University of South-Eastern Norway
Faculty of Technology, Natural Sciences, and Maritime Sciences

Doctoral dissertation no. 153
2023

Thida Swe

# The ecology of phytoplankton with focus on cyanobacteria and of aquatic macrophytes in selected lakes and reservoirs in Myanmar

Thida Swe

# The ecology of phytoplankton with focus on cyanobacteria and of aquatic macrophytes in selected lakes and reservoirs in Myanmar

A PhD dissertation in
**Ecology**

Faculty of Technology, Natural Sciences, and Maritime Sciences
**University of South-Eastern Norway**
Bø, 2023

**Doctoral dissertations at the University of South-Eastern Norway no. 153**

ISSN: 2535-5244 (print)
ISSN: 2535-5252 (online)

ISBN: 978-82-7206-741-9 (print)
ISBN: 978-82-7206-742-6 (online)




Print: University of South-Eastern Norway

# Dedication

To five infinites venerable-

1. The Buddha
2. The Dhamma {his teachings}
3. Sangha {the entire order of Monks}
4. Parents, our very first teachers, and
5. Our teacher's mural and extra mural

# Preface

This thesis has been a part of the project “Integrated Water Resources Management – Institutional Building and Training (IWRM) (Phase I & Phase II)”, funded by the Ministry of Foreign Affairs, Norway. It is a collaboration project between Norwegian Institute for Water Research (NIVA) and the Forest Department (FD), the Ministry of Natural Resources and Environmental Conservation (MONREC).

# Acknowledgements

Firstly, I would like to express my sincere gratitude to my wonderful supervisor, Andreas Ballot, for his invaluable guidance, his patience, motivation, enthusiasm, and immerse knowledge. I will always be grateful for all support he provided along my Ph.D journey. I also wish to extend my special thanks to Marit Mjelde who brought me to the interesting world of aquatic macrophyte. You both taught me a lot of valuable things which I can use in my carrier and contribute to my country. I will never forget the times we spent together during our field work in Myanmar.

My sincere thanks go to my co-supervisor, Synne Kleiven, for taking over as my co-supervisor and for your kind support throughout my Ph.D work.

My special thanks go to everyone who actively participated in our collaborative IWRM project.

I would also like to thank my beloved friends, Ikumi, for moral support, understanding, and giving extra classes for genetic course. Also, I thank my close friend, Odko, for being a good friend since our first course work in Greece till now. I want to thank Joel Tebbenkamp for editing the first draft of my thesis. I will never forget all the fun we have had in Ola's apartment, Bø. I also thank Julia who shared the office, being my gym trainer and saving me from lonely long-hour work by being such a nice friend. Thanks to my other Ph.D mates, Peter, Rasmus, Daniel, Frank, Hanna and Angelina for being good friends and making my time in Norway fantastic and memorable especially during the Coronavirus pandemic.

I would like to show my appreciation to Karin Brekke Lee and Frode Bergan for technical support for my lab work.

Moreover, I would like to express my profound gratitude to Dr. Nyi Nyi Kyaw, former Director General of Forest Department, for granting me the tremendous opportunity of studying in Norway and encouragement and kindness throughout my employment.

I would like to acknowledge to the staffs and officers from the regional Forest Department and the Irrigation Department, Myanmar. Data collection would have been impossible without their support and cooperation.

I would also like to thank my college, my friend, and like my sister, Dr. Thida Cho, an assistant research officer at Water Quality Laboratory, Forest Research Institute, Yezin, Myanmar, who worked hard and struggled for our Lab survive while I was in Norway.

Lastly, and most importantly, I would like to thank my parents. I will forever be indebted to you for always supporting me, believing me, and allowing me to take every major life decision on my own.

Very special thanks from the bottom of my heart to my husband, Ko Myo Thein, without whom this effort would have been worth nothing.

I humbly extend my thanks to all persons, who have been directly or indirectly involved in the completion of this thesis.

# Abstract

Myanmar's freshwater systems are increasingly subjected to degradation, eutrophication, and anthropogenic impacts. The most visible effects of eutrophication in lakes and rivers are dense blooms of noxious, foul-smelling phytoplankton (mainly cyanobacteria), increased organic loading and sedimentation, fish mortalities, reduction of water clarity and degraded water quality and quantity. Many cyanobacterial species are known to produce toxic compounds, cyanotoxins, which can affect terrestrial and aquatic organisms. The control of cyanobacteria, therefore, is vital for freshwater resource management. Many studies have pointed out that aquatic macrophytes can suppress the structure and variation of phytoplankton communities and enhance water clarity by competing for nutrients, releasing allelopathic compounds and shifting environmental light levels. However, no detailed study on the presence of cyanobacteria and their potential toxin production has been conducted so far in Myanmar's water bodies. There is also limited or no information on the influence of macrophytes on the function of freshwater systems. More broadly, the interaction between macrophytes and phytoplankton is poorly understood in Myanmar's freshwater lakes. The overall goal of this study is therefore to improve knowledge about the composition and abundance of phytoplankton and macrophytes, and their interaction in Myanmar's freshwater systems.

The presence of toxin-producing cyanobacteria and cyanotoxins were investigated at Meiktila Lake and Yezin Dam, while an assessment of the importance of aquatic macrophytes as a stabilizing factor in a tropical shallow lake was conducted at Inlay Lake. The distribution of Chara spp. and their ecological requirements was investigated in six water bodies: two natural lakes and four man-made reservoirs. Water sampling and field surveys were conducted in each lake and reservoir 2 to 8 times from 2014 to 2019.

Compared to Inlay Lake, higher phytoplankton biomasses were found in Meiktila Lake and Yezin Dam, of which cyanobacteria were the most dominant groups. Among cyanobacteria species, Raphidiopsis raciborskii was the most dominant taxa (0.2 – 1.9 mg L-1) in Meiktila Lake, while lower biomass (0.0002 - 0.2 mg L-1) was recorded in Yezin Dam. Dolichospermum smithii was only found in Yezin Dam with the highest biomass of 19.18 mg L-1. Neither of these

species were observed in Inlay Lake. Low concentrations of Microcystis were found in all the lakes and reservoirs studied in Myanmar. Microcystis has been reported from only a few water bodies in Myanmar before, but is described from many Asian countries.

This study clearly demonstrates the presence of CYN- and deoxy-CYN producing R. raciborskii and MC-producing Microcystis in the cyanobacteria communities of the investigated lakes and reservoirs in Myanmar, confirmed by Enzyme-linked immunosorbent assay (ELISA) and liquid chromatography with tandem mass spectrometry (LC-MS/MS) (LC-HRMS/MS). Although Dolichospermum smithii was the most dominant species in Yezin Dam, none of the Dolichospermum strains isolated appear to produce any toxins, unlike strains from other locations worldwide.

Although 22 Microcystin (MC) congeners were produced by the Microcystis strain (AB2017/08) of Yezin Dam, MC-LR and [D-Asp3] MC-LR were the dominant MC congeners, comprising 76.9 % and 15.7 % of the total MCs detected. Microcystis strain (AB2017/14) from Meiktila Lake with 52 MC congeners produced a considerably higher number of MCs, but MC-LR and [D-Asp3] MC-LR together made up only 20 % of the total MC concentrations. Due to the presence of CYN-and MC-producing cyanobacteria in these lakes and dams, harmful effects on humans, domestic and wild animals cannot be excluded.

This study also fills the knowledge gap on the current distributions of Chara species in lakes and reservoirs in Myanmar. Two Chara species, Chara zeylanica and C. fibrosa, were recorded in our lake survey in 2014 – 2019. Both species were recorded in low-impacted lakes only, with total phosphorous (TP) concentrations below 20 µg L−1. Increased human impact on freshwater habitats must therefore be considered as a factor reducing Chara biodiversity in Myanmar.

Regression analysis showed that the submerged macrophyte abundance and phytoplankton biomass were inversely correlated in the heavily vegetated northern lake area. Our survey suggests a great importance of the submerged macrophytes to the general water quality and the clear water state in Inlay Lake. Maintaining high macrophyte abundances should therefore

be a goal in lake management strategies. It is highly desirable to include macrophytes and phytoplankton in the lake monitoring in Myanmar.

Keywords: Myanmar, cyanobacteria, Raphidiopsis raciborskii, Microcystis, Dolichospermum smithii, Microcystin, cylindrospermopsin, deoxycylindrospermopsin aquatic macrophytes, submerged macrophyte, Chara zeylanica, Chara fibrosa, Lake ecology, biotic interaction

# List of papers

## Paper 1

Ballot, A., Swe, T., Mjelde, M., Cerasino, L., Hostyeva, V. & Miles, C. O. (2020). Cylindrospermopsin- and Deoxycylindrospermopsin-Producing Raphidiopsis raciborskii and Microcystin-Producing Microcystis spp. in Meiktila Lake, Myanmar. Toxins, 12, 232.

## Paper 2

Swe, T., Miles, C. O., Cerasino, L., Mjelde M., Kleiven, S. & Ballot, A., (2021). Microcystis aeruginosa, Raphidiopsis raciborskii and Dolichospermum smithii, toxin producing and non-toxigenic cyanobacteria in Yezin Dam, Myanmar. Limnologica, 90, 125901.

## Paper 3

Mjelde, M., Swe, T., Langangen, A. & Ballot, A. (2020). A contribution to the knowledge of charophytes in Myanmar; morphological and genetic identification and ecology notes. Botany Letters, 168:1, 102-109.

## Paper 4

Swe, T., Lombardo, P., Ballot, A., Thrane Jan-Erik, Sample, J. Eriksen, T. E., & Mjelde, M., (2021). The importance of aquatic macrophytes in a eutrophic tropical shallow lake. Limnologica, 90, 125910.

# Papers summary

## Paper 1

Ballot, A., Swe, T., Mjelde, M., Cerasino, L., Hostyeva, V. & Miles, C. O. (2020). Cylindrospermopsin- and Deoxycylindrospermopsin-Producing Raphidiopsis raciborskii and Microcystin-Producing Microcystis spp. in Meiktila Lake, Myanmar. Toxins, 12, 232.

Meiktila Lake is a shallow reservoir located close to Meiktila city in central Myanmar. Its water is used for irrigation, domestic purposes and drinking water. No detailed study of the presence of cyanobacteria and their potential toxin production has been conducted so far. To ascertain the cyanobacterial composition and presence of cyanobacterial toxins in Meiktila Lake, water samples were collected in March and November 2017 and investigated for physico-chemical and biological parameters. Phytoplankton composition and biomass determination revealed that most of the samples were dominated by the cyanobacterium Raphidiopsis raciborskii. In a polyphasic approach, seven isolated cyanobacterial strains were classified morphologically and phylogenetically as R. raciborskii, and Microcystis spp. and tested for microcystins (MCs), cylindrospermopsins (CYNs), saxitoxins and anatoxins by enzyme-linked immunosorbent assay (ELISA) and liquid chromatography–mass spectrometry (LC–MS). ELISA and LC–MS analyses confirmed CYNs in three of the five Raphidiopsis strains between 1.8 and 9.8 µg mg-1 fresh weight. Both Microcystis strains produced MCs, one strain 52 congeners and the other strain 20 congeners, including 22 previously unreported variants. Due to the presence of CYN- and MC-producing cyanobacteria, harmful effect on humans, domestic and wild animals cannot be excluded in Meiktila Lake.

Keywords: Meiktila Lake; Raphidiopsis; Microcystis; cylindrospermopsin; Deoxy-cylindrospermopsin; microcystin

## Paper 2

Swe, T., Miles, C. O., Cerasino, L., Mjelde M., Kleiven, S. & Ballot, A., (2021). Microcystis aeruginosa, Raphidiopsis raciborskii and Dolichospermum smithii, toxin producing and non-toxigenic cyanobacteria in Yezin Dam, Myanmar. Limnologica, 90, 125901.

Yezin Dam is a man-made reservoir located close to Yezin village in Myanmar. Its water is used for irrigation, domestic purposes, and as drinking water for many urban communities in the watershed area. In recent years, increased pollution due to the concurrent development around the dam has led to water quality deterioration. No detailed study on the distribution of cyanobacteria and toxin production has been conducted so far. In order to provide insight into the extent of cyanobacteria and cyanotoxins in the dam, water samples were collected once in January 2014 for the isolation of cyanobacterial strains and eight times between March 2017 and June 2018 for the investigation of physical, chemical, and biological parameters. A total of 99 phytoplankton taxa belonging to 50 genera were recorded from Yezin Dam. Microscopic examination showed that a Dolichospermum sp. was the dominant cyanobacterium followed by small numbers of Microcystis, and Raphidiopsis raciborskii in all samples throughout the sampling period. 15 isolated cyanobacterial strains were classified morphologically and phylogenetically as Dolichospermum smithii, R. raciborskii and Microcystis and tested for microcystins (MCs), cylindrospermopsins (CYNs), saxitoxins (STXs) and anatoxins (ATXs) by liquid chromatography-tandem mass spectrometry (LC–MS/MS) and enzyme-linked immunosorbent assay (ELISA). The toxin analysis of all isolated Dolichospermum strains by ELISA and LC–MS did not indicate the presence of ATXs, STXs, CYNs nor MCs. Four of the five isolated Raphidiopsis strains produced CYN and deoxyCYN. One of the isolated Microcystis strains (AB2017/08) from Yezin Dam produced 22 MC congeners. Concentrations of 0.12 µg L-1 CYNs and 0.34 µg L-1 MCs were also found in an environmental sample from Yezin Dam by ELISA. The potential therefore exists for the use of untreated water from Yezin Dam to cause harmful effects on humans, domestic and wild animals.

Keywords: Yezin Dam, Myanmar, Dolichospermum Raphidiopsis Microcystis Cylindrospermopsin, Deoxycylindrospermopsin, Microcystin

## Paper 3

Mjelde, M., Swe, T., Langangen, A. & Ballot, A. (2020). A contribution to the knowledge of charophytes in Myanmar; morphological and genetic identification and ecology notes. Botany Letters, 168:1, 102-109.

