fitoplâncton adaptadas para essas condições. Diante disso, no terceiro capítulo dessa dissertação, exploramos a dinâmica temporal de cianobactéria, focando na descrição da formação de DCM e na distribuição espacial de *Planktothrix* e *Aphanizomenon* no Lago Stechlin, Alemanha (**Capítulo III**). Para atingir esses objetivos, usamos métricas estruturais e funcionais junto com dados de longo prazo.

Nosso estudo evidenciou o aumento da dominância de cianobactérias no Lago Stechlin nos últimos anos, caracterizado principalmente pelo aumento de cianobactérias filamentosas potencialmente tóxicas. Desde 2020, *Planktothrix rubescens* tem sido a principal espécie a compor a comunidade fitoplanctônica, responsável por mais de 70% da biomassa total em 2021. Além disso, *Planktothrix rubescens* alcançou biomassa extremamente alta em camadas profundas na parte superior do hipolímnio, enquanto a biomassa de *Aphanizomenon* raramente excedeu a abundância de *Planktothrix rubescens* e foi encontrada principalmente no epilímnio. O aumento súbito de cianobactérias reflete os a eutrofização ocorrendo no Lago Stechlin e aumenta a preocupação tendo em vista a qualidade da água e o aumento da proliferação de cianobactérias no cenário de aquecimento global (**Capítulo III**).

1. GENERAL INTRODUCTION

Oxygenic photosynthesis was pioneered by cyanobacteria around 2.3 billion years ago, representing the most ancient surviving group of photosynthetic organisms. The eventual rise in oxygen concentrations led to the Great Oxygen Event, changing Earth's atmosphere to aerobic conditions similar to what we know at present (Bekker et al., 2004). The endosymbiosis between early eukaryote protists and cyanobacteria gave origin to all eukaryotic algae and higher plants now populate every sunlit space. Phytoplankton is the fraction of unicellular and colonial photosynthetic organisms adapted to suspension in turbulent water masses (Naselli-Flores et al., 2021a). Due to the short generation time and large populations, phytoplankton evolved many strategies through the geological time for adapting to very diverse environmental conditions, even the ones we may qualify now as extreme may have been normal when they evolved (Padisák & Naselli-Flores, 2021). As a result of several divergent events in phytoplankton evolutionary history, trait conservatism differs according to the level of phylogenetic aggregation. For example, large clades have a lower level of conservatism in traits which leads to species diverging in adaptive strategies. On the other side, smaller clades present high conservatism, and consequently, species are more similar regarding traits and adaptive strategies (Martiny et al., 2013).

The functional approach aimed at grouping species with similar adaptive strategies, thus ecological roles, into groups diminishing redundancy to simplify real ecosystems (Salmaso et al., 2015) considering this cannot always be achieved through the taxonomic approach due to many large clades (i.e., broader taxonomic groups). Colin Reynolds' unprecedented contribution (Naselli-Flores et al., 2021b) to phytoplankton ecology and the development of the functional approach focuses on the selection of phytoplankton assemblages based on habitat properties. Hence, this approach increases the understanding and predictability of phytoplankton community responses, by condensing information of similar species into groups without losing their key responses, such as the mechanisms and effects as occur when general metrics are applied, such as chlorophyll and total phytoplankton biomass. Moreover, phytoplankton is strongly linked to the aquatic food web, thus any slight environmental change directly influences its composition and dynamics, which may interfere with organic matter transfer to higher trophic levels.

The famous hypothesis by Baas-Becking (1934) "Everything is everywhere, but the environment selects" has been used and contested many times in phytoplankton ecology to explain spatial patterns, especially when considering the endemism of species, ongoing invasions, and the absence of species in a whole continent, while frequent elsewhere (Padisák et al., 2016). Nevertheless, a general pattern is that the environmental filters will select the best fit of phytoplankton species under given conditions. These filters could be spatial (e.g. dispersal or transportation), abiotic (e.g. light availability, temperature, and nutrients), and biotic (e.g. herbivory, competition, and parasitism). Hence, phytoplankton patterns are controlled by the interaction of these filters along with trait selection to maximize fitness.

A wide variety of studies have focused on the abiotic drives of phytoplankton dynamics, as eutrophication has been still pervasive in many lakes due to anthropogenic activities. Artificial eutrophication leading to cyanobacterial blooms is the most common cause of freshwater quality impairment, yet, these blooms have been increasing in magnitude, frequency, and duration worldwide (Le Moal et al., 2019). The expansion of cyanobacteria has also been evidenced by the ongoing invasions, when invasive species outside of their native habitat can cause major biodiversity losses threatening ecosystem functioning, economy, and human health (Litchman, 2010). A typical example is *Raphidiopsis raciborskii* (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique, and Salerno, which has a tropical origin, but has extended its native range towards northern temperate regions and can be found currently in many climatic zones (Padisák, 1997; Wilk-Woźniak et al., 2016).

Cyanobacteria can form dense, potentially toxic blooms on the surface and/or at deeper depths in lentic water bodies with extensive impacts on the aquatic ecosystem. Cyanobacteria overgrowth can attain large biomasses reducing light penetration and shading phototrophic species below. After the collapse, their degradation by bacteria leads to the depletion of oxygen, or even the emission of toxic compounds causing the death of aquatic organisms. Besides, anoxic conditions reduce the redox potential and the pH, thus leading to the release of phosphorous trapped in the sediment back into the water column, which functions as positive feedback intensifying eutrophication. In addition, cyanobacteria can produce cyanotoxins, which impact water safety for humans, aquatic organisms, and farmed animal stocks, along with the impairment of the use of water bodies. However, when these blooms occur at deep depths it seldom leads to public response.

Besides eutrophication, climate change act as a catalyst for cyanobacteria blooms and their global expansion (Paerl, 2017; Huisman et al., 2018). Warmer temperatures favour cyanobacteria because their maximal growth rates occur at high temperatures, around 25°C, outcompeting most eukaryotic algae under elevated temperatures (Paerl & Huisman, 2008). Moreover, warming reduces ice cover duration and can modify the mixing regime, leading to the elongation of the stratified period (Råman Vinnå et al., 2021). This series of events can reshape phytoplankton composition in favour of cyanobacteria, which thrive during stratified periods due to buoyancy regulation. In addition, extreme weather events, such as the intensity and frequency of storms, and extreme droughts, have already been reported to promote cyanobacteria blooms (Reichwaldt & Ghadouani, 2012; Mosley, 2015). Therefore, cyanobacteria profit from many physiological adaptations making them successful competitors under diverse scenarios, especially in the climate change era.

In this dissertation, we explore temporal and spatial patterns of phytoplankton in response to apparent conditions including lakes from both tropical and temperate regions. We evaluated the influence of a prolonged drought in man-made shallow mixed lakes from the semi-arid region, ranging from meso- to eutrophic state. The comprehension of cyanobacteria patterns under prolonged drought in the tropical semi-arid region provides a panorama regarding the possible consequences for global water quality in future drylands. The temperate Lake Stechlin is a deep, mostly dimictic, largely

mesotrophic lake of glacial origin with ongoing eutrophication. Hence, we focus on the surface and deep layers of cyanobacteria blooms using the functional and taxonomic approach aiming to understand varying patterns in bloom dynamics.

2. SCIENTIFIC ACHIVEMENTS

3. CHAPTER I

THIS CHAPTER IS BASED ON THE FOLLOWING PAPER:

**THE INFLUENCE OF TROPHIC STATUS AND SEASONAL ENVIRONMENTAL
VARIABILITY ON MORPHO-FUNCTIONAL TRAITS IN TROPICAL MAN-MADE
SHALLOW LAKES**

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Cover photo: Dr. Vanessa Becker (Santa Cruz Lake, Brazil)

3.1 INTRODUCTION

Phytoplankton is a vastly polyphyletic group comprising an immense diversity of species with different adaptative strategies, hence a wide range of functional traits (Litchman & Klausmeier, 2008). According to the most widely used definition, a functional trait is any morpho-physiological or phenological measurable characteristics at the individual level that impact fitness *via* their effects on the growth, reproduction, and survival of the species (Violle et al., 2007). Moreover, species sharing functional traits, the so-called functional groups, are selected by the environmental filters and may similarly affect the ecosystem functioning (Reynolds et al., 2002; Salmaso & Padisák, 2007; Brasil & Huszar, 2011; Salmaso et al., 2015; Kruk et al., 2020). Therefore, functional traits can be used to identify and understand the environmental drivers of phytoplankton assemblage dynamics.

The initial attempts to develop a functional classification (Margalef, 1978; Reynolds, 1984; Padisák & Reynolds, 1998) were accompanied by multiple efforts to refine, improve and validate such classifications (Reynolds et al., 2002; Salmaso & Padisák, 2007; Padisák et al., 2009; Kruk et al., 2010; Salmaso et al., 2015; Derot et al., 2020). The selection of the appropriate approach depends on the ecological question and may include different features (i.e., morphological, physiological, and ecological such as phenology and even taxonomy), along with different levels of complexity and taxonomic knowledge required to apply (Salmaso et al., 2015). Therefore, functional approaches have become widespread and recommended to understand phytoplankton responses to environmental variability, as they are often more reliable than the taxonomic classical approach (Reynolds et al., 2002; Salmaso & Padisák, 2007; Padisák et al., 2009; Kruk et al., 2010). Besides, when the habitat template along with environmental thresholds of functional groups' dominance is determined, this link allows for the assessment of lake conditions based on the pool of species (Reynolds et al., 2002; Padisák et al., 2009; Kruk & Segura, 2012; Kruk et al., 2020)

Trophic status and seasonality have key roles in resource availability, thus shaping phytoplankton composition and dynamics (Naselli-Flores, 2000; Pacheco et al., 2010; Bortolini & Bueno, 2017). In semi-arid tropical regions, these drivers interact because the annual hydrological cycle (i.e., rainy and dry seasons) modifies chemical and physical water parameters favouring different phytoplankton taxa (Rangel et al., 2016; Bortolini & Bueno, 2017; Azevedo et al., 2020; Braga & Becker, 2020). Dry periods leading to the reduction of water volume and depth have been associated with increasing nutrient concentrations, water residence time, and phytoplankton biomass, mainly cyanobacteria, which lead to a decline in water transparency (Padisák et al., 1999; Naselli-Flores, 2003; Aldridge, 2011; Mosley, 2015). On the other hand, short rains may increase nutrient availability *via* short-term runoff events, while long rainy periods promote dilution effects on water bodies (Brasil et al., 2016; Jargal et al., 2021). Therefore, the increase in water volume and depth diluting nutrients concentrations promote the decline of phytoplankton biomass and increases water transparency (Braga & Becker, 2020; Stockwell et al., 2020). Overall, the effects of rainy and dry periods on lakes depend on characteristics

such as their duration and intensity, along with lake morphometry (such as depth, volume) and trophic status (Medeiros et al., 2015; Costa et al., 2016; Stockwell et al., 2020).

Despite the dry season being part of the hydrological cycle in semi-arid regions, the duration and intensity of such periods have been increasingly associated with the recurrence and persistence of toxic or potentially toxic cyanobacterial bloom events. The outcome of further dry periods for phytoplankton remains an open question. Studies on lakes in the semi-arid region have reported that drought may favour either cyanobacterial blooms (McGregor & Fabbro, 2000; Bouvy et al., 2003; Brasil et al., 2016) or mixotrophic organisms and diatoms depending on the intensity of drought and lake morphological characteristics (Medeiros et al., 2015; Costa et al., 2016, 2019; Crossetti et al., 2019). Overall, a general understanding of the effects of drought and eutrophication has been still a major challenge, but essential in order to manage cyanobacterial blooms under the global change *scenario* (Paerl et al., 2020).

Here, we used the Morphologically Based Functional Groups (MBFG) approach to link environmental filters of tropical shallow lakes with phytoplankton dynamics. This approach was established through cluster analysis using data from different climate zones and continents (Kruk et al., 2010). The classification relies exclusively on easily observable morphological traits, such as cell size and volume, presence of mucilage, flagella, aerotopes, heterocytes, and siliceous structures. Furthermore, these morphological traits were correlated to physiological and demographic traits and thus can be used as proxies. Morphological traits are often selected due to being easily observed and measured and influence phytoplankton ecological functions, including reproduction, resource acquisition, and predator avoidance (Naselli-Flores et al., 2007b; Litchman & Klausmeier, 2008; Litchman et al., 2010; Naselli-Flores, 2014). The result was the creation of seven MBFGs, subsequently, the environmental thresholds promoting each group's dominance were identified *via* machine learning techniques (Kruk et al., 2010; Kruk & Segura, 2012). The follow-up classification was updated to eight groups, with nitrogen-fixing cyanobacteria species being separated from the filamentous with the aerotopes due to their different function which confers the opportunity to dominate in different environmental conditions (Reynolds et al., 2014).

MBFG has a close link to environmental conditions and has been efficiently applied to a variety of freshwater environments, however, only a few cases applied this approach in tropical semi-arid regions with prolonged droughts. Hence, in this chapter, we applied the MBFG approach to six man-made lakes in a Brazilian semi-arid region with the aim of (i) evaluate MBFG's response to trophic state, (ii) determine the environmental filters threshold linked with each MBFG's dominant, and (iii) assess the influence of dry and rainy seasons on environmental filters and MBFG's selection.

3.2 METHODOLOGY

3.2.1 STUDY SITE

We studied six man-made lakes located in the northeast region of Brazil in the Rio Grande do Norte state (Fig 1). These lakes vary greatly regarding their morphometric parameters, such as area, maximum capacity, and depth (Table 1). Santa Cruz is the largest regarding the area, maximum capacity, and depth, followed by Tabatinga and Boqueirão, however, they presented the lowest current storage level, i.e., > 34% of maximum capacity. Prata and Encanto have smaller areas and maximum capacities, yet they reinterned their volumes presenting the highest storage level i.e., < 84% of maximum capacity during the study period. Despite that, all lakes displayed current depths extremely reduced compared to their maximum depths.

These lakes were created to retain water during the rainy season to overcome water shortages in the dry season. Seasonal water inflow to these lakes occurs *via* intermittent rivers and precipitation (Barbosa et al., 2012). They provide several essential economic, subsidence, and recreational services to the population in the surroundings, such as crop irrigation, fishing, livestock maintenance, and, for Santa Cruz, and Boqueirão, also the domestic water supply. Furthermore, this study was developed as part of a monthly monitoring program (twelve months) designed by the Institute of Water and Management of the State of Rio Grande do Norte (IGARN) to evaluate whether such lakes may be suitably used for fish cage culture.

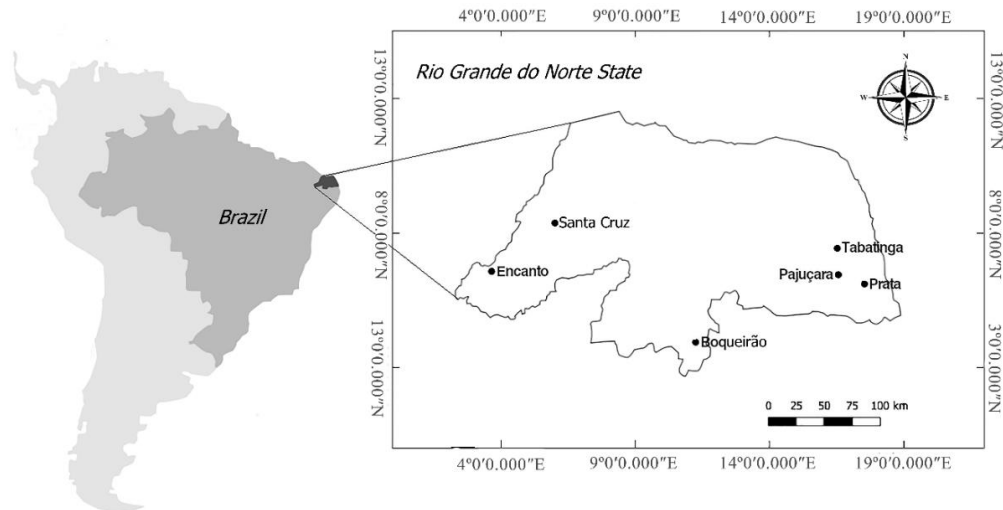


Fig. 1 Map of Brazil and Rio Grande do Norte State showing the location of the six lakes studied.

Table 1 Lakes morphometric variables, storage level means the volume retained in 2017. Maximum depth refers to the depth at full capacity and current depth is the average of all measures done in 2017. Data was not available (NA) for Pajuçara lake. Data source: State Department of the Environment and Water Resources.

Man-made lakes	Basin	Area (ha)	Maximum Capacity (m ³)	Storage level (%)	Maximum depth (m)	Current depth (m)
Santa Cruz	Apodi/Mossoró	3.413	599.712.000	24.75	57.5	16.6
Prata	Jacú	151	9.321.149	81.87	19.5	3.9
Tabatinga	Potengi	1.090	89.835.678	11.76	27.8	5
Encanto	Apodi/Mossoró	124	5.192.538	74.98	16.9	5.9
Boqueirão	Piranhas/Assu	1.267	84.792.119	33.22	29.0	5
Pajuçara	Rio Trairi	NA	NA	NA	NA	4.5

3.2.2 CLIMATIC SCENARIO

The regional climate for the region where the lakes are located is tropical Semi-arid (Alvares et al., 2013), this climate is typical of the hinterland, however, Rio Grande do Norte state cover also part of the coastline. It is characterized by low and irregular annual rainfall (average rainfall of 550 mm. year⁻¹), high average annual temperature (slightly higher than 26.5°C), and evapotranspiration rates above 1500 mm year⁻¹, which makes this region the driest and warmest in the cost of Brazil (Menezes et al., 2012; Alvares et al., 2013; Braga et al., 2015). The high temperature is constant with low oscillations throughout the year; consequently, the irregular rainfall is used to delimit seasons, which divides the year into two main periods: the rainy season with concentrated rainfall from January to June, and the dry season with a negative water balance during the rest of the year, from July to December.

Dry periods are characteristic of the Brazilian semi-arid region, however, the intensity, and duration of this “natural phenomenon” has been increasing over the past few years, hence the mean annual precipitation has already halved from the 1980s to the 2000s (Marengo et al., 2017, 2018). The most recent drought started in 2012 and lasted until mid-2016 representing one of the longest and most severe droughts in the Brazilian semi-arid region in the last decades with serious social and economic impacts (Marengo et al., 2018). Its classification varied from moderately to extremely dry according to the Standard Precipitation Index (SPI) (Figueiredo & Becker, 2018; Braga & Becker, 2020; Marengo et al., 2020) with rainfall deficits ranging from 20 to 60% below the historical mean. Within this period, 2012 and 2016 were the driest years with rainfall 300 - 400 mm year⁻¹ below the expected for this region (Marengo et al., 2018). Our sampling period took place in 2017; thus, we sampled lakes with low storage levels and depths as shown in Table 1.

Pluviometric data from two meteorological stations located in the vicinity of the lakes were provided by the Agricultural Research Company of Rio Grande do Norte (EMPARN), and the average of both was used to characterize the rainfall in the region during our study period. The historical time series before (1963-2011) and during (2012-2016) the prolonged drought was also used to contrast the climatic *scenario* before, during, and shortly after the prolonged drought. In 2017, the annual rainfall was 31% lower than the historical average (1963-2011), but it was 21% above the prolonged drought average (2012-2016) (Fig. 2).

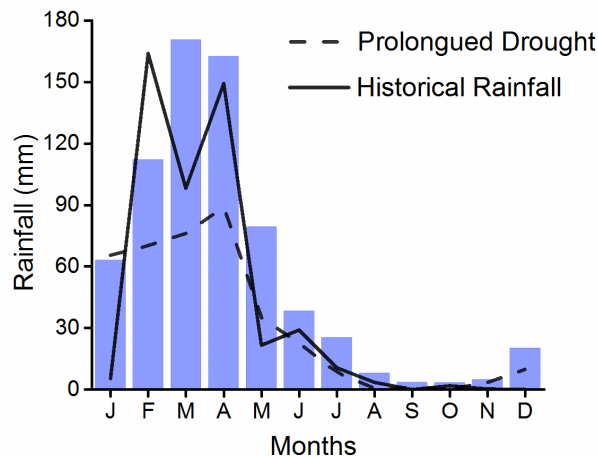


Fig 2. Monthly rainfall between Jan and Dec 2017 (blue bars); historical monthly average rainfall between 1963 and 2011 (solid black line); and monthly average rainfall during the prolonged drought (2012-2016) in the Rio Grande do Norte semi-arid region. (Data source: Agricultural Research Company of Rio Grande do Norte State—EMPARN)

3.2.3 WATER SAMPLING AND ANALYSIS

Water samples were collected monthly on two consecutive days from January to December of 2017 in each lake, except April in Boqueirão and June in Santa Cruz due to technical problems. The sampling took place at the deepest part near the dam of each lake. Water samples were collected with a PVC tube in each lake (2 m in length) integrating the first two meters of the water column beneath the surface. To include spatial variation, this procedure was repeated five times spaced around the collecting point with ~2 meters horizontal distance between them. Water samples (~30L/sample) were integrated and stored in pre-washed bottles. The euphotic depth was defined as 2.7 times the measure obtained by Secchi transparency (Cole, 1994). Water temperature was measured at the bottom and top of the water column. Phytoplankton samples were immediately fixed with an acetic Lugol solution. Samples for analyses were transported to the laboratory in a heat-isolated cooling box.

Aliquots of unfiltered lake water were stored in a freezer (-20°C) for total phosphorus, nitrogen, and carbon analyses. Aliquots of water (250-500 mL) were filtered onto GF/C glass fiber filters (1.2 μm pore size) for chlorophyll-a, and total suspended solids (volatile and fixed). Following, the aliquots of filtered lake water were used to measure total dissolved nitrogen and carbon and soluble reactive phosphorus. TN, TDN, TC, and TDC were analysed by standard techniques using SHIMADZU TOC_{VCPN} sampler with the SSM-5000A solid sample combustion unit by chemiluminescence. TP and SRP were determined using the persulphate oxidation method (Valderrama, 1981). Chl-a was quantified by spectrophotometry using ethanol 95% as a solvent (Wintermans & De Mots, 1965; Jespersen & Christoffersen, 1987), while TSS, VSS, and FSS were determined using gravimetric analyses (Chanlett, 1947). We used the criteria established by (Thornton & Rast, 1993) to determine the trophic states of semi-arid man-made lakes. According to this criteria, waters of semi-arid lakes with TP concentrations lower than $50 \mu\text{g L}^{-1}$ and Chl-a lower than $15 \mu\text{g L}^{-1}$ may be classified as mesotrophic while those

samples with TP and Chl-a higher than $50 \mu\text{g L}^{-1}$ and $15 \mu\text{g L}^{-1}$, respectively, may be considered as eutrophic.

The identification and quantification of the phytoplankton community were performed with an optic light and inverted microscope (400x magnification) until species level (or the lowest taxonomic level possible) *via* morphologic and morphometric characteristics. The units (cells, colonies, and filaments) were enumerated in random fields (Utermöhl, 1958; Uhelinger, 1964; Lund et al., 1958). At least 100 units of the most frequent species were counted. The volume of the sedimentation chamber was selected depending on the number of algae and/or detritus in the sample. The biovolume ($\text{mm}^3 \text{L}^{-1}$) of phytoplankton was obtained based on approximated geometric forms (Hillebrand, 1999; Fonseca et al., 2014) after counting 40-60 units. Phytoplankton biomass (mg L^{-1}) was calculated by assuming that the unit of fresh weight is equivalent to a mass of $1 \text{ mm}^3 \text{L}^{-1} = 1 \text{ mg L}^{-1}$ (Wetzel & Likens, 2000). Species representing more than 5% of the total biomass were included in the statistical analyses.

3.2.4 MBFG CLASSIFICATION

Species representing more than 5% of the total biomass per sample were classified into eight morphologically based functional groups (MBFG). Seven of them were proposed by (Kruk et al., 2010): small organisms with high surface/volume (s/v) (MBFG I); small flagellated organisms with a siliceous exoskeleton (MBFG II); large filaments with aerotopes (except nitrogen-fixing species) (MBFG III); organisms of medium size without specialized traits (MBFG IV); unicellular flagellated organisms (MBFG V); non flagellated organisms with siliceous exoskeleton (MBFG VI) and presence of mucilage along with aerotopes (MBFG VII). Additionally, we included a group of nitrogen-fixing cyanobacteria (MBFG VIII), as proposed by Reynolds et al. (2014).

3.2.5 STATISTICAL ANALYSES

A hierarchical cluster analysis was performed *via* Ward's method to evaluate the similarity between samples based on limnological variables (Z_{max} , Z_{eu} , TP, TN, TC, and Chl-a). Previously, the data were scaled and converted to Euclidian distance. Considering the parametric assumptions were not met, the Mann-Whitney U test was applied to test the differences between dry and rainy seasons regarding abiotic and biotic data; while Kruskal–Wallis test was used to evaluate significant variances of each MBFG among trophic states. Following, we did a classification tree (CART) to identify the environmental thresholds leading to the dominance of each MBFG according to Kruk et al. (2012). To evidence dominance, we included only MBFG with relative biomass equal to or above 70% of the total phytoplankton biomass, hence, each sample had only one MBFG as an outcome. All environmental filters measured were included in the classification tree, except the dissolved nutrient forms to their high variability as a result of phytoplankton and bacteria consumption, which may confuse the establishment of cause-effect relationships (Kruk and Segura, 2012). All statistical analyses were performed using R software (R Core Team, 2018).

3.3 RESULTS

3.3.1 LIMNOLOGICAL SCENARIO

Except for water temperature, physical, chemical, and biological parameters varied considerably among but also within lakes (Fig. 3). With a single exception, the water temperature was always equal to or higher than 25°C but never above 31°C; the average ranged from 27 to 28 °C (Supplementary material, Table S1). Considering the difference in surface and deepest layer temperature, it was less than 1°C with a single exception; therefore, the lakes were considered well mixed). The lakes displayed a gradient of trophic status, according to the eutrophication threshold values for TP (Fig. 3c) and Chl-a (Fig. 3k), Santa Cruz and Prata were mesotrophic, while Encanto, Boqueirão, and Pajuçara were eutrophic. Tabatinga had TP concentration below the eutrophication threshold and Chl-a above it (Fig. 3c; 4k), and for this reason, was classified as meso-eutrophic. Following the trophic tendency, from now on we present the lakes from the less enriched (Santa Cruz) to the most enriched (Pajuçara).

The Z_{\max} ranged from 3.9 m to 16.6 m in Prata and Santa Cruz lakes, respectively, the other lakes had Z_{\max} of around 5.0 m (Fig. 3a). As expected, TP, SRP, and phytoplankton biomass increased towards the eutrophic lakes (Fig. 3c; 3d; 3j). Contrary, Z_{eu} presented the inverse pattern, being deepest in mesotrophic, followed by Prata lake, while in the eutrophic lakes, the Z_{eu} were characterized by mean values equal to or below 2.2 m (Fig. 3b). Prata was the only lake where the whole water column belonged to the euphotic zone during most of the study, i.e., 8 out of 12 months ($Z_{\text{eu}} \geq Z_{\max}$) (Table S1). Low concentrations of TN, TDN, TC, TDC, FSS, VSS, and Chl-a were observed in the mesotrophic lakes, Santa Cruz and Prata (Fig. 3e; 3f; 3g; 3h, 3i, 3k), on the other hand, for the eutrophic lakes, the highest concentrations of TN, TDN, TC, and Chl-a were recorded at Pajuçara (Fig. 3e; 3f; 3g; 3k), TDC and FSS were observed in Boqueirão (Fig. 3h; 3i) and VSS in Tabatinga (Fig. 3j). SRP was always above 3 $\mu\text{g L}^{-1}$, except for one single month in Santa Cruz when SRP was 1.66 $\mu\text{g L}^{-1}$ and TDN was always above 450 $\mu\text{g L}^{-1}$.

The hierarchical cluster grouped lake samples into five major clusters (Fig. 4). Samples were grouped mostly according to their trophic state, still, some seasonal patterns were observed in the eutrophic lakes. Cluster 1 grouped some Pajuçara's samples from the dry season (January to April), this cluster differed the most from the others because contained the highest TP, TN, Chl-a, and the lowest Z_{eu} values recorded in this study. Clusters 2 and 3 were composed of Santa Cruz and Prata samples without seasonal differentiation. Interestingly, the mesotrophic lake Prata was more related to eutrophic lakes than to another mesotrophic lake (Santa Cruz). Cluster 4 included all samples from Tabatinga, the samples from the dry season in Pajuçara, Boqueirão and, Encanto, while cluster 5 contain the samples from the rainy season in Boqueirão and Encanto.

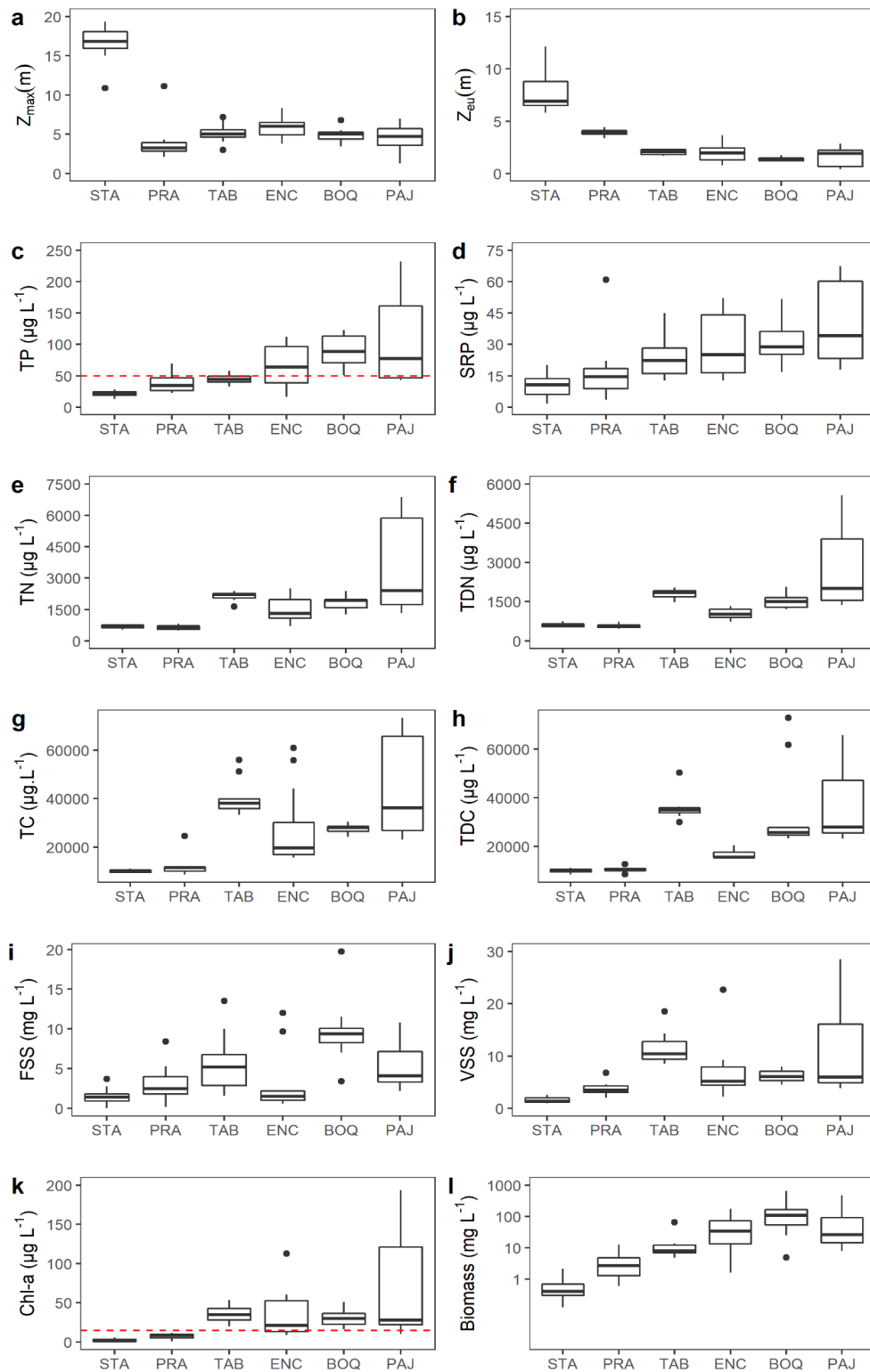


Fig. 3 Boxplot of limnological variables of each lake from Jan-Dec 2017; mesotrophic: **(STA)** Santa Cruz; **(PRA)** Prata; meso-eutrophic: **(TAB)** Tabatinga; eutrophic: **(ENC)** Encanto; **(BOQ)** Boqueirão; **(PAJ)** Pajuçara. The dotted line indicates trophic state thresholds according to Thornton & Rast (1993); TP is 50 $\mu\text{g L}^{-1}$, and Chl-a is 15 $\mu\text{g L}^{-1}$. Bands inside the boxplots represent their medians and vertical bars represent minimum and maximum values. Black dots indicate values that fall outside 1.5 times the interquartile range.

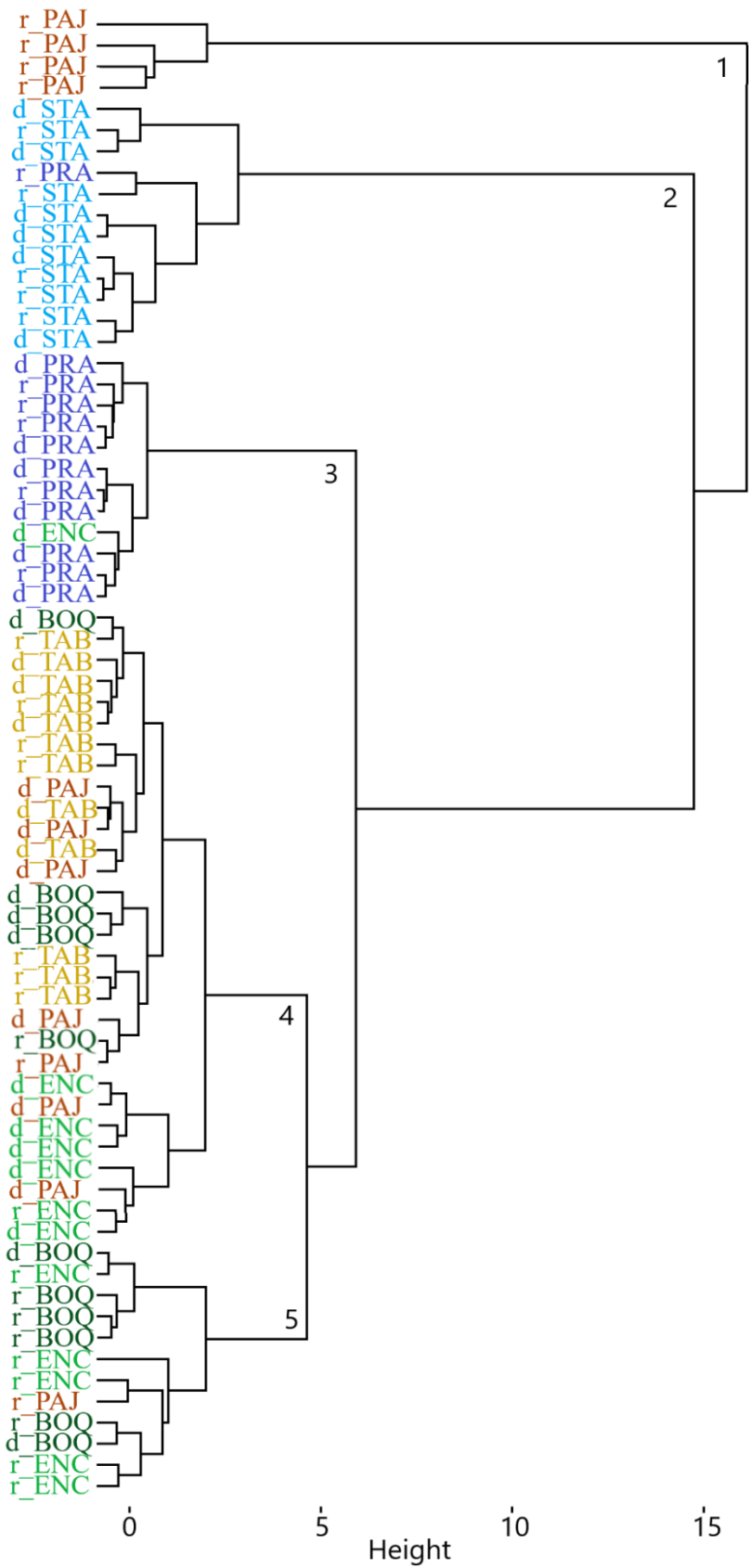


Fig. 4 Dendrogram from hierarchical cluster analysis of water samples from the six lakes; (STA) Santa Cruz; (PRA) Prata; (TAB) Tabatinga; (ENC) Encanto; (BOQ) Boqueirão; (PAJ) Pajuçara. The letters before the lake's name represent seasonality (d -dry: July to December) and (r_- rainy: January to June). The numbers on the corner of the branches indicate the order of the clusters. The database for this dendrogram derives from Fig. 3.

3.3.2 PHYTOPLANKTON COMMUNITY STRUCTURE

A total of 343 species were recorded during the study, from it, 25 were descriptors and placed into five MBFGs, IV, V, VI, VII, and VIII (Table 2). Santa Cruz (mesotrophic) was mostly dominated by MBFG VI, yet, MBFG V and VII also contributed to the phytoplankton community (Fig 5a). In Prata (mesotrophic), the dominant group alternated through time (MBFG IV and VII), still, MBFG V and VII were also found (Fig 5b). Tabatinga lake (meso-eutrophic) was mostly co-dominated by MBFG IV and VI, save in April when MBFG VII bloomed (Fig. 5c). In Encanto lake (eutrophic), MBFG VI, VII, and VIII were the most representatives (Fig. 5d). Boqueirão was virtually dominated by group MBFG VII, except in June, when MBFG VIII dominated at significantly low biomass compare to other months (Fig. 5e). Pajuçara lake (eutrophic) was dominated mainly by MBFG VII and VIII (Fig. 5f). Overall, only MBFG VII dominated in all lakes (i.e., at least one month in each lake) and was also the group which attained the highest biomass on the majority of lakes, except for Santa Cruz lake, where was MBFG VI (Fig. 5).

Table 2 Representative taxa and respectively morphologically based functional groups (MBFG).

MBFG	Definition	Species
IV	Organisms of medium size lacking specialized traits	<i>Coelastrum reticulatum</i> (Dangeard) Senn <i>Monoraphidium minutum</i> (Nägeli) Komárková-Legnerová <i>Planktolyngbya limnetica</i> (Lemmermann) Komárková-Legnerová and Cronberg <i>Pseudanabaena catenata</i> Lauterborn <i>Pseudanabaena</i> spp.
V	Unicellular flagellates of medium to large size	<i>Trachelomonas armata</i> Ehrenberg <i>Trachelomonas</i> spp. <i>photosynthetic flagellates</i> <i>Phacus</i> spp. <i>Peridinium</i> spp. <i>Cryptomonas</i> spp.
VI	Nonflagellated organisms with siliceous exoskeletons	<i>Cyclotella</i> spp. <i>Aulacoseira granulata</i> (Ehrenberg) Simonsen <i>Aulacoseira granulata</i> var. <i>angustissima</i> (O. Müller) Simonsen
VII	Large mucilaginous colonies	<i>Aphanocapsa</i> cf. <i>nubilum</i> Komárek and H.J.Kling <i>Aphanocapsa delicatissima</i> West and G.S.West <i>Aphanocapsa incerta</i> (Lemmermann) G. Cronberg and Komárek <i>Aphanocapsa</i> spp. <i>Botryococcus braunii</i> Kützing <i>Microcystis panniformis</i> Komárek <i>Microcystis aeruginosa</i> (Kützing) Kützing <i>Oocystis</i> spp. <i>Chroococcus</i> spp.
VIII	Nitrogen-fixing cyanobacteria	<i>Raphidiopsis raciborskii</i> (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique and Salerno

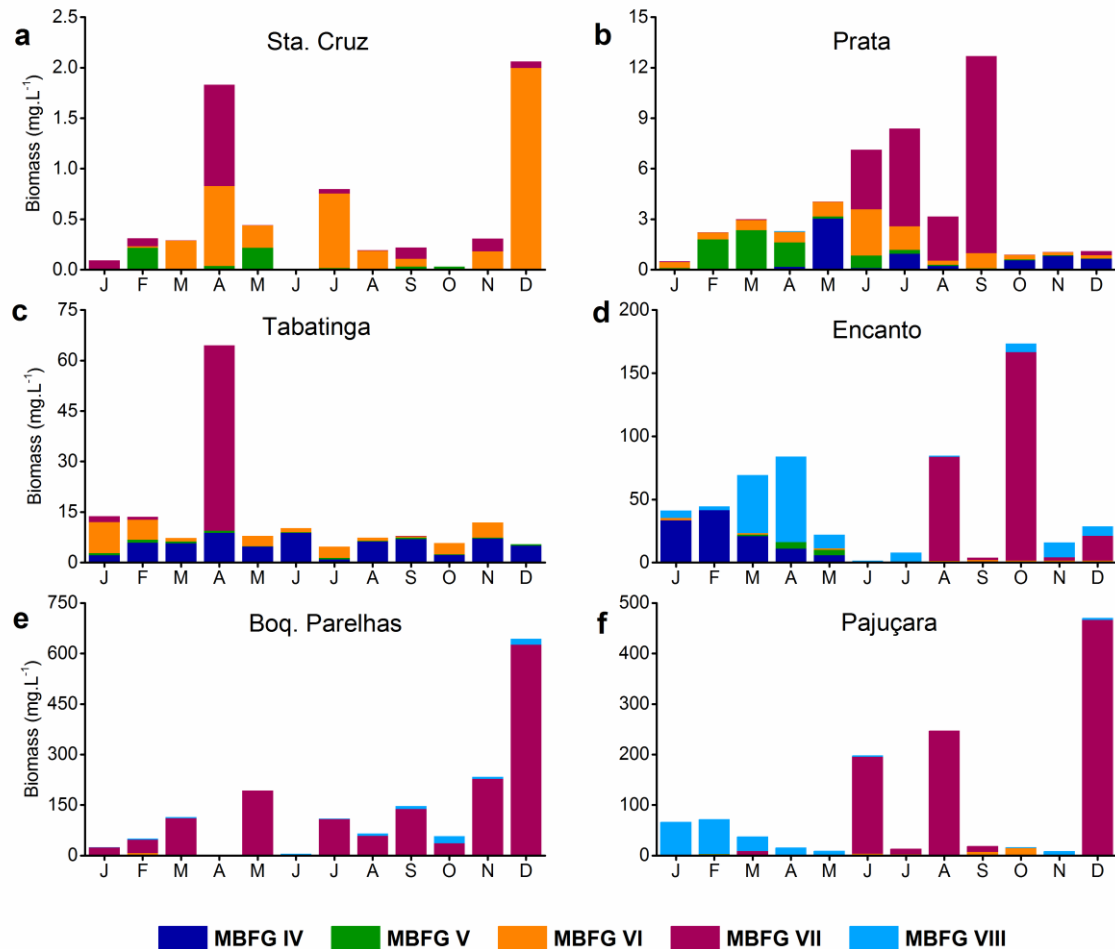


Fig. 5 Total phytoplankton and MBFG biomass composition for each studied lake during the year 2017 (January to December). Data were not available for Santa Cruz in May and Boqueirão in April. Note the different y-axis scales for each lake.

3.3.3 PHYTOPLANKTON RESPONSE TO TROPHIC STATUS

Regarding MBFGs association with trophic status, all MBFGs significantly differed according to the lake's trophic state (IV, VII, and VIII $p < 0.000$; V $p < 0.005$; VI $p < 0.05$). MBFG V was mostly associated with mesotrophic conditions, while MBFG VIII was exclusively found in eutrophic conditions. MBFG VI was dominant in meso- and meso-eutrophic conditions, while MBFG IV was mostly associated with meso-eutrophic conditions. Despite being observed in all lakes, MBFG VII was mostly favoured in eutrophic conditions.

According to the classification tree, Z_{eu} and TP were the most important environmental threshold to distinguish MBFGs dominance (Fig. 6). From the top, Z_{eu} was selected for the root node (i.e., the highest node) with a threshold of 4 m, subsequently by the internal nodes (i.e., a node which has a successor node or nodes) that subdivided Z_{eu} once again with a threshold of 1.2 m. Followed by, a TP threshold of $47 \mu\text{g L}^{-1}$ ends in the leaf nodes (i.e., a node without successor). Starting from the left side of the root node, samples with $Z_{eu} \geq 4\text{m}$ were mostly dominated by MBFG VI; although MBFG V also dominated under this condition, the classification tree neglected this group as a class due to the insufficient amount of data to classify samples in (see histograms on Fig. 5). Moving to the right side

of the root node, MBFG VIII dominates when $Z_{eu} < 1.2$ m. Conversely, MBFG IV and VII dominated when Z_{eu} was between 1.2 to 4 m, still, MBFG IV dominated when TP was below $47 \mu\text{g L}^{-1}$ and MBFG VII dominated when TP was above this threshold.

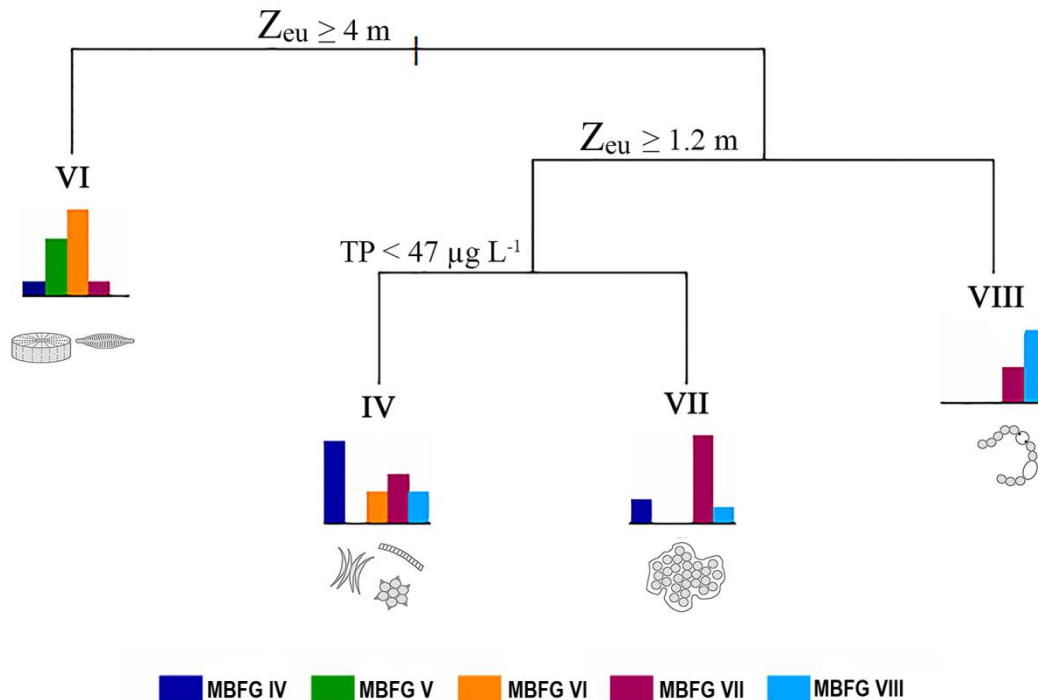


Fig. 6 Classification tree displaying the main environmental threshold factor and its value for the dominant MBFG under each specific environmental condition. Only MBFGs with $>70\%$ of the total biomass were included. Among these groups, the MBFG which most dominated in a certain condition is displayed at the end of each branch with roman numerals. The histogram shows the frequency of MBFG dominance (IV-VIII: left to right) under each of the environmental filters identified by the classification tree. MBFG V was neglected as a class because there was an insufficient amount of data to classify samples.

3.3.4 PHYTOPLANKTON RESPONSE TO SEASONALITY

The seasonal variability of environmental parameters selecting distinct MBFGs was not a general pattern among the lakes, with half of them (Santa Cruz, Prata, and Boqueirão) presenting significant seasonal differences in only one parameter measured, either environmental or MBFG. In Santa Cruz, TN concentration was during the rainy season, followed by a decrease in the dry season (Fig. 7a). In Prata, MBFG V biomass was slightly higher than in the dry season (Fig. 7b), while in Boqueirão, MBFG VIII was favoured in the dry season (Fig. 7c). Besides, concentrations of TP (Fig. 7d), TDN (Fig. 7e), and phytoplankton biomass (Fig. 7f) were higher in the rainy season in Tabatinga lake, followed by a decline in the dry season. Despite phytoplankton biomass significantly differing between rainy and dry seasons in Tabatinga, there was no significant difference regarding MBFG selections.

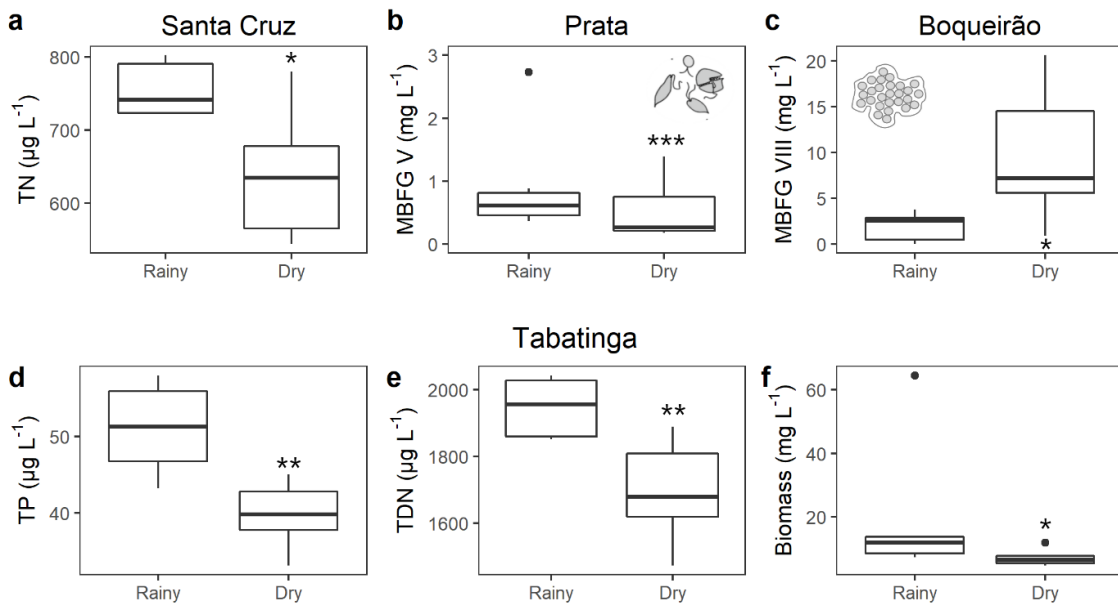


Fig. 7 Boxplot of abiotic and biotic variables with significant differences between rainy and dry seasons in lakes. Bands inside the boxplots represent their medians and vertical bars represent minimum and maximum values. Black dots indicate values that fall outside 1.5 times the interquartile range. All graphs displayed are statistically different in rainy vs. dry seasons (p -value <0.05). Asterisks indicate statistical significance level. The drawings at the corner characterize the MBFG present in the graph.

Seasonal differences in environmental parameters leading to MBFG selection were observed only in two lakes; In Pajuçara during the rainy season, the euphotic zone (Fig. 8a) was shallower, while TP (Fig. 8b), TN (Fig. 8c), TDN (Fig. 8d), TDC (Fig. 8e), FSS (Fig. 8f), Chl-a (Fig. 8g) were higher benefiting MBFG VIII biomass (Fig. 8h). Contrarily, in the dry season the concentration of these variables decreased and the euphotic zone deepened resulting on a drastic decline in the biomass of MBFG VIII. A similar pattern was found in Encanto, during the rainy season the euphotic zone was shallower (Fig. 9a), along with high concentrations of TP (Fig. 9b), TN (Fig. 9c), TDN (Fig. 9d) benefiting MBFG IV (Fig. 9e). While in the dry season, the concentrations of these nutrients and the biomass of MBFG IV declined associated with the deepening of the euphotic zone, increasing MBFG VII biomass (Fig. 9f). Regarding the water level, no significant difference was observed in maximum water depth between the rainy and dry season (data not shown).

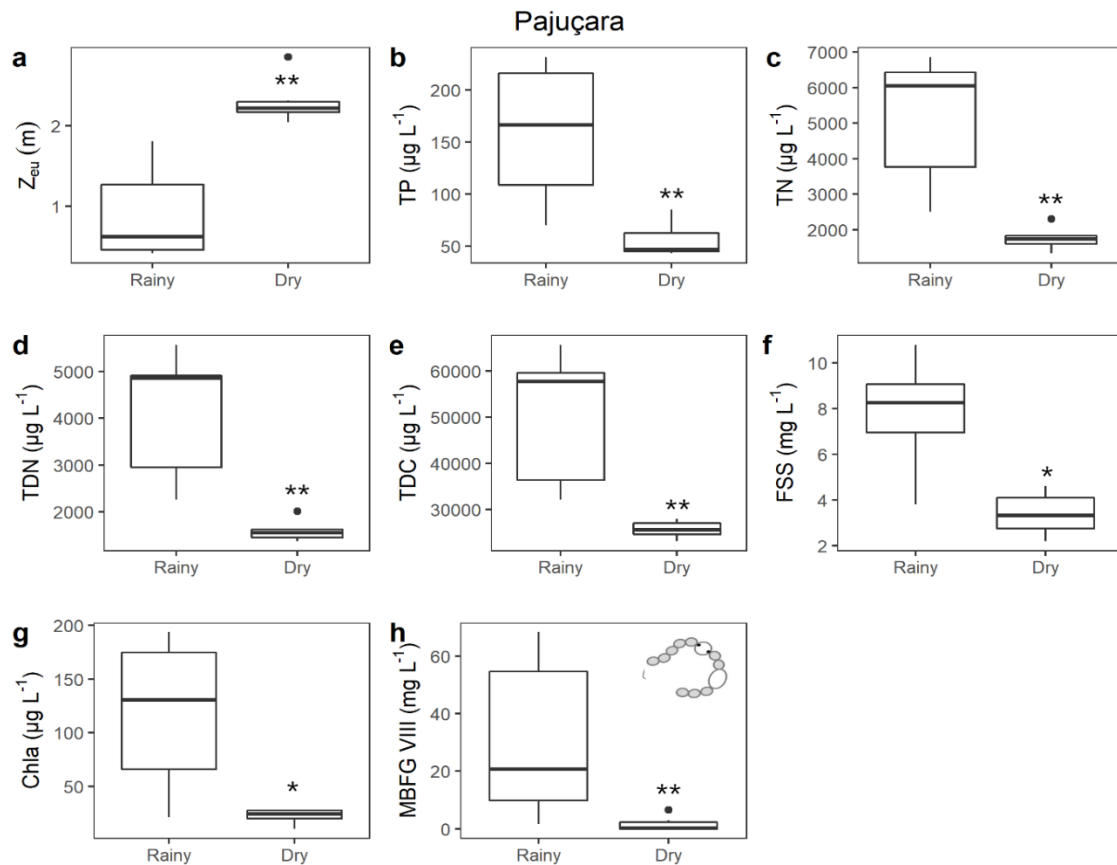


Fig. 8 Boxplot of abiotic and biotic variables with significant differences between rainy and dry seasons in Pajuçara. Bands inside the boxplots represent their medians and vertical bars represent minimum and maximum values. Black dots indicate values that fall outside 1.5 times the interquartile range. All graphs displayed are statistically different in rainy vs. dry seasons (p -value <0.05). Asterisks indicate statistical significance level. The drawings at the corner characterize the MBFG present in the graph.

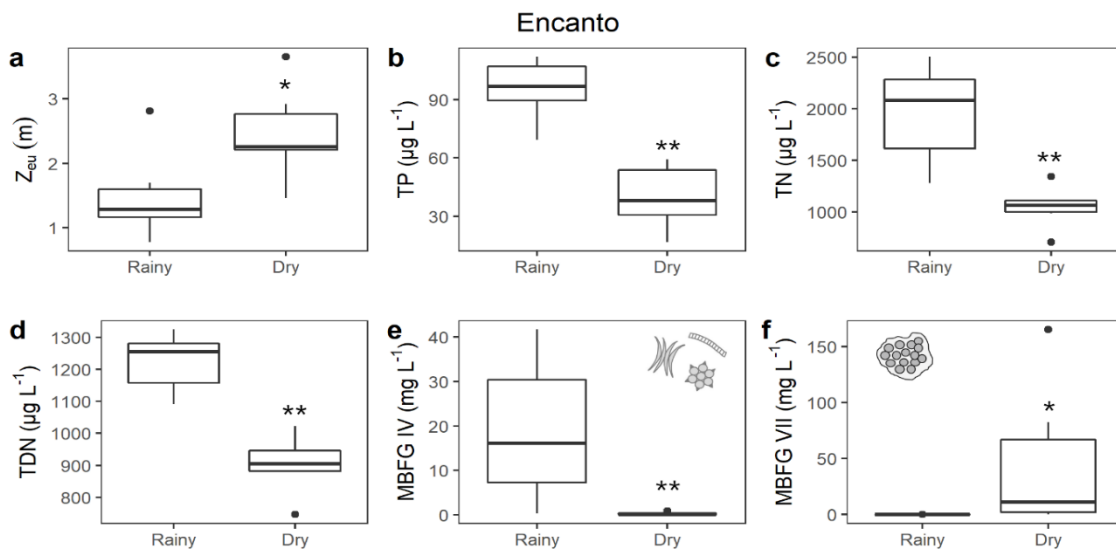


Fig. 9 Boxplot of abiotic and biotic variables with significant differences between rainy and dry seasons in Encanto. Bands inside the boxplots represent their medians and vertical bars represent minimum and maximum values. Black dots indicate values that fall outside 1.5 times the interquartile range. All graphs displayed are statistically different in rainy vs. dry seasons (p -value <0.05). Asterisks indicate statistical significance level. The drawings at the corner characterize the MBFG present in the graph.

3.4 DISCUSSION

MBFGs showed a tight association with the gradient of light and phosphorous concentrations observed in the lakes. We observed that MBFGs with traits to cope with low resources conditions were selected in mesotrophic and meso-eutrophic lakes, such as MBFG IV, V, and VI, these groups possess features to thrive in environments with relatively low nutrient concentrations, such as having flagella and mixotrophy. Contrasting, MBFG VII and VIII were selected in eutrophic environments, the high GALD and *s/v* characteristics from MBFG VIII and great buoyant properties from MBFG VII enhance light interception by the phytoplankton cells being favoured in low light conditions. Furthermore, the contrasting effects of the dry and rainy seasons on environmental parameters selecting distinct MBFGs were not a generalized pattern among the studied lakes, as expected. For the majority of the lakes (i.e., 4 out of 6) seasonality did not present a clear influence on environmental filters and/or MBFG selection.

Nutrient availability is expected to be a stronger driver of phytoplankton dynamics at low trophic state lakes, while light availability became more important towards hypertrophy (Naselli-Flores et al., 2007b). Thus, a different selection of traits to cope with the limiting condition, nutrients, or light would be favoured according to the trophic status. Our findings corroborate this view. Species from MBFG IV have high growth rates, moderate tolerance to low nutrients concentrations, and are favoured under low light attenuation environments (Kruk & Segura, 2012; Segura et al., 2013), hence, this group is frequently associated with low trophic status waters or during transitional successional stages (Reynolds et al., 2002; Kruk & Segura, 2012; Marengo et al., 2020) in agreement with the conditions found in the mesotrophic and meso-eutrophic lakes. The environmental thresholds also evidenced that MBFG IV dominated when TP concentrations were equal to or below $47 \mu\text{g L}^{-1}$, which corresponds to meso- and meso-eutrophic conditions in the study lakes.

The presence of flagella characteristic for MBFG V enables these species to active foraging resources and adjust their position through the water column. Many species in this group produce resting stages cells, which increase their tolerance under unfavorable conditions (Litchman & Klausmeier, 2008; Kruk & Segura, 2012). Besides, some species within MBFG V are capable of mixotrophy (either phagotrophy or osmotrophy). This adaptative strategy provides alternative resources when the environment is limited by nutrients and/or light, thus increasing survival chances compare to strict autotrophs (Saad et al., 2016; Costa et al., 2019). Although nutrient concentrations never fall below the values determined as limited, SRP ($< 3 \mu\text{g L}^{-1}$, except month in Santa Cruz) or DIN ($< 100 \mu\text{g L}^{-1}$) (Reynolds, 2006; Chorus & Spijkerman, 2021), mixotrophic species were often observed in the mesotrophic lakes, Santa Cruz and Prata (*Peridinium* spp., *Cryptomonas* spp., and *Trachelomonas* spp.). Hence, mixotrophy may represent an important trait for benefit MBFG V in these lakes. Contrary to our findings, studies with lakes from the same semi-arid region, including Boqueirão lake studied here, have shown the association of mixotrophic species to severe low light conditions (Medeiros et al.,

2015; Costa et al., 2016, 2019), thus further studies are necessary to unravel the role of mixotrophy in semi-arid lakes.

MBFG VI contains species with a siliceous exoskeleton, lack of flagella and low light requirements frequently found in turbulent and low light environments (Kruk et al., 2010; Izaguirre et al., 2012; Trindade et al., 2021). Here, however, this group (mainly small Centrales) thrived in a low trophic status environment with a relatively deeper euphotic zone compared to the other lakes, as evidenced by the environmental threshold to MBFG VI dominance (i.e., ≥ 4 m). Under well-mixing conditions in a tropical semi-arid region, small Centrales may benefit from a diurnal convective mixing pattern within the epilimnion (partial atelomixis) (Barbosa & Padisák, 2002). Furthermore, diatoms are known to be good competitors for phosphorus, and this group is also frequently associated with low trophic states with low light attenuation in the water column (Kruk & Segura, 2012; Segura et al., 2013; Bortolini et al., 2019; Magalhães et al., 2020).

Towards hypertrophy, traits able to cope with light limitation increase their survival chances, therefore, are selected under eutrophic conditions. Our results also corroborate this view. MBFG VII (large mucilaginous colonies) and VIII (nitrogen-fixing cyanobacteria) dominated in the eutrophic lakes, still, MBFG VIII dominated in shallow euphotic zones (i.e., $Z_{eu} \geq 1.1$ m) compared to MBFG VII. Higher trophic states associated with the increase of phytoplankton biomass, diminish water transparency and select traits, such as those having gas vesicles, typical from MBFG VII. The species from this group can control buoyancy according to light availability and even accumulate on the surface intercepting the influx of light, thus reducing light penetrations on below depths, which provides a competitive advantage over other phytoplankton species (Huisman et al., 2018). Despite MBFG VII being dominant mostly in eutrophic conditions, this group also dominated, for at least one month, in all lakes. Nevertheless, the main species and biomass attained vary regarding the trophic state. The low s/v characteristic of MBFG VII makes them sensitive to low nutrient concentration, which is evidenced by the low biomass achieved for MBFG VII in mesotrophic conditions compared to other groups. Besides, the inclusion of species from environments with different trophic statuses into this group limits the effectiveness to assess lake conditions (Pacheco et al., 2010; Bortolini et al., 2016).

In this study, MBFG VIII was exclusively found in eutrophic lakes. This group thrives under enriched, turbid with low water transparency (Padisák & Reynolds, 1998; Brasil & Huszar, 2011; Bonilla et al., 2012; Magalhães et al., 2020). The elongated shape of the algae belonging to this group increases light-harvesting capacity and competitiveness under light-limiting conditions. Species from MBFG VIII also can fix atmospheric nitrogen via heterocytes and thrives under nitrogen-limited conditions. However, nitrogen was always above values considered limiting during the study, and this group was favoured in lakes with high nitrogen concentrations, evidencing that N_2 fixation is not the trait benefiting these species in eutrophic tropical shallow lakes.

Water temperatures were constantly high during our study, with negligible annual temperature oscillations, and did not play a significant role in explaining MBFG variability. As mentioned earlier,

rain instead of temperature is the most important natural forcing factor determining seasonality in tropical semi-arid regions. Nevertheless, the typical contrasting effects of the rainy and dry seasons on environmental filters selecting distinct MBFGs were not generalized among the studied lakes: Out of six lakes studied, only two showed seasonal differences in environmental filters selecting distinct MBFGs.

The groups MBFG VIII and MBFG IV were linked to the rainy season, at Pajucara and Encanto lakes, respectively, when the higher concentration of nutrients (phosphorous, nitrogen), inorganic suspended solids, and chlorophyll-a led to a shallower euphotic zone. In Encanto lake, the representative species was *Pseudanabaena* spp. (MBFG IV), typically found in low-light environments, due to its elongated shape, similar to MBFGs III and VIII. This pattern was followed by the collapse of MBFG VIII in the dry season, when the concentration of nutrients declined, resulting in the deepening of the euphotic zone, highlighting the association of MBFG VIII with enriched, turbid, and low water transparency conditions (Padisák & Reynolds, 1998; Brasil & Huszar, 2011; Bonilla et al., 2012; Kruk & Segura, 2012; Magalhães et al., 2020). Meanwhile, in Encanto lake, MBFG VII benefited from the decline of nutrients and deepening of the euphotic zone in the dry season, which is expected considering this group was already linked with low light attenuation conditions (Kruk & Segura, 2012). In addition, despite the higher concentrations of total phosphorous and dissolved nitrogen during the rainy season in Tabatinga resulted in higher phytoplankton biomass, it was not related to the selection of any particular MBFG.

The other three lakes did not show a clear seasonal difference regarding environmental filters and MBFG selection; for them, seasonal differences were observed either on environmental filters or MBFGs. In Santa Cruz, the higher total nitrogen concentration in the rainy season did not reflect the distinct selection of MBFG. Although the biomass of MBFG V and VIII differ among seasons in Prata and Boqueirão, respectively, these changes were not associated with the seasonality of any environmental filters included in the scoop of this study.