Information on the distribution and species composition of charophytes in Myanmar is scarce. Only a few studies on charophytes in ponds were conducted in Myanmar at the end of the nineteenth and first half of the twentieth century and lake habitats were not included in these studies. To increase the knowledge, we investigated Chara spp. from seven Myanmar lakes and reservoirs. In a polyphasic approach using morphological traits and DNA barcoding the specimens found were classified as Chara zeylanica and Chara fibrosa. Chara zeylanica is the most common of the two species found in Myanmar and was observed in five lakes, while Chara fibrosa was only found in three lakes. Chara zeylanica seems to prefer calcareous lakes while C. fibrosa was found in both highly and moderate alkaline lakes. Both species were recorded in low-impacted lakes only, with total phosphorous (TP) concentrations below 20 µg L−1. Increased human impact on freshwater habitats must therefore be considered as a factor reducing Chara biodiversity in Myanmar.

Keywords: Myanmar; Chara zeylanica; Chara fibrosa; DNA barcoding; matK; rbcl

## Paper 4

Swe, T., Lombardo, P., Ballot, A., Thrane Jan-Erik, Sample, J. Eriksen, T. E., & Mjelde, M., (2021). The importance of aquatic macrophytes in a eutrophic tropical shallow lake. Limnologica, 90, 125910

Inlay Lake is the second largest natural lake in Myanmar. Located in Shan State, in the eastern part of the country, it is a known biodiversity hotspot. The lake is negatively affected by an increasing local human population and rapid growth in both agriculture and tourism. In recent decades, several studies have listed faunistic and floristic groups in Inlay Lake, but there is still a general lack of knowledge about the aquatic macrophyte and phytoplankton community composition and abundance, and their interactions. To fill this knowledge gap, field surveys

of biological and physical and chemical parameters were carried out in the period 2014 - 2017. They show that Inlay Lake is a shallow, clear water and calcareous lake, with nutrient concentrations indicating mesotrophic-eutrophic conditions. However, close to the shore, nutrient concentrations are generally higher, reflecting pollution from inflowing rivers, shoreline villages and floating gardens. Both the richness and abundance of aquatic macrophytes in Inlay Lake were high, with several species forming extensive stands in most of the lake over the whole survey period. Total phytoplankton and cyanobacterial biomass were low, but cyanobacteria included toxin-producing strains of Microcystis, suggesting that cyanobacterial and total phytoplankton biomass need to be kept low to avoid potentially harmful cyanobacterial blooms. Submerged macrophyte abundance and phytoplankton biomass were inversely correlated in the heavily vegetated northern lake area. Our survey suggests a great importance of the submerged macrophytes to the general water quality and the clear water state in Inlay Lake. Maintaining high macrophyte abundances should therefore be a goal in management strategies, both for Inlay Lake and other lakes in Myanmar. It is highly desirable to include macrophytes and phytoplankton in the lake monitoring in Myanmar.

Keywords: Lake ecology, Biodiversity, Biotic interactions, Water quality, Lake management

# Abbreviations

| | |
|---|---|
| 7-deoxy-CYN | 7-deoxy-cylindrospermopsin |
| 7-epi-CYN | 7-epi-cylindrospermopsin |
| a.s.l | Above sea level |
| ANOVA | Analysis of Variance |
| ATX-a | Anatoxin-a |
| bp | Base pair |
| Ca | Calcium |
| Chl-a | Chlorophyll-a |
| CYNs | Cylindrospermopsins |
| deoxyCYN | deoxy-cylindrospermopsin |
| DNA | Deoxyribonucleic acid |
| ELISA | Enzyme-linked immunosorbent assay |
| ENA | European Nucleotide Archive |
| EU WFD | European Water Framework Directive |
| FW | Fresh weight |
| HANTX | Homoanatoxin-a |
| HSD | Honestly Significant Difference |

| | |
|---|---|
| $Km^2$ | Square kilometer |
| LC-MS/MS | liquid chromatography with tandem mass spectrometry |
| $LD_{50}$ | Lethal Dose, the amount of a material, given all at once, which causes the death of 50% of a group of test animals |
| m | meter |
| MC-LA | Leucine and arginine in the positions of X and Z of microcystin |
| MC-LR | Leucine and arginine in the positions of X and Y of microcystin |
| MC-RR | Arginine and arginine in the positions of X and Z of microcystin |
| MCs | Microcystins |
| ML | Maximum likelihood |
| mm | millimeter |
| MP | Maximum parsimony |
| NCBI | National Center for Biotechnology Information |
| NIVA | Norwegian Institute for Water Research |
| NJ | Neighbor-joining |
| NWFD | National Water Framework Directive |
| PCA | Principal component analysis |
| PCR | Polymerase chain reaction |
| PSP | Paralytic Shellfish Poisoning |

| | |
|---|---|
| PSTs | Paralytic shellfish toxins |
| RDA | Redundancy analysis |
| rRNA | Ribosomal nucleic acid |
| STXs | Saxitoxins |
| TN | Total Nitrogen |
| TOC | Total Organic Carbon |
| TP | Total phosphorous |
| VFDF | Very fast death factor |
| WHO | World Health Organization |

# Table of contents

**1 Introduction ..... 1**

1.1 Phytoplankton ..... 2

1.2 Cyanobacteria ..... 2

1.3 Cyanobacterial toxins ..... 3

1.3.1 Microcystins ..... 4

1.3.2 Cylindrospermopsin ..... 5

1.3.3 Anatoxin ..... 6

1.3.4 Saxitoxins ..... 8

1.3.5 Monitoring of Cyanotoxins ..... 9

1.4 Aquatic macrophytes ..... 9

**2 Objectives ..... 13**

**3 Materials and Methods ..... 15**

3.1 Study Areas ..... 15

3.2 Sampling ..... 19

3.2.1 Physical measurements and water quality ..... 19

3.2.2 Water Sampling for phytoplankton and cyanotoxin analysis ..... 19

3.2.3 Aquatic macrophytes survey ..... 20

3.3 Phytoplankton Analysis ..... 20

3.4 Isolation of strains and morphological characterization ..... 21

3.5 Genomic DNA extraction, PCR amplification and sequencing ..... 21

3.6 Phylogenetic Analysis ..... 22

3.7 Toxin analyses ..... 24

3.7.1 ELISA for MCs, CYNs, ATXs and STXs ..... 24

3.7.2 Microcystin analysis by LC-HRMS ..... 25

3.7.3 CYNs, ATXs and STXs analysis by LC-MS/MS ..... 25

3.8 Statistical Analyses ..... 25

**4 Results and Discussion ..... 27**

4.1 Physical and chemical properties of selected water bodies ..... 27

4.2 Phytoplankton and Cyanobacteria in Myanmar Lakes ..... 28
4.3 Phylogenetic studies on cyanobacteria ..... 30
4.4 Cyanotoxins ..... 31
4.5 Aquatic macrophytes ..... 35
4.6 Correlation between phytoplankton and aquatic macrophytes ..... 37
**5 Conclusions and future perspectives ..... 43**
**6 References ..... 47**

# 1 Introduction

Myanmar covers 676,590 $km^2$, which makes it the largest country in Southeast Asia. Its landscape includes mountainous areas, lowlands, deltas, coastal areas, and numerous natural and man-made bodies of freshwater. Availability of freshwater, however, varies regionally as well as seasonally. Most freshwater used for agricultural and household purposes comes from rivers, lakes, man-made reservoirs, and to a lesser degree, groundwater (Mjelde et al., 2017). The internal total renewable water resources in Myanmar are about 1,000 $km^3$ per year, including surface water (lakes and rivers) and groundwater (with river base flow) (FAO, 2011). As an agriculture-based country, Myanmar has heavily invested in dam construction throughout the country to increase water availability in order to promote agricultural production and socio-economic development (Aung et al., 2017). Because of the relative scarcity of natural lakes in Myanmar, reservoirs are the predominant lake type in many regions.

Access to clean freshwater is important for human health, a healthy environment and poverty reduction, and it is also critical for a sustainable economy. However, similar to trends in other countries, Myanmar's water systems are increasingly subject to degradation, eutrophication, and anthropogenic impacts (Ansari et al., 2010). Common sources for water pollution in Myanmar include use of laundry detergents and soaps in lakes and rivers, poor sanitation and wastewater treatment systems, intrusion of chemical fertilizers and pesticides from agriculture, industrial waste, sedimentation and erosion due to deforestation/forest degradation, and unsustainable agricultural practices in watershed areas (Swe, 2019). The most visible effects of eutrophication in lakes and rivers are dense blooms of noxious, foul-smelling phytoplankton, increased organic loading and sedimentation, fish mortalities, reduction of water clarity, and degraded water quality and quantity (Klapper, 1991).

Facing increased degradation of Myanmar`s freshwater systems, the National Water Resources Committee in Myanmar decided in 2014 to develop a National Water Framework Directive (NWFD) (NWFD, 2014), similar to the EU Water Framework Directive (EU WFD) (EC, 2000). Both the EU WFD and the NWFD have an ecosystem-based approach for characterizing

and classifying water quality, an approach designed to protect, preserve and improve the aquatic environment. To assess the ecological status of surface waters, the directive requires data and indices for biological elements, supported by physical and chemical elements. Indices are important tools to assess the biological quality in lake and river habitats and are applied worldwide (Lyche-Solheim et al., 2013, Friberg et al., 2011). Indices based on aquatic macrophytes are widely applied, especially in Europe (Poikane et al., 2018, Hellsten et al., 2014, Mjelde et al., 2013, Penning et al., 2008a). Both in the NWFD and EU WFD, phytoplankton is also recognized as one of the most important bioindicators for assessing the ecological status of lakes (e.g. Nixdorf et al. 2008, Phillips et al. 2013, Nesheim et al., 2016).

## 1.1 Phytoplankton

Phytoplankton are microscopic organisms comprising prokaryotic cyanobacteria and eukaryotic algae. They are free-floating organisms moving mainly with water currents. The predominant groups of phytoplankton are Bacillariophyceae, Chrysophyceae, Chlorophyceae, Cyanobacteria and Dinophyceae. The availability of carbon dioxide, sunlight, and nutrients such as nitrogen and phosphorous, determine their growth and some cyanobacterial taxa can even sequester atmospheric nitrogen allowing them to grow in areas with limited nitrogen concentrations (Lindsey et al., 2010). Because they are sensitive to changes in environmental conditions, phytoplankton biomass and community composition can be used as indicators of water quality (Brettum and Andersen, 2005, Reynolds et al., 2002, Reynolds, 1997). The greatest biodiversity of phytoplankton organisms is often observed under low nutrient concentrations (Barcelos and Ramos et al., 2017). When nutrient concentrations are elevated, phytoplankton diversity decreases and, consequently, the phytoplankton community is mainly dominated by a few phytoplankton taxa (e.g. cyanobacteria) that are adapted to eutrophic conditions (González and Roldán, 2019, Jia et al., 2017).

## 1.2 Cyanobacteria

Cyanobacteria, also known as blue-green algae, blue-green bacteria, cyanoprokaryota, or cyanophytes, are mainly responsible for harmful algae blooms in lakes and reservoirs worldwide. They represent a single phylogenetic group within the domain Bacteria, with each

species in this group capable of photosynthesis (Castenholz et al., 2001). Their long evolutionary history (~3.5 billion years) has enabled them to live in different environments and occupy distinct niches (Ferrão-Filho and Kozlowsky-Suzuki, 2011). They can be found in almost every terrestrial and aquatic system. Like eukaryotic algae and plants, cyanobacteria have photosystems I and II. Typically, they produce oxygen during photosynthesis by using water as an electron donor (Sánchez - Baracaldo and Cardona, 2020). Under anaerobic conditions, some species can also conduct photosynthesis by using only photosystem I if hydrogen sulphide is present as an electron donor (Cohen, 2006).

Cyanobacteria cells are spheroidal, rod-shaped, or tube-shaped. They can grow as single cells, in colonies or as straight or spiral filaments. Various cyanobacterial species are able to produce toxic compounds, called cyanotoxins. If they occur in mass developments (blooms), they can become harmful to aquatic ecosystems, the economy, drinking water supplies, recreational activities, and they can directly harm animals and humans (Paerl and Otten, 2013). Bloom-forming cyanobacteria mainly belong to the genera *Anabaenopsis*, *Aphanizomenon*, *Arthrospira*, *Dolichospermum* (*Anabaena*), *Microcystis*, *Nodularia*, *Oscillatoria*, *Planktothrix* and *Raphidiopsis* (*Cylindrospermopsis*) (Oliver and Ganf, 2000, Reynolds and Walsby, 1975). It has been estimated from water blooms and mass developments of planktic and benthic cyanobacteria in different countries that 25% to 90% of the blooms are toxic (Bláha et al., 2009, Baker and Humpage, 1994, Carmichael, 1988). Cyanobacterial strains from the same species and even from the same waterbodies can either be toxin producing or non-producing (Kurmayer et al., 2017, Sivonen and Jones, 1999). About 40 out of 150 described genera of cyanobacteria are known to produce cyanotoxins, which can affect terrestrial and aquatic organisms (Bernard et al., 2017, Van Apeldoorn et al., 2007).

## 1.3 Cyanobacterial toxins

Cyanotoxins are bioactive secondary metabolites produced by cyanobacteria. Humans may be exposed to cyanotoxins via contact through recreational activities such as bathing in contaminated water and by consuming untreated, or unsuitably treated drinking water, food, or some dietary supplements (Roy-Lachapelle et al., 2017, Testai et al., 2016, Funari and

Testai, 2008). These toxins can cause gastroenteritis, skin reactions and liver failure in humans (Kubickova et al., 2019, Zanchett and Oliveira-Filho, 2013, Drobac et al., 2013, Funari and Testai, 2008, Kuiper-Goodman et al., 1999), and cause skin lesions, nervous system problems, and liver damage in domestic and wild animals. Lethal effects of cyanotoxins have been reported in humans and animal such as sheep, cattle, horses, pigs and dogs (Huisman and Hulot, 2005, Cox et al., 2003, Carmichael, et al., 2001, Kuiper-Goodman et al., 1999, Jochimsen et al., 1998).