Our results showed that not all lakes presented seasonal environmental differences associated with MBFG selection, highlighting lake-dependent responses. Nevertheless, when indeed seasonal differences were evidenced in the environmental filters, the rainy season was always linked with higher concentrations of nutrients and suspended solids, along with a reduced euphotic zone contrasting to the dry season. Here, it is important to highlight that our study does not refute several others, within the same region, which shows that the rainy season dilutes nutrients concentration resulting in the deepening of the euphotic zone, while the dry season intensifies eutrophication promoting cyanobacteria dominance (Braga et al., 2015; Brasil et al., 2016; Costa et al., 2016; Figueiredo & Becker, 2018; Rocha Junior et al., 2018; Braga & Becker, 2020). Instead, our results evidenced that other effects than nutrient dilution may be expected when the rainy season is not associated with the increase in water depth or volume. In fact, less intense periods of rain increase nutrient concentration via short-term runoff, which does not increase water depth and subsequently dilution effects, in contrast

to long rainy periods (Stockwell et al., 2020). Despite the amount of rainfall during the studied year 2017 was in general above the average of the prolonged drought period (2012-2016), it remained below the historical average, thus did not lead to significant differences in water levels among seasons. Furthermore, the majority of lakes (i.e., 5 out of 6) were very shallow and rain events might have resuspended sediment through increased water column mixing (Søndergaard et al., 2003; Marion et al., 2017).

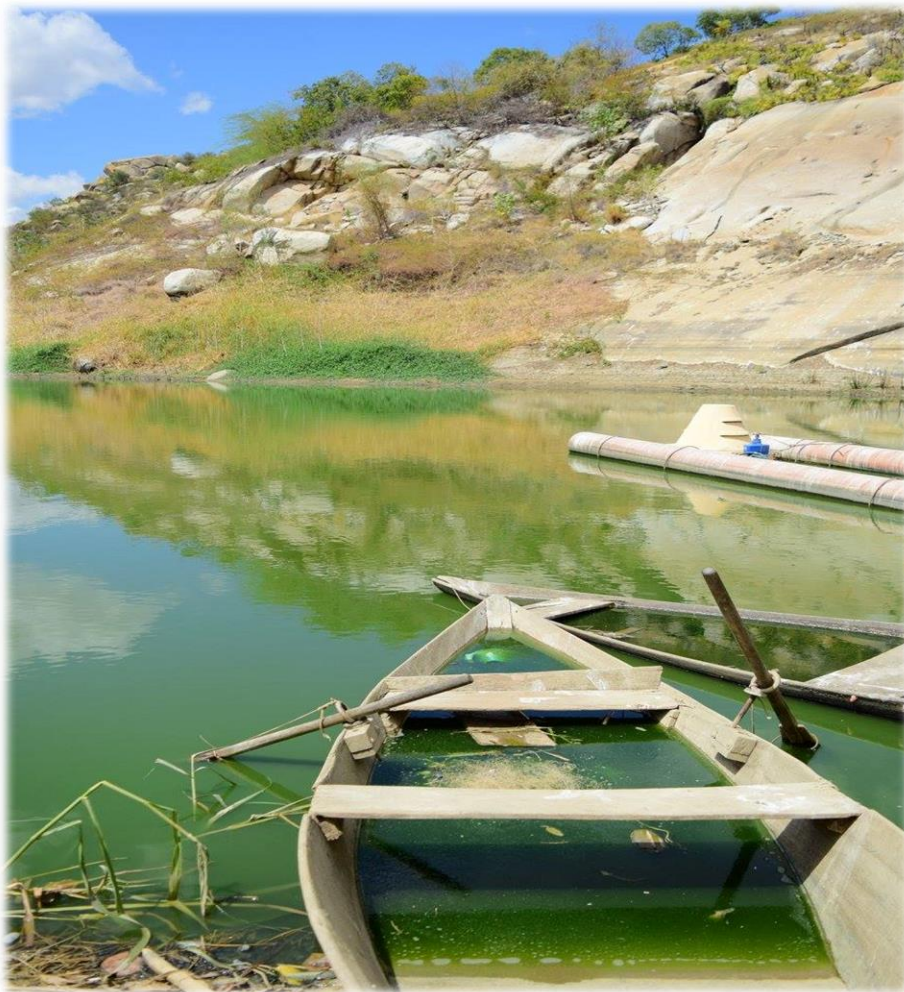
In summary, the application of the functional approach associated with the identification of environmental thresholds improves our knowledge of environmental drivers structuring the phytoplankton assemblage and increases the capacity to manage and mitigate water quality under ongoing environmental changes. Our results showed a tight association between morpho-functional traits and the gradient of light and phosphorous concentrations displayed by the lakes, highlighting that MBFG could be used as an effective tool to assess lake conditions. Like all functional approaches, the MBFG (Kruk et al., 2010) has advantages and limitations. It is beneficial for being objective and easy to apply; however, this simplification reduces its sensitivity to capture important phytoplankton traits that would be necessary to fully comprehend the functionality of the system, including mixotrophy, potential nitrogen fixation, and inclusion of species indicating other trait selective drivers within the same group, such as MBFG IV. Overall, MBFGs association with the trophic status along with the determination of environmental thresholds via machine learning methodology (CART) could offer a handy tool, mainly for environmental agencies, to monitor, manage, and even predict cyanobacterial blooms in Brazilian semi-arid regions.

4. CHAPTER II

THIS CHAPTER IS BASED ON THE FOLLOWING PAPER:

ABIOTIC FACTORS DRIVING CYANOBACTERIAL BIOMASS AND COMPOSITION UNDER PERENNIAL BLOOM CONDITIONS IN TROPICAL LATITUDES

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Cover photo: Gargalheiras Lake, Brazil by Dr. Vanessa Becker

4.1 INTRODUCTION

The role of eutrophication triggering harmful cyanobacterial blooms has long been recognized as a critical threat to freshwater ecosystems (Paerl, 1988). Cyanobacteria blooms alter energy flux in aquatic food webs, leading to a loss of biodiversity and threatening ecosystem function and services (Paerl & Otten, 2013). These blooms are frequently associated with the production of cyanotoxins, presenting a potential health hazard to several organisms *via* contact or ingestion, including pets, livestock, wildlife, and also humans, especially when drinking water supplies are affected (Carmichael, 1992; Merel et al., 2013; Huisman et al., 2018). Besides, cyanobacteria bloom promotes great economic losses, such as the increase in water treatment cost for drinking supply, decline of commercial and subsistence fishing and aquaculture, and impairment of recreational use of freshwaters (Aylward et al., 2005; EPA, 2015; Le Moal et al., 2019).

Cyanobacterial blooms have been magnifying worldwide regarding their distribution, recurrence, and magnitude. Besides eutrophication, other environmental pressures also benefit their global expansion, such as the combined and even interactive effects of eutrophication and climate change, especially warming (Wagner & Adrian, 2009; Moss, 2011; Paerl & Paul, 2012; Burford et al., 2016; Harke et al., 2016; Salmaso et al., 2018). Cyanobacteria dominance in freshwaters is triggered by the combination of drivers well described in the literature, including anthropogenic nutrient over-enrichment, namely from excessive loads of phosphorus and nitrogen, high temperatures and light intensity, shallow depth, low flushing events, high inorganic turbidity (Soares et al., 2009; Moss, 2011; Kosten et al., 2012; Rigosi et al., 2014; Costa et al., 2016; González-Madina et al., 2019; Havens et al., 2019). Selective grazing pressure may also favour cyanobacteria bloom as many species possess grazing defences against zooplankton, such as morphology when exceeding the size of filter-feeding apparatus inhibiting the uptake and/or intracellular toxic metabolites, thus, zooplankton tends to avoid cyanobacteria and selectively feed on eukaryotic species in readily edible size range (i.e., <30 μm) (Chislock et al., 2013; Leitão et al., 2018).

Seasonal droughts associated with the reduction of water levels also have a key role in cyanobacteria bloom dynamics (Naselli-Flores, 2003; Medeiros et al., 2015; Brasil et al., 2016; Tilahun & Kifle, 2019), especially in semi-arid regions where dry periods are part of the hydrological cycle. The negative water balance during dry periods reduces lake water depth and volume and subsequently affects several physical, and chemical parameters, thus consequently phytoplankton composition and dynamics (Olds et al., 2011; Mosley, 2015). The majority of studies linked to dry periods reported on cyanobacteria dominance (Bouvy et al., 2000, 2003; McGregor & Fabbro, 2000; Naselli-Flores, 2003; Figueiredo & Becker, 2018; Havens et al., 2019; Braga & Becker, 2020). However, mixotrophic organisms were favoured under extremely dry periods in shallow lakes as a consequence of the high inorganic turbidity and extremely reduced euphotic zone which limited cyanobacterial overgrowth

(Costa et al., 2016, 2019). Overall, there is little doubt that droughts result in the decline of not only water quantity but also quality.

In tropical semi-arid regions, cyanobacterial blooms are more recurrent and tend to persist longer compared to higher latitudes where key drivers, such as light availability and temperature fluctuate seasonally (Lind et al., 2016). Hence, perennial cyanobacterial blooms have already been reported in several eutrophic waterbodies with the absence of such seasonality (Figueredo & Giani, 2009; Muir & Perissinotto, 2011; Figueredo et al., 2016; Batista et al., 2018; Giani et al., 2020). However, the drivers regulating the phytoplankton assemblage composition and dynamics during persistent blooms are less explored. In order to achieve successful water quality management, the identification of local drivers regulating cyanobacterial assemblage is required (Mantzouki et al., 2016; Moura et al., 2018; Le Moal et al., 2019). Yet, only a few studies have reported the environmental factors regulating cyanobacteria compositions and dynamics under perennial blooms in the tropical region.

Despite several traits being shared, cyanobacteria are a heterogeneous group responding differently to environmental conditions and biotic interactions. Therefore, the link between traits and environmental drivers is necessary to target lake-specific management actions which lead to water quality improvement. As an example, *Microcystis* and *Raphidiopsis* are typical bloom forming genera in tropical waters (Soares et al., 2013; Brasil et al., 2016; Tilahun & Kifle, 2019). Despite both *Microcystis* and *Raphidiopsis* being associated with eutrophic waterbodies (Soares et al., 2013) they present different habitat preferences. *Raphidiopsis* is shade-tolerant typically thriving in mixed, shallow and hypertrophic environments (Padisák, 1997; Reynolds et al., 2002; Soares et al., 2013; Bonilla et al., 2016; Recknagel et al., 2019). Contrary, *Microcystis* is adapted to higher light intensity and low turbulence (Paerl et al., 1985; Huisman et al., 2004; Soares et al., 2009, 2013; Mowe et al., 2015; Torres et al., 2016). Additionally, *Raphidiopsis* is capable of nitrogen fixation and is anticipated to become dominant under nitrogen-limited conditions, whereas *Microcystis* is expected to prevail when nitrogen availability is not a growth-limiting factor (Spröber et al., 2003; Schindler et al., 2008; Paterson et al., 2011; Mowe et al., 2015). These genera may produce distinct toxins, *Microcystis* mostly produces the hepatotoxin microcystin, on the other side, *Raphidiopsis* may synthesize a diverse array of neurotoxins including saxitoxin, neosaxitoxin, gonyautoxins, and cylindrospermopsin (McGregor & Fabbro, 2000; Fonseca et al., 2015; Cirés & Ballot, 2016; Cirés et al., 2017).

Here in this chapter, we aim to (i) describe the cyanobacteria assemblage composition, (ii) evaluate the effect of dry periods associated with the reduction of water depths on environmental parameters and cyanobacterial dominance, and (iii) identify environmental preference of the two most common genera *Microcystis* and *Raphidiopsis* during a perennial bloom. To answer these, we evaluated six shallow multi-use lakes in the Brazilian semi-arid tropical region during a prolonged drought.

4.2 METHODOLOGY

The study site, climatic *scenario*, and sampling analysis were the same as described in the methodology of **Chapter I** in sections 3.2.1, 3.2.2, and 3.2.3 respectively.

4.2.1 “*RAPHIDIOPSIS* INDEX (R-INDEX)”

To identify the environmental filters regulating *Raphidiopsis* or *Microcystis* dominance during a perennial bloom, we developed the “*Raphidiopsis* index” (i.e., R-index), which is the ratio of *Raphidiopsis* biomass and the sum of *Raphidiopsis* plus *Microcystis* biomass (see index in Fig.10). Here, 0.5 means equal biomass of both taxa, 1.0 represents absolute dominance of *Raphidiopsis*, and 0 (zero) indicates absolute dominance of *Microcystis* (see example in Fig. 10). The R-index was only applied when the bloom was dominated by *Raphidiopsis* and/or by *Microcystis* occurring in three of the eutrophic lakes. The R-index was applied to all abiotic variables measured (Z_{eu} , Z_{max} , TP, SRP, TN, TDN, TSS, VSS, and FSS) to identify the environmental preferences of *Microcystis* or *Raphidiopsis*.

$$R - index = \frac{Raphidiopsis}{Raphidiopsis + Microcystis}$$

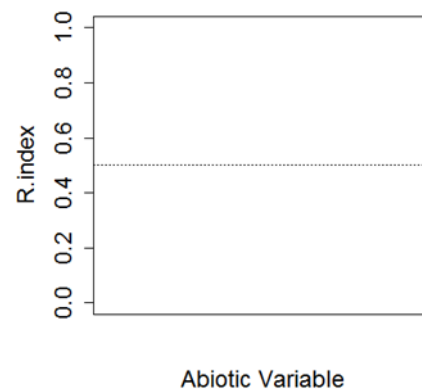


Fig. 10 R-index equation and a graph exemplifying R-index results. 0.5 (dotted line) means equal biomass for both taxa, 1.0 represents absolute dominance of *Raphidiopsis* and 0 (zero) indicates absolute dominance of *Microcystis*.

4.2.2 STATISTICAL ANALYSES

A detrended correspondence analysis (DCA) was applied to identify the most suitable constrained ordination method for the biotic data (Ter Braak & Prentice, 1988). Following, a Redundancy Analysis (RDA) was performed to investigate the relationship between cyanobacteria assemblage (i.e., using the relative biomass of each genus) and environmental filters. The abiotic variables used were selected based on their variance inflation factor (VIF); only variables with a VIF below 10 were used to avoid multi-collinearity (Hair et al., 1995). Hence, only Z_{eu} , Z_{max} , Temp, SRP, TDN, VSS, and FSS were included in the analysis. Abiotic data were $\log_{10}(x+1)$ transformed while the relative biomass were converted *via* Hellinger transformation (Legendre & Gallagher, 2001). A post-hoc test (ANOVA) was performed to test the significance of the RDA axis. Here, we evaluate all lakes together to identify the relation between Z_{max} , total and dissolved nutrients, phytoplankton, and cyanobacterial biomass were carried out *via* single-factor generalized linear models (GLM). The deviance value (%D) provided by the GLM analysis indicates the variability of the dependent variable which is explained by the

independent variable(s). The normality of the data was evaluated by the Shapiro-Wilk test and when necessary, variables were $\log_{10}(x)$ or $\log_{10}(x+1)$ transformed to improve the assumptions of linearity: Concentrations of TP, SRP, TN, and TDN were $\log_{10}(x)$ transformed; the biomass of cyanobacteria and phytoplankton were $\log_{10}(x+1)$ transformed. The criterion of $p < 0.05$ was considered statistically significant. All statistical analyses were carried package in the R software

4.3 RESULTS

The dominance of cyanobacteria increased towards hypertrophy (Fig. 11). In the mesotrophic lakes, different taxonomic groups contributed to the phytoplankton assemblage. In Santa Cruz, Bacillariophyceae dominated mostly, yet cyanobacteria, Euglenophyceae, and Cryptophyceae dominated at least one month each (Fig. 11a). Meanwhile, Prata was mainly characterized by Chlorophyceae dominance, still cyanobacteria and Dinophyceae also contributed to the phytoplankton assemblage (Fig 11b). In Tabatinga, the meso-eutrophic lake, cyanobacteria had a more expressive dominance and co-dominated with Bacillariophyceae (Fig 11c). In the eutrophic lakes, Encanto, Boqueirão, and Pajuçara, cyanobacteria dominated all year around, save one month in Encanto and Boqueirão each when Bacillariophyceae dominated (Fig 11d, 11e, Fig 11f).

Five genera of cyanobacteria were found during the study, nevertheless, the dominant genera differed among lakes and over time (Fig. 12). The lowest biomass of cyanobacteria was found in the mesotrophic lakes, Santa Cruz followed by Prata being characterized by *Aphanocapsa* and *Planktolyngbya* (Fig. 12a; 12b). In the meso-eutrophic lake, Tabatinga, cyanobacteria biomass was closer and even equal to the total biomass in some months, and was mainly composed of *Planktolyngbya* and *Microcystis* (Fig. 12c). Regarding the eutrophic lakes, cyanobacteria biomass was nearly equal to total phytoplankton biomass in Encanto, being mainly composed of *Pseudoanabaena*, *Raphidiopsis*, and *Microcystis* (Fig. 12d), while in Boqueirão and Pajuçara, cyanobacteria biomass was equal to the total phytoplankton biomass and the main genera were *Raphidiopsis* and *Microcystis*, but they rarely co-occurred (12f; 12f).

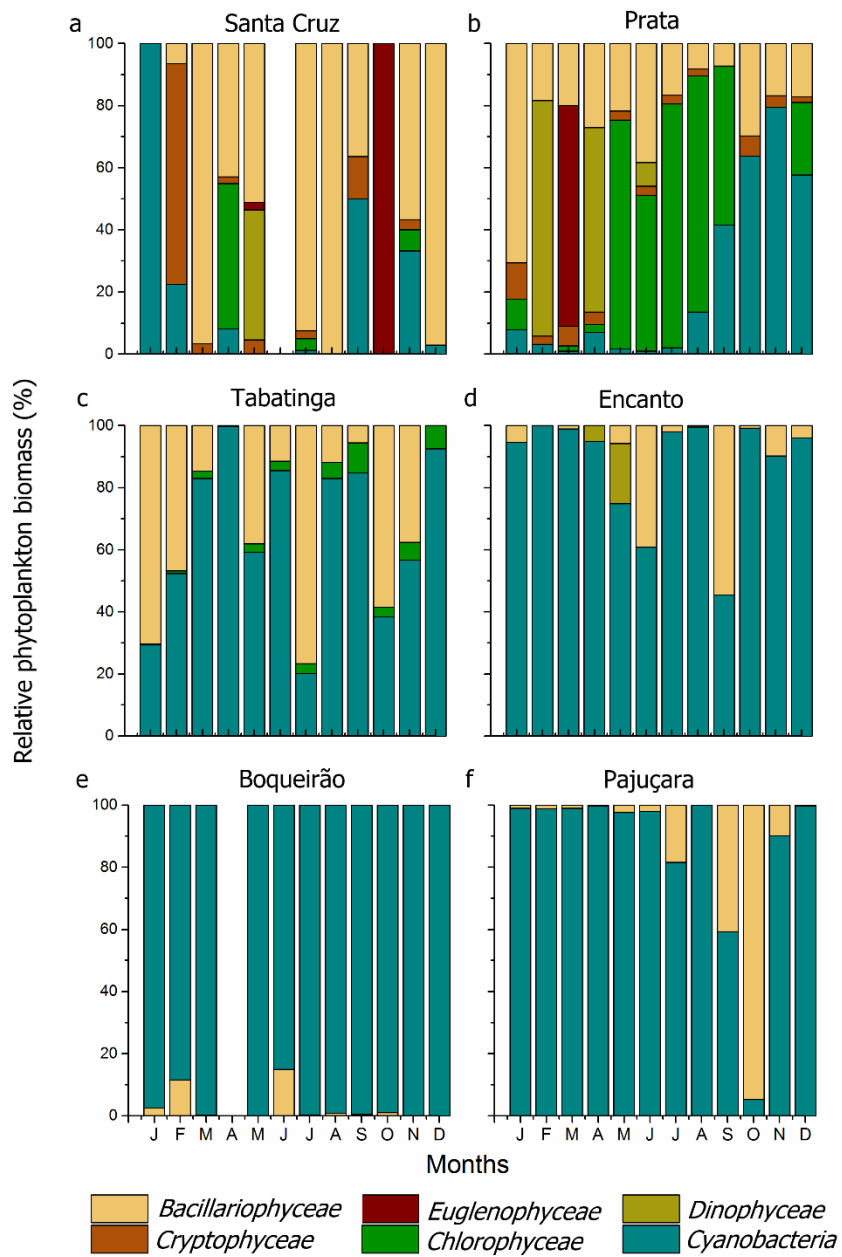


Fig. 11 Phytoplankton taxonomic composition shown as relative biomass of each phytoplankton group in 2017 for each studied lake.

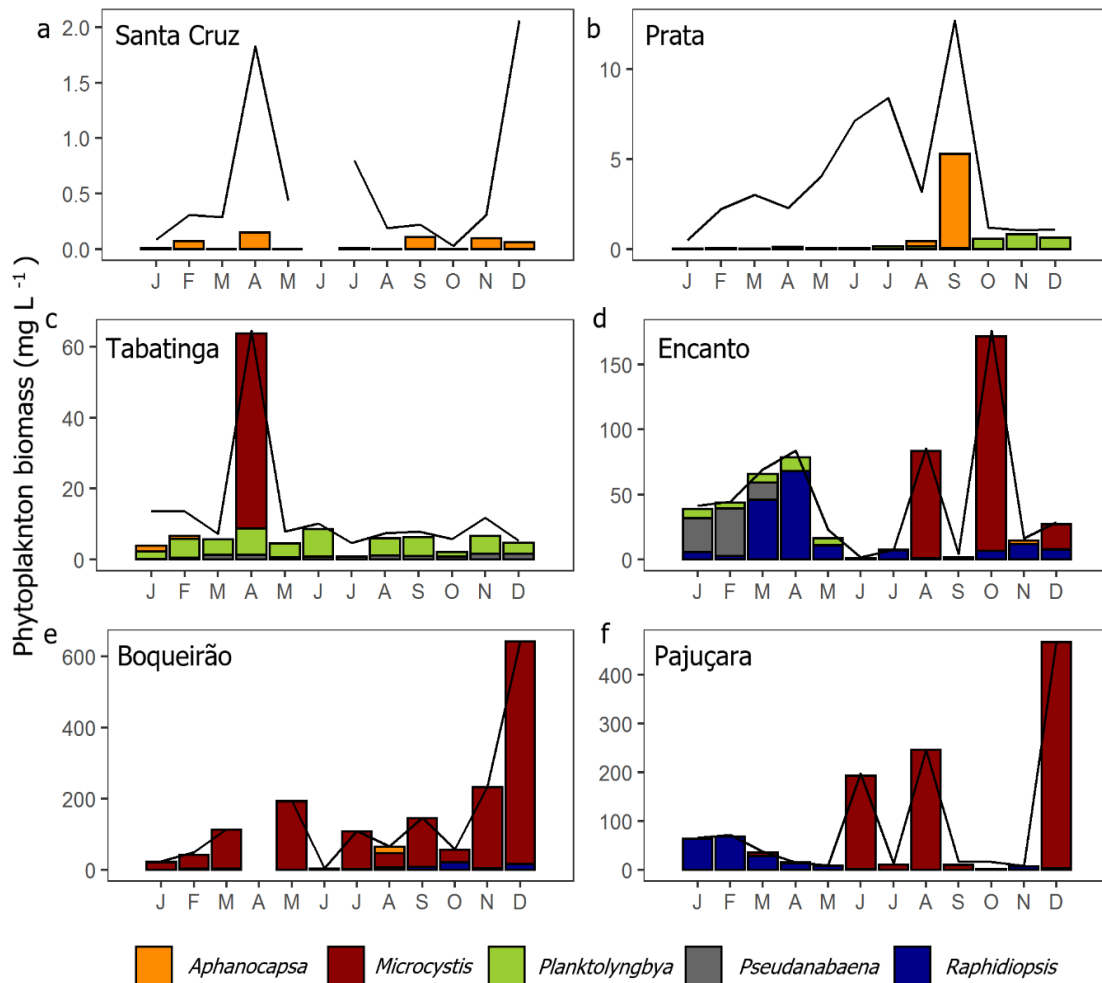


Fig. 12 Cyanobacteria dominance in each studied lake study during 2017 (colour bars) and phytoplankton total biomass (solid black line).

The seven abiotic variables considered in the RDA analysis explained 35% of the biomass variation of five cyanobacteria genera, *Raphidiopsis*, *Microcystis*, *Planktolyngbya*, *Aphanocapsa*, and *Pseudoanabaena* (Fig. 13, $R^2 = 0.35$; R^2 adjusted = 0.25). Axes 1 and 2 explained 12.7% and 6.2%, respectively. The Monte Carlo test indicated that the eigenvalue of both axes was statistically significant ($p < 0.001$). The RDA coefficients indicated that Temp (0.41), SRP (-0.51), were mostly correlated to axis 1, while Z_{\max} (-0.94), TDN (0.58), VSS (0.72), and FSS (0.48), were correlated to axis 2. On the negative side of axis 1, the biomass of *Raphidiopsis* and *Microcystis* was related to a higher concentration of SRP in samples from the eutrophic lakes (Boqueirão, Encanto, and Pajuçara). On the positive side of axis 1, *Planktolyngbya* biomass was associated with lower concentrations of SRP in Tabatinga (meso-eutrophic) and some samples from Prata (mesotrophic). On the negative side of axis 2, *Aphanocapsa* biomass was related to higher Z_{\max} and low concentration of suspended solids (VSS and FSS). Axis 2 separated the lake according to the trophic status. According to the RDA, among all abiotic variables, Z_{\max} , Z_{eu} , and VSS explained most of the variation in cyanobacterial biomass and composition.

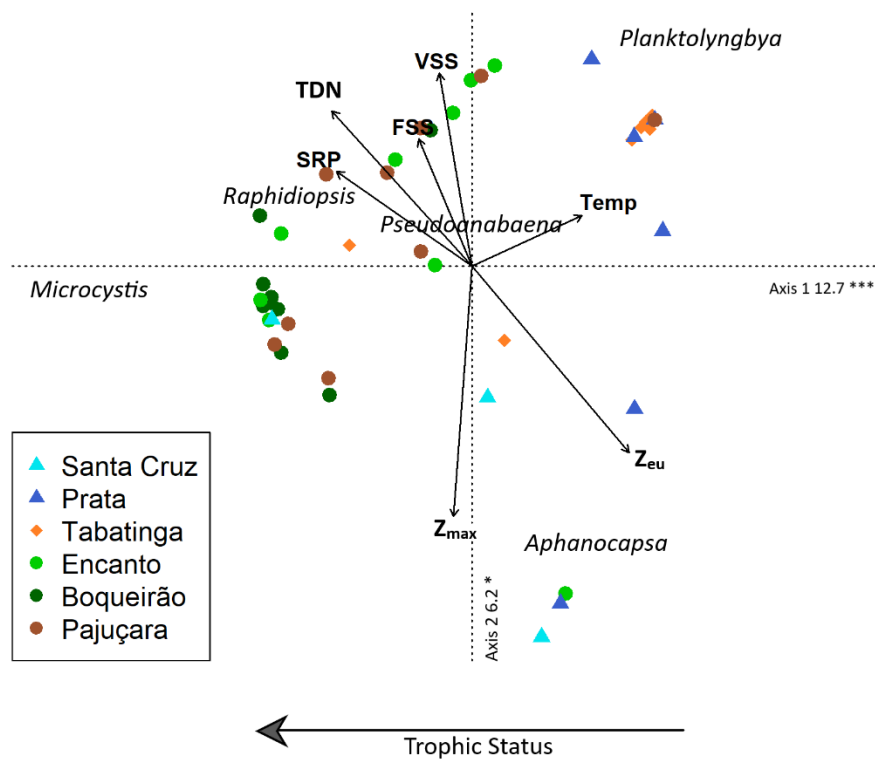


Fig. 13 Redundancy Analysis (RDA) applied to the relative biomass of cyanobacteria genera and abiotic variables in the six studied lakes. Symbols indicate the lake's trophic status: Triangle is mesotrophic; the diamond is meso-eutrophic and the circle is eutrophic. Arrow indicates the trophic tendency. Degree of significance: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Generalized linear regressions indicated that Z_{max} was significantly and negatively correlated to TP concentration ($p < 0.001$, Fig. 14a), SRP, TDN, TN (Table 4), and total phytoplankton biomass ($p < 0.001$, Fig. 14b). Based on the deviance values, the maximum depth zone explained 28% and 22% of the variability in TP concentration and phytoplankton biomass, respectively. Moreover, cyanobacterial and total phytoplankton biomass were significantly and positively related to TP ($p < 0.001$, Fig. 14c; 14d) but also to TDP, TN, and TDN (Table 4). Based on the deviance value, TP explained 40% of the variability in cyanobacteria and 38% in phytoplankton biomass.

Table 4 Summary results of the generalized linear models (GLM). The explanatory and response variable for each GLM is shown together with the regression equation and % deviance. All regressions were significant at $p < 0.001$.

Response Variable	Explanatory Variable	Equation	D ²
SRP	Z_{max}	$\log_{10}(y+1) = -0.03x + 1.52$	18.34
TN	Z_{max}	$\log_{10}(y+1) = -0.02x + 3.32$	18.81
TDN	Z_{max}	$\log_{10}(y+1) = -0.03x + 3.23$	21.45
TP	Z_{max}	$\log_{10}(y) = -0.03x + 1.91$	27.91
Phytoplankton	Z_{max}	$\log_{10}(y+1) = -0.07x + 1.63$	22.38
Cyanobacteria	TP	$\log_{10}(y+1) = 1.84\log_{10}(x+1) - 2.18$	36.98
Phytoplankton	TP	$\log_{10}(y+1) = 1.62\log_{10}(x+1) - 1.64$	37.73

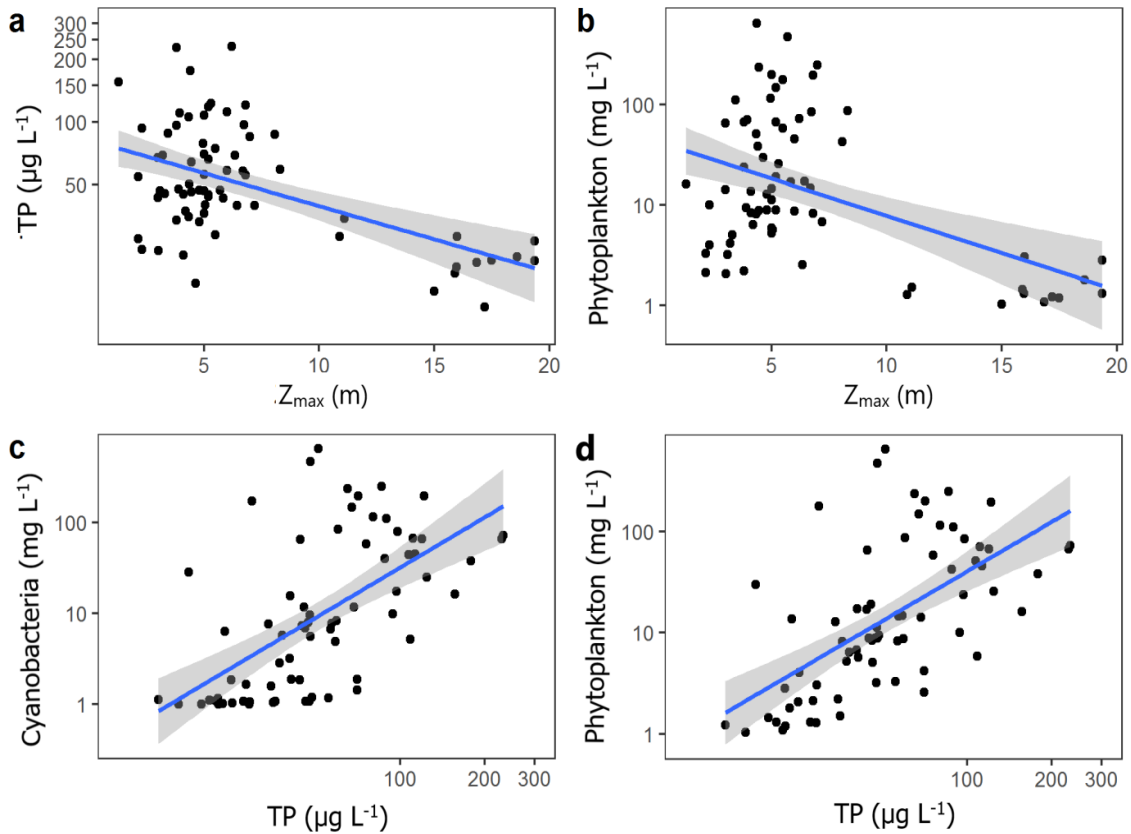


Fig. 14 GLM regressions showing the relationship between the maximum depth (Z_{\max}) and (a) log-transformed total phosphorus ($\mu\text{g L}^{-1}$), (b) log-transformed phytoplankton biomass (mg L^{-1}), and between (c) total phosphorus ($\log(x)$) and total cyanobacteria biomass (mg L^{-1}) $\log(x+1)$ transformed and (d) total phosphorus ($\log(x)$) and log-transformed phytoplankton biomass (mg L^{-1}). Dots represent all samples from the six study lakes from January to December 2017. The line shows the fit while the shaded area shows the 95% confidence interval for each regression model.