Cyanobacterial toxins are classified by how they affect the human body or their toxicological properties – e.g. hepatotoxins, neurotoxins, dermatotoxins (Codd et al., 2005, Sivonen and Jones, 1999) and according to their chemical structure such as cyclic peptides, alkaloids and lipopolysaccharides (LPS) (Chorus and Welker, 2021, Chorus and Bartram, 2005, Codd et al., 1999, Chorus and Bartram, 1999, Carmichael, 1992).

### 1.3.1 Microcystins

The microcystins (MCs) are cyclic oligopeptides and are the most frequently found hepatotoxins in fresh and brackish waters worldwide. These toxins were named microcystins since they were first isolated from the cyanobacterium Microcystis aeruginosa (Kützing) Kützing (WHO, 1999, Carmichael et al., 1988). An estimated 66 % of Microcystis blooms produce MCs (Green, 2011). In addition to members of the genus *Microcystis*, MCs can be produced by planktic and benthic cyanobacteria taxa such as *Aphanizomenon*, *Aphanocapsa*, *Calothrix*, *Dolichospermum/Anabaena*, *Fischerella*, *Geitlerinema*, *Gloeotrichia*, *Leptolyngbya*, *Limnothrix*, *Merismopedia*, *Nostoc*, *Planktothrix* (*Oscillatoria*), *Phormidium*, *Pseudanabaena*, *Spirulina*, *Synechococcus*, *Trichodesmium* as well as the terrestrial cyanobacterium *Hapalosiphon* (Chorus, 2012, Furtado et al., 2009, Gantar et al., 2009, Myers et al., 2007, Richardson et al., 2007, Mohamed et al., 2006, Oksanen et al., 2004, Codd et al., 1999, Beattie et al., 1998, Mez et al., 1997, Sivonen 1996, Prinsep et al., 1992, Sivonen et al., 1990b). MCs can bioaccumulate in aquatic vertebrates and invertebrates such as fish, mussels, and zooplankton. MCs mainly affect the liver but can also affect the kidneys and reproductive system (Liu et al., 2018). MCs are inhibitors of eukaryotic protein phosphatases, which are

important for cell growth and tumor suppression. Therefore, MCs are carcinogenic promoters (Carmichael, 1997, Carmichael, 1994, Luukkainen et al., 1993).

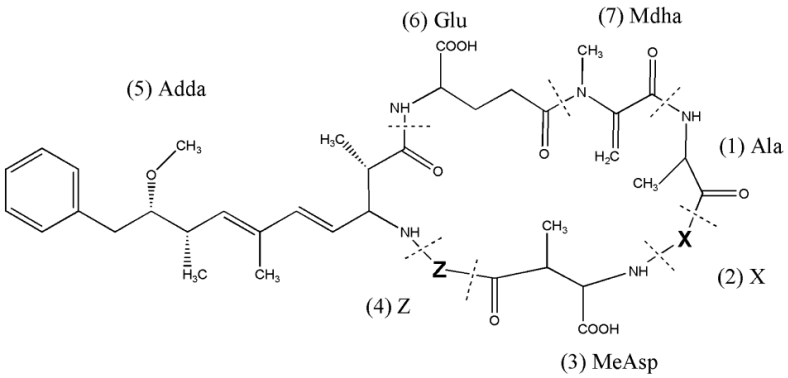



Figure (1). Chemical structure of microcystin (Kaloudis et al., 2013), cyclo (D-Ala[1]-X[2]-D-MeAsp[3]-Y[4]-Adda[5]-D-Glu[6]-Mdha[7]). X and Y represent variable L-amino acids at positions 2 and 4. MeAsp is D-erythro-ß methylaspartic acid and Mdha is N-methyldehydroalanine. Adda is (2S, 3S, 8S, 9S)-3-amin-9-methox-2, 6, 8-triethyl-10-phenyl- 4, 6-decadienoic acid.

Variation in the chemical structure of MCs is very common. Approximately 25 % of microcystin variants are due to the variations in the X and Y position, but substitutions have been reported for each amino acid (Codd et al., 1999, Sivonen, 1996). To date more than 279 variants have been structurally characterized (Bouaïcha et al., 2019) with molecular weights varying from 909 g/mol to 1115 g/mol (Bláha et al., 2009). Microcystin-LR (MC-LR) is the most common microcystin variant and is possibly carcinogenic to humans (IARC, 2010).

### 1.3.2 Cylindrospermopsin

The cyclic alkaloid cylindrospermopsin (CYN) was first isolated from a culture of *Cylindrospermopsis raciborskii* (now *Raphidiopsis raciborskii* (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno strains from a reservoir in tropical northern Australia (Kuiper-Goodman et al., 1999) and chemically characterized by Ohtani et al. (1992). Toxicity of CYN was first reported in 1979 on Palm Island, Australia where more

than 100 persons were hospitalized. This incident was associated with the consumption of drinking water contaminated by *C. raciborskii* (Griffiths and Saker, 2003, Hawkins et al., 1997, Byth, 1980).

CYN production has now been confirmed in other cyanobacteria, including *Aphanizomenon*, *Chrysosporum*, *Dolichospermum* (*Anabaena*), *Lyngbya*, *Raphidiopsis*, *Oscillatoria*, and *Umezakia* (Akcaalan et al., 2014, Cirés et al., 2014, McGregor et al., 2011, Mazmouz et al., 2010, Brient et al., 2009, Seifert et al., 2007, Preußel et al., 2006, Li et al., 2001, Schembri et al., 2001, Banker et al., 1997, Harada et al., 1994). There are four structural variants of cylindrospermopsin: 7-epi-cylindrospermopsin (7-epi-CYN), 7-deoxy-cylindrospermopsin (7-deoxy-CYN), 7-deoxy-desulpho-cylindrospermopsin and 7-deoxy-desulpho-12-acetylcylindrospermopsin (Wimmer et al., 2014, Banker et al., 2000, Norris et al., 1999). CYN is a protein synthesis inhibitor, genotoxin (Humpage et al., 2000) and carcinogen (Falconer and Humpage, 2006) which primarily affects the kidneys, lungs, heart, thymus, spleen and intestine in mammals (Falconer et al., 1999, Hawkins et al., 1997).

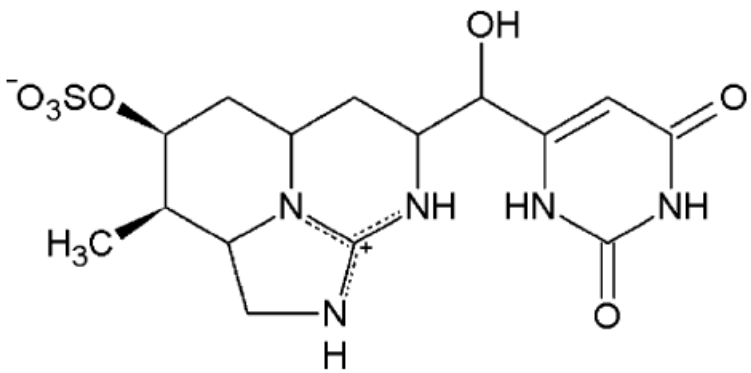



Figure (2) Chemical structure of the cylindrospermopsins (Žegura et al., 2011)

### 1.3.3 Anatoxin

The neurotoxin anatoxin-a (ATX-a) is one of the most frequently found cyanotoxins and was called the very fast death factor (VFDF) due to its capacity of rapid death for animals (Bruno et al., 2017) with an LD50 for mice of 250 µg $kg^{-1}$ body weight (Devlin et al., 1977). It binds to neuronal nicotinic acetylcholine receptors affecting the central nervous system. ATX-a is a

bicyclic secondary amine, 2-acetyl-9-azabicyclo- [4.2.1] non-2-ene (Figure 3) (Dow and Swoboda, 2000, Carmichael, 1988, Devlin et al., 1977, Huber, 1972). Homoanatoxin-a (HANTX) is a toxic analogue of ATX-a and has an additional methyl group (CH) on carbon atom 12 (C12) (Figure 3). They both can sometimes be found together in cyanobacterial or water samples (Bruno et al., 2017, Harland et al., 2014, Mejean et al., 2009, Cadel-Six et al., 2007, Namikoshi et al., 2003) and share the same toxicological properties (WHO, 2019). These toxins are associated with *Anabaena* sp., *Aphanizomenon flos-aquae*, *Aphanizomenon sp*, *Arthrospira fusiformis*, *Cuspidothrix issatschenkoi*, *Dolichospermum circinale*, *D. flos-aquae*, *D. macrosporum*, *D. mendotae*, *D. planctonicum*, *D. spiroides*, *Microcoleus autumnalis*, *Microcystis aeruginosa*, *Microcystis sp.*, *Oscillatoria sp.*, *Phormidium favosum*, *P. formosum*, *Phormidium sp.*, *Pseudanabaena limnetica*, *Tychonema bourrellyi* (Shams et al. 2015, Harland et al., 2014, Hodoki et al., 2013, Ballot et al., 2010b, Osswald et al., 2009, Cadel-Six et al., 2007, Selwood et al., 2007, Wood et al., 2007a, Ballot et al., 2005, Gugger et al. 2005, Aráoz et al., 2005, Ballot et al., 2004, Namikoshi et al. 2003, Watanabe et al., 2003, Lakshmana Rao et al., 2002, Park et al., 1993, Rapala et al., 1993, Edwards et al., 1992, Sivonen et al., 1989a, Carmichael et al., 1979, Devlin et al. 1977)

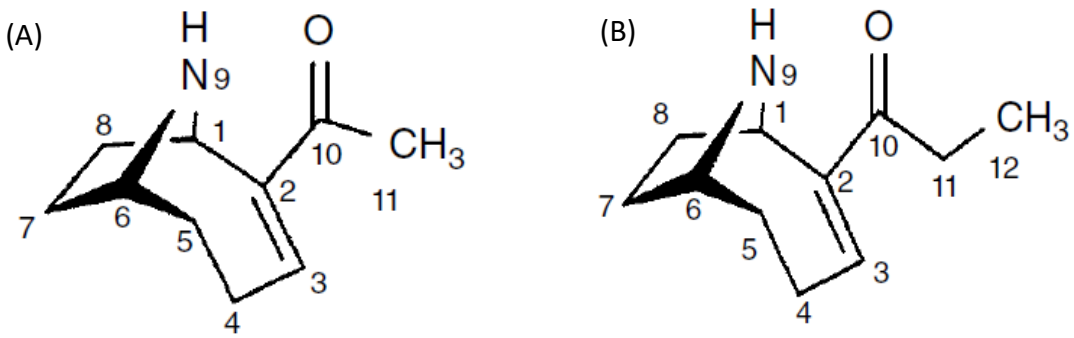



Figure (3) Chemical structures of (A) anatoxin-a (ATX-a) and (B) homoanatoxin-a (HANTX) (Bruno et al., 2017)

### 1.3.4 Saxitoxins

Saxitoxins (STXs), also called Paralytic Shellfish Poisoning (PSP) toxins or Paralytic shellfish toxins (PSTs), are neurotoxic alkaloids, which are produced by marine eukaryotic dinoflagellates belonging to the genera *Alexandrium*, *Gymnodinium* and *Pyrodinium* (Lefebvre et al., 2008, Usup et al., 1994, Oshima et al., 1993) and freshwater prokaryotic cyanobacteria such as *Aphanizomenon gracile*, *Aphanizomenon sp*., *Cuspidothrix issatschenkoi*, *Dolichospermum circinale*, *Lyngbya wollei*, *Raphidiopsis brookii*, *Cylindrospermum sp*., *Phormidium uncinatum*., *Geitlerinema sp*., *Limnothrix redekei*, *Scytonema* cf. *crispum* (Borges et al., 2015, Casero et al., 2014, Smith et al., 2012, Soto-Liebe et al., 2012, Smith et al., 2011, Ledreux et al., 2010, Ballot et al., 2010a, Yunes et al., 2009, Pomati et al., 2006, Liu et al., 2006, Gkelis et al., 2005, Pereira et al., 2004, Nogueira et al., 2004, Li et al., 2003, Testé et al., 2002, Dias et al., 2002, Ferreira et al., 2001, Li et al., 2000, Pomati et al., 2000, Pereira et al., 2000, Carmichael 1997, Negri et al., 1997, Humpage et al., 1994, Mahmood and Carmichael, 1986a, Ikawa et al., 1982). Saxitoxin (STX) was the first identified saxitoxin analogue. It was first isolated in pure form from the Alaskan butter clam, Saxidomus giganteus in 1957 (Schantz et al. 1957).

STX has the molecular formula $C_{10}H_{17}N_7O_4$ and is composed of a 3, 4-propinoperhydropurine tricyclic system and two guanidinium groups (Figure 4), which are responsible for its high polarity (Shimizu, 2000). The alkaloids block the sodium channel in the neurons, impair the action potential, and cause paralysis of muscles (Sivonen, 1996, Kao, 1993). To date, 57 naturally occurring STX analogs have been identified (Chorus, 2020, Wiese et al., 2010) and about 20 structural variants are known to be produced by cyanobacteria (Codd et al., 2005).

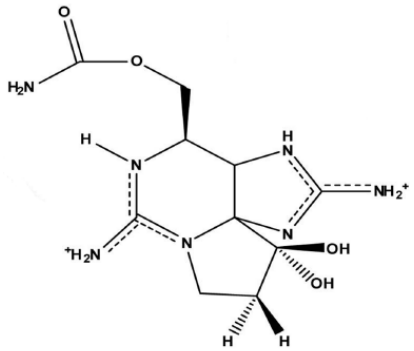


Figure (4) Chemical structure of saxitoxins (Cusick and Sayler, 2013)

### 1.3.5 Monitoring of Cyanotoxins

Cyanotoxins have been confirmed as a causative agent in numerous reports on poisoning and mortality of animals and humans, and the prevalence of these cases is increasing through time (Codd et al., 2005, Krienitz et al., 2003, Kuiper-Goodman et al., 1999, Christoffersen, 1996, Bell and Codd, 1994). The control of Cyanobacteria therefore is vital for freshwater resource management. Aquatic phytoremediation technology is crucial for restoration of eutrophic water bodies (Yasin et al., 2017b, Straile 2015) with low cost, high ability of removing nitrogen and phosphorus and minimal disturbance to the environment (Ali et al., 2017, Wu et al, 2015). Numerous studies also demonstrated that many aquatic macrophytes could significantly suppress some cyanobacteria species (Jiang et al., 2015, Dong et al., 2014, Hilt and Gross, 2008, De Figueiredo et al., 2004). Prioritizing submerged macrophyte restoration, therefore, is an effective approach for the sustainability of lake ecosystems (Zhang et al., 2020, Hilt et al., 2006).

## 1.4 Aquatic macrophytes

Aquatic macrophytes are plants growing in the water submerged, floating-leaved or emergent. They can be divided into helophytes, which are semi-aquatic and emergent plants, and aquatic macrophytes (hydrophytes), which include submerged plants (elodeids, charophytes) or plants with floating leaves (nymphaeids and lemnids) (Ballot et al., 2017). Aquatic macrophytes play a vital role in the structure and function of aquatic freshwater

systems, providing refuge and food for aquatic organisms (Jeppesen et al., 2012, Timms and Moss, 1984), and improving water quality by absorbing and sequestering nutrients. Macrophytes can suppress structure and variation of phytoplankton communities and enhance water clarity through competition for nutrients (Dou et al., 2015, Mjelde and Faafeng, 1997), releasing allelopathic compounds (Gross et al., 2007), and shifting environmental light levels (Cunha et al., 2012). Aquatic macrophytes also influence the sediment deposition via wind protection (Brix, 1997), and both submerged and free-floating macrophyte species can break down pollutants introduced by humans and animals (Srivastava et al., 2008).

High macrophyte abundance can increase a lake's resistance to increasing nutrient load (Scheffer et al. 1993a, Phillips et al., 1978). Shallow lakes without, or with only limited, macrophyte cover are more susceptible to high nutrient loads than lakes with macrophyte cover (Kuiper et al., 2017). In addition, high macrophyte diversity seems to have some influence on inhibiting the shift to phytoplankton dominance (Sayer et al., 2010) and can improve the stability and biodiversity of aquatic systems (Siu Yeon Tan et al., 2017). Scheffer et al. (1993a) and Jeppesen et al. (1998) suggested two alternative stable states in temperate eutrophic lakes: a clear water state dominated by submerged macrophytes, and a turbid and phytoplankton-dominated state. The turbid state dominated by phytoplankton is considered undesirable, while water dominated by submerged macrophytes is regarded as better quality (EC, 2000). However, massive growth of macrophytes can lead to accumulation of organic material and decreased oxygen content in the water, which may be followed by fish mortalities (Cooke et al., 2016, Karim et al., 2013, Martin et al., 1993, Gopal, 1990). When macrophytes decay, nutrients are released back into the water, which can lead to increased phytoplankton growth (Mitchell et al., 1988).

Knowledge about aquatic macrophyte growth, and understanding the mechanisms behind community shifts, is of considerable importance for management aimed at promoting good ecological status of lakes and reservoirs. Aquatic macrophyte overgrowth and their management have long been a concern in Asia and other continents (Haller, 1990). The dynamics between physical and chemical water qualities and biological communities has been

well studied in temperate lakes (Phillips et al., 2016, Lyche-Solheim et al., 2013), however, there is limited information for tropical lakes. It is unclear if the knowledge and conclusions from temperate systems apply to tropical lakes (Lewis, 2000).

Seasonal rains, which cause water level fluctuations and variations in sediment and nutrient loads, are also an important determinant of macrophyte growth and decomposition in tropical lakes (Thomaz et al., 2008). Submerged macrophyte communities can be severely reduced by large water level changes (Xie et al., 2009, Yu and Yu, 2009), while phytoplankton tends to be less affected and may even increase in biomass (Pan et al., 2018). It seems that tropical lakes are more sensitive to eutrophication and may more easily shift to a turbid water state than temperate lakes (Lewis, 2000). In tropical water systems, free-floating species, e.g. *Eichhornia spp*., *Salvinia spp*., are often dominant because they have high growth rates and clonal reproduction (Albertoni et al., 2014, Thomaz et al., 2008). However, their capacity for lake stability in high nutrient loads seem lower than that of submerged macrophytes (Meerhoff et al., 2003).

# 2 Objectives

Development of indices for phytoplankton and aquatic macrophytes in Myanmar requires taxonomic identification knowledge and knowledge of biological community and species response to environmental stress. However, the diversity and distribution of freshwater species in Myanmar is poorly investigated and documented (Allen et al. 2012). There is also limited or no information on the influence of macrophytes on the function of freshwater systems. More broadly, the interaction between macrophytes and phytoplankton is poorly understood in Myanmar's freshwater lakes. Various cyanobacterial species forming blooms are potential producers of hepatotoxic or neurotoxic compounds, which can be a threat to human and animal health. Toxic compounds are therefore included in the quality assessments. The use of these biological parameters should be considered in management strategies for lakes in Myanmar.

The overall goal of this study is, therefore, to provide important knowledge about the composition and abundance of phytoplankton and macrophytes and their interaction in Myanmar's freshwater systems. We focus specifically on cyanobacteria, production and presence of cyanotoxins, and aquatic macrophytes (i.e. hydrophytes, species belonging to the submerged, floating-leaved and free-floating groups as they depend largely on water quality) (Mjelde et al., 2018). This work will be part of the basic knowledge for the development of *a biological monitoring system based on phytoplankton and aquatic macrophytes in freshwater lakes in Myanmar.* This has been accomplished by addressing the following sub-goals:

- identify and classify phytoplankton with focus on cyanobacteria and aquatic macrophyte taxa in selected waterbodies in Myanmar using classical morphological and genetic methods (**I**, **II**, **III**)
- isolate and culture potentially harmful cyanobacteria from selected waterbodies in Myanmar (**I**, **II**)
- investigate the presence of toxin producing cyanobacteria and their toxins in selected waterbodies (**I**, **II**)
- study the *Chara* distribution in lakes and reservoirs in Myanmar and identify ecological requirements for *Chara* species (**III**)

- assess the importance of aquatic macrophytes as a stabilizing factor in a tropical shallow lake (**IV**)

# 3 Materials and Methods

## 3.1 Study Areas

The field survey was conducted in six water bodies: two natural lakes (Inlay Lake, Wethtigan Lake) and four man-made reservoirs (Meiktila Lake, Yezin Dam, Kyet Mauk Taung Reservoir, Ngaliak Reservoir) (Figure 5, Table 1). The presence of toxin producing cyanobacteria and cyanotoxins were investigated in Meiktila Lake and Yezin Dam (**I**, **II**), and the assessment of the importance of aquatic macrophytes as a stabilizing factor in a tropical shallow lake was conducted in Inlay Lake (**IV**). The distribution of *Chara* spp. and their ecological requirements was investigated in all six water bodies (**III)**.

Inlay and Wethtigan Lakes are shallow natural lakes with maximum depths of 3.5 m and 1.2 m, respectively. Inlay Lake is situated in Southern Shan State at high altitude (884 m) on the elevated Shan plateau, while Wethtigan Lake is located in the central dry zone area at 66 m above sea level (a.s.l). Inlay Lake is the second largest natural lake in Myanmar and has a surface area of 116 $km^2$. Wethtigan Lake has a surface area of around 1.7 $km^2$.

Meiktila Lake, Yezin Dam, Kyet Mauk Taung Reservoir and Ngalaik Reservoir are situated in the Mandalay region in central Myanmar. Meiktila Lake is a shallow reservoir, divided into two separate basins by a dam. The lake basins are located at an altitude of 230 m with a surface area of around 9.1 $km^2$ and a maximum water depth of 10 m. Kyet Mauk Taung Reservoir, which is situated at 279 m a.s.l., has a surface area of about 7.3 $km^2$. Yezin Dam has a surface area of about 6.4 $km^2$ and is located at 128 m a.s.l. Yezin Dam is a low-moderate alkaline and turbid lake with large water level fluctuations. Ngalaik Dam is also a medium-large reservoir, situated in a boreal area in Ottara Thiri Township, Nay Pyi Taw. The reservoir has a surface area of around 5.5 $km^2$ and is situated at 163 m a.s.l. All reservoirs are mainly used for irrigation while Yezin Dam is also a drinking water source for local communities. Ngalaik Dam is also used for recreation. Figure 5 presents a map showing the location of the studied lakes and reservoirs. The geomorphological features and background information of the investigated lakes and reservoirs in this study are displayed in Table 1.


Figure (5) Map of Myanmar with the location of the selected water bodies. (Source: overview-map: https://www.nationsonline.org/oneworld/map/Myanmar-administrative-map.htm

Table 1. The geomorphological features and background information of the evaluated lakes and reservoirs in Myanmar.

| | Inlay Lake | Wethigan Lake | Meiktila Lake (South & North) | Kyet Mauk Taung Dam | Yezin Dam | Ngalaik Dam |
|---|---|---|---|---|---|---|
| **Manuscript** | **III, IV** | **III** | **I, III** | **III** | **II, III** | **III** |
| **Geographical position** | N 20° 65'<br>E 96° 92' | N 20° 57'<br>E 94° 64' | N 20° 52'<br>E 95° 51' | N 20° 81'<br>E 94° 64' | N 19° 50'<br>E 96° 16' | N 19° 86'<br>E 96° 00' |
| **Division/State** | Nyaung Shwe tsp., Southern Shan State | Salin tsp., Magway Division | Meiktila tsp., Mandalay Division | Kyaukpadaung tsp., Mandalay Division | Zay Yet Thiri tsp., Nay Pyi Taw, Mandalay Division | Ottara Thiri tsp., Nay Pyi Taw, Mandalay Division |
| **Altitude a.s.l. (m)** | 884 | 66 | 230 | 279 | 128 -133 | 163 |
| **Type** | Natural Lake | Natural lake | Semi-natural | Man-made | Man-made | Man-made |
| | Clear, very calcareous | Clear, Calcareous | Moderate alkaline, slightly turbid | Calcareous, slightly humic water body | Low-moderate alkaline, turbid | Clear, Calcareous |
| **Established Year** | - | - | 1173-1210 | 1961 - 1967 | 1973-74 | 1978-87 |
| **Surface area ($km^2$)** | 94.4 – 126.1* | 1.7 | 9,1 - 54* | 7.3 | 6.4 - 9.3* | 5.5 |
| **Max. Water Depth (m)** | 3.2 | 1.6 | 10 | >10 | 21 | > 25 |
| **Mainly water usage** | Drinking, domestic | Domestic | Drinking water, domestic, irrigation | Irrigation, domestic | Irrigation, domestic, drinking | Irrigation, recreation |
| **No. of sampling points** | 15 | 3 | 5 (3 in Northern; 2 in Southern) | 3 | 3 | 3 |
| **Sampling period** | 2014 - 2017 | November 2019 and March 2020 | March and November, 2017 | November 2019 and March 2020 | November 2014, 2017-2018 | March and November, 2017 |
| **Impact** | Sedimentation, domestic waste, fertilizers and pesticide from floating gardens, dye from weaving, oil spillage from boats, poor sanitation, waste/ garbage from in-site stilt houses | Domestic waste | Sedimentation due to deforestation, domestic waste, street runoff and solid waste | Agricultural runoff, low rainfall, and high temperature | Sedimentation, domestic waste, detergent and laundry soap, unknown waste from upstream areas | Waste from recreational park |
| **Yearly rainfall (mm)** | 1370 | 848 | 808 | 825 | 980 | 1167 |
| **Remarks** | Wildlife Sanctuary<br>Biosphere Reserve<br>Ramsar site | Wildlife Sanctuary | Southern and Northern parts were divided by a dam | - | - | Recreational Reservoir |

Table 2 presents the materials and methods used in the field and laboratory, which are described in the respective manuscript.

Table 2. Materials and methods used to study the physical and chemical conditions, phytoplankton, and aquatic macrophytes in freshwater lakes and reservoirs in Myanmar between 2014 and 2019.

| Methods | Manuscript |
|---|---|
| In-situ measurement of water temperature, pH, Conductivity, Dissolved Oxygen | I, II, III, IV |
| Integrated samples for chemical analysis | I, II, III, IV |
| Water samples for phytoplankton | I, II, IV |
| Water samples for cyanotoxins | II |
| Quantitative and qualitative analysis of phytoplankton using light microscopy and inverse microscopy | I, II, IV |
| Aquatic macrophytes survey and morphological Identification of aquatic macrophytes | I, II, III, IV |
| Isolation and cultivation of cyanobacterial strains | I, II, IV |
| Extraction of genomic DNA | I ,II, IV |
| Molecular analysis of toxin producing cyanobacteria (PCR of 16S rRNA region) | I, II |
| Molecular analysis of macrophyte (*Chara* spp.) species | III |
| Construction of phylogenetic trees of cyanobacterial strains using maximum likelihood algorithm | I, II |
| Construction of phylogenetic trees of macrophyte (*Chara* spp.) species using maximum likelihood (ML), maximum parsimony (MP) and distance (neighbour-joining (NJ)) | III |
| Analyses of microcystins, anatoxin-a, cylindrospermopsins, saxitoxins by ELISA | I, II, IV |
| Analyses of microcystins, anatoxin-a, cylindrospermopsins, saxitoxins by LC-MS/MS | I, II |
| Statistical analysis (univariate, bivariate and multivariate analysis) | IV |

## 3.2 Sampling

Each lake and reservoir was investigated 2 to 8 times in the period from 2014 to 2019. Physical measurements, water samples, phytoplankton and aquatic macrophytes were collected at 3 to 15 sites in each study area.