The eutrophic lakes displayed a perennial dominance of cyanobacteria, being mostly represented by the species *Raphidiopsis raciborskii* and *Microcystis aeruginosa*. The “R-index” was applied to all environmental variables measured to identify the range of environmental conditions that corresponded to the dominance of each species in Encanto, Boqueirão, and Pajuçara lakes. Based on the index we could find the environmental thresholds of each species and the differences in environmental preferences of each. Hence, we found that *Microcystis* only dominated when the concentrations of TP was $< 123 \mu\text{g L}^{-1}$ (Fig. 15a), TDN was $< 2260 \mu\text{g L}^{-1}$ (Fig. 15b), and TSS was $< 16.5 \text{mg L}^{-1}$ (Fig. 15c). Meanwhile, *Raphidiopsis* dominated in environments within the same range, but also environments with higher nutrient concentrations from $39 - 231 \mu\text{g L}^{-1}$ TP (Fig. 15a), $953 - 4910 \mu\text{g L}^{-1}$ TDN (Fig. 15b), and $3 - 47 \text{mg L}^{-1}$ TSS (Fig. 15c). Furthermore, *Microcystis* dominated in environments with a relatively deeper Z_{eu} and was never dominant when $Z_{\text{eu}} < 1.1 \text{m}$ (Fig. 15d). Conversely, *Raphidiopsis* dominated at a wider range of Z_{eu} , including those with a shallower $Z_{\text{eu}} < 1.1 \text{m}$ (Fig. 15d). *Microcystis* and *Raphidiopsis* dominance only appeared in lakes with Z_{\max} less than 5 m (data not shown). Temporal co-dominance between these two genera of bloom-forming cyanobacteria was seldom observed, i.e., out of a total of 36 samples only four had an R-index between 0.1-0.9, indicating that most of the time

only one of these species dominated and only once the R-index was between 0.4-0.6, which represents co-dominance.

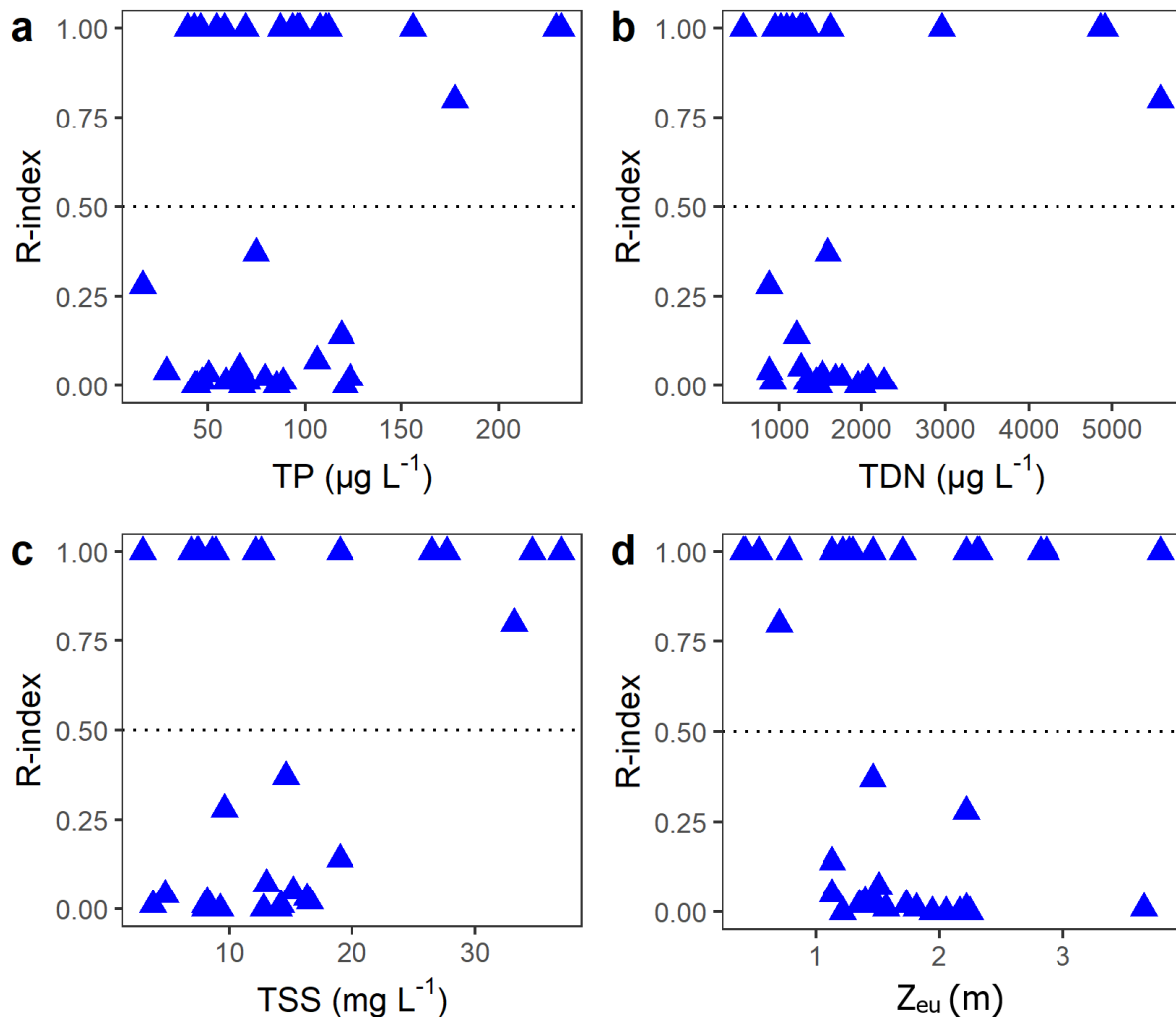


Fig. 15 *Raphidiopsis* Index about abiotic variables. Horizontal black dash lines represent equal biomass for both genera, 0 means 100% dominance of *Microcystis* and 1 means 100% dominance of *Raphidiopsis*. The environmental variables showed were the ones in which there were differences regarding the presence of the genera.

4.4 DISCUSSION

Phytoplankton and cyanobacteria biomass increased following the tendency to hypertrophy in tropical lakes. The frequency of cyanobacterial blooms changed according to trophic status, in the mesotrophic and meso-eutrophic lakes were episodic, while perennial in the eutrophic lakes. The increase of cyanobacteria dominance subsequently declined the relative contribution of eukaryotic organisms to the phytoplankton assemblage. Cyanobacteria blooms are driven by a variety of factors, including chemical and physical parameters, along with biological interactions, still, under no limiting light conditions, the concentration of nutrients, mainly P and N, is often the key driver of cyanobacteria blooms (Paerl, 2017). In accordance, perennial blooms were observed in all the eutrophic lakes studied, highlighting the potential for cyanobacterial last-long dominance in tropical lakes with favourable conditions all year round (Figueredo & Giani, 2009; Soares et al., 2009; Figueredo et al., 2016; Lind et

al., 2016; Batista et al., 2018). Low water depth associated with a high concentration of nutrients positively influenced phytoplankton and cyanobacteria biomass, which is evidence of the role of drought associated with water level reduction in intensifying the effects of eutrophication (Naselli-Flores, 2003; Aldridge, 2011; Bakker & Hilt, 2016; Brasil et al., 2016; Costa et al., 2016; Havens et al., 2019). This pattern is frequently observed in arid and semi-arid regions, with drastic changes in water levels due to seasonality (rainy and dry) which modify physical and chemical water parameters influencing phytoplankton composition and dynamics (Braga et al., 2015; Brasil et al., 2016; Costa et al., 2016; Braga & Becker, 2020). Several studies have already reported that the reduction of water depth induced by drought often leads to a higher concentration of nutrients, favoring cyanobacteria dominance and leading to a decline in water quality (Naselli-Flores, 2003; Aldridge, 2011; Brasil et al., 2016; Moura et al., 2018; Rocha Junior et al., 2018; Havens et al., 2019). Our study supports them, as we obtained similar results, shallow lakes had higher concentrations of nutrient concentrations (total and dissolved) and a larger dominance of cyanobacteria over the phytoplankton community. However, when drought periods are not associated with water level reductions, other effects than dilution may be expected (as described in **Chapter I**).

Droughts are often associated with a reduction in water level, which increases the concentration of nutrients and consequently the dilution capacity in freshwater systems (Mosley, 2015). Moreover, drought increases the water retention time diminishing hydrologic flushing events, which may temporarily disrupt cyanobacteria blooms, thus, blooms tend to persist in the absence of these events (Paerl, 2018). The longer retention time also allows species with a slow growth rate to attain dense populations (Reichwaldt & Ghadouani, 2012). Furthermore, the reduction of water depth enhances internal processes, including sediment resuspension and internal phosphorus load. A study within the same region showed that semi-arid lakes are likely to release P trapped in the sediment under certain environmental conditions including low depths, wind drive mixing suspending the sediment, and anoxia on the sediment (Cavalcante et al., 2018).

The changes in cyanobacteria composition, besides biomass, were substantially influenced by environmental parameters. Our results show TP promotes phytoplankton and cyanobacteria biomass; while, light availability regulates cyanobacterial composition in the eutrophic lakes. The eutrophic lakes presented perennial cyanobacterial dominance, yet the dominance alternated between the two main bloom-forming genera (*Raphidiopsis* and *Microcystis*) which rarely co-occurred in time. For example, *Raphidiopsis* dominated in the rather turbid and eutrophic waters and was found in a wider range of environmental conditions contrasting with *Microcystis*. *Raphidiopsis* was observed up to the maximum total phosphorous concentration recorded in our study ($\sim 230 \mu\text{g L}^{-1}$), compared to *Microcystis* which did not occur at concentrations above $170 \mu\text{g L}^{-1}$. These results corroborate previous studies which already associated *Raphidiopsis* dominance with a broad range of total phosphorus concentrations (Padisák, 1997; Soares et al., 2013; Chislock et al., 2014; Bonilla et al., 2016; Burford et al., 2016). In accordance, a laboratory study has shown that *Raphidiopsis* has higher photosynthetic

activity and even more effective phosphorous uptake compared to other cyanobacteria, including *Microcystis* (Wu et al., 2009). In our study, both species were found on a similar gradient of SRP, which implies that turbidity, instead of TP, regulated the switch between both species.

Raphidiopsis was also observed at a high concentration of total dissolved nitrogen (i.e., $< 2500 \mu\text{g L}^{-1}$) at which *Microcystis* was not found. Despite being expected to thrive in nitrogen-limiting conditions due to the capacity to fix nitrogen (Reynolds et al., 2002), *Raphidiopsis* dominated under relatively N-rich conditions. Hence, N-fixation is not expected to give a competitive advantage to *Raphidiopsis* over *Microcystis* in tropical lakes under no limiting nitrogen concentrations. Complementary, *Raphidiopsis raciborskii* was shown to be an efficient facultative diazotroph, with high competitive strength under fluctuating levels of nitrogen (Hoffman et al., 2011; Burford et al., 2016)

The switch between *Microcystis* and *Raphidiopsis* in the perennial bloom was linked to water transparency ($Z_{\text{eu}} < 1.1\text{m}$). The increase of TSS associated with the decrease of the euphotic zone benefited *Raphidiopsis*, which is long recognized as a shade-tolerant species that can form blooms that persist despite self-shading (Padisák, 1997; Karadžić et al., 2013; Burford et al., 2016). Contrariwise, *Microcystis* forms surface blooms and tolerate high levels of UV radiation without the symptoms of photoinhibition due to the ability to synthesize photoprotective pigments and carotenoids (Paerl et al., 1985). Hence, *Microcystis* benefits in high-insolation environments and grows poorly in turbid environments (Reynolds et al., 2002; Reynolds, 2006; Batista et al., 2018). Our results support previous reports associating *Microcystis* dominance with elevated water transparency during perennial blooms (Soares et al., 2009; Batista et al., 2018). Moreover, the intraspecific variation is crucial for *Raphidiopsis*' success in a broad spectrum of environmental conditions and explains its successful expansion to different geographical areas highlighting the challenge of managing this species (Bonilla et al., 2012; Burford et al., 2016; Wilk-Woźniak et al., 2016; Recknagel et al., 2019). Overall, *Raphidiopsis* may be expected to replace *Microcystis* following the increase of nutrients and turbidity concentrations in eutrophic shallow tropical lakes during perennial blooms.

Microcystis and *Raphidiopsis* non-simultaneous occurrence during our study might also be due to biotic factors. For example, allelopathic compounds produced by cyanobacteria, including *Microcystis* and *Raphidiopsis*, are known to inhibit the growth of competing primary producers (Leflaive & Ten-Hage, 2007; Rzymiski & Poniedziałek, 2014). Among these two cyanobacteria, laboratory studies have shown that *Raphidiopsis* is a better competitor compared to *Microcystis* (Jia et al., 2020) with specific allelopathic effects inhibiting *Microcystis* growth when competition for resources is high (Mello et al., 2012; Rzymiski et al., 2014). Thus, understanding the drivers of cyanobacterial species composition during permanent tropical blooms would benefit from integrating both abiotic and biotic factors.

The other genera had a minor contribution to the phytoplankton community and were associated with the less enriched lakes. The shade-adapted filamentous cyanobacterium, *Planktolyngbya* (Fabbro & Duivenvoorden, 2000), was related to higher concentrations of suspended solids (VSS and FSS) and

lower SRP concentration, co-dominating with *Microcystis* year-round in the meso-eutrophic Tabatinga lake. *Planktolyngbya* is often found in mixed and turbid environments, with relatively lower values of phosphorous in Brazilian semi-arid lakes (Figueredo et al., 2016; Barroso et al., 2018). Meanwhile, *Aphanocapsa*, a coccoid cyanobacterium typical of eutrophic environments (Padisák et al., 2009), was observed in low trophic status lakes with a lower concentration of suspended solids and deeper water depth. Although *Aphanocapsa* was rare in the studied lakes, microcystin-producing variants from this genus have been frequently reported in other semi-arid lakes in Brazil (Domingos et al., 1999; Marcon et al., 2017), evidencing the potential threat considering these lakes are used for multipurpose.

Global warming triggering cyanobacteria dominance is not new novel in phytoplankton ecology (Paerl & Huisman, 2009; Rigosi et al., 2014; Visser et al., 2016; Paerl et al., 2020), even in the tropical region this association has been already evidenced (Bouvy et al., 2000; Huszar et al., 2000; Soares et al., 2009). However, we did not find any relation between water temperature and cyanobacteria biomass or composition. Furthermore, water temperatures were constantly high and often exceeding 25°C, which is considered optimal for cyanobacterial growth (Paerl, 2018). Complementarily, a microcosm experiment with samples from one of the eutrophic lakes studied here, Boqueirão, showed that the abundance of cyanobacteria (Reynolds functional groups **H1** and **M**) did not increase in the warm and nutrient-enriched treatment (Souza et al., 2018). Hence, the temperature is not a main driver of phytoplankton assemblage in tropical semi-arid lakes, where temperatures are already optimal and thus, non-limiting.

Overall, our results underpin the sensitivity of tropical semi-arid lakes to eutrophication due to year-round favourable conditions, high temperatures, and light availability, to cyanobacteria blooms and urge effective efforts to improve water quality. Equally important, we show that shallower lakes and subsequent increases in nutrient concentration are key factors leading to cyanobacteria dominance, which in the absence of constraints persist year-round. We also pointed out that reduced water transparency regulates the switch from *Microcystis* to *Raphidiopsis* dominance during a perennial in shallow tropical lakes. Complementarily, *Raphidiopsis* thrived under higher nutrients and suspended solids concentration (low light) in lakes where the temperature is not a constraint. Furthermore, reports on the dynamics of truly perennial cyanobacterial blooms are scarce in the literature and the present study provides support for the development of successful strategies for controlling harmful blooms in shallow lakes under warm and dry climates. We suggest future studies use the “*Raphidiopsis* index”, or similar indices, as an informative and easy tool to account for potential switches among species dominance.

5. CHAPTER III

INTER-SPECIFIC COMPETITION BETWEEN *PLANKTOTHRIX* AND *APHANIZOMENON* IN LAKE STECHLIN, GERMANY



Cover photo: Lake Stechlin, Germany by Dr. Peter Casper

5.1 INTRODUCTION

Spatial processes are key drivers in structuring phytoplankton distribution, especially within a lake considering that resources are not uniformly distributed (Naselli-Flores & Padisák, 2016). Hence, the water column structure has a primary role in affecting the phytoplankton community because it provides a major axis for spatial heterogeneity explaining the vertical distribution of phytoplankton (Mellard et al., 2011). Phytoplankton distribution in the water column is likely controlled by the combination of environmental factors, including light and nutrient availability, wind-induced mixing, and thermal stratification (Fee, 1976; Frempong, 1981; Gervais et al., 1997; Klausmeier & Litchman, 2001; Barbosa & Padisák, 2002; Becker et al., 2009), and biological factors as predation, herbivory avoidance, and parasitism (Lampert et al., 2003; Hampton et al., 2014; Van den Wyngaert et al., 2022). Besides, phytoplankton evolved many adaptive strategies, such as the ability to entrainment, along with a high degree of phenotypic plasticity to cope with the heterogeneity of microhabitats provided by the water column in order to maximize resource use efficiency in the competitive pelagic arena (Naselli-Flores & Barone, 2011; Naselli-Flores et al., 2021a).

Deep Chlorophyll Maximum (DCM) is a subsurface layer enriched in chlorophyll, which can be explained by many mechanisms, including *in situ* phytoplankton growth and or increase of Chl-a per cell (photoacclimation). It is frequently found in stratified marine and freshwater ecosystems (Camacho, 2006; Mignot et al., 2014; Leach et al., 2018; Cornec et al., 2021). This phenomenon is of great ecological importance due to being a hot spot for primary production, nutrient cycling, influencing the vertical movement of grazers, and providing vertical habitat gradients for primary consumers (Fee, 1976; Lampert et al., 2003; Ardyna et al., 2013; Leach et al., 2018; Zastepa et al., 2021). Although DCM has a wide distribution regarding latitude, lake size, and trophic status (Grigorszky et al., 2003; Padisák et al., 2003a; Ardyna et al., 2013; Cullen, 2015; Baldry et al., 2020; Scofield et al., 2020), the mechanism of its formation, characteristics (i.e., depth, magnitude, shape), and duration may differ across lakes due to differences in physical and biological factors (Cullen, 1982, 2015; Camacho, 2006; Mignot et al., 2011). Hence, a large knowledge gap remains in identifying the environmental drivers responsible for DCM formation and maintenance and the consequences in particular lake ecosystems (Brentrop et al., 2016).

Certain conditions are compulsory for DCM development, such as stratification: establishment of an epi-, meta- and hypolimnion system that regulates the input of nutrients. The epilimnion is frequently nutrient-limited or depleted in the euphotic zone due to the photosynthetic activities while deep layers are relatively nutrient-rich but lack light. Hence, the interface is limited to a narrow stratum rich in nutrients, typically cold, still with enough light availability for adapted phytoplankton species to grow under such conditions (Camacho, 2006; Cuypers et al., 2011; Selmeczy et al., 2016). Moreover, DCM is often observed in low trophic status environments, with nutrient limitation, and low productivity, which results in elevated water transparency, allowing light to penetrate and reach deep strata

supporting *in situ* growth. Despite this phenomenon being most common in oligotrophic environments, which frequently lasts all over the stratified period, it has been also observed in meso- and eutrophic conditions which may present different dynamics, thus, with phototrophic bacteria involved (Simmonds et al., 2015; Brentrup et al., 2016; Leach et al., 2018; Scofield et al., 2020)

Cyanobacteria blooms are increasing worldwide due to anthropogenic forces, and their detrimental consequences for water quality and ecosystem functioning in freshwaters are readily recognized (Paerl, 1988, 2018; Cottingham et al., 2015; Huisman et al., 2018). Many, if not all, planktic cyanobacterial taxa, can modify their buoyancy and form dense populations in deeper waters (Reynolds et al., 1987; Carraro et al., 2012; Selmeczy et al., 2016; Hamre et al., 2018). Contrary to surface cyanobacteria blooms, deep cyanobacteria blooms are not easy to observe since requiring a specific sampling procedure and these seldom reach the public interest (Erratt et al., 2022; Mi et al., 2022). Yet, these blooms present an additional human health threat when potentially toxic taxa occur at drinking water systems because water is often drained from depths where DCM may occur (Salmaso et al., 2014; Chorus & Welker, 2021; Zastepa et al., 2021). Furthermore, most of the research focussed on surface blooms, as deep blooms remain often seen as uncommon phenomena or a monitoring bias in freshwater environments, hence even less is known about toxin production at deeper blooms (Zastepa et al., 2021; Erratt et al., 2022).

In Lake Stechlin, the DCM has been widely explored, since after the DCM recognition by autotrophic picoplankton (*Cyanobium* spp.) a regular monitoring program has been implemented to unravel the mechanisms regulating such phenomena (Padisák et al., 1997). Accordingly, deep-layer populations of cyanobacteria occur regularly in the lake, formed mainly at the beginning of the monitoring program by *Cyanobium* and this pattern was recurrent for 15 years (Padisák et al., 1997, 2010). However, it changed reflecting the process of eutrophication driven by global warming ongoing within the lake. Recent studies showed the gradual decline of the *Cyanobium* population along with the increase of filamentous cyanobacteria's contribution to DCM, mainly *Planktothrix rubescens* (De Candolle ex. Gomont) Anagnostidis & Komárek, *Aphanizomenon flos-aquae* Ralfs ex. Bornet & Flahault and *Dolichospermum* spp. (Selmeczy et al., 2016, 2019). Moreover, on a unique occasion, *Cyanobium*, *Planktothrix rubescens* and *Aphanizomenon flos-aquae* appeared at the same time forming DCM but spatially segregated (Selmeczy et al., 2016).

The sudden increase in potentially toxic filamentous cyanobacteria since the beginning of the twenty-first century in Lake Stechlin raises concern regarding future scenarios for water quality and urges efforts to identify the key drivers along with management options. *Planktothrix rubescens* is typically found forming metalimnetic blooms in stratified deep oligo-mesotrophic. Blooms from this species have been increasing in frequency and magnitude not only in Lake Stechlin but also in several other lakes in the temperate region (Dokulil & Teubner, 2012; Gallina et al., 2017; Selmeczy et al., 2019; Knapp et al., 2021). Meanwhile, *Aphanizomenon flos-aquae* and *Dolichospermum* spp. are nitrogen fixer cyanobacteria frequently found to form surface blooms in eutrophic lakes with low

nitrogen concentrations, yet, since 2015 these species started to reach high biomass in the oligo-mesotrophic Lake Stechlin (Selmeczy et al., 2019).

Although the number of studies on deep maximum populations increased, only a few cases explore the temporal scale of the development, maintenance, and erosion *via* microscopic investigation, which would increase the ability to assess the ecological importance of DCM and provide important information for efficient water management. Hence, the evaluation of species distribution and changes in key gradients are essential to understand the ecological importance of this phenomenon. Based on this, the present chapter aims to evaluate i) the long-term temporal dynamics of cyanobacteria, and ii) to characterize the DCM formation, with a focus on the spatial distribution of *Planktothrix rubescens* and *Aphanizomenon flos-aquae* in Lake Stechlin, Germany.

5.2 METHODOLOGY

5.2.1 STUDY SITE

Lake Stechlin is a deep oligo-mesotrophic lake located on the northern border of Brandenburg in the Mecklenburg Lake District, North-Eastern part of Germany (Fig. 16) ($53^{\circ}10'/13^{\circ}02'$). The lake was formed during the last glacial period approximately 12,000 years ago. Due to its glacial origin, there is no regular water inflow, hence, the water level is regulated by the balance between precipitation, groundwater exchange, and evaporation. The typical stratification pattern is dimictic with the onset of the thermal stratification period frequently developing from April to June with the most stable thermocline occurring late in July. Inverse stratification is found during winter if the lake is covered by ice. However, inverse stratification is not a general pattern in Lake Stechlin since, depending on winter temperatures, it can remain mixed during winter presenting characteristics of warm monomictic lakes.

Lake Stechlin has a maximum and a mean depth of 69.5 m and 23.3 m, respectively, and covers a surface area of 4.25 km², while the catchment area consists of 12.4 km² (Casper & Koschel, 1995). Nutrient concentrations are given in Table 3. The lake is situated in a conservation park and 80% of its catchment area is covered by forest, mainly pine (*Pinus sylvestris* L.) (Selmeczy et al., 2019). The Stechlin-Ruppiner Land Nature Park was created in 2001 and protects around 862 km² including different types of protected areas (i.e., landscape protection areas and fauna-flora-habitat-areas).

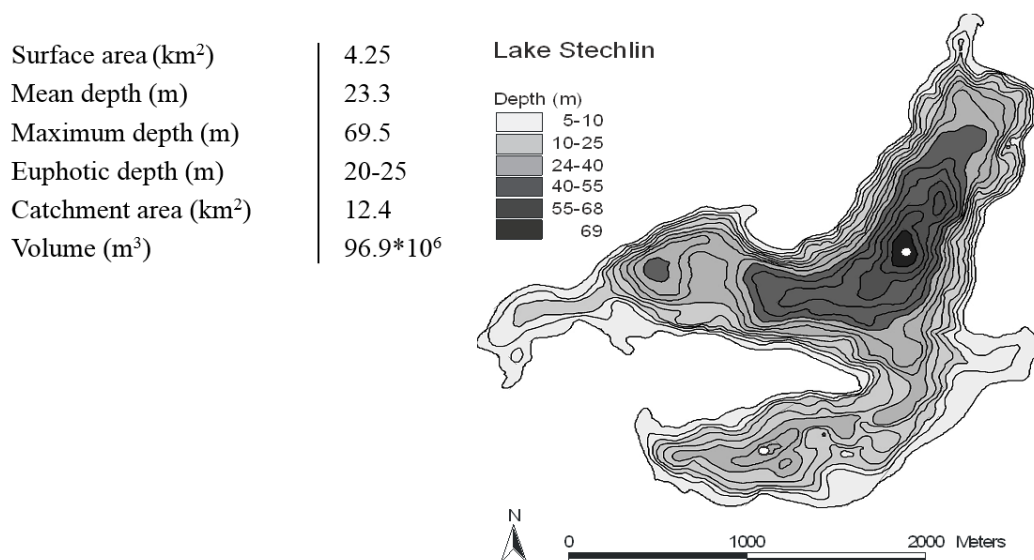


Fig. 16 Bathymetric map with morphological data of Lake Stechlin. The white dot on the deepest part of the lake indicates the sampling point. Figure modified from (Selmeczy et al., 2019).

Table 3 Nutrient concentrations (average, maximum and minimum values between parentheses) in the euphotic zone of Lake Stechlin from 2018 to 2019. Data provided by The Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB).

Nutrients	Concentrations (mg L ⁻¹)
Total Phosphorous (TP)	0.026 (0.01-0.056)
Soluble Reactive Phosphorous (SRP)	0.011 (0.001-0.042)
Total Nitrogen (TN)	0.519 (0.425-0.635)
Nitrite (NO ₂)	0.001 (0-0.003)
Nitrate (NO ₃)	0.032 (0-0.113)
Ammonium (NH ₄)	0.020 (0.001- 0.062)

Lake Stechlin went through several anthropogenic changes, which reflected its trophic status, such as the construction of the Polzow-canal connecting it to the River Havel, and the installation of the Nuclear Power Plant Rheinsberg (NPP), the latter one is well described in the literature. The NPP operated from 1966 to 1989. During this period, cooling water and domestic sewage were discharged into the originally oligotrophic Lake Stechlin (Casper, 1985; Casper & Koschel, 1995). Besides, water from the mesotrophic Lake Nehmitz was used as cooling water for the NPP and was then released into Lake Stechlin flowing back to Lake Nehmitz via an interconnecting canal. Despite the external input of nutrients from this long-lasting anthropogenic activity, Lake Stechlin remained oligotrophic due to different processes, which counteracted eutrophication, such as an increase in phosphorous binding capacity of the sediment and the reduction of the water retention time (Koschel et al., 1983, 2002; Koschel & Adams, 2003). Electricity generation by the NPP was terminated in 1989, but the cooling water system remained in operation until 1995. Yet, the effects on the lake's limnological parameters and phytoplankton composition still are being studied and evidenced.

The phytoplankton community of Lake Stechlin has been the subject of study since 1959, which makes it one of the most well-known lakes in Europe. After DCM recognition by the pico-sized cyanobacterium *Cyanobium*, a continuous monitoring sampling program was implemented in 1994 including measurements of water's physical and chemical parameters and primary production (Padisák et al., 1998). However, other species of cyanobacteria have also been reported to compose DCM in Lake Stechlin, namely *Planktothrix rubescens*, and *Aphanizomenon flos-aquae* (Selmeczy et al., 2016).

5.2.2 WATER SAMPLING

In order to explore the vertical distribution of *Planktothrix rubescens* and *Aphanizomenon flos-aquae*, water samples were collected monthly (March to October) from 2018 to 2020 in Lake Stechlin, except in March of 2018. The sampling point is located at the deepest part of Lake Stechlin (as indicated in Fig. 16). Water samples were taken with Van Dorn bottles every 5 m from the surface (0 m) to the bottom (65 m) resulting in measurements at 14 evenly distributed different depths. The samples were immediately preserved in Lugol's solution and stored in a dark place at room temperature. Temperature and Secchi disk data were provided by the Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB). The euphotic depth was determined as 2.7 times the measure obtained by the Secchi disk (Cole, 1994). *Planktothrix rubescens* and *Aphanizomenon flos-aquae* filaments were counted according to methodologies by Utermöhl (1958) and Lund et al. (1958). At least 200 units were counted per sample using an inverted microscope. Dimensions of at least 20 filaments/species/sample were measured and phytoplankton biomass was estimated based on cells' volume according to the geometric form of cylinders (Hillebrand et al., 1999) using cell counting software (OPTICOUNT, 2008).

To assess the dynamics of cyanobacteria in Lake Stechlin, we utilized long-term phytoplankton datasets from 1994 to 2021. Water samples were collected using a standardized methodology, from depths of 0-25m, allowing for comparison with previous years. The data and methodology, including phytoplankton counting, are detailed in Padisák et al. (2003b, 2010) and Selmeczy et al. (2019). The complete dataset for Lake Stechlin's phytoplankton from 1994-2020 can be found in the Leibniz Institute of Freshwater Ecology and Inland Fisheries Database (Padisák et al., 2023).

5.3 RESULTS

5.3.1 LONG-TERM PHYTOPLANKTON DATA SETS IN LAKE STECHLIN

The phytoplankton community and dynamics of Lake Stechlin have been continuously studied from 1994 until the current day. Since then, cyanobacteria's contribution to the total phytoplankton biomass has been gradually increasing over the years in Lake Stechlin (Fig. 17). From 1994 until 2009, cyanobacteria seldom contributed more than 30% of the total phytoplankton biomass, save in 1998 when reached cyanobacteria nearly 70% of the total phytoplankton biomass. Since 2010, cyanobacteria contributions have shown a growth tendency, with some years representing the main group to dominate the community (more than 50% of total biomass). In recent years, the dominance of cyanobacteria has

significantly increased, reaching unprecedented levels in Lake Stechlin. In 2020 and 2021, they accounted for nearly all of the phytoplankton biomass, representing over 85%.

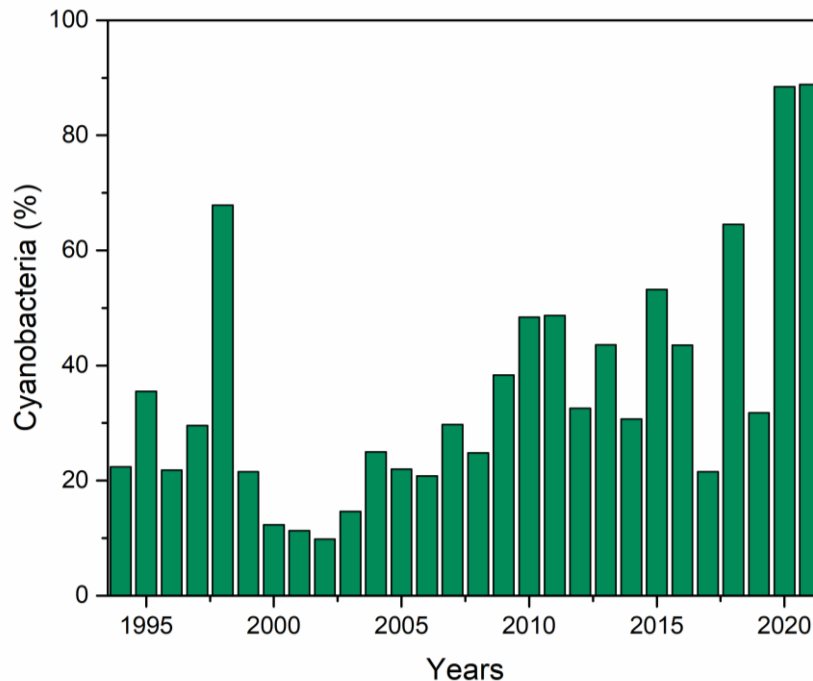


Fig. 17 Annual average of cyanobacteria contribution to the total phytoplankton shown as relative biomass in Lake Stechlin from 1994 to 2021.

Phytoplankton total biomass has been increasing over time in Lake Stechlin, more and more associated with the dominance of *Planktothrix rubescens*, *Aphanizomenon flos-aquae*, and *Dolichospermum* spp. Since 1994, *Planktothrix rubescens* and *Dolichospermum* spp. have mostly been found every year as a part of the phytoplankton community of Lake Stechlin, while *Aphanizomenon flos-aquae* was first recorded only in 2001 (Fig. 18). Until 2005, these species were observed in sparse biomass, except in 1998 when *Planktothrix rubescens* bloomed ($524.5 \mu\text{g L}^{-1}$). *Dolichospermum* spp. started to gradually increase its biomass after 2005. After that, *Aphanizomenon flos-aquae* biomass started to increase with peaks in 2010 and 2012 (453.3 and $400.9 \mu\text{g L}^{-1}$), with an intermittent peak of *Dolichospermum circinale* (Rabenhorst ex Bornet & Flahault) Wacklin, Hoffmann & Komárek in 2011 ($208.3 \mu\text{g L}^{-1}$). In the following years, *Planktothrix rubescens* biomass started to increase, however, *Dolichospermum lemmermannii* (P.G.Richter), P. Wacklin, L. Hoffmann & J. Komárek. bloom overcame *Planktothrix rubescens* biomass ($655.3 \mu\text{g L}^{-1}$). In 2017, *Planktothrix rubescens* reached almost a monodominance and since reached extremely high annual average values of biomass (i.e., 1626.5 and $1704.0 \mu\text{g L}^{-1}$ in 2020 and 2021, Fig. 18) with surface blooms mostly in winter/early spring (Fig.19). Moreover, more recent samples from 2023 have shown that *Planktothrix rubescens* monodominance have prevailed in Lake Stechlin (data not shown).

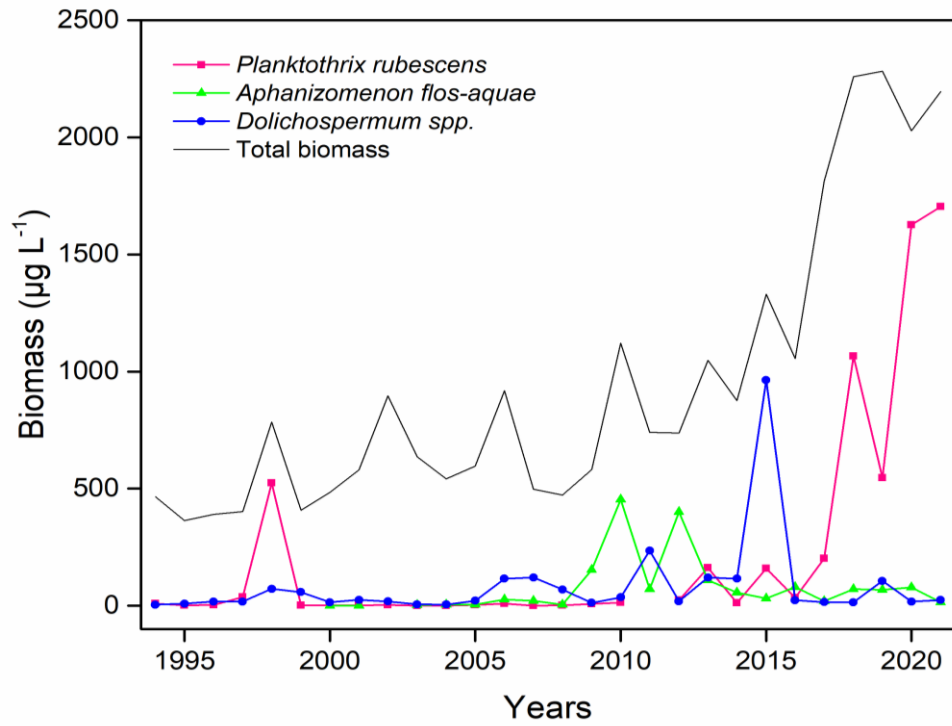


Fig. 18 Annual average of total phytoplankton biomass and the main species of cyanobacteria (*Planktothrix rubescens*, *Aphanizomenon flos-aquae*, and *Dolichospermum* spp.) in Lake Stechlin from 1994 to 2021.

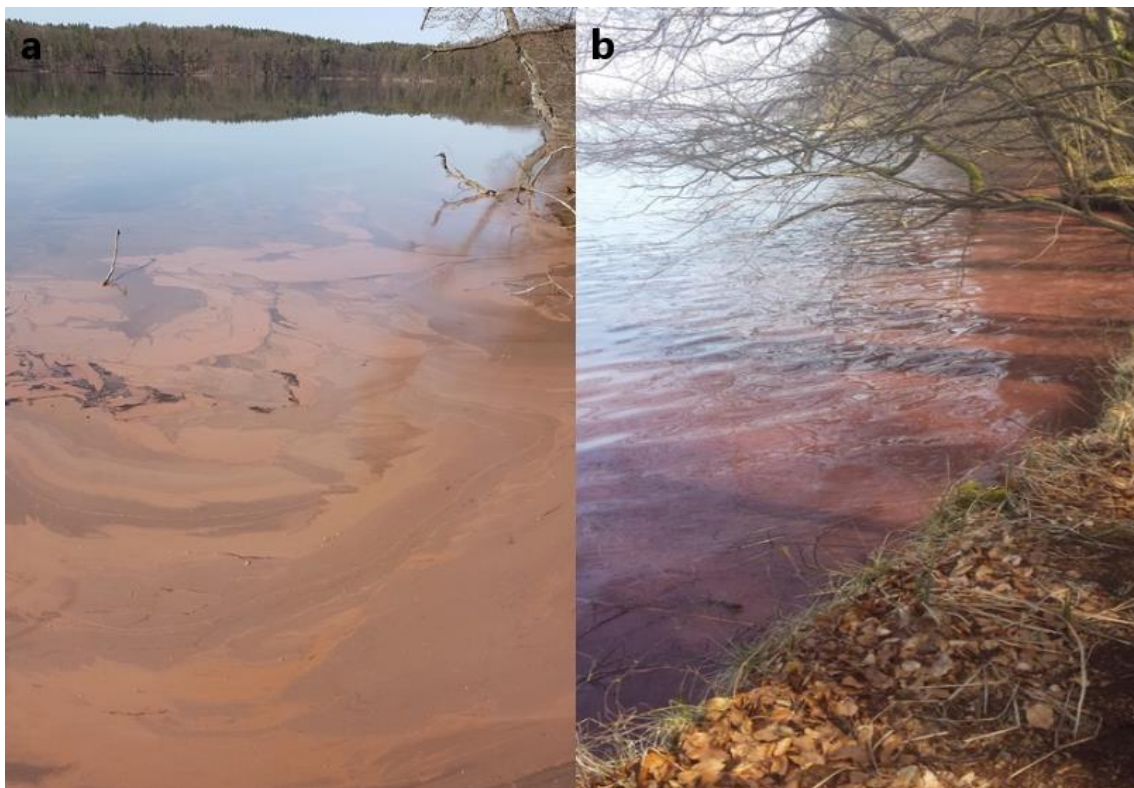


Fig. 19 *Planktothrix rubescens* spring surface bloom in Lake Stechlin (a) May 2020; (b) April 2021. Source: Dr. Peter Casper

The proportion of filamentous N₂-fixers and non-N₂-fixers cyanobacteria changed throughout the year in Lake Stechlin from 2018 to 2020 (Fig. 20). Non-N₂-fixers' biomass started to increase from the beginning of the year until April when reached the maximum biomass. This pattern was consistent for 2018 and 2020, during these years a bimodal pattern was observed with a second peak in November/December. In contrast, the non-N₂-fixers presented a unimodal pattern in 2019, characterized by small biomass oscillations and only a peak in December. After April, non-N₂-fixers' biomass started to decline until August/September. In the meantime, N₂-fixers remained in lower biomass most of the year, presenting a single peak in June, when overcame non-N₂-fixers biomass. This unimodal pattern was regular for three consecutive years. Later, non-N₂-fixers started to increase again overgrowing N₂-fixers for the rest of the year. The main species representing the non-N₂-fixers was *Planktothrix rubescens*, while N₂-fixers were *Dolichospermum* spp. and *Aphanizomenon flos-aquae*.

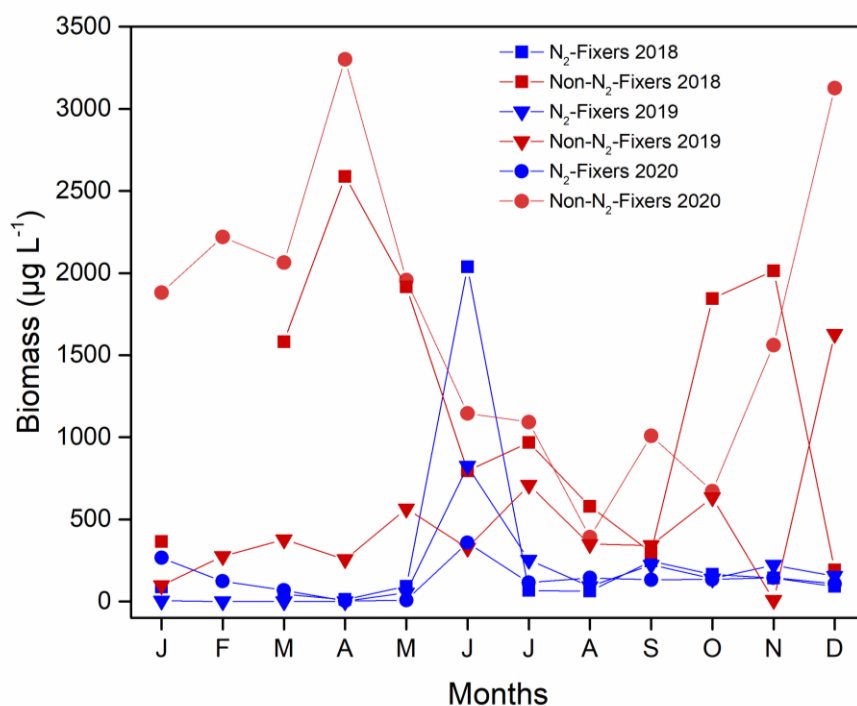


Fig. 20 Monthly biomass of nitrogen fixers and non-nitrogen fixers cyanobacteria in Lake Stechlin from 2018 to 2020.

Z_{eu} depth changed greatly during these three years (Fig. 21), in general, Z_{eu} was deeper in 2018, with the deepest being observed in October (19.3 m; Fig. 21a). Despite in 2019 average monthly biomass was lower compared to the other years, the Z_{eu} was shallower of which the shallowest was recorded in June (5.7 m; Fig. 21b), Contrastingly, Z_{eu} gradually increased from March 2020 following the decrease in *Planktothrix rubescens* average monthly biomass (Fig. 21c). Z_{eu} was found deepest in October regarding all years.

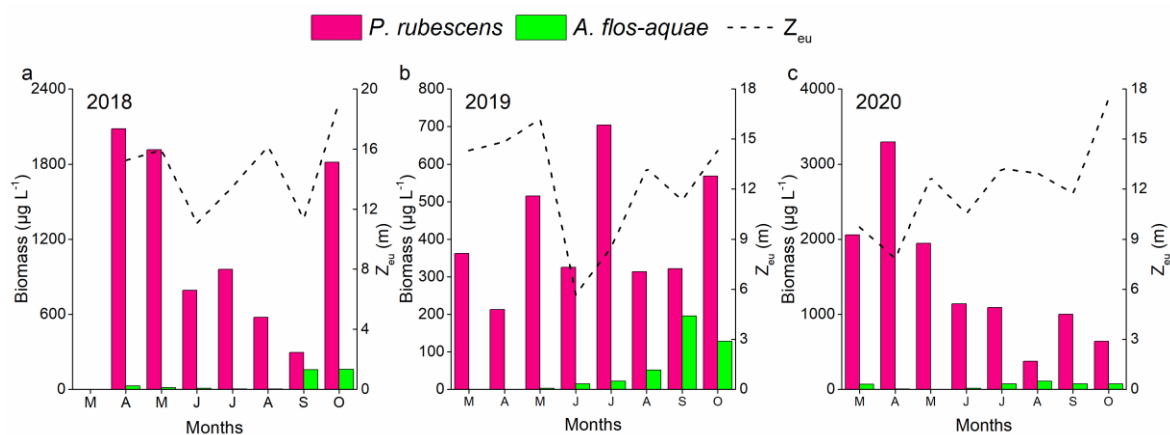


Fig. 21 Average monthly biomass of *Planktothrix rubescens* and *Aphanizomenon flos-aquae* biomass along with the euphotic zone in Lake Stchelin, from March to October during the studied period, 2018 to 2020, except in March of 2018 when sampling did not occur.

5.3.2 DCM FORMATION AND VERTICAL DISTRIBUTION OF PLANKTOTHRIX RUBESCENS AND APHANIZOMENON FLOS-AQUAE

In 2018, *Planktothrix rubescens* dominated from April to July, followed by *Aphanizomenon flos-aquae* dominance in the coming months (Fig. 22). The onset of the stratification started in April and lasted until September and the Z_{mix} depth was 7.5 m. *Planktothrix rubescens* and *Aphanizomenon flos-aquae* were still distributed throughout the whole water column in April, in which *Planktothrix rubescens* displayed several peaks at different depths (Fig. 22a). In May, *Planktothrix rubescens* started to accumulate in the upper hypolimnion at 20 m ($1135 \mu\text{g L}^{-1}$; Fig. 22b), meanwhile, *Aphanizomenon flos-aquae* only appeared below the metalimnion (i.e., 10 m) and concentrated at 20 m ($50 \mu\text{g L}^{-1}$; Fig. 22b). In June, *Planktothrix rubescens* formed a distinct DCM at 20 m reaching the maximum biomass recorded for 2018 ($2587 \mu\text{g L}^{-1}$; Fig. 22c). By July, the biomass of both species decreased and they were absent in the uppermost layer (i.e., 0-5 m), yet *Planktothrix rubescens* remained accumulated at 20 m ($206 \mu\text{g L}^{-1}$), with a smaller peak at 55 m (Fig. 22d). In August, when the thermocline was the most stable, both genera remained absent from the epilimnion, however, *Aphanizomenon flos-aquae* started to grow in the metalimnion where presented a peak at 10 m ($116 \mu\text{g L}^{-1}$; Fig. 22e), while *Planktothrix rubescens* biomass declined even more, but still concentrated at 20 m ($42 \mu\text{g L}^{-1}$; Fig. 22e). In September, *Aphanizomenon flos-aquae* reappeared and grew in the epilimnion reaching its maximum biomass of 2018 at 0m ($489 \mu\text{g L}^{-1}$; Fig. 22f). By October, the thermocline started deepening and the Z_{mix} was 12.5 m. Meanwhile, *Planktothrix rubescens* reappeared on the epilimnion, where *Aphanizomenon flos-aquae* maintained its high biomass (Fig. 22g).

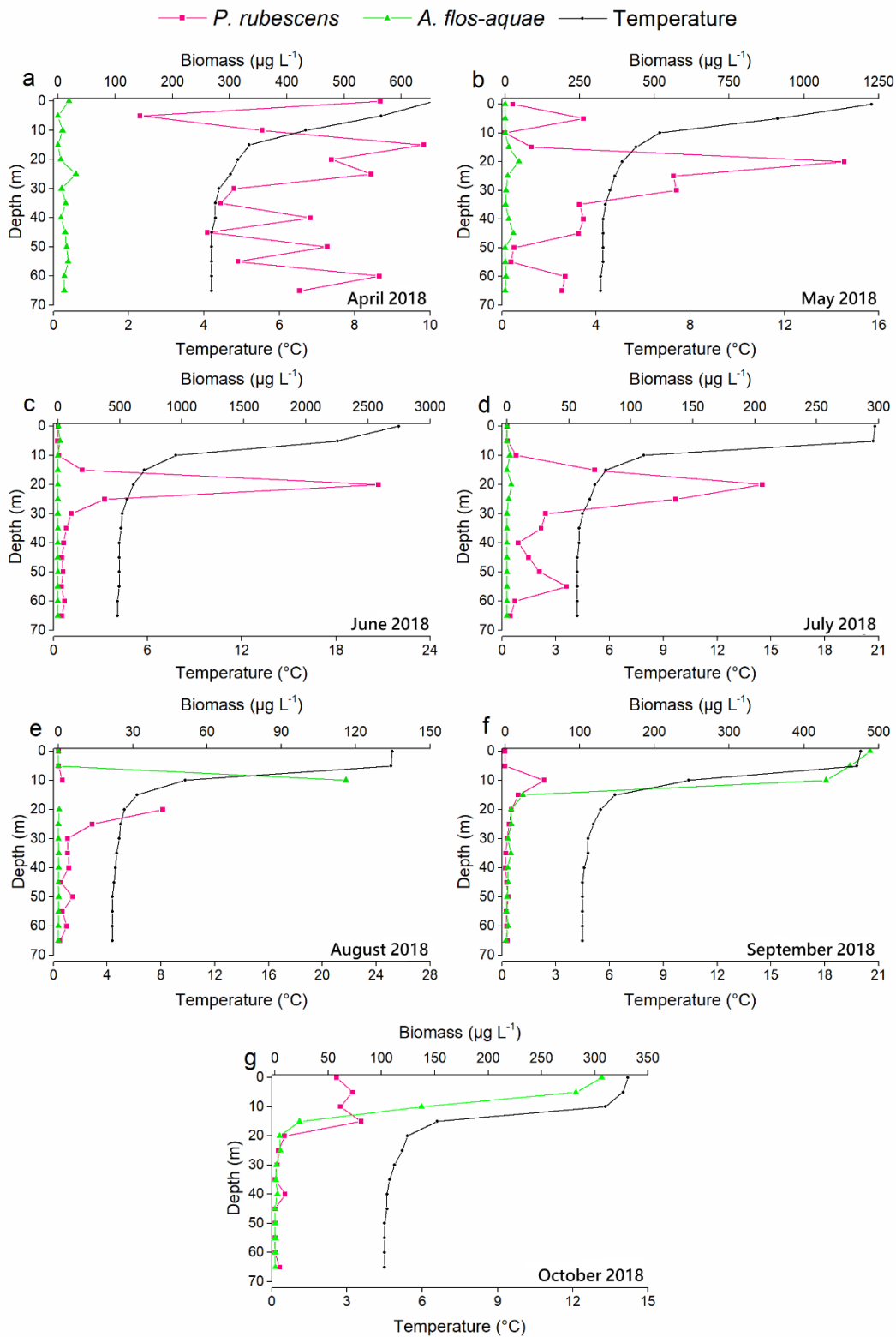


Fig. 22 Biomass vertical distribution of *Planktothrix rubescens* and *Aphanizomenon flos-aquae* in the water column (0-65m depth) along with the temperature gradient in Lake Stechlin from April to October 2018. Note that the biomass scales are different due to the highly different values.

In 2019, *Planktothrix rubescens* exceeded *Aphanizomenon flos-aquae* biomass in all months (Fig. 23). In March, during the isothermal period, both species were distributed all over the water column (Fig. 23a). In April, when the surface temperature started to warm up, *Planktothrix rubescens* and *Aphanizomenon flos-aquae* increased in biomass at 0 m ($418 \mu\text{g L}^{-1}$ and $171 \mu\text{g L}^{-1}$, respectively; Fig. 23b). In May, when the lake was already stratified and Z_{mix} was 7.5m, *Planktothrix rubescens* and *Aphanizomenon flos-aquae* grew below the thermocline at 15 m (834 and $119 \mu\text{g L}^{-1}$, respectively; Fig. 23c). In June, *Planktothrix rubescens* formed a DCM at 15 m reaching the highest biomass of 2019 ($12,887 \mu\text{g L}^{-1}$). In contrast, the biomass of *Aphanizomenon flos-aquae* declined and disappeared from many depths (Fig. 23d). In July, *Planktothrix rubescens* persisted with maximum accumulation at 15 m ($10,013 \mu\text{g L}^{-1}$) and *Aphanizomenon flos-aquae* increased its biomass in the epilimnion at 5 m ($285 \mu\text{g L}^{-1}$; Fig. 23e). By August, *Aphanizomenon flos-aquae* biomass declined, but *Planktothrix rubescens* drastically collapsed. Besides, spatially segregated small peaks were observed: *Aphanizomenon flos-aquae* peak deepened to the metalimnion (10 m) and *Planktothrix rubescens* in the upper part of hypolimnion (15 m; Fig. 23e). In September, both species increased in biomass, yet remained spatially segregated. During this month, *Aphanizomenon flos-aquae* reached its highest biomass of 2019 at 10 m ($295 \mu\text{g L}^{-1}$, Fig. 23g). In October, the thermocline deepened to 12.5 m, while *Planktothrix rubescens* biomass increased in the 0-15 m layer, on the other hand, *Aphanizomenon flos-aquae* biomass declined there (Fig. 23h).

In 2020, *Planktothrix rubescens* biomass was significantly higher than that of *Aphanizomenon flos-aquae* all over the year (Fig. 24). In March, *Planktothrix rubescens* already accumulated biomass 50 times higher compared with the same period in the previous year. In this month, during the isothermal period, *Planktothrix rubescens* had several peaks and the highest was at 60 m ($6066 \mu\text{g L}^{-1}$, Fig. 24a). In April, when the surface temperature started to warm up, *Planktothrix rubescens* started to concentrate between 15 and 20 m (Fig. 24b). By May, when the lake was already stratified and the Z_{mix} was 7.5 m, *Planktothrix rubescens* grew below the thermocline in the upper hypolimnion at 15 m ($10,194 \mu\text{g L}^{-1}$; Fig. 24c). In June, *Aphanizomenon flos-aquae* started to grow in the epilimnion and *Planktothrix rubescens* maximum biomass deepened to 20 m reaching the highest value recorded in 2020 ($10,314 \mu\text{g L}^{-1}$; Fig. 24d). By July, *Aphanizomenon flos-aquae* continued its growth in the epilimnion, where reached its highest peak of the year at 0 m ($532 \mu\text{g L}^{-1}$), while *Planktothrix rubescens* biomass declined, and the peak emerged to 15 m ($5012 \mu\text{g L}^{-1}$; Fig. 24e). In August, *Planktothrix rubescens* biomass collapsed but remained concentrated with a small peak at 15 m ($128 \mu\text{g L}^{-1}$; Fig. 24f). By September, when the thermocline started to deepening to 12.5 m, both species increased their biomass. *Aphanizomenon flos-aquae* dominated in the epilimnion, while *Planktothrix* remained accumulated at 15 m ($2596 \mu\text{g L}^{-1}$; Fig. 24g) By October, the biomass of both species declined and *Planktothrix rubescens* peak deepened to 20 m ($621 \mu\text{g L}^{-1}$; Fig. 24h).

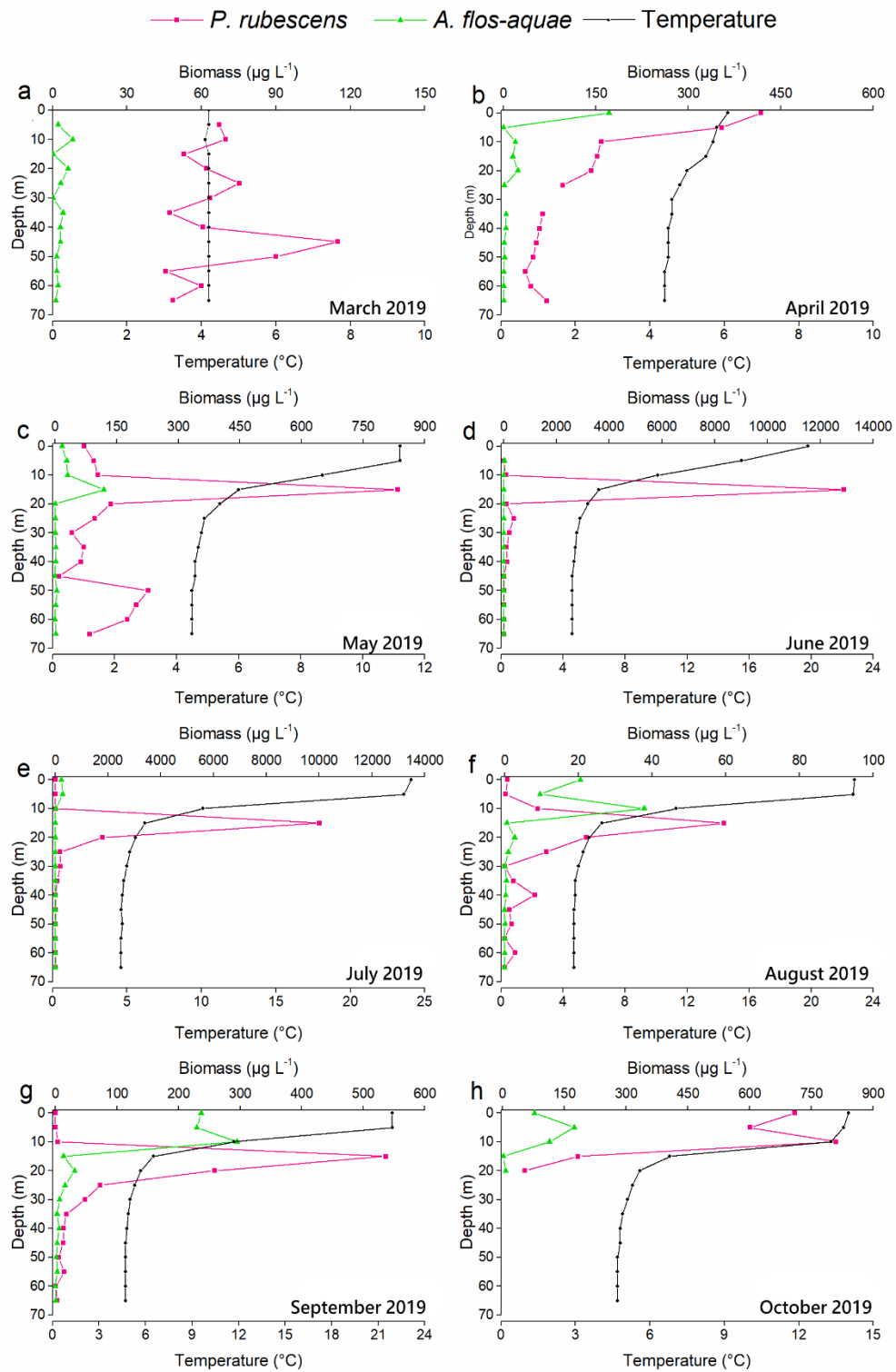


Fig. 23 Biomass vertical distribution of *Planktothrix rubescens* and *Aphanizomenon flos-aquae* in the water column (0-65m depth) along with the temperature gradient in lake Stechlin from March to October 2019. Noticed that the biomass scales are different due to the high differentiation of values. In October samples were taken only from 0 to 20 m due to technical reasons.

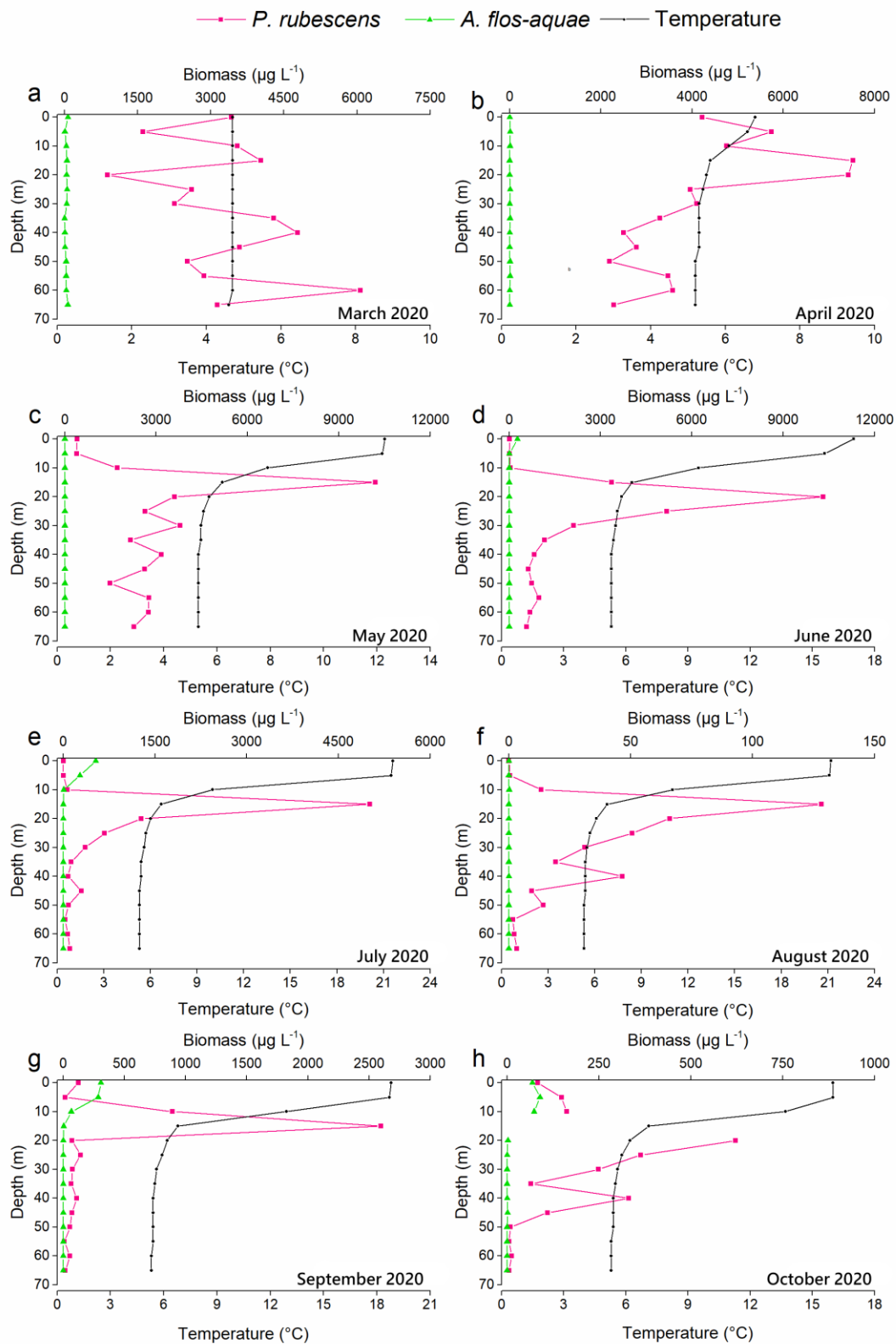


Fig. 24 Biomass vertical distribution of *Planktothrix rubescens* and *Aphanizomenon flos-aquae* in the water column (0-65m depth) along with the temperature gradient in Lake Stechlin from March to October 2020. Note that the biomass scales are different due to the highly different values.

5.4 DISCUSSION

Phytoplankton biomass and cyanobacterial dominance have been increasing in Lake Stechlin, mostly represented by the increase of *Planktothrix rubescens* followed by *Aphanizomenon flos-aquae* and *Dolichospermum* spp. contribution to the phytoplankton community. Since 2020, *Planktothrix rubescens* has been the main species to compose the phytoplankton community, responsible for more than 70 % of the total biomass in 2021. Besides, *Planktothrix rubescens* attained extremely high biomass in deep layers in the upper part of the hypolimnion (15-20 m). Meanwhile, *Aphanizomenon flos-aquae* biomass seldom exceeded *Planktothrix rubescens* biomass and was mostly found in epilimnion (0-5 m), after the decline of the *P. rubescens* population. DCM development started in May and lasted until September, coinciding with the stratification period in Lake Stechlin, following the deepening of the thermocline in October, *P. rubescens* emerged from deeper depths and started to accumulate near the surface.

Stratified conditions (permanent or transient) are obligatory for DCM establishment because it controls the flux of nutrients and the depth of the mixed layer where these nutrients will be available for phytoplankton growth (Leach et al., 2018). The metalimnion or the upper hypolimnion represents an intermediary environment regarding light and nutrients where adapted species can attain high biomass due to the mixing time being longer than the algal generation time, allowing growth and accumulation (Cullen, 2015). These conditions particularly benefit *P. rubescens*, due to being a shade-adapted species with low maximum growth rate (i.e., $\mu_m < 0.35 \text{ d}^{-1}$), in this way, the metalimnion serves as a refuge for avoiding the high irradiances in the epilimnion, where its photosynthesis can be or inhibited, and being outcompeted by species with higher maximum growth rate, such as *Aphanizomenon flos-aquae* (i.e., $\mu_m = 0.8 \text{ d}^{-1}$) (Reynolds, 1984; Davis & Walsby, 2002; Walsby et al., 2004; Knapp et al., 2021).

In order to grow at deeper depths, species need to control their position in the water column *via* buoyancy or flagella and be adapted to relatively low light conditions in thermally stratified systems to maximize access to nutrients while avoiding herbivory from above (Fee, 1976; Reynolds et al., 1987; Camacho, 2006; Lofton et al., 2020). *Planktothrix rubescens* and *Aphanizomenon flos-aquae* are shade-tolerant species, which can control their buoyancies through gas vesicles to adjust to irradiance levels. At low irradiance levels, they increase the gas vesicle volume along with the decrease of dense components, while at high irradiance; buoyancy is lost because of the relative decrease in gas vesicle volume and increase in carbohydrates and other dense components. Hence, buoyancy regulation in response to irradiance has been observed for *Aphanizomenon*, but it is mostly studied for *Planktothrix rubescens* and explains the depth where these species form populations in stratified lakes (Reynolds et al., 1987; Porat et al., 2001; Walsby et al., 2001, 2004).

P. rubescens was the main species to compose DCM during the study period (2018-2020). This species is well known to form metalimnetic populations in deep and stable stratified lakes due to the

low irradiance and temperature requirements (Gallina et al., 2017; Knapp et al., 2021). Despite the frequent association with the metalimnion, *P. rubescens* can also be found at the epilimnion and upper part of the hypolimnion as shown here and already reported in Lake Stechlin (Selmeczy et al., 2016, 2018). *P. rubescens* maximum biomass was mostly observed at 20 m below the surface, except in 2019 when was at 15 m, while *Aphanizomenon flos-aquae* maximum biomass was mostly observed at the surface at 0 m, but also on the metalimnion at 10 m. Due to being a better competitor under low light conditions, *Planktothrix rubescens* was always found at deeper depths than *Aphanizomenon flos-aquae*. Besides, *Planktothrix rubescens* contain both phycoerythrin and phycocyanin as accessory pigments, these enhance light absorption in the green spectra in deep water which gives a competitive advantage at deeper depths (Reynolds, 2006).