### 3.2.1 Physical measurements and water quality

At each survey point, we took in-situ measurements of water temperature, pH, conductivity, and dissolved oxygen using a Hach HQd Portable Meter (Hach, Loveland, CO, USA) and water depth using a Hondex PS-7 handheld portable water depth sounder gauge (Honda Electronics Co., Ltd, Japan) (**I**, **II**, **IV**). Water transparency was measured with a Secchi disk (Bertoni, 2011) at each survey point. Additionally, integrated water samples (1 m step) were collected from the trophogenic zone (two times the Secchi depth) for chemical analyses using a Limnos water sampler (Limnos, Komorów, Poland) (**I**, **II**, **IV**). One water sample for nutrient analysis was always preserved with 4M $H_2SO_4$ (to 1% final concentration) and one sample was not preserved for other physical and chemical properties. Water samples were analysed for Total Phosphorous (TP), Total Nitrogen (TN), Total Organic Carbon (TOC), turbidity, total alkalinity, color and calcium (Ca) at the Water Quality Laboratory, Forest Research Institute, Yezin, Myanmar (**I**, **II**) and the Norwegian Institute for Water Research (NIVA), Norway (**IV**).

### 3.2.2 Water Sampling for phytoplankton and cyanotoxin analysis

We took 50 ml subsamples from the integrated samples for quantitative phytoplankton analyses (phytoplankton composition, biomass) which were preserved with Lugol's solution (**I**, **II**, **IV**). We used a phytoplankton net with 20 µm mesh size to collect and concentrate phytoplankton samples which were preserved with formaldehyde (to 4 % final concentration) for qualitative phytoplankton analyses. A 50 ml water sample was also collected from the surface layer at each sampling point to isolate cyanobacteria (**I**, **II**, **IV**). These samples were kept in a cool shady place and gently shaken twice per day until processing at the Norwegian Institute for Water Research (NIVA) in Norway after 3 – 10 days depending on the time of sampling and return to Norway.

### 3.2.3 Aquatic macrophytes survey

Hydrophyte species are highly sensitive to water quality, which permits their use in indices such as those developed and used in the EU Water Framework Directive, Northern Intercalibration Group (Hellsten et al., 2014). Therefore, during aquatic macrophyte surveys for this study we focused exclusively on hydrophytes. At each sampling point, we identified and recorded plants in an area of approximately 1 $m^2$ using an aqua scope, while plant samples were collected by dredging with a casting rake from the boat (**I, II, III**, **IV**) (Kolada et al., 2012).

The abundance of each macrophyte species was scored according to a semi-quantitative scale, where 1 = rare, 2 = scattered, 3 = common, 4 = locally dominant, and 5 = dominant. The estimated bottom cover of aquatic macrophytes was based on these semi-quantitative scores for all localities. In paper **III**, abundances at each locality were approximated by summarizing the cubed five-level values for each species. The five-level scale used for abundance estimation of aquatic macrophytes is non-linear and using cubed 5-level values for total abundance is a method commonly used for submerged macrophytes (Melzer, 1999) and is regarded as the "best possible" approximation for comparing abundances among algal groups and sites (Schneider et al., 2018). All macrophytes were identified to species level when possible by using floras for the region including La-Ongsri (2009), Wieglet & Kaplan (1998), Cook (1996), Wiegleb (1990), and Triest (1988) (**I**, **II**, **IV**). Charophytes were identified based on Wood and Imahori (1965) and verified later by genetic analysis (**I**, **III**, **IV**).

## 3.3 Phytoplankton Analysis

All Lugol-fixed samples were analysed for phytoplankton composition and biomass using Utermöhl sedimentation chambers (Utermöhl, 1958) and an inverted microscope (Leica DMi8, Ortomedic, Oslo, Norway) (**I**, **II**, **IV**). Phytoplankton biomasses were calculated by geometrical approximations using the computerized counting program Opticount (SequentiX-Digital DNA Processing, Klein Raden, Germany). For calculations the specific density of phytoplankton cells was assumed as 1 g $cm^{-3}$.

## 3.4 Isolation of strains and morphological characterization

Single colonies of *Microcystis*, and single filaments of *Dolichospermum* and *Raphidiopsis* from Meiktila Lake, Yezin Dam and Inlay Lake were isolated using a microcapillary (**I**, **II** & **IV)**. They were washed five times and placed in wells on microtiter plates containing 300 µL Z8 medium (Kótai, 1972). After successful growth, samples were placed in 50 mL Erlenmeyer flasks containing 20 mL Z8 medium and incubated at 22 °C. Strains were then classified based on morphological traits (Komárek, 2013, Komárek and Anagnostidis, 1999) using a Leica DM2500 light microscope, Leica DFC450 camera and Leica Application Suite software (LAS) (Leica, Oslo, Norway). Morphological identification was based on criteria, including (i) size of vegetative cells and heterocytes and (ii) nature and shape of filaments or colonies. Length and width of 50-250 vegetative cells or filaments and of 20-50 heterocytes were measured for the calculation of mean cell biomass.

## 3.5 Genomic DNA extraction, PCR amplification and sequencing

For papers **I** and **II**, genomic DNA of all isolated strains were extracted following the protocols of Ballot et al. (2016). All PCRs were performed on a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Oslo, Norway) using the iProof High-Fidelity PCR Kit (Bio-Rad Laboratories, Oslo, Norway). The 16S rRNA gene of strains isolated in this study were amplified using the primers described by Ballot et al. (2016). The amplified PCR products were purified with Qiagen PCR purification columns (Qiagen, Hilden, Germany) in accordance with the manufacturer's protocol and then sequenced by using the same primers as for PCR and intermediate primers as used in Ballot et al. (2016). The PCR products were sequenced on an ABI 3730 Avant genetic analyser using the BigDye terminator V.3.1 cycle sequencing kit (Applied Biosystems, Thermo Fisher Scientific Oslo, Norway) according to the manufacturer's instructions.

For the molecular analysis of *Chara* species in paper **III**, we extracted genomic DNA from 11 *Chara* specimens followed by the protocols of Schneider et al. (2016). PCR of the *matK* and *rbcl* genes was performed using the iProof High-Fidelity PCR Kit (Bio-Rad Laboratories, Oslo, Norway). The PCR products were sequenced on an ABI 3730 Avant genetic analyser using the BigDye

terminator V.3.1 cycle sequencing kit (Applied Biosystems, Thermo Fisher Scientific Oslo, Norway) according to the manufacturer's instructions.

## 3.6 Phylogenetic Analysis

The Seqassem (version 04/2008) and Align (version 03/2007) software packages (Sequenti X-Digital DNA Procession, Klein Raden Germany) were used for the analyses of 16S rRNA gene sequences of isolated cyanobacterial strains (**I**, **II).** In paper **I**, we used 1135 base pairs of the 16S rRNA gene to construct a phylogenetic tree for *Cylindrospermopsis*/ *Raphidiopsis*. Five *Raphidiopsis* strains from Meiktila Lake and 35 additional *Cylindrospermopsis*/ *Raphidiopsis* sequences from GenBank were included in this analysis and *Sphaerospermopsis aphanizomenoides* (LN846954) was used as the outgroup. The *Microcystis* phylogeny was constructed using 1426 base pairs of the16S rRNA genes*. Chroococcus subviolaceus* (MF072353) was used as the outgroup, two strains from Meiktila Lake and 38 additional *Microcystis* sequences derived from GenBank were included in the analysis. In paper **II**, we used 1136 base pairs of the 16S rRNA gene from *Anabaena/ Dolichospermum* to construct a phylogenetic tree. Eight *Dolichospermum* sequences from Yezin Dam and 23 additional *Dolichospermum* sequences derived from GenBank were included in the analysis. *Cuspidothrix issatschenkoi* (FN689797) was employed as the outgroup. The phylogenetic trees were constructed using the Maximum Likelihood (ML) algorithm in Mega v. 7 (Kumar et al., 2016). The sequence data produced for Paper **I** and **II** were submitted to the European Nucleotide Archive (ENA) under the accession numbers listed in Table 3.

For phylogenetic analysis of *Chara* species, we constructed phylogenetic trees using 935 base pairs of the *matK* gene and 567 base pairs of the *rbcl* gene (**III**). For the *matK* tree, we used 11 *Chara* sample sequences from Myanmar and 24 *Chara* sequences from GenBank. The *rbcl* tree used 7 *Chara* samples from Myanmar and 32 other *Chara* sequences obtained from GenBank. *Nitellopsis obtusa* (AY170447) and *Lamprothamnium succinctum* (KX431014) were used as the outgroups in the *matK* and *rbcl* phylogenetic trees, respectively. Maximum likelihood (ML), maximum parsimony (MP) and distance (neighbor-joining (NJ)) were performed for analysis with 1000 bootstrap replicates in MEGA version X (Kumar et al., 2018). The sequence data produced for Paper **III** were submitted to GenBank (NCBI) under the accession numbers listed in Table 4.

Table 3. Cyanobacteria species and strains isolated from Meiktila Lakes (**I**) and Yezin Dam (**II**) in Myanmar, strain codes, and ENA accession numbers.

| Species | Strain | Lake/Reservoir | *Accession Number* 16S rRNA |
|---|---|---|---|
| ***Dolichospermum*** | | | |
| ***D. smithii*** | AB2017/01 | Yezin Dam | LR794159 |
| ***D. smithii*** | AB2017/02 | Yezin Dam | LR794160 |
| ***D. smithii*** | AB2017/03 | Yezin Dam | LR794161 |
| ***D. smithii*** | AB2017/04 | Yezin Dam | LR794162 |
| ***D. smithii*** | AB2017/05 | Yezin Dam | LR794163 |
| ***D. smithii*** | AB2017/06 | Yezin Dam | LR794164 |
| ***D. smithii*** | AB2017/17 | Yezin Dam | LR794165 |
| ***D. smithii*** | AB2017/18 | Yezin Dam | LR794166 |
| ***Raphidiopsis*** | | | |
| ***R. raciborskii*** | AB2017/05 | Meiktila Lake | LR590626 |
| ***R. raciborskii*** | AB2017/09 | Meiktila Lake | LR590627 |
| ***R. raciborskii*** | AB2017/12 | Meiktila Lake | LR590628 |
| ***R. raciborskii*** | AB2017/13 | Meiktila Lake | LR590629 |
| ***R. raciborskii*** | AB2017/16 | Meiktila Lake | LR746263 |
| ***R. raciborskii*** | AB2017/14 | Yezin Dam | LR590626 |
| ***R. raciborskii*** | AB2017/14 | Yezin Dam | LR590627 |
| ***R. raciborskii*** | AB2017/14 | Yezin Dam | LR590628 |
| ***R. raciborskii*** | AB2017/14 | Yezin Dam | LR590629 |
| ***R. raciborskii*** | AB2017/14 | Yezin Dam | LR746263 |
| ***Microcystis*** | | | |
| ***Microcystis*** | AB2017/14 | Meiktila Lake | LR590630 |
| ***Microcystis*** | AB2017/15 | Meiktila Lake | LR590631 |
| ***Microcystis*** | AB2017/14 | Yezin Dam | LR794160 |
| ***Microcystis*** | AB2017/15 | Yezin Dam | LR794160 |

Table 4. *Chara* samples, origin and GenBank accession numbers (**III**)

| Strain | Habitat | *Accession Number* | |
|---|---|---|---|
| | | ***matK*** | ***rbcl*** |
| **MMYA-1** | Inlay Lake | MT739758 | MT739769 |
| **MMYA-2** | Inlay Lake | MT739759 | MT739774 |
| **MY-32** | Yezin Dam | MT739768 | - |
| **MY-33** | Yezin Dam | MT739760 | - |
| **MY-45** | Meiktila Lake (North) | MT739767 | - |
| **MY-34** | Meiktila Lake (South) | MT739761 | - |
| **MY-35** | Ngalaik Reservoir | MT739762 | MT739772 |
| **MY-58** | Kyet Mauk Taung Reservoir | MT739765 | MT739773 |
| **MY-59** | Kyet Mauk Taung Reservoir | MT739763 | MT739775 |
| **MY-60** | Wethtigan lake | MT739766 | MT739771 |
| **MY-61** | Wethtigan lake | MT739764 | MT739770 |

"-" = not analysed

## 3.7 Toxin analyses

### 3.7.1 ELISA for MCs, CYNs, ATXs and STXs

Presence, types and amounts of cyanobacterial toxins from strains isolated from Meiktila Lake, Yezin Dam and Inlay Lake were investigated by enzyme-linked immunosorbent assay (ELISA) (**I**, **II**, **IV**). Prior to analysis, the fresh culture materials of all isolated strains from Meiktila Lake (**I**), Yezin Dam (**II**) and Inlay Lake (**III**) were frozen and thawed three times. All *R. raciborskii* were tested for cylindrospermopsins (CYNs) using the Abraxis Cylindrospermopsin ELISA kit (Abraxis LLC, Warminster, PA, USA) following the manufacturer's instructions. The *Microcystis* strains were tested for MCs using the Abraxis Microcystins/Nodularin (ADDA) ELISA kits (Abraxis LLC, Warminster, PA, USA). The same procedures were also used to test all isolated *Dolichospermum* strains for CYNs and MCs. All strains from each taxon were also tested for saxitoxins and anatoxin-a using the Abraxis Saxitoxins (PSP) and Abraxis Anatoxin (VFDF) ELISA kits (Abraxis LLC, Warminister, PA, USA). Water samples from Yezin Dam collected in February 2020 were also tested for STXs, ATXs, CYNs and MCs with the above-mentioned ELISA kits. The color reaction of all ELISA tests was evaluated on a Perkin Elmer 1420 Multilabel counter Victor3 (Perkin Elmer,

Waltham, MA, USA) at 450 nm. The toxin concentrations were evaluated by manual analysis of the absorbance data as recommended by the vendor.

### 3.7.2 Microcystin analysis by LC-HRMS

The *Microcystis* strains from Meiktila Lake (**I**) and Yezin Dam (**II**) were investigated for their MC profiles with high resolution LC-MS/MS (LC-HRMS/MS). Fresh culture materials of all *Microcystis* strains from Meiktila Lake (**I**) and Yezin Dam (**II**) were prepared by freezing and thawing 3 times, diluting with an equal volume of MeOH and filtering with a 0.22 µm filter (Miles et al., 2012). A Q Exactive-HF Orbitrap mass spectrometer equipped with a HESI-II heated electrospray ionization interface (ThermoFisher Scientific, Waltham, MA, USA) on an Agilent 1200 LC system was used to perform LC-HRMS/MS analysis. The MS was operated in positive and negative ion modes calibrated from m/z 74-1622 and m/z 69-1780, respectively. The detailed analysis procedures are described in Papers **I** and **II**.

### 3.7.3 CYNs, ATXs and STXs analysis by LC-MS/MS

Cyanobacterial toxins were extracted from freeze-dried cultures (40 ml), following procedures described by Cerasino et al. (2017). LC-MS/MS analyses were performed using a Waters Acquity UPLC system (Waters, Milford, MA, USA) coupled to a SCIEX 4000 QTRAP mass spectrometer (AB Sciex Pte. Ltd., Singapore). A HILIC column (Ascentis Express OH5, 2.7 µm, 50 × 2.1 mm; Merck Life Science S.r.l., Milan, Italy) was used for Chromatographic separation of analytes while MS detection was made using positive electrospray ionization by scheduled Multiple Reaction Monitoring. Details of the experimental set up are fully described by Cerasino et al. (2017) (**I, II**).

## 3.8 Statistical Analyses

In paper **IV**, univariate, bivariate and multivariate analyses were performed to assess the relationship between environmental variables and biology, to describe the relationship between phytoplankton and macrophytes, and to detect possible differences between seasons and/or lake areas. Relationships between community composition and environmental variables were assessed using redundancy analysis (RDA) by using the Vegan library (Oksanen et al., 2020) in R (RCoreTeam, 2020).

In order to support the multivariate analyses, the Uni- and bi-variate analyses were performed with Addinsoft XLSTAT®©, with significance assumed as $p < 0.05$. Univariate analyses included two-way type I ANOVA to detect differences in average abundance/biomass among lake areas (North, Middle, and South) and between sampling seasons (November, corresponding to the end of the rainy summer season, and February/March, corresponding to the dry winter season). Turkey HSD (Honestly Significant Difference) post-hoc multiple comparisons were performed after significant ANOVA. to find out which specific average values of abundance/biomass are different from which values of abundance/biomass.

We also performed linear regressions between macrophyte abundance and phytoplankton biomass to check if the mutual exclusion of macrophytes and phytoplankton, typical in nutrient-rich, shallow temperate lakes (Jeppesen et al., 1998), exists also in tropical Inlay Lake (**IV**).

# 4 Results and Discussion

## 4.1 Physical and chemical properties of selected water bodies

The natural lakes, Inlay Lake and Wethtigan Lake, are clear (Turbidity < 1.5 FNU) and calcareous (Ca concentrations > 40 mg/L) (**III**). In both, water transparency was high (secchi depth to bottom) so that sunlight can reach the bottom, as in a typical shallow lake with abundant macrophytes (Scheffer and Jeppesen, 1998). Ngalaik reservoir is also clear (Turbidity < 2.5 FNU) and calcareous (Ca concentrations > 20 mg/L), while Kyet Mauk Taung reservoir is calcareous (Ca concentrations > 40 mg/L) and slightly humic (TOC 5.1 mg $L^{-1}$) (**III**). Meiktila Lake is moderate alkaline (pH > 8.5; Ca = 14 – 19 mg $L^{-1}$) and turbid (Turbidity ≥ 83 FNU), the southern basin being more turbid than the northern (**I**, **III**). Yezin Dam is a low to moderate alkaline (pH 7.5 - 8.8) and turbid reservoir (Secchi depth = 1.5 m: Turbidity = 5.75 FNU) with large water level fluctuations (**II, III**). All studied lakes were characterized by average TP values of less than 20 µg $L^{-1}$ (**III**), except Yezin Dam where higher TP values between 20 – 57 µg $L^{-1}$ were observed during our study period (**II**). In general, the TP values of all studied lakes and reservoirs indicate oligo- to eutrophic conditions (8 – 57 µg $L^{-1}$) according to Salas and Martino (1991).

Except from Inlay and Meiktila Lakes, only few other lakes have been described in detail in Myanmar. In contrast to earlier water chemistry studies of Inlay Lake (Pradhan et al., 2015, Akaishi et al., 2006), we found relatively low nutrient concentrations in the lake's waters. It can be assumed that a large amount of nutrients is bound in the rich macrophyte vegetation and stored in the sediments (**IV**, Mjelde and Faafeng, 1997, Schneider et al., 2014, Van Donk et al., 1993). Data from the tributaries show periodically a very high nutrient input (TP= 57 – 93 µg P $L^{-1}$, TN = 530 – 1600 µg N $L^{-1}$), indicating potentially eutrophic conditions (**IV**). In a recent study, higher nitrate concentrations were observed during the whole year in floating garden areas of Inlay Lake compared to the inlet streams of the lake. It was therefore concluded that lower amounts of nutrients are discharging from the watershed areas than from floating gardens (Khaung et al., 2021). We found that Inlay Lake's waters were categorized by lower electrolytic conductivity (EC) values (<300 µScm$^{-1}$) in the rainy season and higher EC values (> 400 µScm$^{-1}$) in the dry season (**IV**). This is in accordance with the findings of Thin et al. (2016) and Khaung et al

(2021) who observed lower EC (236 µScm$^{-1}$ and 270 µScm$^{-1}$) in wet seasons and higher EC values (489 µScm$^{-1}$ and 310 µScm$^{-1}$) in dry seasons.

In Meiktila Lake, we measured pH values between 8.5 and 9.3, TP concentrations between 12–23 µg L$^{-1}$ (Phosphate = 0.4 –7.6 µg P L$^{-1}$) and TN concentrations between 360–570 µg L$^{-1}$ (Nitrate = 0.83 – 8.33 µg N L$^{-1}$) (**II**). Our findings differ considerably from those in a previous study in Meiktila Lake, where lower pH values (6.5 – 7.5), higher phosphate (up to 860 µg P L$^{-1}$) and nitrate concentrations (up to 860 µg N L$^{-1}$), and wide temperature variations (23-30˚C) were recorded (Hlaing, 2014). The contradicting results between our survey and the above-mentioned studies (Khaung et al 2021, Thin et al 2016, Hlaing 2014) indicate large temporal and spatial variations of nutrient concentrations in the lakes of Myanmar. They can be explained by seasonal changes of nutrient input via inlet rivers from the upstream watershed areas and varying fertilization regimes in floating gardens in the agricultural season and off-season (Inlay Lake). Water temperature and pH of the lakes and reservoirs investigated in our study were in the same range as values observed in some tropical lakes and reservoirs in Thailand, India, Vietnam and China (Zhu et al., 2021, Quevedo-Castro et al., 2019, Bhat et al., 2015, Duong et al., 2013), while higher nutrient concentrations (N and P) were observed in the selected lakes of Myanmar (**I**, **II**, **III**, **IV**).

## 4.2 Phytoplankton and Cyanobacteria in Myanmar Lakes

Fourteen phytoplankton groups were identified in Inlay Lake (**IV**) while 13 groups were recorded in Meiktila Lake (**I**) and 10 groups in Yezin Dam (**II**). Bacillariophyceae, Cryptophyceae, Chlorophyceae, Euglenophyceae and Cyanobacteria were common phytoplankton groups in these water bodies (**I**, **II**, **IV**).

A significant relation between phytoplankton and water quality was observed in Inlay Lake (**IV**). Inlay Lake was characterized by a diverse phytoplankton composition while the phytoplankton biomass was generally very low, with average biomasses of less than 1 mg L$^{-1}$ in all areas. The exceptions were some localities near to the shores and floating gardens where higher phytoplankton biomasses (more than 2 mg L$^{-1}$) of taxa like *Cryptomonas* spp., *Aphanocapsa* spp., *Merismopedia* spp. and *Monoraphidium spp.* were observed (**IV**). High abundance of *Cryptomonas sp*., *Aphanocapsa sp*., *Merismopedia sp*. and *Monoraphidium sp.* is described as typical for eutrophic waters (Komárek and Anagnostidis, 1986, Huber-Pestalozzi, 1983). The

floating gardens and shore areas at Inlay Lake were characterized by higher concentrations of nutrients (TP & TN) and organic matter resulting from use of fertilizers, the waste and wastewater from in-lake stilt houses, and inflowing rivers (**IV**, Khaung et al., 2021, Pradhan et al. 2015, Rosén, 1981).

Our RDA analysis showed that TOC and TN significantly explain variability in phytoplankton community composition found in November 2015 in Inlay Lake (**IV**). A similar positive correlation between phytoplankton and TN was observed in West Lake, a subtropical shallow lake in China (Zeng et al., 2017), while a negative correlation between TN and cyanobacteria was found in Tri An reservoir, Vietnam (Dao et al., 2016). In Baiyangdian Lake, a typical macrophyte-dominated lake in northern China and in 6 tropical lakes in Brazil, however, positive correlations between TP and cyanobacteria and phytoplankton were observed (Zhu et al., 2021; Vanderley et al., 2021). These correlations suggest that nutrients (TN or TP) are the main limiting factors of phytoplankton (including cyanobacteria) growth.

Compared to Inlay Lake the phytoplankton biomasses found in Meiktila Lake and Yezin Dam were considerably higher and ranged in Meiktila Lake from 1.24 mg $L^{-1}$ to 3.68 mg $L^{-1}$ (**I**), while the highest phytoplankton biomasses of up to 35.79 mg $L^{-1}$ were recorded in Yezin Dam (**II**).

In Inlay Lake, cyanobacteria were present only in smaller numbers, most likely due to the high abundance of aquatic macrophytes (**IV**). Although a cyanobacteria-phytoplankton regression could not be performed due to overall low biomass values and a high datapoint scatter, our observations suggest that cyanobacteria biomass in Inlay Lake may be directly related to total phytoplankton biomass, as has been described for subtropical (Canfield et al., 1989) and temperate lakes (Downing et al., 2001).

In Meiktila Lake and Yezin Dam cyanobacteria were the most dominant taxa (**I, II**), indicating eutrophic conditions (Ke et al., 2009, Zhang and Zang, 2015). In Meiktila Lake, *R. raciborskii* was the most dominant cyanobacterial species and accounted for 27 % to 91 % (0.2 – 1.9 mg $L^{-1}$) of the total cyanobacterial biomass (**I**), while in Yezin Dam the amount of *R. raciborskii* was much lower with biomasses between 0.0002 and 0.2 mg $L^{-1}$. This species was not observed in Inlay Lake (**II, IV**). *R. raciborskii* has not been described from Myanmar water bodies before but has been described from various other subtropical and tropical Southeast Asian freshwater habitats and

other temperate water bodies worldwide (Nguyen et al. 2017, Antunes et al. 2015, Dao et al. 2010, Li et al. 2001).

Another cyanobacterial species *Dolichospermum smithii* (Komárek) Wacklin, L. Hoffmann & Komárek was the dominating taxon in Yezin Dam, with the highest biomass of 19.18 mg $L^{-1}$ (**II**). This species was not observed in Meiktila Lake and Inlay Lake. *D. smithii* is reported of mesotrophic to slightly eutrophic ponds, reservoirs, and lakes in temperate areas but also from tropical Brazil and Senegal (Komárek, 2013, Berger et al. 2005, Sant'Anna, 1991). In Meiktila Lake and Yezin Dam, *Microcystis* was commonly found together with *R. raciborskii* which contrasts with Vanderley (2021) who stated that *R. raciborskii* and *M. aeruginosa* rarely co-occurred in eutrophic lakes in Brazil. Low amounts of *Microcystis* were also found in Inlay Lake and the other studied lakes and reservoirs in Myanmar (**I**, **II**, **IV**). *Microcystis* has been reported from only a few water bodies in Myanmar before but is described from many Asian countries (Naw et al., 2020, Harke et al., 2016, Mowe 2015, Green 2011). Other common cyanobacteria species present in the studied water bodies were *Oscillatoria*, *Limnothrix*, *Aphanocapsa*, *Aphanothece*, and *Merismopedia* (**I**, **II**, **IV**).

The dominant phytoplankton showed a spatial and temporal distribution in the investigated lakes throughout the study period. Patchiness on small and bigger scales has been described for phytoplankton distribution in lake and ocean environments, especially for species that have well developed buoyancy-regulating mechanisms like cyanobacteria or dinoflagellates (Breier et al., 2018, Borics et al., 2011). It is therefore difficult to relate the nutrient values to phytoplankton biomass because of the variable distribution.

## 4.3 Phylogenetic studies on cyanobacteria

Seven potentially toxin-producing cyanobacteria strains from Meiktila Lake (**I**) and 15 strains from Yezin Dam (**II**) were isolated and identified based on morphology and molecular phylogeny using 16S rRNA gene sequences. Based on morphological features, eight of the isolated strains from Yezin Dam were classified as *D. smithii (***II**) while five strains from Meiktila Lake and five strains from Yezin Dam were identified as *R. raciborskii* (**I, II**). Two strains from Meiktila Lake were identified as *M. aeruginosa* and *M. novacekii* (Komárek - Compere) (**I**) and two strains isolated from Yezin Dam were identified as *M. aeruginosa* (**II**), respectively. As genetic methods

do not support the morphologically based assignment of *Microcystis* spp., Harke et al. (2016) suggested all *Microcystis* spp. warrant placement into the same species complex. In the following parts of the thesis, we use therefore "*Microcystis*" instead of species names.