The water column profiles of *P. rubescens* and *Aphanizomenon flos-aquae* showed vertical segregation similar to previous years in Lake Stechlin, however, during our study, these populations were recorded in shallower depths. *Planktothrix rubescens* was observed to accumulate at the upper part of the hypolimnion 20-25 m, while *Aphanizomenon flos-aquae* was observed in the middle of the thermocline at 10 m (Padisák et al., 2003a; Selmeczy et al., 2016). Our study showed *Planktothrix rubescens* formed a sharp upper hypolimnetic maximum (15 – 20 m), while *Aphanizomenon flos-aquae* was mostly found at the epilimnion (0 - 5 m). This may be justified by the reduction of the euphotic zone considering that the thermocline depth did not change much. The euphotic zone in Lake Stechlin was previously reported to be in the range of 20-25 m, contrasting, during our study Z_{eu} was always below 20 m (i.e., an average of 13 m), with a month when reached only 5.7 m. Hence, both species adjusted their position to remain in the euphotic zone where the irradiance supports phototrophic growth. Many factors may be associated with the reduction of the euphotic zone in Lake Stechlin, including *Planktothrix rubescens* overgrowth since the deepening of the euphotic depth in 2020 followed the decrease in biomass. In 2011, extreme weather events, including strong winds, led to a bloom of *Dolichospermum* spp. in Lake Stechlin, resulting in a drastic reduction of the euphotic zone. The disruption of the stratification pattern allowed for the entrainment and overgrowth of cyanobacteria from the deep chlorophyll maximum into the mixed layer (Kasprzak et al., 2017).

Different processes may influence DCM, including depth-differential sinking, high herbivore pressure in the epilimnetic zone, and photoinhibition, while these can affect phytoplankton distribution; *in situ* growth and photoacclimation are often indicated as primary causes of DCM formation (Camacho, 2006; Cullen, 2015; Leach et al., 2018). During our study, we could observe *in situ* growth mostly of *Planktothrix rubescens* and sometimes of *Aphanizomenon flos-aquae* populations at deeper depths, however, other processes may also regulate their vertical distribution. Nevertheless, herbivory pressures do not seem to be a key factor regulating these species' distribution. *Planktothrix rubescens* and *Aphanizomenon flos-aquae* possess traits that act as grazer defences, such as the morphology, long filaments, or filament clusters that can exceed the size of the feeders' apparatus of filter feeding, thus inhibiting the ingestion and intracellular storage of toxic metabolites. Besides, the role of grazing

pressure on cyanobacterial blooms is regulated by the traits of the zooplankton community co-existing within the lake. While generalist grazers (i.e., cladocerans) may either decline cyanobacterial blooms despite toxicity or facilitate these blooms through grazing on smaller-sized edible algae, selective grazing (i.e., copepods) also may favour these blooms by feeding on eukaryotic species, therefore, reducing competition (Chislock et al., 2013; Leitão et al., 2018). Furthermore, the decline of zooplankton biomass in Lake Stechlin, (mostly copepods, *Eudiaptomus gracilis* Sars G.O., 1863 and *Eurytemora lacustris* Poppe, 1887) was already evidenced to parallel the increase of filamentous cyanobacterial biomass, mainly *Dolichospermum* spp., and *Aphanizomenon flos-aquae* (Selmeczy et al., 2019).

Since 2020, *Planktothrix rubescens* reached almost a monodominance competitively excluding other native species, hence reducing the compositional diversity of phytoplankton. The expansion of *P. rubescens*, along with the increase in frequency and duration of blooms have been observed in several lakes from the temperate region (Carraro et al., 2012; Dokulil & Teubner, 2012; Salmaso et al., 2015; Gallina et al., 2017; Knapp et al., 2021; Mi et al., 2022). *Planktothrix rubescens* blooms are characterized by a pink-red colour and have been occurring for a long time in Lake Stechlin. The first official report of *P. rubescens* was recorded nearly 100 years ago (Krieger, 1927), however, it seems that these blooms date further back than the scientific reports and gave origin to „The Red Cock” legend in the region (Padisák et al., 2010). Since then, *P. rubescens* became a permanent element of the flora in Lake Stechlin remaining in low abundance until some outstanding events favour its overgrowth, i.e., the long-lasting ice duration in 1998 and 2013. In these years, *P. rubescens* population started to grow during the autumn turnover and continued until the establishment of the thermocline in spring, when a large part of the biomass continued growth in the upper hypolimnion composing DCM, while the other part sank to the sediment (Padisák et al., 2003b; Selmeczy et al., 2016).

We observed a similar pattern in our study. During the isothermal period in March 2020, *P. rubescens* accumulated biomass 50 times higher compared with the same period in the previous year. It was distributed throughout the whole water column, in April. With the onset of the stratification, part of the biomass accumulated and increased in the hypolimnion. However, the duration of the ice cover did not change much in the winter of 2019/2020. *P. rubescens* grew during the winter season while accumulating directly under the ice cover was already observed in lakes with seasonal ice cover (Halstvedt et al., 2007; Lenard, 2015). Besides, strains of *P. rubescens* from Lake Zürich showed great tolerance to dark periods surviving on a range of 8–11 weeks stored at 4–5 °C in the dark (Holland & Walsby, 2008). Complementary, *P. rubescens* populations presented high survival rates of overwintering within the same lake (Knapp et al., 2021). In Lake Stechlin, during the *P. rubescens* outbreak in 1998, filaments did not decompose during winter remaining senescent and accumulating near the sediment (Padisák et al., 2003b). In addition, some filaments can be regarded as non-specialized dormant forms and can be resuspended to the water column thus providing *P. rubescens* a competitive advantage with probable assistance of building up phosphorous storage reserves during the sedimentary

phase (so-called luxury uptake). Overall, *P. rubescens* thrives during winter, when conditions are harsh for most phototrophic phytoplankton species, which directly influences spring recruitment, thus DCM formation.

Aphanizomenon flos-aquae has a more recent history in Lake Stechlin. It appeared first in 2001 remaining seasonal until 2006 and then became perennial. In 2009, this species peaked contributing more than 80% to the total biomass and even formed an unexpected bloom under the ice (Padisák et al., 2010). *A. flos-aquae* is typically found to form surface blooms in eutrophic lakes (Dokulil & Teubner, 2000; Yamamoto & Nakahara, 2005; Wagner & Adrian, 2009), however, this species was first described to form DCM at the metalimnion in Lake Stechlin (Selmeczy et al., 2016). Similar to *Planktothrix rubescens*, *Aphanizomenon flos-aquae* contains phycoerythrin and phycoerythrocyanin, which enhance light absorption (Huisman et al., 1999) and enable this species to develop in the metalimnion. During our study, *Aphanizomenon flos-aquae* was predominantly associated with the epilimnion, with rare occasions when its biomass exceeded that of *Planktothrix rubescens* were also observed near the surface. *Aphanizomenon flos-aquae* presents great ecophysiological plasticity, which enables this species to grow in different conditions, either in cold and low light (i.e., winter bloom) or warm and high light (i.e., at the surface) due to its tolerance of a wide range of light and temperature (Üveges et al., 2012). *A. flos-aquae* was found in low biomass during most of the study period (March-July), its population only started to increase around August/September following the collapse of the *Planktothrix rubescens* population. Despite nutrient concentrations were not measured here, Stechlin has a history of N limitation during the stratified period, thus the collapse of the *P. rubescens* population for three consecutive years in August may indicate nitrogen limitation in the system. *Aphanizomenon flos-aquae* is sensitive to low phosphorus but tolerant to low nitrogen due to fix atmospheric nitrogen (Reynolds et al., 2002). Hence, nitrogen-limiting conditions favour *Aphanizomenon flos-aquae* as evidenced by the sudden increase in biomass after the decline of *Planktothrix rubescens*, and even a switch in dominance in favour of *Aphanizomenon flos-aquae* was observed from August to October 2018. Besides, the increase of N₂ fixing species, namely *Dolichospermum lemmermannii*, after the decline of the *Planktothrix rubescens* population has been already observed in Lake Stechlin and interpreted as the ecosystem's need for more nitrogen (Padisák et al., 2003b). Our finding supports this view; nitrogen-fixing cyanobacteria (mainly *Dolichospermum* spp. and *Aphanizomenon. flos-aquae*) increased biomass in June for three consecutive years coinciding with the decline of non-nitrogen-fixing cyanobacteria (*Planktothrix rubescens*). Furthermore, a hypothetically plausible mechanism could be a synergistic relationship between these species. After the collapse of *P. rubescens*, the biomass sank to the sediment where aerobic decomposition takes place, consuming the oxygen available, thus reducing the pH and the redox potential, which in turn deliberate phosphorous trapped in the sediment (Lee et al., 1977; Nürnberg, 1987; Selmeczy et al., 2019). This way, phosphorous becomes available and can be uptaken by *Aphanizomenon flos-aquae*, on the other hand, *Aphanizomenon flos-aquae* can fix

nitrogen which could be used for *Planktothrix rubescens* renewed growth. However, additional research is needed to further elucidate the relationship.

The ongoing eutrophication in Lake Stechlin is evidenced by the sudden increase of filamentous cyanobacteria blooms since the early 21st century, with many species considered recent immigrants, including *Aphanizomenon flos-aquae* (Padisák et al., 2010). The ecosystem in Lake Stechlin was strongly influenced by the implementation of a nuclear power plant when cooling water from the mesotrophic Lake Nehmitz was released on the initially oligotrophic Lake Stechlin. For a long time, no substantial change in trophic status was observed because of a decrease in retention time and external phosphorous co-precipitation in the sediment counteracted eutrophication effects (Koschel et al., 1983). However, climate change has modified this scenario and delayed consequences from this activity have been observed. The current increase in TP concentrations, thus, the carrying capacity in Lake Stechlin has been associated with the rise in water temperature along with the elongation of the stratification period as also evidenced for other temperate lakes (Jane et al., 2021; 2023; Selmeczy et al., 2019). Considering that no source of external phosphorous is known in Lake Stechlin, it is likely that comes from internal loading. The increase in phosphorous pulse led to a decrease in the DIN/SRP ratio in lake Stechlin favouring nitrogen fixer cyanobacteria in the past (Padisák et al., 2003b). However, a more recent study exploring 25 years of temporal variability in Lake Stechlin showed that no trend in the concentration of DIN was found and that cyanobacterial biomass in Lake Stechlin was not associated with changing NO₃-/SRP ratios (a rough proxy for N/P ratios), but with the increase of phosphorus concentrations (Kröger et al., 2023). Despite nitrogen concentrations are not the main driver of cyanobacteria increases in Lake Stechlin, filamentous buoyant cyanobacteria have been on the rise, and further blooms may be expected under the scenario of global warming. The outcome of species that thrive under these new conditions may depend on their competitive success in acquiring nitrogen, considering the anticipated decrease in DIN/SRP concentrations. Therefore, further studies are needed to elucidate the role of nitrogen concentrations in promoting nitrogen-fixing cyanobacteria in Lake Stechlin.

6. GENERAL DISCUSSION AND GLOBAL CHANGE SCENARIOS

Earth has been facing significant climatic changes, in which warming is the most prevalent symptom with effects not only at individual and species levels including shifts in species survival and changes in the distribution but also on biotic interactions and ecosystem services (Walther et al., 2002; Walther, 2010; Osland et al., 2021). Given that climate warming enhanced the occurrence of cyanobacteria blooms, warmer temperatures are the catalyst in the global expansion of cyanobacteria blooms in freshwater environments due to many eco-physiological traits, which provide them advantage under these conditions, such as maximum growth rates higher than other eukaryotic algae at elevated temperatures (Paerl & Huisman, 2008, 2009; Paerl & Paul, 2012). Moreover, the reduction of ice cover and/or the elongation of the stratification period favour buoyant cyanobacteria, as already evidenced in northern Europe lakes and American reservoirs (Wiedner et al., 2007; Wagner & Adrian, 2009; Smucker et al., 2021). Besides, the rise in surface water temperature in the temperate region is also one of the explanations for the ongoing invasion of species, of which *Raphidiospis raciborskii* is the most widespread case, since earlier warming and the subsequent germination of akinetes advances the population establishment (Wiedner et al., 2007; Sukenik et al., 2012; Recknagel et al., 2019; Sidelev et al., 2020).

Besides warming, other symptoms of climatic changes are also expected and in some parts of the globe already evidenced, such as changes in rainfall patterns. Precipitation is likely to increase in high-latitude regions, while a decrease is expected in the subtropical regions associated with intensified drought in many regions (IPCC, 2021). These extreme weather events, either storms or drought, have been reported to trigger cyanobacterial blooms (Reichwaldt & Ghadouani, 2012; Mosley, 2015; Costa et al., 2016; Kasprzak et al., 2017; Havens et al., 2019). Furthermore, climatic model predictions also forecast the expansion of lands affected by drought worldwide, including in North and South America, Africa, southern Europe, and Australia (Feng & Fu, 2013; Huang et al., 2016). Here, we used data from lakes in the Brazilian semi-arid region as a model to trace the effects of prolonged drought on phytoplankton dynamics, which gives a view regarding the possible consequences for water quality globally in future drylands. This region is vulnerable to extremes of climate variability due to the hydrological cycle, which is characterized by periods of negative water balance, often lasting more than half of the year, and precipitation concentrated in only a few months. A further increase in rainfall deficit is predicted for the next century in this region (Marengo et al., 2017, 2020), yet, severe reductions in precipitation have already been evidenced, such as the prolonged drought explored here, which was classified as the most severe drought in the Brazilian semi-arid region in the last decades (Marengo et al., 2018)

Several studies explored phytoplankton response during this prolonged drought, and most of them pointed to the role of drought associated with the reduction of water levels in triggering eutrophication and cyanobacterial blooms (Braga et al., 2015; Medeiros et al., 2015; Brasil et al., 2016; Costa et al.,

2016; Figueiredo & Becker, 2018; Braga & Becker, 2020). Our results underlined that when the dry season within the annual hydrological cycle does not result in a reduction in water levels, the effects of seasonality were diminished and did not lead to an increase in the effects of eutrophication, yet, different cyanobacteria taxa benefited in the dry and rainy season. It is also important to highlight that these lakes faced the accumulative effects of this prolonged drought period, with rainfall deficit varying between 20-60% below the historical mean for the rainy season (Marengo et al., 2017, 2018), hence they already displayed current depths extremely reduced compared to their maximum depths. Moreover, our results also show that lakes with lower water depths presented higher concentrations of nutrients promoting cyanobacteria bloom. Overall, our findings uphold that the effects of drought-triggering eutrophication *via* reduction of water level may enhance cyanobacterial bloom duration and favor *Raphidiopsis raciborskii*, at least in shallow semi-arid lakes.

R. raciborskii blooms became a worldwide issue as has been reported in almost all continents and various climate zones (Wilk-Woźniak et al., 2016). The combination of different ecotypes along with global warming, explains its successful spread from the tropics to many other regions of the globe (Padisák, 1997; Sukenik et al., 2012; Wilk-Woźniak et al., 2016; Sidelev et al., 2020). In the past years, *R. raciborskii* rapidly widened its distribution across Europe where became an invasive species, with several impacts on biodiversity and water quality (Borics et al., 2010; O’Neil et al., 2012; Svirčev et al., 2014; Wilk-Woźniak et al., 2016; Sidelev et al., 2020). This species is challenging to manage due to its wide ecological tolerance and high phenotypic plasticity (Bonilla et al., 2012; Antunes et al., 2015) and dispersal capacity (Padisák, 1997). Besides, *R. raciborskii* produces a high diversity of potential toxins, which may increase management costs regarding toxins analyses and water treatment (Paerl & Otten, 2016). Therefore, a model using species distribution was proposed for the early detection of *R. raciborskii* in European lakes, through the identification of potentially suitable areas for the colonization and survival of the specie to prevent further expansion (Meriggi et al., 2022).

We also call attention to the use of the semi-arid lakes for fish cage culture as initially intended. The input of fish food increases nutrient concentrations (Gorlach-Lira et al., 2013; Henry-Silva et al., 2019), which may increase the risk of eutrophication in the mesotrophic lakes, Santa Cruz and Prata. Although we did not measure cyanotoxins, microcystins from *Microcystis*, and saxitoxins from *Raphidiopsis*, cyanotoxicity is widely reported in other lakes within the same region (Molica et al., 2005; Bittencourt-Oliveira et al., 2014; Fonseca et al., 2015; Lorenzi et al., 2018; Moura et al., 2018), hence, is very likely that cyanotoxins are present in the study lakes as well. Besides, fish may bioaccumulate cyanotoxins (Lee et al., 2017) raising a public health concern regarding fish consumption from these lakes. The ingestion of cyanotoxins has been associated with liver and skin diseases, digestive problems, neurological symptoms, and even death (Lee et al., 2017; Chorus & Welker, 2021). Moreover, the increase of saxitoxin in the drinking water supply in the Northern region of Brazil during this prolonged drought was linked to the highest incidence of microcephaly in newborns linked with the Zika virus (Pedrosa et al., 2020), evidencing an even greater threat to human health.

Here, we applied the taxonomic and functional approaches to better understand phytoplankton dynamics in space and time. Morphological traits associated with trophic status and the identification of environmental thresholds for the dominance of different phytoplankton groups offer a practical tool for biomonitoring due to being straightforward to apply. Equally important the study of the species dynamics, *Raphidopsis raciborskii* and *Microcystis aeruginosa* during a perennial bloom provided valuable information, such as the environmental preferences accessed *via* R-index. Studies at the species level result in a higher level of information, complementary, the functional approach simplifies real systems and included a higher number of species decreasing redundancy (Salmaso et al., 2015). Moreover, the classification tree identified the same value for the euphotic zone to disguise MBFG VII and VIII dominance, as the R-index and for *Microcystis* and *Raphidiopsis*. This occurred because, in eutrophic semi-arid tropical lakes, the phytoplankton community is often dominated by a single species, monodominant. Despite the switch between dominance, both approaches could access the environmental preferences of each one. Therefore, both approaches are recommended to identify the environmental preference of the dominant species or MBFG and could be used for developing successful management strategies.

Despite the typical link between eutrophication and cyanobacteria blooms, there are also widespread reports of cyanobacteria blooms in oligo- to mesotrophic lakes worldwide, some of them even underpin the increase in cyanobacteria relative abundance in these systems in the past few years (Winter et al., 2011; Ewing et al., 2020; Freeman et al., 2020; Reinl et al., 2021). Our results endorse these: the increase in cyanobacteria dominance in Lake Stechlin has been evidenced despite the oligo-mesotrophic status. Moreover, a great fraction of the “recent immigrant species” was composed of cyanobacteria, for example, *Aphanizomenon flos-aquae* which was first found in 2001 and by 2009 bloomed dominating nearly all the phytoplankton biomass (Padisák et al., 2010; Üveges et al., 2012; Selmeczy et al., 2019). Furthermore, an outstanding change in the phytoplankton community of Lake Stechlin was the increase in *Planktothrix rubescens* dominance resulting in a decline in compositional diversity. This species has been a permanent component of the flora, which used to be found in low biomass until some outstanding events promote its overgrowth such as long ice cover (Padisák et al., 1998; Selmeczy et al., 2016). Nevertheless, the abiotic conditions promoting the recent increase in *P. rubescens* have not been fully unveiled.

In other temperate low trophic lakes, global warming was underpinned as the main driver to promote *P. rubescens* blooms, either due to the earlier onset of the stratification or elongation of the stratification period, which prolongs the persistence of the metalimnion (Jacquet et al., 2005; Dokulil & Teubner, 2012; Knapp et al., 2021). Changes in stratification patterns were also reported to influence *P. rubescens* population due to interfering with resources availability; the deepening of the nutrient-depleted layer was suggested to favour *P. rubescens* which can use organic phosphorus giving a competitive advantage over other species in Lake Lac du Bourget, France (Jacquet et al., 2005). On the other hand, the decrease of nutrients as a consequence of recurrent incomplete water column mixing led

to the decline of *P. rubescens* in favour of mixotrophic organisms in Lake Garda, Italy (Salmaso et al., 2018). A mesocosm experiment with the deepening of the thermocline in Lake Stechlin did not seem to affect the development of *P. rubescens*, yet, a further increase in thermocline depth may displace the other species composing DCM (i.e., *Cyanobium* spp. and *Aphanizomenon flos-aquae*) in favour of *Planktothrix rubescens* due to be a better competitor under low light (Selmeczy et al., 2018).

The presence of *P. rubescens* or other species from the *Planktothrix* genus in waterbodies is worrisome not only for the potentially toxigenic but because when this genus is present, microcystins are indeed detected in the majority of the samples (i.e., >80%) (Chorus & Welker, 2021). Microcystin from *P. rubescens* has been detected either in metalimnetic or surface blooms, although higher concentrations were recorded during surface blooms (Jacquet et al., 2005; Naselli-Flores et al., 2007a; Ibelings et al., 2014; Salmaso et al., 2014). In addition, *P. rubescens* microcystin producers' genotypes were already identified in Lake Stechlin along with microcystin detection which highlights attention considering the lake is widely used for recreative activities (Dadheech et al., 2014). Since the raising concern regarding *P. rubescens* blooms, many efforts have been applied to manage it, however, even in lakes with successful ongoing re-oligotrophication, *P. rubescens* blooms persisted due to the increase in light availability in the metalimnion (Jacquet et al., 2005; Ernst et al., 2009). Furthermore, a series of metalimnetic withdrawals along with an increase in light scenarios pointed out the required optimal withdrawal volume (10% of the reservoir volume) and light extinction coefficient ($< 0.55 \text{ m}^{-1}$) to effectively suppress *P. rubescens* in Rappbode Reservoir, the biggest drinking water reservoir in Germany (Mi et al., 2022).

Overall, global change influences environmental conditions, which directly affect phytoplankton composition and dynamics. Therefore, there is very little doubt that global changes will lead to a switch in species composition and that a further increase in cyanobacterial blooms is expected in response to rapid global changes (Wiedner et al., 2007; Kosten et al., 2012; Paerl & Paul, 2012; Gallina et al., 2017; Kasprzak et al., 2017). Cyanobacteria are an extremely diverse group that profit from different sets of ecophysiological traits making them successful competitors under the most diverse scenarios, yet, not all traits are shared among taxa, thus different cyanobacterial taxa will respond to different aspects of climate change (Carey et al., 2012; Paerl & Paul, 2012; Mantzouki et al., 2016).

7. CONCLUSION

Despite the differences between the lakes studied here, such as latitude, origin, depth, and trophic status, climatic changes promoting cyanobacteria blooms were a common issue among them. In tropical semi-arid lakes, rainfall rather than temperature regulates seasonality, which often results in contrasting effects of the rainy and dry seasons on environmental filters selecting distinct sets of traits. Though this pattern was not generalized during our study, we could observe that the effects of seasonality varied across the lake. Our results underpin eutrophication as the main environmental factor for MBFG selection suggesting that reduced seasonality affects phytoplankton patterns during dry years in the tropics. In contrast to what was expected, the rainy season was associated with a higher concentration of nutrients and suspended solids along with a shallower euphotic zone compared to the dry season, evidencing that other effects than nutrient dilution may be expected when the rainy season is not associated with the increase in water depth or volume.

Equally important that we also show that shallow lakes and the subsequent increase in nutrient concentration are key factors leading to cyanobacteria dominance, which in the absence of constraints such as light and temperature tend to persist year-round in eutrophic lakes. Hence, we reinforce the sensitivity of tropical semi-arid lakes to eutrophication. We also pointed out that reduced water transparency regulates the switch from *Microcystis* to *Raphidiopsis* dominance during their perennial presence in shallow tropical lakes. Complementary, *Raphidiopsis* thrived under higher nutrients and suspended solids concentration and lower light availability than *Microcystis*. Overall, our findings underpin the role of drought-triggered eutrophication *via* the reduction of water level in enhancing cyanobacterial bloom. Besides, *Raphidiopsis raciborskii* has a wider ecological tolerance, and a further increase in nutrient concentrations linked with reductions in the euphotic zone may favour *R. raciborskii*, at least in shallow semi-arid lakes.

We applied the taxonomic and functional approaches to track environmental demands for the dominance of MBFG or species. Morphological traits associated with trophic status could offer a practical tool for biomonitoring due to being straightforward to apply. Nevertheless, the study of the species dynamics, of *Raphidiopsis raciborskii* and *Microcystis aeruginosa* provided a higher level of information. The use of techniques to identify environmental thresholds, such as classification tree and R-index proved to be successful and could be used to develop successful management strategies, especially under perennial blooms.

In the tropics, cyanobacterial blooms were not observed in mesotrophic lakes, contrariwise, cyanobacteria dominance increased in Lake Stechlin despite being originally oligo-mesotrophic. *Planktothrix rubescens* was the main species to compose DCM during the study period, forming dense populations in the upper part of the hypolimnion. Since 2020, *P. rubescens* reached almost a monodominance, maintained high biomass in deep layers during most of the year, and even accumulated biomass over winter. Meanwhile, *Aphanizomenon flos-aquae* was found mostly near the surface in low biomass compared to *Planktothrix rubescens*, still, *Aphanizomenon flos-aquae* biomass started to increase following the collapse of the *Planktothrix rubescens* population. The drastic increase of *P. rubescens* in

Lake Stechlin is worrisome and raises concern. Here we suggest that warming (i.e., earlier onset of stratification and the elongation of the stratified period) along with ecophysiological traits (i.e., buoyancy control and shade-adaptation) explain *P. rubescens*' success in Lake Stechlin. Nevertheless, the relation between warming and *P. rubescens* dominance in Lake Stechlin requires further studies to be better understood.

In conclusion, cyanobacterial blooms are becoming more prevalent in response to climatic changes, thus, urging effective efforts to improve water quality in freshwater bodies. Our results showed perennial dominance of cyanobacteria in tropical shallow eutrophic lakes, and also in a deep temperate oligo-mesotrophic lake. Despite the lower biomass attained in mesotrophic conditions, the driver of cyanobacteria blooms in low trophic systems remains unexplored compared to eutrophic ones. Similarly, cyanobacteria blooms in deeper layers have also been overlooked in many freshwater ecosystems. These gaps in literature lead to an incomplete understanding of blooms, hence identifying the mechanisms that initiate and sustain cyanobacterial blooms across a broader range of environmental conditions assists in comprehending the global increase of cyanobacterial dominance. In closing, the present dissertation explored cyanobacteria dominance in various habitats, which provided not only useful information for the development of mitigation strategies but also serves as a basis for further predictions, especially under climatic change scenarios, contributing with a piece to assist to filling the gaps in the fascinating puzzle in phytoplankton ecology.

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9. REFERENCES

- Aldridge, K., 2011. Impact of a drought on nutrient concentrations in the Lower Lakes (Murray Darling Basin, Australia). *Inland Waters* 1: 159–176.
- Alvares, C. A., J. L. Stape, P. C. Sentelhas, J. L. De Moraes Gonçalves, & G. Sparovek, 2013. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift* 22: 711–728.
- Antunes, J. T., P. N. Leão, & V. M. Vasconcelos, 2015. *Cylindrospermopsis raciborskii*: Review of the distribution, phylogeography, and ecophysiology of a global invasive species. *Frontiers in Microbiology* 6: 473.
- Ardyna, M., M. Babin, M. Gosselin, E. Devred, S. Bélanger, A. Matsuoka, & J. E. Tremblay, 2013. Parameterization of vertical chlorophyll a in the Arctic Ocean: Impact of the subsurface chlorophyll maximum on regional, seasonal, and annual primary production estimates. *Biogeosciences* 10: 4383–4404.
- Aylward, B., J. Bandyopadhyay, J.-C. Belausteguigotia, P. Börkey, A. Cassar, L. Meadors, L. Saade, M. Siebentritt, R. Stein, S. Tognetti, C. Tortajada, T. Allan, C. Bauer, C. Bruch, A. Guimaraes-Pereira, M. Kendall, B. Kiersch, C. Landry, E. M. Rodriguez, R. Meinzen-Dick, Suzanne Moellendorf, S. Pagiola, I. Porras, B. Ratner, A. Shea, B. Swallow, T. Thomich, N. Voutchkov, C. Lead, A. Bruce, L. Authors, P. Bo, C. Authors, S. Moellendorf, R. Editors, R. Constanza, P. Jacobi, & F. Rijsberman, 2005. *Freshwater Ecosystem Services. Ecosystems and human well-being: current state and trends.* Washington DC, Island Press. 3: 213-255.
- Azevedo, A. D. S., A. do N. Moura, N. K. C. Aragão-Tavares, & Ê. W. Dantas, 2020. Taxonomic and functional approaches to phytoplankton in ecosystems with different coverage of aquatic plants. *Revista Brasileira de Botânica* 43: 665–675.
- Baas-Becking, L. G. M., 1934. *Geobiologie, of Inleiding Tot de Milieukunde. Met Literatuurlijst en Ind.* Van Stockum.
- Bakker, E. S., & S. Hilt, 2016. Impact of water-level fluctuations on cyanobacterial blooms: options for management. *Aquatic Ecology* 50: 485–498.
- Baldry, K., P. G. Stratton, N. A. Hill, & P. W. Boyd, 2020. Subsurface chlorophyll-a maxima in the Southern Ocean. *Frontiers in Marine Science* 7: 671.
- Barbosa, F. A. R., & J. Padišák, 2002. The forgotten lake stratification pattern: atelomixis, and its ecological importance. *Verhandlungen der Internationale Vereinigung für theoretische und angewandte* 28: 1385–1395.
- Barbosa, J. E. de L., E. S. F. Medeiros, J. Brasil, R. da S. Cordeiro, M. C. B. Crispim, & G. H. G. da Silva, 2012. Aquatic systems in semi-arid Brazil: limnology and management. *Acta Limnologica Brasiliensia Associação Brasileira de Limnologia* 24: 103–118.
- Barroso, H. S., J. A. dos Santos, R. V. Marins, & L. D. de Lacerda, 2018. Assessing temporal and spatial variability of phytoplankton composition in a large reservoir in the Brazilian northeastern region under intense drought conditions. *Journal of Limnology* 77: 130–146.

- Batista, A. M. M., C. C. Figueredo, & A. Giani, 2018. Variability in a permanent cyanobacterial bloom: species-specific responses to environmental drivers. *FEMS Microbiology Ecology* 94: 197.
- Becker, V., L. Cardoso, & V. L. M. Huszar, 2009. Diel variation of phytoplankton functional groups in a subtropical reservoir in southern Brazil during an autumnal stratification period. *Aquatic Ecology* 43: 285–293.
- Bekker, A., H. D. Holland, P. L. Wang, D. Rumble, H. J. Stein, J. L. Hannah, L. L. Coetzee, & N. J. Beukes, 2004. Dating the rise of atmospheric oxygen. *Nature* 427: 117–120.
- Bittencourt-Oliveira, M. D. C., V. Piccin-Santos, A. N. Moura, N. K. C. Aragão-Tavares, & M. K. Cordeiro-Araújo, 2014. Cyanobacteria, microcystins and cylindrospermopsin in public drinking supply reservoirs of Brazil. *Anais da Academia Brasileira de Ciências* 86: 297–309.
- Bonilla, S., L. Aubriot, M. C. S. Soares, M. González-Piana, A. Fabre, V. L. M. Huszar, M. Lüring, D. Antoniades, J. Padisák, & C. Kruk, 2012. What drives the distribution of the bloom-forming cyanobacteria *Planktothrix agardhii* and *Cylindrospermopsis raciborskii*? *FEMS Microbiology Ecology* 79: 594–607.
- Bonilla, S., M. González-Piana, M. C. S. Soares, V. L. M. Huszar, V. Becker, A. Somma, M. M. Marinho, M. Kokociński, M. Dokulil, D. Antoniades, & L. Aubriot, 2016. The success of the cyanobacterium *Cylindrospermopsis raciborskii* in freshwaters is enhanced by the combined effects of light intensity and temperature. *Journal of Limnology* 75: 606–617.
- Borics, G., I. Grigorszky, S. Szabó & J. Padisák, 2000. Phytoplankton associations in a small hypertrophic fishpond in East Hungary during a change from bottom-up to top-down control. *Hydrobiologia* 424: 79-90.
- Bortolini, J. C., & N. C. Bueno, 2017. Temporal dynamics of phytoplankton using the morphology-based functional approach in a subtropical river. *Revista Brasileira de Botânica* 40: 741–748.
- Bortolini, J. C., P. R. L. da Silva, G. Baumgartner, & N. C. Bueno, 2019. Response to environmental, spatial, and temporal mechanisms of the phytoplankton metacommunity: comparing ecological approaches in subtropical reservoirs. *Hydrobiologia* 830: 45–61.
- Bortolini, J. C., G. A. Moresco, A. C. M. de Paula, S. Jati, & L. C. Rodrigues, 2016. Functional approach based on morphology as a model of phytoplankton variability in a subtropical floodplain lake: a long-term study. *Hydrobiologia* 767: 151–163.
- Bouvy, M., D. Falcão, M. Marinho, M. Pagano, & A. Moura, 2000. Occurrence of *Cylindrospermopsis* (Cyanobacteria) in 39 Brazilian tropical reservoirs during the 1998 drought. *Aquatic Microbial Ecology* 23: 13–27.
- Bouvy, M., S. M. Nascimento, R. J. R. Molica, A. Ferreira, V. Huszar, & S. M. F. O. Azevedo, 2003. Limnological features in Tapacurá reservoir (northeast Brazil) during a severe drought. *Hydrobiologia* 493: 115–130.
- Braga, G. G., & V. Becker, 2020. Influence of water volume reduction on the phytoplankton dynamics in a semi-arid man-made lake: A comparison of two morphofunctional approaches. *Anais da Academia Brasileira de Ciências* 92: 1–17.

- Braga, G. G., V. Becker, J. N. P. de Oliveira, J. R. de Mendonça Junior, A. F. de M. Bezerra, L. M. Torres, Â. M. F. Galvão, & A. Mattos, 2015. Influence of extended drought on water quality in tropical reservoirs in a semi-arid region. *Acta Limnologica Brasiliensia* 27: 15–23.
- Brasil, J., J. L. Attayde, F. R. Vasconcelos, D. D. F. Dantas, & V. L. M. Huszar, 2016. Drought-induced water-level reduction favors cyanobacteria blooms in tropical shallow lakes. *Hydrobiologia* 770: 145–164.
- Brasil, J., & V. L. M. Huszar, 2011. O papel dos traços funcionais na ecologia do fitoplâncton continental. *Oecologia Australis* 15: 799–834.
- Brentrup, J. A., C. E. Williamson, W. Colom-Montero, W. Eckert, E. de Eyto, H. P. Grossart, Y. Huot, P. D. F. Isles, L. B. Knoll, T. H. Leach, C. G. McBride, D. Pierson, F. Pomati, J. S. Read, K. C. Rose, N. R. Samal, P. A. Staehr, & L. A. Winslow, 2016. The potential of high-frequency profiling to assess vertical and seasonal patterns of phytoplankton dynamics in lakes: An extension of the Plankton Ecology Group (PEG) model. *Inland Waters* 6: 565–580.
- Burford, M. A., J. Beardall, A. Willis, P. T. Orr, V. F. Magalhaes, L. M. Rangel, S. M. F. O. E. Azevedo, & B. A. Neilan, 2016. Understanding the winning strategies used by the bloom-forming cyanobacterium *Cylindrospermopsis raciborskii*. *Harmful Algae* 54: 44–53.
- Camacho, A., 2006. On the occurrence and ecological features of deep chlorophyll maxima (DCM) in Spanish stratified lakes. *Limnetica* 25: 453–478.
- Carey, C. C., B. W. Ibelings, E. P. Hoffmann, D. P. Hamilton, & J. D. Brookes, 2012. Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. *Water Research* 46: 1394–1407.
- Carmichael, W. W., 1992. Cyanobacteria secondary metabolites—the cyanotoxins. *Journal of Applied Bacteriology* 72: 445–459.
- Carraro, E., N. Guyennon, D. Hamilton, L. Valsecchi, E. C. Manfredi, G. Viviano, F. Salerno, G. Tartari, & D. Copetti, 2012. Phytoplankton responses to human impacts at different scales. Coupling high-resolution measurements to a three-dimensional lake model to assess the spatial and temporal dynamics of the cyanobacterium *Planktothrix rubescens* in a medium-sized lake. *Hydrobiologia* 698: 77–95.
- Casper, P., & R. Koschel, 1995. Description of Lake Stechlin. *Limnologica* 25: 281–284.
- Casper, S. J., 1985. Lake Stechlin: a temperate oligotrophic lake. 58.
- Cavalcante, H., F. Araújo, & V. Becker, 2018. Phosphorus dynamics in the water of tropical semi-arid reservoirs in a prolonged drought period. *Acta Limnologica Brasiliensia* 30.
- Chanlett, E. T., 1947. Standard Methods for the Examination of Water and Sewage. *American Journal of Public Health and the Nations Health American Public Health Association* 37: 1053–1054.
- Chislock, M. F., O. Sarnelle, L. M. Jernigan, & A. E. Wilson, 2013. Do high concentrations of microcystin prevent *Daphnia* control of phytoplankton? *Water Research* 47: 1961–1970.

- Chislock, M. F., K. L. Sharp, & A. E. Wilson, 2014. *Cylindrospermopsis raciborskii* dominates under very low and high nitrogen-to-phosphorus ratios. *Water Research* 49: 207–214.
- Chorus, I., & E. Spijkerman, 2021. What Colin Reynolds could tell us about nutrient limitation, N:P ratios and eutrophication control. *Hydrobiologia* 848: 95–111.
- Chorus, I., & M. Welker, 2021. Exposure to cyanotoxins. In: *Toxic Cyanobacteria in Water*: 295–400, WHO.
- Cirés, S., & A. Ballot, 2016. A review of the phylogeny, ecology and toxin production of bloom-forming *Aphanizomenon spp.* and related species within the Nostocales (cyanobacteria). *Harmful Algae* 54: 21–43.
- Cirés, S., M. C. Casero, & A. Quesada, 2017. Toxicity at the edge of life: A review on cyanobacterial toxins from extreme environments. *Marine Drugs*. 7: 233.
- Cole, G., 1994. *Textbook of Limnology*. Waveland Press., Illinois.
- Cornec, M., H. Claustre, A. Mignot, L. Guidi, L. Lacour, A. Poteau, F. D’Ortenzio, B. Gentili, & C. Schmechtig, 2021. Deep Chlorophyll Maxima in the Global Ocean: Occurrences, Drivers and Characteristics. *Global Biogeochemical Cycles* 35: e2020GB006759.
- Costa, M. R. A., J. L. Attayde, & V. Becker, 2016. Effects of water level reduction on the dynamics of phytoplankton functional groups in tropical semi-arid shallow lakes. *Hydrobiologia* 778: 75–89.
- Costa, M. R. A., R. F. Menezes, H. Sarmiento, J. L. Attayde, L. da S. L. Sternberg, & V. Becker, 2019. Extreme drought favors potential mixotrophic organisms in tropical semi-arid reservoirs. *Hydrobiologia* 831: 43–54.
- Cottingham, K. L., H. A. Ewing, M. L. Greer, C. C. Carey, & K. C. Weathers, 2015. Cyanobacteria as biological drivers of lake nitrogen and phosphorus cycling. *Ecosphere* 6: 1.
- Crossetti, L. O., D. de C. Bicudo, L. M. Bini, R. B. Dala-Corte, C. Ferragut, & C. E. de Mattos Bicudo, 2019. Phytoplankton species interactions and invasion by *Ceratium furcoides* are influenced by extreme drought and water-hyacinth removal in a shallow tropical reservoir. *Hydrobiologia* 831: 71–85.
- Cullen, J. J., 1982. The deep chlorophyll maximum: comparing vertical profiles of chlorophyll-a. *Canadian Journal of Fisheries and Aquatic Sciences* 39: 791–803.
- Cullen, J. J., 2015. Subsurface chlorophyll maximum layers: Enduring enigma or mystery solved? *Annual Review of Marine Science* 7: 207–239.
- Cuypers, Y., B. Vinçon-Leite, A. Groleau, B. Tassin, & J. F. Humbert, 2011. Impact of internal waves on the spatial distribution of *Planktothrix rubescens* (cyanobacteria) in an alpine lake. *ISME Journal* 5: 580–589.
- Dadheech, P. K., G. B. Selmechzy, G. Vasas, J. Pádisak, W. Arp, K. Tapolczai, P. Casper, & L. Krienitz, 2014. Presence of potential toxin-producing cyanobacteria in an oligo-mesotrophic lake in Baltic Lake District, Germany: An ecological, genetic and toxicological survey. *Toxins* 6: 2912–2931.

- Davis, P. A., & A. E. Walsby, 2002. Comparison of measured growth rates with those calculated from rates of photosynthesis in *Planktothrix* spp. isolated from Blelham Tarn, English Lake District. *New Phytologist* 156: 225–239.
- Derot, J., A. Jamoneau, N. Teichert, J. Rosebery, S. Morin, & C. Laplace-Treyture, 2020. Response of phytoplankton traits to environmental variables in French lakes: New perspectives for bioindication. *Ecological Indicators* 108: 105659.
- Dokulil, M. T., & K. Teubner, 2000. Cyanobacterial dominance in lakes. *Hydrobiologia*. 438: 1-14.
- Dokulil, M. T., & K. Teubner, 2012. Deep living *Planktothrix rubescens* modulated by environmental constraints and climate forcing. *Hydrobiologia* 698: 29–46.
- Domingos, P., T. K. Rubim, R. J. R. Molica, S. M. F. O. Azevedo, & W. W. Carmichael, 1999. First report of microcystin production by picoplanktonic cyanobacteria isolated from a Northeast Brazilian drinking water supply. *Environmental Toxicology* 14: 31–35.
- EPA, 2015. A Compilation of Cost Data Associated with the Impacts and Control of Nutrient Pollution. EPA 820-F-15-096.
- Ernst, B., S. J. Hoeger, E. O'Brien, & D. R. Dietrich, 2009. Abundance and toxicity of *Planktothrix rubescens* in the pre-alpine Lake Ammersee, Germany. *Harmful Algae* 8: 329–342.
- Erratt, K. J., I. F. Creed, E. C. Freeman, C. G. Trick, J. Westrick, J. A. Birbeck, L. C. Watson, & A. Zastepa, 2022. Deep cyanobacteria layers: an overlooked aspect of managing risks of cyanobacteria. *Environmental Science and Technology* 56: 17902-17912
- Ewing, H. A., K. C. Weathers, K. L. Cottingham, P. R. Leavitt, M. L. Greer, C. C. Carey, B. G. Steele, A. U. Fiorillo, & J. P. Sowles, 2020. “New” cyanobacterial blooms are not new: two centuries of lake production are related to ice cover and land use. *Ecosphere* 11: e13170.
- Fabbro, L. D., & L. J. Duivenvoorden, 2000. A two-part model linking multidimensional environmental gradients and seasonal succession of phytoplankton assemblages. *Hydrobiologia* 438: 13–24.
- Fee, E. J., 1976. The vertical and seasonal distribution of chlorophyll in lakes of the experimental lakes area, Northwestern Ontario: Implications for primary production estimates. *Limnology and Oceanography* 21: 767–783.
- Feng, S., & Q. Fu, 2013. Expansion of global drylands under a warming climate. *Atmospheric Chemistry and Physics* 13: 10081–10094.
- Figueiredo, A. do V., & V. Becker, 2018. Influence of extreme hydrological events in the quality of water reservoirs in the semi-arid tropical region. *Brazilian Journal of Water Resources* 23: 1–8.
- Figueredo, C. C., & A. Giani, 2009. Phytoplankton community in the tropical lake of Lagoa Santa (Brazil): Conditions favoring a persistent bloom of *Cylindrospermopsis raciborskii*. *Limnologia* 39: 264–272.

- Figueredo, C. C., R. M. Pinto-Coelho, A. M. M. B. Lopes, P. H. O. Lima, B. Gücker, & A. Giani, 2016. From intermittent to persistent cyanobacterial blooms: Identifying the main drivers in an urban tropical reservoir. *Journal of Limnology* 75: 445–454.
- Fonseca, B. M., C. Ferragut, A. Tucci, L. O. Crossetti, F. Ferrari, D. de C. Bicudo, C. L. Sant’Anna, & C. E. de M. Bicudo, 2014. Biovolume de cianobactérias e algas de reservatórios tropicais do Brasil com diferentes estados tróficos. *Hoehnea* 41: 9–30.
- Fonseca, J. R., P. C. S. Vieira, P. Kujbida, & I. A. S. da Costa, 2015. Cyanobacterial occurrence and detection of microcystins and saxitoxins in reservoirs of the Brazilian semi-arid. *Acta Limnologica Brasiliensia* 27: 78–92.
- Freeman, E. C., I. F. Creed, B. Jones, & A. K. Bergström, 2020. Global changes may be promoting a rise in select cyanobacteria in nutrient-poor northern lakes. *Global Change Biology* 26: 4966–4987.
- Frempong, E., 1981. Diel variation in the abundance, vertical distribution, and species composition of phytoplankton in a eutrophic english lake. *The Journal of Ecology* 69: 919.
- Gallina, N., M. Beniston, & S. Jacquet, 2017. Estimating future cyanobacterial occurrence and importance in lakes: a case study with *Planktothrix rubescens* in Lake Geneva. *Aquatic Sciences* 79: 249–263.
- Gervais, F., J. Padišák, & R. Koschel, 1997. Do light quality and low nutrient concentration favour picocyanobacteria below the thermocline of the oligotrophic Lake Stechlin? *Journal of Plankton* 19: 771–781.
- Giani, A., Z. E. Taranu, G. von Rückert, & I. Gregory-Eaves, 2020. Comparing key drivers of cyanobacteria biomass in temperate and tropical systems. *Harmful Algae* 97: 101859.
- González-Madina, L., J. P. Pacheco, L. Yema, P. de Tezanos, P. Levrini, J. Clemente, C. Crisci, J. J. Lagomarsino, G. Méndez, C. Fosalba, G. Goyenola, & N. Mazzeo, 2019. Drivers of cyanobacteria dominance, composition and nitrogen fixing behavior in a shallow lake with alternative regimes in time and space, Laguna del Sauce (Maldonado, Uruguay). *Hydrobiologia* 829: 61–76.
- Gorlach-Lira, K., C. Pacheco, L. C. T. Carvalho, H. N. Melo Júnior, & M. C. Crispim, 2013. The influence of fish culture in floating net cages on microbial indicators of water quality. *Brazilian Journal of Biology* 73: 457–463.
- Grigorszky, I., J. Padišák, G. Borics, C. Schitschen, & G. Borbély, 2003. Deep chlorophyll maximum by *Ceratium hirundinella* (O. F. Müller) Bergh in a shallow oxbow in Hungary. *Hydrobiologia* 506–509: 209–212.
- Hair, J. F. J., R. E. Anderson, R. L. Tatham, & W. C. Black, 1995. *Multivariate Data Analysis*. Macmillan, New York.
- Halstvedt, C. B., T. Rohrlack, T. Andersen, O. Skulberg, & B. Edvardsen, 2007. Seasonal dynamics and depth distribution of *Planktothrix* spp. in Lake Steinsfjorden (Norway) related to environmental factors. *Journal of Plankton* 29: 471–482.

- Hampton, S. E., D. K. Gray, L. R. Izmet'eva, M. V. Moore, & T. Ozersky, 2014. The rise and fall of plankton: Long-term changes in the vertical distribution of algae and grazers in Lake Baikal, Siberia. *PLoS ONE* 9: e88920.
- Hamre, K. D., M. E. Lofton, R. P. McClure, Z. W. Munger, J. P. Doubek, A. B. Gerling, M. E. Schreiber, & C. C. Carey, 2018. In situ fluorometry reveals a persistent, perennial hypolimnetic cyanobacterial bloom in a seasonally anoxic reservoir. *Freshwater Science* 37: 483–495.
- Harke, M. J., T. W. Davis, S. B. Watson, & C. J. Gobler, 2016. Nutrient-controlled niche differentiation of western Lake Erie cyanobacterial populations revealed via metatranscriptomic surveys. *Environmental Science and Technology* 50: 604–615.
- Havens, K. E., G. Ji, J. R. Beaver, R. S. Fulton, & C. E. Teacher, 2019. Dynamics of cyanobacteria blooms are linked to the hydrology of shallow Florida lakes and provide insight into possible impacts of climate change. *Hydrobiologia* 829: 43–59.
- Henry-Silva, G. G., H. D. N. Melo-Junior, & J. L. Attayde, 2019. Extreme drought events and the sustainability of fish farming in net cages in reservoirs of the semi-arid northeastern region in Brazil. *Acta Limnologica Brasiliensia* 31: e112.
- Hillebrand, H., 1999. Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology* 424: 403–424.
- Hoffman, M., H. W. Paerl, M. F. Piehler, J. Braddy, P. H. Moisaner, L. A. Cheshire, & E. S. Calandrino, 2011. Facultative diazotrophy increases *Cylindrospermopsis raciborskii* competitiveness under fluctuating nitrogen availability. *Microbiology Ecology* 79: 800–811.
- Holland, D. P., & A. E. Walsby, 2008. Viability of the cyanobacterium *Planktothrix rubescens* in the cold and dark, related to over-winter survival and summer recruitment in Lake Zürich. *European Journal of Phycology* 43: 179–184.
- Huang, J., H. Yu, X. Guan, G. Wang, & R. Guo, 2016. Accelerated dryland expansion under climate change. *Nature Climate Change* 6: 166–171.
- Huisman, J., G. A. Codd, H. W. Paerl, B. W. Ibelings, J. M. H. Verspagen, & P. M. Visser, 2018. Cyanobacterial blooms. *Nature Reviews Microbiology* 16: 471–483.
- Huisman, J., J. Sharples, J. M. Stroom, P. M. Visser, W. E. A. Kardinaal, J. M. H. Verspagen, & B. Sommeijer, 2004. Changes in turbulent mixing shift competition for light between phytoplankton species. *Ecology* 85: 2960–2970.
- Huisman, Richard R. Jonker, Cor Zonneveld, & Franz J. Weissing, 1999. Competition for light between phytoplankton species: Experimental tests of mechanistic theory. *Ecology* 80: 211–222.
- Huszar, V. L. M., L. H. S. Silva, M. Marinho, P. Domingos, & C. L. Sant'Anna, 2000. Cyanoprokaryote assemblages in eight productive tropical Brazilian waters. *Hydrobiologia* 424: 67–77.
- IPCC Climate Change, 2021. The Physical Science Basis. Working Group I Contribution to the IPCC Sixth Assessment Report.

- Ibelings, B. W., L. C. Backer, W. E. A. Kardinaal, & I. Chorus, 2014. Current approaches to cyanotoxin risk assessment and risk management around the globe. *Harmful Algae* 40: 63–74.
- Izaguirre, I., L. Allende, R. Escaray, J. Bustingorry, G. Pérez, & G. Tell, 2012. Comparison of morpho-functional phytoplankton classifications in human-impacted shallow lakes with different stable states. *Hydrobiologia* 698: 203–216.
- Jacquet, S., J. F. Briand, C. Leboulanger, C. Avois-Jacquet, L. Oberhaus, B. Tassin, B. Vinçon-Leite, G. Paolini, J. C. Druart, O. Anneville, & J. F. Humbert, 2005. The proliferation of the toxic cyanobacterium *Planktothrix rubescens* following restoration of the largest natural French lake (Lac du Bourget). *Harmful Algae* 4: 651–672.
- Jane, S.F., Hansen, G. J. A., Kraemer, B. M., Leavitt, P. R., Mincer, J. L., North, R. L., Pilla, R. M., Stetler, J. T., Williamson, C. E., Woolway, R. I., Arvola, L., Chandra, S., DeGasperi, C. L., Diemer, L., Dunalska, J., Erina, O., Flaim, G., Grossart, H. P., Hambright, K. D., Hein, C., Hejzlar, J., Janus, L. L., Jenny, J. P., Jones, J. R., Knoll, L. B., Leoni, B., Mackay, E., Matsuzaki, S. S., McBride, C., Müller-Navarra, D. C., Paterson, A. M., Pierson, D., Rogora, M., Rusak, J. A., Sadro, S., Saulnier-Talbot, E., Schmid, M., Sommaruga, R., Thiery, W., Verburg, P., Weathers, K. C., Weyhenmeyer, G. A., Yokota, K., Rose, K. C., 2021. Widespread deoxygenation of temperate lakes. *Nature* 594 :66-70.
- Jane, S. F., Mincer, J. L., Lau, M. P., Lewis, A. S. L., Stetler, J. T., & Rose, K. C., 2023. Longer duration of seasonal stratification contributes to widespread increases in lake hypoxia and anoxia. *Global Change Biology* 29: 1009– 1023.
- Jargal, N., U. Atique, M. Mamun, & K. G. An, 2021. Seasonal and long-term connections between trophic status, sestonic chlorophyll, nutrients, organic matter, and monsoon rainfall in a multipurpose reservoir. *Water* 13: 1720.
- Jespersen, A. M., & K. Christoffersen, 1987. Measurements of chlorophyll-a from phytoplankton using ethanol as extraction solvent. *Archiv für Hydrobiologie* 109: 445-454.
- Jia, N., Y. Yang, G. Yu, Y. Wang, P. Qiu, H. Li, & R. Li, 2020. Interspecific competition reveals *Raphidiopsis raciborskii* as a more successful invader than *Microcystis aeruginosa*. *Harmful Algae* 97: 101858.
- Karadžić, V., G. S. Simić, D. Natić, A. Ržaničanin, M. Ćirić, & Z. Gačić, 2013. Changes in the phytoplankton community and dominance of *Cylindrospermopsis raciborskii* (Wolosz.) Subba Raju in a temperate lowland river (Ponjavica, Serbia). *Hydrobiologia* 711: 43–60.
- Kasprzak, P., T. Shatwell, M. O. Gessner, T. Gonsiorczyk, G. Kirillin, G. Selmezy, J. Padisák, & C. Engelhardt, 2017. Extreme weather event triggers cascade towards extreme turbidity in a clear-water lake. *Ecosystems* 20: 1407–1420.
- Klausmeier, C. A., & E. Litchman, 2001. Algal games: The vertical distribution of phytoplankton in poorly mixed water columns. *Limnology and Oceanography* 46: 1998–2007.
- Knapp, D., B. Fernández Castro, D. Marty, E. Loher, O. Köster, A. Wüest, & T. Posch, 2021. The red harmful plague in times of climate change: blooms of the cyanobacterium *Planktothrix rubescens* triggered by stratification dynamics and irradiance. *Frontiers in Microbiology* 12: 705914.

- Koschel, R., & D. D. Adams, 2003. An approach to understanding a temperate oligotrophic lowland lake (Lake Stechlin, Germany). *Advances in Limnology* 58: 1–9.
- Koschel, R., J. Brenndorf, G. Proft, & R. Recknagel, 1983. Calcite precipitation as a natural mechanism of eutrophication. *Archiv für Hydrobiologie* 98: 380–408.
- Koschel, R. H., T. Gonsiorczyk, L. Krienitz, J. Padisák, & W. Scheffler, 2002. Primary production of phytoplankton and nutrient metabolism during and after thermal pollution in a deep, oligotrophic lowland lake (Lake Stechlin, Germany). *SIL Verhandlungen der internationale Vereinigung für theoretische und angewandte Limnologie* 28: 569–575.
- Kosten, S., V. L. M. Huszar, E. Bécares, L. S. Costa, E. van Donk, L. A. Hansson, E. Jeppesen, C. Kruk, G. Lacerot, N. Mazzeo, L. De Meester, B. Moss, M. Lürling, T. Nöges, S. Romo, & M. Scheffer, 2012. Warmer climates boost cyanobacterial dominance in shallow lakes. *Global Change Biology* 18: 118–126.
- Krieger, 1927. Die Gattung *Centronella* Voigt. *Ber. Deutsch. Bot. Ges* 45: 281–290.
- Kröger, B., G. B. Selmecky, P. Casper, J. Soininen & J. Padisák, 2023. Long-term phytoplankton community dynamics in Lake Stechlin (NE Germany) under sudden and heavily accelerating eutrophication *Freshwater Biology* 68: 737-751.
- Kruk, C., M. Devercelli, & V. L. Huszar, 2020. Reynolds Functional Groups: A trait-based pathway from patterns to predictions. *Hydrobiologia* 848: 113–129.
- Kruk, C., V. L. M. Huszar, E. T. H. M. Peeters, S. Bonilla, L. Costa, M. Lürling, C. S. Reynolds, & M. Scheffer, 2010. A morphological classification capturing functional variation in phytoplankton. *Freshwater Biology* 55: 614–627.
- Kruk, C., & A. M. Segura, 2012. The habitat template of phytoplankton morphology-based functional groups. *Hydrobiologia* 698: 191–202.
- Lampert, W., E. McCauley, & B. F. J. Manly, 2003. Trade-offs in the vertical distribution of zooplankton: ideal free distribution with costs? *Proc Biol Sci.* 270: 765–773.
- Le Moal, M., C. Gascuel-Oudou, A. Ménesguen, Y. Souchon, C. Étrillard, A. Levain, F. Moatar, A. Pannard, P. Souchu, A. Lefebvre, & G. Pinay, 2019. Eutrophication: A new wine in an old bottle? *Science of the Total Environment* 651: 1–11.
- Leach, T. H., B. E. Beisner, C. C. Carey, P. Pernica, K. C. Rose, Y. Huot, J. A. Brentrup, I. Domaizon, H. P. Grossart, B. W. Ibelings, S. Jacquet, P. T. Kelly, J. A. Rusak, J. D. Stockwell, D. Straile, & P. Verburg, 2018. Patterns and drivers of deep chlorophyll maxima structure in 100 lakes: The relative importance of light and thermal stratification. *Limnology and Oceanography* 63: 628–646.
- Lee, G. F., Sonzogni, W. C., & Spear, R. D., 1977. Significance of oxic vs anoxic conditions for Lake Mendota sediment phosphorus release. *Interactions Between Sediments and Freshwater* 294-306.
- Lee, J., S. Lee, & X. Jiang, 2017. Cyanobacterial toxins in freshwater and food: Important sources of exposure to humans. *Annual Review of Food Science and Technology* 8: 281–304.

- Leflaive, J., & L. Ten-Hage, 2007. Algal and cyanobacterial secondary metabolites in freshwaters: A comparison of allelopathic compounds and toxins. *Freshwater Biology* 52: 199–214.
- Legendre, P., & E. D. Gallagher, 2001. Ecologically meaningful transformations for ordination of species data. *Oecologia* 129: 271–280.
- Leitão, E., K. A. Ger, & R. Panosso, 2018. Selective grazing by a tropical copepod (*Notodiaptomus iheringi*) facilitates microcystis dominance. *Frontiers in Microbiology* 9: 301.
- Lenard, T., 2015. Winter bloom of some motile phytoplankton under ice cover in a mesotrophic lake: Vertical distribution and environmental factors. *Oceanological and Hydrobiological* 44: 164–171.
- Lind, O., L. Dávalos-Lind, C. López, M. López, & J. Dyble Bressie, 2016. Seasonal morphological variability in an in situ Cyanobacteria monoculture: example from a persistent *Cylindrospermopsis* bloom in Lake Catemaco, Veracruz, Mexico. *Journal of Limnology* 75: 66–80.
- Litchman, E., 2010. Invisible invaders: Non-pathogenic invasive microbes in aquatic and terrestrial ecosystems. *Ecology Letters* 13: 1560–1572.
- Litchman, E., P. de Tezanos Pinto, C. A. Klausmeier, M. K. Thomas, & K. Yoshiyama, 2010. Linking traits to species diversity and community structure in phytoplankton. *Hydrobiologia* 653: 15–28.
- Litchman, E., & C. A. Klausmeier, 2008. Trait-based community ecology of phytoplankton. *Annual Review of Ecology, Evolution, and Systematics* 39: 615–639.
- Lofton, M. E., T. H. Leach, B. E. Beisner, & C. C. Carey, 2020. Relative importance of top-down vs. bottom-up control of lake phytoplankton vertical distributions varies among fluorescence-based spectral groups. *Limnology and Oceanography* 65: 2485–2501.
- Lorenzi, A. S., M. K. Cordeiro-Araújo, M. A. Chia, & M. do C. Bittencourt-Oliveira, 2018. Cyanotoxin contamination of semi-arid drinking water supply reservoirs. *Environmental Earth Sciences* 77: 595.
- Lund, J. W. G., C. Kipling, & E. D. Le Cren, 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia* 11: 143–170.
- Magalhães, L., L. M. Rangel, A. Melo Rocha, S. J. Cardoso, & L. H. Sampaio da Silva, 2020. Responses of morphology-based phytoplankton functional groups to spatial variation in two tropical reservoirs with long water-residence time. *Inland Waters* 11: 29–43.
- Mantzouki, E., P. M. Visser, M. Bormans, & B. W. Ibelings, 2016. Understanding the key ecological traits of cyanobacteria as a basis for their management and control in changing lakes. *Aquatic Ecology* 50: 333–350.
- Marcon, A. E., J. A. Navoni, M. F. de Oliveira Galvão, A. C. F. S. Garcia, V. S. do Amaral, R. A. Petta, T. F. da C. Campos, R. Panosso, A. L. Quinelato, & S. R. B. de Medeiros, 2017. Mutagenic potential assessment associated with human exposure to natural radioactivity. *Chemosphere* 167: 36–43.

- Marengo, J. A., L. M. Alves, R. C. S. Alvala, A. P. Cunha, S. Brito, & O. L. L. Moraes, 2018. Climatic characteristics of the 2010-2016 drought in the semi-arid northeast Brazil region. *Anais da Academia Brasileira de Ciencias* 90: 1973–1985.
- Marengo, J. A., A. P. M. A. Cunha, C. A. Nobre, G. G. Ribeiro Neto, A. R. Magalhaes, R. R. Torres, G. Sampaio, F. Alexandre, L. M. Alves, L. A. Cuartas, K. R. L. Deusdará, & R. C. S. Alvala, 2020. Assessing drought in the drylands of northeast Brazil under regional warming exceeding 4 °C. *Natural Hazards* 103: 2589–2611.
- Marengo, J. A., R. R. Torres, & L. M. Alves, 2017. Drought in Northeast Brazil—past, present, and future. *Theoretical and Applied Climatology* 129: 1189–1200.
- Margalef, R., 1978. Life-forms of phytoplankton as survival alternatives in an unstable environment. *Oceanologica Acta* 1: 493–509.
- Marion, J. W., F. Zhang, D. Cutting, & J. Lee, 2017. Associations between county-level land cover classes and cyanobacteria blooms in the United States. *Ecological Engineering* 108: 556–563.
- Martiny, A.C., Treseder, K., Pusch, G., 2013. Phylogenetic conservatism of functional traits in microorganisms. *ISME J.* 7: 830-8.
- McGregor, G. B., & L. D. Fabbro, 2000. Dominance of *Cylindrospermopsis raciborskii* (Nostocales, Cyanoprokaryota) in Queensland tropical and subtropical reservoirs: Implications for monitoring and management. *Lakes and Reservoirs: Research and Management* 5: 195–205.
- Medeiros, L., A. Mattos, M. Lüring, & V. Becker, 2015. Is the future blue-green or brown? The effects of extreme events on phytoplankton dynamics in a semi-arid man-made lake. *Aquatic Ecology* 49: 293–307.
- Mellard, J. P., K. Yoshiyama, E. Litchman, & C. A. Klausmeier, 2011. The vertical distribution of phytoplankton in stratified water columns. *Journal of Theoretical Biology* 269: 16–30.
- Mello, M. M. E., M. C. S. Soares, F. Roland, & M. Lüring, 2012. Growth inhibition and colony formation in the cyanobacterium *Microcystis aeruginosa* induced by the cyanobacterium *Cylindrospermopsis raciborskii*. *Journal of Plankton* 4: 987–994.
- Menezes, R. S. C., E. V. S. B. Sampaio, V. Giongo, & A. M. Pérez-Marin, 2012. Ciclagem biogeoquímica em ecossistemas terrestres do bioma Caatinga. *Brazilian Journal of Biology* 72: 643–653.
- Merel, S., D. Walker, R. Chicana, S. Snyder, E. Baurès, & O. Thomas, 2013. State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. *Environment International* 59: 303–327.
- Meriggi, C., S. Drakare, E. Polaina Lacambra, R. K. Johnson, & A. T. Laugen, 2022. Species distribution models as a tool for early detection of the invasive *Raphidiopsis raciborskii* in European lakes. *Harmful Algae* 113: 102202.
- Mi, C., D. P. Hamilton, M. A. Frassl, T. Shatwell, X. Kong, B. Boehrer, Y. Li, J. Donner, & K. Rinke, 2022. Controlling blooms of *Planktothrix rubescens* by optimized metalimnetic water withdrawal: a modelling study on adaptive reservoir operation. *Environmental Sciences Europe* 34: 102.