Phylogenetic analyses using maximum likelihood (ML) trees of 16S rRNA gene confirmed the morphological determination of the isolated *Raphidiopsis*, *Microcystis* and *Dolichospermum* strains (**I, II**). All *Raphidiopsis* strains from Meiktila Lake and Yezin Dam were placed as a separate monophyletic clade based on 16S rRNA gene sequences (1135 bp) of *Cylindrospermopsis* and *Raphidiopsis* strains from Asia, Europe, Australia, and North America (**I, II**). All *Microcystis* strains from Myanmar clustered together with 16S rRNA gene sequences (1426 bp) of *Microcystis* from Europe, Asia, and Africa. The 16S rRNA gene similarity between *Microcystis* strains from Meiktila Lake and Yezin Dam was ≤ 99.65%.

All isolated *Dolichospermum* strains from Yezin Dam were grouped in the same subcluster in the 16S rRNA gene tree and were assigned to *D. smithii* (**II**). The phylogenic analysis confirms the assignment to *D. smithii.* However, the strains from Yezin Dam are clustered together with several other *Dolichospermum* species, including *D. smithii*, *D. viguieri*, *D. mucosa*, *D. spiroides*, *D. circinale* and *D. ucrainicum*. It is unclear if correct species names are assigned to the *Dolichospermum* 16S rRNA gene sequences in GenBank. This shows the difficulty to assign *Dolichospermum* sp. from Yezin Dam to a particular species based on publicly available genetic data only. The reliable use of phylogenetic data from public databases (e.g., NCBI, ENA) depends strongly on their unambiguity.

## 4.4 Cyanotoxins

During the study period, *Dolichospermum smithii* was the dominant taxon in the phytoplankton communities of Yezin Dam. However, none of the *Dolichospermum* strains isolated from Yezin Dam appeared to produce any toxins (**II**). Likewise, no strains of *Dolichospermum smithii* from other locations worldwide have been shown to produce cyanobacterial toxins such as MCs, STXs, ATXs or CYNs.

ELISA confirmed that three of four *Raphidiopsis* strains from Meiktila Lake (**I**) and four of five *Raphidiopsis* strains from Yezin Dam (**II**) produce variable amounts of CYNs. None of the strains,

however, produced STXs, ATXs or MCs. Based on ELISA analyses the concentrations of CYNs varied from 1.84 to 4.3 µg $mg^{-1}$ fresh weight (FW) in the *Raphidiopsis* strains from Meiktila Lake (**I**) and from 0.89 to 3.5 µg $mg^{-1}$ FW in the *Raphidiopsis* strains from Yezin Dam (**II**). By using LC-MS/MS, it was shown that the CYN producing strains produced the two variants: CYN and deoxy-CYN. The *Raphidiopsis* strains from Meiktila Lake produced CYN and deoxy-CYN concentrations between 1.7 and 2.5 µg $mg^{-1}$ FW and 1.3 – 7.3 µg $mg^{-1}$ FW, respectively (**I**), while the strains from Yezin Dam produced between 0.17 and 1.03 µg $mg^{-1}$ FW and 0.11 – 2.19 µg $mg^{-1}$ FW (Table 4) (**II**). A recent study reported that *Raphidiopsis* strains from Australia produce CYN in similar amounts (up to 3.5 µg $mg^{-1}$ FW). These, however, comprised only intracellular CYNs (Saker and Griffiths, 2000).

Table 4. Cylindrospermopsin (CYN) and deoxycylindrospermopsin (deoxyCYN) concentrations in cultured *Raphidiopsis raciborskii* strains isolated from Meiktila Lake (**I**) and Yezin Dam (**II**)

| Lake/ Reservoir | Strain | ELISA | LC-MS/MS | | | |
|---|---|---|---|---|---|---|
| | | µg CYN/mg FW | µg CYN/mg FW | µg deoxyCYN /mg FW | CYNs %[#] | deoxyCYNs % |
| **Meiktila** | AB2017/09 | 2.18 | -* | -* | 38 | 62 |
| | AB2017/05 | n.d. | n.d. | n.d. | n.d. | n.d. |
| | AB2017/16 | 1.84 | 1.65 | 1.25 | 57 | 43 |
| | AB2017/12 | n.d. | n.d. | n.d. | n.d. | n.d. |
| | AB2017/13 | 4.31 | 2.46 | 7.29 | 25 | 75 |
| **Yezin** | AB2017/03 | 0.89 | 0.17 | 0.11 | 61 | 39 |
| | AB2017/04 | 3.50 | 0.2 | 0.11 | 65 | 35 |
| | AB2017/06 | - | - | n.d | n.d. | n.d. |
| | AB2017/19 | 3.18 | 0.75 | 1.84 | 29 | 71 |
| | AB2017/20 | 1.86 | 1.03 | 2.19 | 32 | 68 |

*=biomass not determined, n.d. = not detected, FW = fresh weight

[#]=Percentages are % of total CYNs measured by LC-MS/MS.

The production of CYNs by *R. raciborskii* has previously only been reported from lakes in China, Japan, Thailand, Vietnam, and Australia (Nguyen et al., 2017, Chonudomkul et al., 2004, Saker and Neilan, 2001, Hawkins et al., 1997). In Brazil, *Raphidiopsis* strains are known to produce only saxitoxins (Hoff-Risseti et al., 2013). *R. raciborskii* is also widely distributed in Europe but not confirmed as a CYN or STX producer. Only one study by Đorđević et al. (2015) has reported the finding of CYN (in Serbian Lake Aleksandrovac) and related it to the presence of *R. raciborskii*, but it was not confirmed by genetic and chemical investigations of isolated strains.

ELISA confirmed that two *Microcystis* strains from Meiktila Lake and one *Microcystis* strain from Yezin Dam are MC producers (**I**, **II**). None of the *Microcystis* strains from Meiktila Lake and Yezin Dam produced CYNs, STXs, or ATXs. The MC concentrations of 1100 µg $g^{-1}$ FW from one *Microcystis* strain from Meiktila Lake (**I**) and 1160 µg $g^{-1}$ FW from Yezin Dam (**II**) are in the same range as described for a *Microcystis* strain from South African Hartbeespoort Dam (Ballot et al., 2014). Higher MC concentration of 14000 µg $g^{-1}$ FW was detected in the other Microcystis strain isolated from Meiktila Lake (**I**). Vézie et al. (2002) also reported higher MC concentrations of up to 5.8 µg $mg^{-1}$ dry weight in *Microcystis* strain (GL060916) from Lake Grand-Lieu, France. The production of MCs was also confirmed by ELISA in four *Microcystis* cultures isolated from Inlay Lake (data not shown), but none of these produced CYNs, STXs and ATXs (**IV**).

According to LC-MS/MS (LC-HRMS/MS) analysis on the MC producing strains, altogether 56 MC congeners including 22 previously undescribed congeners were detected in the *Microcystis* strains from Meiktila Lake (**I**) and 22 MC variants including 6 previously unreported congeners were detected in the *Microcystis* strains from Yezin Dam (**II**). The toxicity of MCs produced by the isolated strains ranges from highly toxic variants like MC-LR or MC-LA to lesser toxic variants such as MC-RR (Rinehart et al., 1994). The MC profile of the strain (AB2017/08) from Yezin Dam differs considerably from the strains isolated from Meiktila Lake (**I**) and Hartbeespoort Dam (Ballot et al., 2014). Although 22 MC congeners were produced by the strain (AB2017/08) of Yezin Dam, MC-LR and [D-Asp3] MC-LR were the dominant MC congeners, comprising 76.9 % and 15.7 % of the total MCs detected (**II**). *Microcystis* strain (AB2017/14) from Meiktila Lake with 52 MC congeners produced a considerably higher number of MCs, but MC-LR and [D-Asp3] MC-LR together made up only 20 % of the total MC concentrations (**I**). As the toxicity of several of the

MC variants found in our study has not yet been investigated, a full risk assessment for these variants is difficult. Accordingly, future research should focus on the toxicity of these new variants.

MCs are the most widely distributed hepatotoxic cyanotoxins and have been reported from many Asian countries including China (Zhu et al., 2014), Korea (Oh et al., 2001, Park et al., 1998), Bangladesh (Welker et al., 2005), Malaysia (Sinang et al., 2015), Singapore (Porojan et al., 2020), India (Ghosh et al., 2008, Agrawal et al., 2006), the Philippines (Cuvin-Aralar et al., 2005), Thailand (Mahakhant et al., 1998, Somdee et al., 2013) and Vietnam (Trung et al., 2018). Prior to our study, however, no investigations of cyanotoxin production had been conducted in Myanmar. This study clearly demonstrates the presence of CYN- and deoxy-CYN producing *R. raciborskii* and MC-producing *Microcystis* in the phytoplankton communities of the investigated lakes and reservoirs in Myanmar.

The ELISA analyses performed for Yezin Dam water in February 2020 showed the presence of both MCs and CYNs in the dam water (**II**). The concentrations of MCs (0.34 µg $L^{-1}$) and CYNs (0.12 µg $L^{-1}$) were well below the provisional short-term drinking-water guideline value (GV) of 3 µg $L^{-1}$ for CYNs and 12 µg $L^{-1}$ for MCs and did not exceed the provisional World Health Organization (WHO) guideline values of 1 µg $L^{-1}$ for MC-LR or 0.7 µg $L^{-1}$ CYN (WHO, 2020a, WHO, 2020b) in drinking water. Higher CYNs concentrations (up to 8.25 µg $L^{-1}$) confirmed by ELISA were reported from 25 urban reservoirs of China (Lei et al., 2014). The low concentrations of MCs and CYNs detected in Yezin Dam can be attributed to the low biomass of *Microcystis* and *R. raciborskii* as well as to the co-existence of non-toxin-producing and toxin-producing *Microcystis* and *Raphidiopsis* strains in the Dam. The coexistence of MC-producing and non-producing *Microcystis* strains has also been reported from Hartbeespoort Dam in South African (Ballot et al., 2014). The biomass of *R. raciborskii* observed in Yezin Dam was also considerably lower than that observed in Meiktila Lake at the time of investigation. However, total biomass and relative abundance of different cyanobacterial taxa in Meiktila Lake and Yezin Dam are expected to vary over time. A future shift from the dominance of non-toxin producing *Dolichospermum*, *Raphidiopsis* or *Microcystis* strains to toxin-producing strains cannot be excluded. A further

increase in biomass of toxin producing *Microcystis* or *Raphidiopsis* could lead to increased concentrations of CYNs and MCs in the Lake and Dam water.

## 4.5 Aquatic macrophytes

The investigated lakes in Myanmar varied considerably in the occurrence and abundance of aquatic macrophytes. The highest abundance of macrophytes was observed in Inlay Lake (**IV**) and the species richness was high when compared to other tropical lakes (Ondiba et al., 2018b, Saluja and Garg, 2017a, Dong et al., 2014, Dalu et al., 2012, Lacoul and Freedman, 2006) and even higher than in other lakes in Myanmar (**I**, **II**, **III, IV**). Twenty-eight aquatic species comprising 16 submerged (including charophytes), 7 floating-leaved and 5 free-floating species were identified in Inlay Lake during our survey period (**IV**). The maximum depth of Inlay Lake is 3.2 m and the water is very clear. This allows growth of aquatic macrophytes in all parts of the lake. The high macrophyte diversity in Inlay Lake is mainly due to submerged species, which comprise 53% of the total diversity (**IV**, Yuasa et al., 2019). The highest richness of macrophytes was recorded close to the shores, while the middle of the lake had the lowest richness. The submerged vegetation, dominated by *Nechamandra alternifolia* and *Potamogeton lucens*, and to a lesser degree, *Ceratophyllum demersum*, *Myriophyllum verticillatum*, *Najas indica* and *Chara zeylanica*, was observed throughout the year in extensive stands in most parts of the lake. The free-floating species, dominated by *Eichhornia crassipes*, formed small- to medium-sized moving islands (**IV**).

The significant association of total macrophyte and submerged species abundance with spatial factors (lake areas and depth) indicate the lower abundance of submerged species in the middle and deepest part of the Lake (IV), which also refer to a maximum growing depth of submerged macrophytes at approximately 3 meters in Inlay Lake. Many studies have investigated how depth affected the growth of submerged macrophytes with the optimal growth depth range of 0.6 m to 1.2 m for *Potamogeton crispus* in Lake Taihu (Zhou et al., 2016), 2.5 - 4.5 m and 1 - 2 m or 5 - 6 m for *P. maackianus* and *Ceratophyllum demersum* in the polydominant communities in Lake Erhai (Ye et al., 2018) and 1.25 m for *P. crispus* and *Hydrilla verticillata* in Lake Gehu (Wu et al., 2021).

Besides the depth, the spatial factors most likely reflect differences in functional traits and habitat preferences, for example, *Nechamandra alternifolia* seems to prefer the northern area while *Chara zeylanica* had highest abundance in the southern part of Inlay Lake. Total abundance of floating-leaved and free-floating species (nympheids and lemnids) was lower than total submerged vegetation and remained similar across lake areas and sampling periods. Luxurious growth of floating leaved vegetation and submerged vegetation dominated by *Chara* was also found in Wethtigan Lake (**III**). Stable water levels, permanent shallow areas, gentle slopes and increased water Secchi disc transparency, combined with radiation and nutrient availability, influence the distribution and abundance of macrophytes (Barbosa et al., 2014, Silva et al.,2014, Pierini & Thomaz, 2009, Bini & Thomaz 2005, Bini et al., 1999). Mäemets and Freiberg (2007) have confirmed the positive correlation between the depth limit of submerged macrophytes and water transparency in Estonian water bodies.

Compared to Inlay Lake, Meiktila Lake was characterized by a lower abundance of aquatic macrophytes. We recorded twelve hydrophyte species in Meiktila Lake (**I**) which is similar to a recent study (Hlaing, 2014). Most notable were the extensive stands of floating leaved *Nelumbo nucifera* and a thick belt of *Potamogeton cf. nodosus* in the northern part of Meiktila Lake (**I**). The waters north of the *Potamogeton* belt were much clearer than the turbid waters south of the *Potamogeton* belt confirming its filtering function (**I**). The southern part was dominated by *Vallisnera australis* and the charophyte *Chara zeylanica* (**I, III**). The lowest macrophyte coverage was observed in Yezin Dam while both Ngalaik and Kyet Mauk Taung Reservoirs had a moderate coverage of a few submerged species including charophytes (**II**, **III**).