- Mignot, A., H. Claustre, F. D'Ortenzio, X. Xing, A. Poteau, & J. Ras, 2011. From the shape of the vertical profile of in vivo fluorescence to chlorophyll-a concentration. *Biogeosciences* 8: 2391–2406.
- Mignot, A., H. Claustre, J. Uitz, A. Poteau, F. D'Ortenzio, & X. Xing, 2014. Understanding the seasonal dynamics of phytoplankton biomass and the deep chlorophyll maximum in oligotrophic environments: A Bio-Argo float investigation. *Global Biogeochemical Cycles* 28: 856–876.
- Molica, R. J. R., E. J. A. Oliveira, P. V. V. C. Carvalho, A. N. S. F. Costa, M. C. C. Cunha, G. L. Melo, & S. M. F. O. Azevedo, 2005. Occurrence of saxitoxins and an anatoxin-a(s)-like anticholinesterase in a Brazilian drinking water supply. *Harmful Algae* 4: 743–753.
- Mosley, L. M., 2015. Drought impacts on the water quality of freshwater systems; review and integration. *Earth-Science Reviews* 203–214.
- Moss, B., 2011. Allied attack: climate change and eutrophication. *Inland Waters* 1: 101–105.
- Moura, A. D. N., N. K. C. Aragão-Tavares, & C. A. Amorim, 2018. Cyanobacterial blooms in freshwater bodies from a semi-arid region, Northeast Brazil: A review. *Journal of Limnology* 77: 179–188.
- Mowe, M. A. D., S. M. Mitrovic, R. P. Lim, A. Furey, & D. C. J. Yeo, 2015. Tropical cyanobacterial blooms: A review of prevalence, problem taxa, toxins and influencing environmental factors. *Journal of Limnology* 74: 205–224.
- Muir, D. G., & R. Perissinotto, 2011. Persistent phytoplankton bloom in Lake St. Lucia (Simangaliso Wetland Park, South Africa) caused by a cyanobacterium closely associated with the genus *Cyanothece* (Synechococcaceae, Chroococcales). *Applied and Environmental Microbiology* 77: 5888–5896.
- Naselli-Flores, L., 2000. Phytoplankton assemblages in twenty-one Sicilian reservoirs : Relationships between species composition and environmental factors. *Hydrobiologia* 424: 1–11.
- Naselli-Flores, L., 2003. Man-made lakes in Mediterranean semi-arid climate: The strange case of Dr. Deep Lake and Mr. Shallow Lake. *Hydrobiologia* 506–509: 13–21.
- Naselli-Flores, L., 2014. Morphological analysis of phytoplankton as a tool to assess ecological state of aquatic ecosystems: The case of Lake Arancio, Sicily, Italy. *Inland Waters* 4: 15–26.
- Naselli-Flores, L., & R. Barone, 2011. Fight on plankton! Or, phytoplankton shape and size as adaptive tools to get ahead in the struggle for life. *Cryptogamie, Algologie* 32: 157–204.
- Naselli-Flores, L., R. Barone, I. Chorus, & R. Kurmayer, 2007a. Toxic cyanobacterial blooms in reservoirs under a semi-arid Mediterranean climate: The magnification of a problem. *Environmental Toxicology* 22: 399-404.
- Naselli-Flores L; M. T. Dokulil, A. J. Elliott & J. Padišák, 2021b. New, old and evergreen frontiers in freshwater phytoplankton ecology: The legacy of Colin S. Reynolds. *Hydrobiologia* 848: 1-6.

- Naselli-Flores, L., & J. Padišák, 2016. Blowing in the wind: How many roads can a phytoplankton walk down? A synthesis on phytoplankton biogeography and spatial processes. *Hydrobiologia* 303–313.
- Naselli-Flores, L., J. Padišák, & M. Albay, 2007b. Shape and size in phytoplankton ecology: Do they matter? *Hydrobiologia* 578: 157–161.
- Naselli-Flores, L., T. Zohary, & J. Padišák, 2021a. Life in suspension and its impact on phytoplankton morphology: An homage to Colin S. Reynolds. *Hydrobiologia* 848: 7–30.
- Nürnberg, G. K., 1987. A comparison of internal phosphorus loads in lakes with anoxic hypolimnia: laboratory incubation versus in situ hypolimnetic phosphorus accumulation 1. *Limnology and Oceanography* 32: 1160–1164.
- O’Neil, J. M., T. W. Davis, M. A. Burford, & C. J. Gobler, 2012. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae* 14: 313–334.
- Olds, B. P., B. C. Peterson, K. D. Koupal, K. M. Farnsworth-Hoback, C. W. Schoenebeck, & W. Wyatt Hoback, 2011. Water quality parameters of a Nebraska reservoir differ between drought and normal conditions. *Lake and Reservoir Management* 27: 229–234.
- OPTICOUNT, 2008. http://science.do-mix.de/software_opticount.php
- Osland, M. J., P. W. Stevens, M. M. Lamont, R. C. Brusca, K. M. Hart, J. H. Waddle, C. A. Langtimm, C. M. Williams, B. D. Keim, A. J. Terando, E. A. Reyier, K. E. Marshall, M. E. Loik, R. E. Boucek, A. B. Lewis, & J. A. Seminoff, 2021. Tropicalization of temperate ecosystems in North America: The northward range expansion of tropical organisms in response to warming winter temperatures. *Global Change Biology* 27: 3009–3034.
- Pacheco, J. P., C. Iglesias, M. Meerhoff, C. Fosalba, G. Goyenola, F. Teixeira-de Mello, S. García, M. Gelós, & F. García-Rodríguez, 2010. Phytoplankton community structure in five subtropical shallow lakes with different trophic status (Uruguay): A morphology-based approach. *Hydrobiologia* 646: 187–197.
- Padišák, J., 1997. *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju, an expanding, highly adaptive cyanobacterium: worldwide distribution and review of its ecology. *Archiv für Hydrobiologie* 107: 563–593.
- Padišák, J., F. Antônio, R. Barbosa, & L. Krienitz, 2003a. Deep layer cyanoprokaryota maxima in temperate and tropical lakes. *Advances in Limnology* 58: 175–199.
- Padišák, J., L. O. Crossetti, & L. Naselli-Flores, 2009. Use and misuse in the application of the phytoplankton functional classification: A critical review with updates. *Hydrobiologia* 621: 1–19.
- Padišák, J., É. Hajnal, L. Krienitz, J. Lakner, & V. Üveges, 2010. Rarity, ecological memory, rate of floral change in phytoplankton-and the mystery of the Red Cock. *Hydrobiologia* 653: 45–64.
- Padišák, J., J. Köhler, & S. Hoeg, 1999. Effect of changing flushing rates on development of late summer *Aphanizomenon* and *Microcystis* populations in a shallow lake, Müggelsee, Berlin, Germany. In: *Theoretical Reservoir Ecology and its Applications*: 411–423, Bachhuys Publishers, Leiden.

- Padisák, J., L. Krienitz, R. Koschel, & J. Nedoma, 1997. Deep-layer autotrophic picoplankton maximum in the oligotrophic Lake Stechlin, Germany: Origin, activity, development and erosion. *European Journal of Phycology* 32: 403–416.
- Padisák, J., L. Krienitz, W. Scheffler, R. Koschel, J. Kristiansen, & I. Grigorszky, 1998. Phytoplankton succession in the oligotrophic Lake Stechlin (Germany) in 1994 and 1995. *Hydrobiologia* 369–370: 179–197.
- Padisák, J., & L. Naselli-Flores, 2021. Phytoplankton in extreme environments: Importance and consequences of habitat permanency. *Hydrobiologia* 848: 157–176.
- Padisák, J., & C. S. Reynolds, 1998. Selection of phytoplankton associations in Lake Balaton, Hungary, in response to eutrophication and restoration measures, with special reference to the cyanoprokaryotes. *Hydrobiologia* 384: 41–53.
- Padisák, J., W. Scheffler, P. Kasprzak, R. Koschel, & L. Krienitz, 2003b. Interannual variability in the phytoplankton composition of Lake Stechlin (1994–2000). *Advances in Limnology* 58: 101–133.
- Padisák, J., G. B. Selmeczy, M. Papke, S. Schmidt, J. Woodhouse, P. Kasprzak, S. Wollrab, U. Beyer, M. Bodenlos, M. Degebrod, R. Degebrod, T. Gonsiorczyk, E. Huth, M. Lentz, E. Mach, U. Mallok, J. C. Nejtgaard, S. Pinnow, R. Roßberg, ... S. A. Berger, 2023. Lake Stechlin phytoplankton 1994–2020. IGB Leibniz-Institute of Freshwater Ecology and Inland Fisheries. Dataset. <https://doi.org/10.18728/igb-fred-824.1>
- Padisák, J., G. Vasas, & G. Borics, 2016. Phycogeography of freshwater phytoplankton: Traditional knowledge and new molecular tools. *Hydrobiologia* 3–27.
- Paerl, H. W., 1988. Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. *Limnology and Oceanography* 33: 823–843.
- Paerl, H. W., 2017. Controlling harmful cyanobacterial blooms in a climatically more extreme world: Management options and research needs. *Journal of Plankton Research* 39: 763–771.
- Paerl, H. W., 2018. Mitigating toxic planktonic cyanobacterial blooms in aquatic ecosystems facing increasing anthropogenic and climatic pressures. *Toxins* 10: 1–16.
- Paerl, H. W., P. T. Bland, N. D. Bowles, & M. E. Haibach, 1985. Adaptation to high-Intensity, low-wavelength light among surface blooms of the cyanobacterium *Microcystis aeruginosa*. *Applied and Environmental Microbiology* 49: 1046–1052.
- Paerl, H. W., K. E. Havens, N. S. Hall, T. G. Otten, M. Zhu, H. Xu, G. Zhu, & B. Qin, 2020. Mitigating a global expansion of toxic cyanobacterial blooms: Confounding effects and challenges posed by climate change. *Marine and Freshwater Research* 7: 579–592.
- Paerl, H. W., & J. Huisman, 2008. Climate: Blooms like it hot. *Science* 320: 57–58.
- Paerl, H. W., & J. Huisman, 2009. Climate change: A catalyst for global expansion of harmful cyanobacterial blooms. *Environmental Microbiology* 1: 27–37.

- Paerl, H. W., & T. G. Otten, 2013. Harmful cyanobacterial blooms: Causes, consequences, and controls. *Microbial Ecology* 65: 995–1010.
- Paerl, H. W., & T. G. Otten, 2016. Duelling “CyanoHABs”: Unravelling the environmental drivers controlling dominance and succession among diazotrophic and non-N₂-fixing harmful cyanobacteria. *Environmental Microbiology* 18: 316–324.
- Paerl, H. W., & V. J. Paul, 2012. Climate change: Links to global expansion of harmful cyanobacteria. *Water Research* 46: 1349–1363.
- Paterson, M. J., D. W. Schindler, R. E. Hecky, D. L. Findlay, & K. J. Rondeau, 2011. Comment: Lake 227 shows clearly that controlling inputs of nitrogen will not reduce or prevent eutrophication of lakes. *Limnology and Oceanography* 56:1545–1547.
- Pedrosa, C. da S. G., L. R. Q. Souza, T. A. Gomes, C. V. F. de Lima, P. F. Ledur, K. Karmirian, J. Barbeito-Andres, M. D. N. Costa, L. M. Higa, Á. D. Rossi, M. Bellio, A. Tanuri, A. Prata-Barbosa, F. Tovar-Moll, P. P. Garcez, F. A. Lara, R. J. R. Molica, & S. K. Rehen, 2020. The cyanobacterial saxitoxin exacerbates neural cell death and brain malformations induced by Zika Virus. *PLoS Neglected Tropical Diseases* 14: e0008060.
- Porat, R., B. Teltsch, A. Perelman, & Z. Dubinsky, 2001. Diel buoyancy changes by the cyanobacterium *Aphanizomenon ovalisporum* from a shallow reservoir. *Journal of Plankton* 23: 753–763.
- R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, www.R-project.org/.
- Råman Vinnå, L., I. Medhaug, M. Schmid, & D. Bouffard, 2021. The vulnerability of lakes to climate change along an altitudinal gradient. *Communications Earth and Environment* 2: 35.
- Rangel, L. M., M. C. S. Soares, R. Paiva, & L. H. S. Silva, 2016. Morphology-based functional groups as effective indicators of phytoplankton dynamics in a tropical cyanobacteria-dominated transitional river-reservoir system. *Ecological Indicators* 64: 217–227.
- Recknagel, F., T. Zohary, J. Rücker, P. T. Orr, C. C. Branco, & B. Nixdorf, 2019. Causal relationships of *Raphidiopsis* (formerly *Cylindrospermopsis*) dynamics with water temperature and N:P-ratios: A meta-analysis across lakes with different climates based on inferential modelling. *Harmful Algae* 84: 222–232.
- Reichwaldt, E. S., & A. Ghadouani, 2012. Effects of rainfall patterns on toxic cyanobacterial blooms in a changing climate: Between simplistic scenarios and complex dynamics. *Water Research* 46: 1372–1393.
- Reinl, K. L., J. D. Brookes, C. C. Carey, T. D. Harris, B. W. Ibelings, A. M. Morales-Williams, L. N. De Senerpont Domis, K. S. Atkins, P. D. F. Isles, J. P. Mesman, R. L. North, L. G. Rudstam, J. A. A. Stelzer, J. J. Venkiteswaran, K. Yokota, & Q. Zhan, 2021. Cyanobacterial blooms in oligotrophic lakes: Shifting the high-nutrient paradigm. *Freshwater Biology* 66: 1846–1859.
- Reynolds, C. S., 1984. Phytoplankton periodicity: the interactions of form, function and environmental variability. *Freshwater Biology* 14: 111–142.

- Reynolds, C. S., 2006. The ecology of phytoplankton. *The Ecology of Phytoplankton*. Cambridge University Press, Cambridge.
- Reynolds, C. S., J. Alex Elliott, & M. A. Frassl, 2014. Predictive utility of trait-separated phytoplankton groups: A robust approach to modeling population dynamics. *Journal of Great Lakes Research* 40: 143–150.
- Reynolds, C. S., V. Huszar, C. Kruk, L. Naselli-Flores, & S. Melo, 2002. Towards a functional classification of the freshwater phytoplankton. *Journal of Plankton Research* 24: 417–428.
- Reynolds, C. S., R. L. Oliver, & A. E. Walsby, 1987. Cyanobacterial dominance: The role of buoyancy regulation in dynamic lake environments. *New Zealand Journal of Marine and Freshwater Research* 21: 379–390.
- Rigosi, A., C. C. Carey, B. W. Ibelings, & J. D. Brookes, 2014. The interaction between climate warming and eutrophication to promote cyanobacteria is dependent on trophic state and varies among taxa. *Limnology and Oceanography* 59: 99–114.
- Rocha Junior, C. A. N., M. R. A. Costa, R. F. Menezes, J. L. Attayde, & V. Becker, 2018. Water volume reduction increases eutrophication risk in tropical semi-arid reservoirs. *Acta Limnologica Brasiliensia* 30: e106.
- Rzymiski, P., & B. Poniedziałek, 2014. In search of environmental role of cylindrospermopsin: A review on global distribution and ecology of its producers. *Water Research* 66: 320–337.
- Rzymiski, P., B. Poniedziałek, M. Kokociński, T. Jurczak, D. Lipski, & K. Wiktorowicz, 2014. Interspecific allelopathy in cyanobacteria: Cylindrospermopsin and *Cylindrospermopsis raciborskii* effect on the growth and metabolism of *Microcystis aeruginosa*. *Harmful Algae* 35: 1–8.
- Saad, J. F., F. Unrein, P. M. Tribelli, N. López, & I. Izaguirre, 2016. Influence of lake trophic conditions on the dominant mixotrophic algal assemblages. *Journal of Plankton Research* 38: 818–829.
- Salmaso, N., A. Boscaini, C. Capelli, & L. Cerasino, 2018. Ongoing ecological shifts in a large lake are driven by climate change and eutrophication: evidences from a three-decade study in Lake Garda. *Hydrobiologia* 824: 177–195.
- Salmaso, N., D. Copetti, L. Cerasino, S. Shams, C. Capelli, A. Boscaini, L. Valsecchi, F. Pozzoni, & L. Guzzella, 2014. Variability of microcystin cell quota in metapopulations of *Planktothrix rubescens*: Causes and implications for water management. *Toxicon* 90: 82–96.
- Salmaso, N., L. Naselli-Flores, & J. Padisák, 2015. Functional classifications and their application in phytoplankton ecology. *Freshwater Biology* 60: 603–619.
- Salmaso, N., & J. Padisák, 2007. Morpho-Functional Groups and phytoplankton development in two deep lakes (Lake Garda, Italy and Lake Stechlin, Germany). *Hydrobiologia* 578: 97–112.

- Schindler, D. W., R. E. Hecky, D. L. Findlay, M. P. Stainton, B. R. Parker, M. J. Paterson, K. G. Beaty, M. Lyng, & S. E. M. Kasian, 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. *Proc. Natl. Acad. Sci. U.S.A.* 105: 11254–11258.
- Scofield, A. E., J. M. Watkins, E. Osantowski, & L. G. Rudstam, 2020. Deep chlorophyll maxima across a trophic state gradient: A case study in the Laurentian Great Lakes. *Limnology and Oceanography* 65: 2460–2484.
- Segura, A. M., C. Kruk, D. Calliari, & H. Fort, 2013. Use of a morphology-based functional approach to model phytoplankton community succession in a shallow subtropical lake. *Freshwater Biology* 58: 504–512.
- Selmečzy, G. B., A. Abonyi, L. Krienitz, P. Kasprzak, P. Casper, A. Telcs, Z. Somogyvári, & J. Padišák, 2019. Old sins have long shadows: climate change weakens efficiency of trophic coupling of phyto- and zooplankton in a deep oligo-mesotrophic lowland lake (Stechlin, Germany)—a causality analysis. *Hydrobiologia* 831: 101–117.
- Selmečzy, G. B., L. Krienitz, P. Casper, & J. Padišák, 2018. Phytoplankton response to experimental thermocline deepening: a mesocosm experiment. *Hydrobiologia* 805: 259–271.
- Selmečzy, G. B., K. Tapolczai, P. Casper, L. Krienitz, & J. Padišák, 2016. Spatial-and niche segregation of DCM-forming cyanobacteria in Lake Stechlin (Germany). *Hydrobiologia* 764: 229–240.
- Sidelev, S., O. Koksharova, O. Babanazarova, J. Fastner, E. Chernova, & E. Gusev, 2020. Phylogeographic, toxicological and ecological evidence for the global distribution of *Raphidiopsis raciborskii* and its Northernmost presence in Lake Nero, Central Western Russia. *Harmful Algae* 98: 101889.
- Simmonds, B., S. A. Wood, D. Özkundakci, & D. P. Hamilton, 2015. Phytoplankton succession and the formation of a deep chlorophyll maximum in a hypertrophic volcanic lake. *Hydrobiologia* 745: 297–312.
- Smucker, N. J., J. J. Beaulieu, C. T. Nietch, & J. L. Young, 2021. Increasingly severe cyanobacterial blooms and deep water hypoxia coincide with warming water temperatures in reservoirs. *Global Change Biology* 27: 2507–2519.
- Soares, M. C. S., V. L. M. Huszar, M. N. Miranda, M. M. Mello, F. Roland, & M. Lürling, 2013. Cyanobacterial dominance in Brazil: Distribution and environmental preferences. *Hydrobiologia* 717: 1–12.
- Soares, M. C. S., M. I. D. A. Rocha, M. M. Marinho, S. M. F. O. Azevedo, C. W. C. Branco, & V. L. M. Huszar, 2009. Changes in species composition during annual cyanobacterial dominance in a tropical reservoir: physical factors, nutrients and grazing effects. *Aquatic Microbial Ecology* 57: 137–149.
- Søndergaard, M., J. P. Jensen, & E. Jeppesen, 2003. Role of sediment and internal loading of phosphorus in shallow lakes. *Hydrobiologia* 506-509: 135–145.

Souza, M. da C., L. O. Crossetti, & V. Becker, 2018. Effects of temperature increase and nutrient enrichment on phytoplankton functional groups in a Brazilian semi-arid reservoir. *Acta Limnologica Brasiliensia* 30.

Spróber, P., H. M. Shafik, M. Présing, A. W. Kovács, & S. Herodek, 2003. Nitrogen uptake and fixation in the cyanobacterium *Cylindrospermopsis raciborskii* under different nitrogen conditions. *Hydrobiologia* 506–509: 169–174.

Stockwell, J. D., J. P. Doubek, R. Adrian, O. Anneville, C. C. Carey, L. Carvalho, L. N. De Senerpont Domis, G. Dur, M. A. Frassl, H. P. Grossart, B. W. Ibelings, M. J. Lajeunesse, A. M. Lewandowska, M. E. Llames, S. I. S. Matsuzaki, E. R. Nodine, P. Nöges, V. P. Patil, F. Pomati, K. Rinke, L. G. Rudstam, J. A. Rusak, N. Salmaso, C. T. Seltmann, D. Straile, S. J. Thackeray, W. Thiery, P. Urrutia-Cordero, P. Venail, P. Verburg, R. I. Woolway, T. Zohary, M. R. Andersen, R. Bhattacharya, J. Hejzlar, N. Janatian, A. T. N. K. Kpodonu, T. J. Williamson, & H. L. Wilson, 2020. Storm impacts on phytoplankton community dynamics in lakes. *Global Change Biology* 26: 2756–2784.

Sukenik, A., O. Hadas, A. Kaplan, & A. Quesada, 2012. Invasion of Nostocales (cyanobacteria) to subtropical and temperate freshwater lakes - physiological, regional, and global driving forces. *Frontiers in Microbiology* 3: 86.

Svirčev, Z. B., N. Tokodi, D. Drobac, & G. A. Codd, 2014. Cyanobacteria in aquatic ecosystems in Serbia: Effects on water quality, human health and biodiversity. *Systematics and Biodiversity* 12: 261–270.

Ter Braak, C. J. F., & Prentice, I. C. 1988. A theory of gradient analysis. *Advances in Ecological Research*, 18: 271-317.

Thornton, J. A., & W. Rast, 1993. A test of hypotheses relating to the comparative limnology and assessment of eutrophication in semi-arid man-made lakes. *Comparative Reservoir Limnology And Water Quality Management* 1–24, Springer.

Tilahun, S., & D. Kifle, 2019. The influence of El Niño-induced drought on cyanobacterial community structure in a shallow tropical reservoir (Koka Reservoir, Ethiopia). *Aquatic Ecology* 53: 61–77.

Torres, C. de A., M. Lürling, & M. M. Marinho, 2016. Assessment of the effects of light availability on growth and competition between strains of *Planktothrix agardhii* and *Microcystis aeruginosa*. *Microbial Ecology* 71: 802–813.

Trindade, R. M. L., S. M. dos Santos, C. A. de Souza, C. R. A. dos Santos, & J. C. Bortolini, 2021. Using morphofunctional characteristics as a model of phytoplankton dynamics in a tropical reservoir. *Brazilian Journal of Botany* 44: 467–477.

Uhelinger, V., 1964. Étude statistique des méthodes de dénombrement planctonique. *Archives Sciences* 17: 121–123.

Utermöhl, H., 1958. Zur Vervollkommung der quantitativen Phytoplankton Methodik. *Mitteilungen der internationale Vereinigung für theoretische und angewandte Limnologie* 9: 1–38.

- Üveges, V., K. Tapolczai, L. Krienitz, & J. Padisák, 2012. Photosynthetic characteristics and physiological plasticity of an *Aphanizomenon flos-aquae* (Cyanobacteria, Nostocaceae) winter bloom in a deep oligo-mesotrophic lake (Lake Stechlin, Germany). *Hydrobiologia* 698: 263–272.
- Valderrama, J. C., 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Marine Chemistry* 10: 109–122.
- Van den Wyngaert, S., L. Ganzert, K. Seto, K. Rojas-Jimenez, R. Agha, S. A. Berger, J. Woodhouse, J. Padisák, C. Wurzbacher, M. Kagami, & H. P. Grossart, 2022. Seasonality of parasitic and saprotrophic zoospore fungi: linking sequence data to ecological traits. *ISME Journal* 16: 2242–2254.
- Violle, C., M.-L. Navas, D. Vile, E. Kazakou, C. Fortunel, I. Hummel, & E. Garnier, 2007. Let the concept of trait be functional! *Oikos* 116: 882–892.
- Visser, P. M., J. M. H. Verspagen, G. Sandrini, L. J. Stal, H. C. P. Matthijs, T. W. Davis, H. W. Paerl, & J. Huisman, 2016. How rising CO₂ and global warming may stimulate harmful cyanobacterial blooms. *Harmful Algae* 54: 145–159.
- Wagner, C., & R. Adrian, 2009. Cyanobacteria dominance: Quantifying the effects of climate change. *Limnology and Oceanography* 54: 2460–2468.
- Walsby, A. E., Z. Dubinsky, J. C. Kromkamp, C. Lehmann, & F. Schanz, 2001. The effects of diel changes in photosynthetic coefficients and depth of *Planktothrix rubescens* on the daily integral of photosynthesis in Lake Zürich. *Aquatic Sciences* 63: 326–349.
- Walsby, A. E., G. Ng, C. Dunn, & P. A. Davis, 2004. Comparison of the depth where *Planktothrix rubescens* stratifies and the depth where the daily insolation supports its neutral buoyancy. *New Phytologist* 162: 133–145.
- Walther, G. R., 2010. Community and ecosystem responses to recent climate change. *Phil. Trans. R. Soc. B.* 365: 2019–2024.
- Walther, G., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. C. Beebee, J. Fromentin, O. Hoegh-Guldberg, & F. Bairlein, 2002. Ecological response to recent climate change. *Nature* 416: 389–395.
- Wetzel, R., & G. Likens, 2000. *Limnological analyses*. Springer Verlag, New York.
- Wiedner, C., J. Rücker, R. Brüggemann, & B. Nixdorf, 2007. Climate change affects timing and size of populations of an invasive cyanobacterium in temperate regions. *Oecologia* 152: 473–484.
- Wilk-Woźniak, E., W. Solarz, K. Najberek, & A. Pocięcha, 2016. Alien cyanobacteria: An unsolved part of the “expansion and evolution” jigsaw puzzle? *Hydrobiologia* 764: 65–79.
- Winter, J. G., A. M. Desellas, R. Fletcher, L. Heintsch, A. Morley, L. Nakamoto, & K. Utsumi, 2011. Algal blooms in Ontario, Canada: Increases in reports since 1994. *Lake and Reservoir Management* 27: 107–114.
- Wintermans, J. F. G. M., & A. De Mots, 1965. Spectrophotometric characteristics of chlorophylls a and b and their phenophytins in ethanol. *Biophysics Including Photosynthesis* 109: 448–453.

Wu, Z., J. Shi, & R. Li, 2009. Comparative studies on photosynthesis and phosphate metabolism of *Cylindrospermopsis raciborskii* with *Microcystis aeruginosa* and *Aphanizomenon flos-aquae*. *Harmful Algae* 8: 910–915.

Yamamoto, Y., & H. Nakahara, 2005. The formation and degradation of cyanobacterium *Aphanizomenon flos-aquae* blooms: The importance of pH, water temperature, and day length. *Limnology* 6: 1–6.

Zastepa, A., T. R. Miller, L. C. Watson, H. Kling, & S. B. Watson, 2021. Toxins and other bioactive metabolites in deep chlorophyll layers containing the cyanobacteria *Planktothrix cf. Isothrix* in two georgian bay embayments, Lake Huron. *Toxins* 13: 45.

10. LIST OF ACADEMIC ACTIVITIES DURING THE DOCTORAL TRAINING

PUBLICATIONS:

- **Vanderley, R. F.**, V. Becker, R. Panosso, K. A. Ger, J. Padisák, 2022. The influence of trophic status and seasonal environmental variability on morpho-functional traits in tropical man-made shallow lakes. *Environmental Monitoring and Assessment* 194: 504. DOI 10.1007/s10661-022-10091-y
- **Vanderley, R. F.**, K. A. Ger, V. Becker, M. G. T. A. Bezerra, & R. Panosso, 2021. Abiotic factors driving cyanobacterial biomass and composition under perennial bloom conditions in tropical latitudes. *Hydrobiologia* 848: 943–960. DOI 10.1007/s10750-020-04504-7

CONFERENCES, ORAL PRESENTATIONS:

- 19th Workshop of the International Association of Phytoplankton Taxonomy and Ecology (IAP), Tiszafüred, Hungary. 23th-30th Sep 2022. *Presentation Title*: The influence of trophic status and temporal patterns on phytoplankton morphofunctional traits during a dry period in tropical shallow lakes.
- 35th Congress of the International Society of Limnology, Republic of Korea (Hybrid), 22nd-27th Aug 2021. *Presentation Title*: "The influence of trophic status and temporal patterns on phytoplankton morphofunctional traits during a dry period in tropical shallow lakes".
- 12th Symposium for European Freshwater Sciences (SEFS), Virtual Conference, 25th -30th July 2021. *Presentation Title*: "Abiotic factors driving cyanobacterial biomass and composition under perennial bloom conditions in tropical latitudes".
- 10th International Shallow Lakes Conference: "Towards a landscape ecology of shallow lakes" online conference. 05th May 2021. *Presentation Title*: "Abiotic factors driving cyanobacterial biomass and composition under perennial bloom conditions in tropical latitudes".
- Pannon Egyetem, Kari Tudományos Konferencián. 2020.07.16. University of Pannonia, Veszprém, Hungary. *Presentation Title*: "Abiotic factors driving cyanobacterial biomass and composition under perennial bloom conditions in tropical latitudes".

OTHER ORAL PRESENTATIONS

- Online presentation for the research group ELISA (Limnologic Studies of Semiarid) at the Laboratory of Water Resources and Sanitation, Universidade Federal do Rio Grande do Norte. 23rd May 2021. *Presentation Title*: "Abiotic factors driving cyanobacterial biomass and composition under perennial bloom conditions in tropical latitudes".

CONFERENCES ATTENDANCE AND WORKSHOPS:

- ParAqua - Training School "Identification and detection of zoosporic parasites". Institute of Freshwater Ecology and Inland Fisheries (IGB), Germany. 16th-17th May 2022.
- Online Symposium of Limnology and Sanitation in the Semi-Arid Region, National Institute of Semi-Arid (INSA_MCTIC). 21st-25th Sep 2020.
- Online seminar: Cyanobacteria and public health. National Institute of Semi-Arid (INSA_MCTIC) 10th Aug 2020
- Budapest Water Summit - Budapest, Hungary. 15th-17th Oct 2019.

TEACHING EXPERIENCE:

- Lecture to students enrolled in the Science Theory and Communication in Science. 14th Nov 2022, University of Pannonia.
- Co-supervisor of the diploma thesis of an MSc student in Environmental Science with a scholarship from Stipendium Hungaricum, University of Pannonia
- Online lecture to students enrolled in the Environmental Microbiology course. 18th Feb 2021, University Federal do Rio Grande do Norte, Brazil.

OTHERS

- International Society of Limnology (SIL) activities to discuss and elaborate a paper entitled “Phosphorus, nitrogen, cyanobacteria, and eutrophication - from molecules to macroscale”.
- Participated in a podcast promoted by the Directorate for International Relations and Communication at the University of Pannonia (NKKI) to launch science communication in a new channel.

11. SUPPLEMENTARY MATERIAL

Table S1. Descriptive statistics (average, maximum and minimum values between parentheses) of limnological variables from each lake during 2017.

Variables	Santa Cruz	Prata	Tabatinga	Encanto	Boqueirão	Pajuçara
Z_{eu} (m)	7.5 (3.5-12.1)	3.8 (3.2-4.3)	2.2 (1.6-2.4)	1.9 (0.8-3.5)	1.3 (0.3-1.6)	1.6 (0.3-2.7)
Z_{max} (m)	16.6 (10.9-19.3)	3.9 (2.1-11)	5 (3-7)	5.9 (3.8-8.3)	5 (3.4-6.8)	4.52 (1.3-7)
Temp (°C)	28 (26-30)	28 (26-31)	28 (25-30)	28 (25-31)	26 (23-28)	28 (25-30)
TP ($\mu\text{g}\cdot\text{L}^{-1}$)	22.7 (12.8-35.8)	38.7 (22.9-69.2)	45.4 (33.1-56.1)	67.6 (16.7-111.9)	97.5 (50.24-170.76)	107.6 (42.9-231.9)
SRP ($\mu\text{g}\cdot\text{L}^{-1}$)	10.7 (1.7-20.2)	17 (3.5-60.8)	23.25 (12.8-45)	29.5 (12.8-52.3)	38.8 (16.78-119.75)	40.7 (17.8-67.4)
TN ($\mu\text{g}\cdot\text{L}^{-1}$)	690.6 (543.7-802.4)	652.6 (500.3-830.3)	2150.2 (1640-2389)	1502.3 (707.9-2502)	1824.83 (1278-2383)	3462.9 (1328-6864)
TDN ($\mu\text{g}\cdot\text{L}^{-1}$)	603.9 (510.3-748.2)	573.7 (456.1- 742.1)	1809.4 (1473-2042)	1046.9 (746.1-1324)	1530 (1212-2070)	2736.3 (1370- 5574)
TSS ($\text{mg}\cdot\text{L}^{-1}$)	3.2 (1.5-6.3)	6.8 (2.8-12.1)	16.9 (10.4-32)	10.4 (3-34.7)	15.9 (8.2-27.7)	16 (8 -37)
FSS ($\text{mg}\cdot\text{L}^{-1}$)	1.6 (0 -3.7)	3.1 (0.2-8.4)	5.5 (1.6-13.5)	3.2 (0.6-12)	9.7 (3.4-19.7)	5.1 (2.2-10.8)
VSS ($\text{mg}\cdot\text{L}^{-1}$)	1.6 (1-2.6)	3.7 (2-6.8)	11.4 (8.5-18.5)	7.2 (2.2-22.6)	6.2 (4.5-8)	10.8 (3 (83-28.5)
Chl-a ($\mu\text{g}\cdot\text{L}^{-1}$)	3 (0.9-10.8)	7.6 (3.7-11.6)	35 (20.2-53.6)	32.6 (9-112.7)	29.1 (4.8-51.2)	70.3 (10.4-193.6)
Phytoplankton ($\text{mg}\cdot\text{L}^{-1}$)	0.60 (0.0-2.1)	3.9 (0.5-12.7)	13.3 (4.7-64.5)	48.4 (1.6-175.8)	149.2 (4.8-643.1)	97.3 (7.8-469.8)
Cyanobacteria ($\text{mg}\cdot\text{L}^{-1}$)	0.05 (0.0-0.11)	0.7 (0.0-5.3)	10 (0.9-63.8)	45.8 (0.9-171.7)	148.2 (4.1- 643)	94.4 (0.8-468.4)