Since *Chara* spp. were found in the investigated lakes in this study, we conducted a more profound study on this genus in the selected lakes. It is obvious that *C. zeylanica* is the most common *Chara* species in Inlay Lake, Meiktila Lake (South), Ngalaik Reservoir, Kyet Mauk Taung Reservoir and Wethtigan Lake (**III**). The other *Chara* species *C. fibrosa* was found in Yezin Dam, Kyet Mauk Taung Reservoir and Wethtigan Lake (**III**). Both species were confirmed by morphological identification and phylogeny-based identification using DNA barcoding (**III**). Studies on charophytes are very scarce in Myanmar and our study presents the first study of *Chara* species distribution in lakes and reservoirs in Myanmar. The only other comprehensive study about charophytes was published in 1932 (Pal, 1932), in which 24 charophyte species,

including 12 *Chara* species from paddy-fields, ponds, drains and marshy areas were reported. Several reasons can explain why we have recognized so few species compared to the survey in the 1930s. Different survey seasons, increased impacts on habitats, and taxonomic changes since the 1930s could be responsible for the discrepancy. Our study also focused on lakes and reservoirs only – habitats which were not included in the survey by Pal (1932). Nevertheless, we believe that increased human impact on freshwater habitats is affecting *Chara* biodiversity in Myanmar, as it is elsewhere worldwide (Dodds et al., 2013, Søndergaard and Jeppesen, 2007).

According to our observations, *C. zeylanica* seems to prefer calcareous lakes. All investigated lakes in our study, except Yezin Dam, were characterized by calcium (Ca) concentrations higher than 19 mg $L^{-1}$. This observation is consistent with Vaidya (1967) who also found *C. zeylanica* primarily in lakes with Ca concentrations of >19 mg$L^{-1}$. *C. fibrosa* appears in both highly and moderately alkaline lakes, but we found larger specimens and more vigorous stands in lakes characterized by a higher alkalinity (Ca > 40 mg $L^{-1}$) (Inlay Lake, Wethtigan Lake, Kyet Mauk Taung Reservoir) **(III**). This agrees with Asaeda et al. (2014) who mentioned 40 – 80 mg Ca $L^{-1}$ as the optimum range for this species, which is also in accordance with observations by Vaidya (1967).

Paper **III** demonstrated that *Chara* species in Myanmar were mostly recorded in lower impacted lakes with TP concentrations below 20 µg $L^{-1}$. The exception was Yezin Dam where higher TP values up to 57 µg $L^{-1}$ were found in different sampling periods (**II**). The EU Water Framework Directive (WFD, 2000) has recognized charophytes as sensitive species. Due to their sensitivity to eutrophication, high abundance in a lake is considered indicative of good trophic conditions and high ecological status (Penning et al., 2008b). High variation of TP values in Yezin Dam (**II, III**), in addition to water level fluctuations (Ellawala et al., 2011), could be the reason for the presence of smaller and very low abundance *C. fibrosa* specimens in the Dam.

## 4.6 Correlation between phytoplankton and aquatic macrophytes

Inlay Lake has been facing natural and manmade threats which have led to decreased water holding capacity, reduced water quality, hindered navigability, and reduced value to livestock (Pradhan et al., 2015, Than, 2007, Su and Jassby, 2000). The phytoplankton community composition of the lake indicates eutrophic conditions (**IV**), however, we have observed very low

phytoplankton biomasses (< 1 mg FW $L^{-1}$) which are most likely indicating a competition for nutrients with aquatic macrophytes. We hypothesize that submerged macrophytes control phytoplankton biomass and composition and hence stabilize a clear water state in Inlay Lake, which is supported by Yuasa et al. (2019). However, climatic conditions can vary considerably in tropical areas between rainy and dry periods, and can affect physical, chemical, and biological properties and the stability of a lake (Sahoo et al., 2016). Inlay Lake is a large, shallow lake and the composition of the macrophyte and phytoplankton communities varies considerably, both from north to south and from the littoral zone towards the center (**IV**). Total phytoplankton biomass was negatively correlated with total macrophyte abundance in the northern lake area ($R = - 0.77$, $p = 0.03$), but positively correlated ($R = + 0.77$, $p = 0.02$) in the central area in November (**IV**). There were no significant correlations ($p > 0.05$) across sampling points in February. A negative effect of submerged macrophytes (elodeids and charophytes) on the total phytoplankton biomass was also observed in the heavily vegetated northern part of the lake at both sampling dates ($R= - 0.83$, $p = 0.01$ in November; $R = 0.84$, $p = 0.02$ in February/March). The dominant submerged macrophyte species may be capable of removing and storing large quantities of nutrients from the water by foliar and root uptake, as has been observed for temperate lakes. Several studies demonstrate how macrophytes affect the nutrient concentrations in the water and suppress phytoplankton biomasses (e.g. Hilt and Lombardo, 2010; Scheffer et al., 1993).

Floating-leaved and free-floating macrophytes (nympheids and lemnids) had a positive relationship ($R = + 0.77$, $p = 0.03$) with total phytoplankton biomass in the central lake area in November and in southern part in February/March ($R = + 0.84$, $p = 0.02$) (**IV**). The negative correlations ($R = - 0.86$, $p = 0.014$; $R = - 0.88$, $p = 0.009$) between cyanobacteria biomass and macrophytes, both submerged and total macrophytes, were pronounced in the northern part of the lake in February, as found in subtropical and temperate lakes (Scheffer 1998, Downing et al. 2001).

In tropical and subtropical aquatic systems, growth of floating macrophytes is high and may compete with phytoplankton for light, nutrients (nitrate, phosphate and dissolved inorganic carbon) and habitat similarly or even more than the submerged plants (Meerhoff et al., 2003,

Van Donk and Van de Bund, 2002). However, we did not find a significant correlation between cyanobacteria and floating-leaved macrophytes in any part of the lake in either season. Cunha et al. (2012) showed a negative influence of free-floating and emerged species on phytoplankton in a subtropical reservoir in Brazil. A possible explanation for this discrepancy may be that, because of the low biomass of submerged macrophytes in the deeper central areas, there are enough nutrients for all macrophyte and phytoplankton groups in these areas of Inlay Lake. The very low phytoplankton biomass in the southern part of the lake and the low abundance of aquatic macrophytes is most likely related to high turbidity due to eroded material transported by the Belui River, and high boat traffic.

Several studies have shown that macrophytes affect nutrient concentrations in the water and then suppress phytoplankton growth (Hilt and Lombardo, 2010, Scheffer et al., 1993a). According to Van Donk and Van de Bund (2002) and Jasser (1995), macrophytes reduce the abundance and biomass of the phytoplankton community by modifying the phytoplankton compositions through decreasing cyanobacteria density and increasing the species diversity. Similar patterns were also observed in shallow sub-tropical lakes in Australia, China and Japan (Zeng et al., 2017, Akhurst et al., 2017, Guo-feng et al., 2000). In Maojiabu Lake in China, macrophyte restoration was used to decrease phytoplankton density and biomass and shift to phytoplankton community structures without or with a smaller number of cyanobacteria species (Zeng et al., 2017).

However, we also suspect that other factors in addition to competitive nutrient uptake are jointly responsible for very low phytoplankton biomass in Inlay Lake. For example, allelopathic compounds released from *Myriophyllum verticillatum, Ceratophyllum demersum* and *Chara zeylanica* (Hilt et al., 2006, Gross et al., 2003) and algae toxic compounds from other species like *Eichhornia crassipes* (Gross, 2003, Sharma et al., 1996) may inhibit the growth of phytoplankton. The negative correlation of cyanobacteria and submerged macrophyte in the northern part of the lake might be due to the year-round dominance of *Nechamandra alternifolia* in that area. The leaves of this species possess large secretory cells (Cook and Lüönd, 1982) and secretions from these may have an allelopathic effect on phytoplankton. However, this hypothesis is not yet confirmed. In the central lake area, the relatively low macrophyte abundance in relation to water volume might prevent the macrophytes from applying control on phytoplankton growth.

The relatively high abundance of cyanobacteria found in the central lake area supports the latter hypothesis, as cyanobacteria are typically more susceptible to macrophyte allelopathy than other phytoplankton groups (Mohamed, 2017, Švanys et al., 2016, Lombardo et al., 2013, Chang et al., 2012, Gross, 2003, Jasser, 1995). This could be because the allelochemicals inhibit the photosystem II in cyanobacteria due to the oxidative stress and damaging the electron transport chain during photosynthesis (Gao et al., 2017, Zhu et al., 2010, Leu et al., 2002). It has been shown that certain macrophyte species (such as *Myriophyllum spicatum*, *Ceratophyllum demersum*, *Potamogeton malaianus*, *P. crispus, Stratiotes aloides*, *Elodea nuttallii*, *Hydrilla verticillata*, *Vallisneria spiralis*, *Najas minor*) exhibit allelopathic activity against certain phytoplankton species and inhibit their growth (such as *Microcystis aeruginosa*, *Pseudokirchneriella subcapitata*, *Anabaena flos-aquae*, *Phormidium tenue*, *Limnothrix redekei*, *Stephanodiscus minutulus*, *Scenedesmus obliquus*, *Chlorella* spp.) (Dong et al., 2018, Liu et al., 2018, Zhou et al., 2017). A similar pattern was observed in Meiktila Lake. The northern part of the lake is divided by a *Potamogeton* belt. The differences in Secchi depth and *Raphidiopsis* biomass on the two sides of the *Potamogeton* belt in the northern part of the Meiktila Lake could, therefore, be attributed to allelopathic effects of the macrophytes (**I**) since water quality and other environmental factors are similar in both areas. Similar differences in Secchi depth values were also recorded by Hlaing (2014) in the same locations in Meiktila Lake.

In Yezin Dam only a few aquatic macrophytes e.g. *Hydrilla verticillata* and *Chara fibrosa* were found with low abundance during this study. This can explain the considerably higher phytoplankton (cyanobacterial) biomasses in Yezin Dam compared to Inlay and Meiktila Lakes (**I**, **II**, **IV**). Since the 1990s, aquatic macrophytes in Yezin Dam have been removed to improve function and increase aesthetics (personal communication with local people). Water level regulations in Yezin Dam also impact the growth of macrophytes. The removal of macrophytes can lead to changes in phytoplankton composition and biomass, favoring the occurrence of potentially toxic cyanobacteria (Wojciechowski et al., 2018, Peretyatko et al., 2007). In Bolivian Lake Laguna it has been shown that the chlorophyll concentration has increased following the artificial removal of macrophytes (Ayala et al., 2007).

Based on the study in Inlay Lake (**IV**), it appears that submerged macrophytes are important for maintaining a clear water state in tropical lakes like Inlay Lake, similar to their effects in temperate lakes (e.g., Scheffer et al. 1993b, Jeppesen et al. 1998, Hilt and Lombardo 2010, Mjelde & Faafeng 1997). Owing to differing phenology of each macrophyte species, Inlay Lake maintains robust macrophyte communities, especially of submerged species, throughout the year, and this contributes to the resilience and stability of the lake ecosystem. The extensive macrophyte abundance can store substantial amounts of nutrients, which will not be available for phytoplankton or periphyton growth. The shallowness of the lake also influences the clear water state, as the submerged vegetation covers almost the whole lake bottom and reduces turbidity.

A knowledge gap of the Inlay Lake ecosystem still exists because there is no information about zooplankton and fish communities and their grazing capacity in the lake. Several studies have shown that phytoplankton is controlled by zooplankton grazing (Williams, 1999, Stephen et al, 1998, Moss et al., 1997, Meijer et al., 1994, Knisely and Geller, 1986). Phytoplankton abundances increase in the presence of higher densities of zoo-planktivorous fish due to reduced grazing of zooplankton (Williams and Moss, 2003). Pomati et al., (2020) has shown that increasing water temperature or total grazing pressure and decreasing phosphorus levels have a positive effect on large phytoplankton groups. Fish, invertebrates, zooplankton and periphyton algae certainly play a role in the stabilization of the Inlay Lake ecosystem. The fish community of Inlay Lake includes both omnivorous and piscivorous fish species (Allen et al. 2012). The omnivorous fish may place a high predation pressure on both zooplankton and submerged macrophytes (Yu et al. 2016). To confirm the influence of zooplankton and fish on phytoplankton abundance and composition, further studies are needed.

At present, Inlay Lake is a clear-water macrophyte-dominated lake. It is stabilized by the extensive growth of submerged macrophytes (**IV**) of which *Chara* is is one of the dominant taxa, especially in the northern part of the lake (**III**). The presence of Charophytes in the macrophyte communities may help to buffer against the impact of nutrient inputs. Despite a high degree of human impact from anthropogenic activities such as urbanization, chemical fertilizers and pesticides, and poor sanitation, Inlay Lake maintains a low nutrient status (particularly nitrogen

and phosphorus). However, lack of comprehensive management plans focussing on decreasing nutrient loads threatens the current state of Inlay Lake. Changes that may increase nutrient inputs including decreased forest cover, extensive development of hotels in watershed areas, and harvesting of macrophytes for floating garden development need to be addressed.

Increasing knowledge about the lake ecosystem is highly needed to critically evaluate the consequences of anthropogenic pressures such as nutrient loading due to increased population, agricultural areas, tourism and extension of hotel areas and sedimentation loads due to forest degradation and deforestation. Additionally, continuation of active management and monitoring of the Inlay Lake is highly recommended.

# 5 Conclusions and future perspectives

This study increases the knowledge about the composition and abundance of phytoplankton and aquatic macrophytes in the freshwater systems of Myanmar that have previously not been well investigated. It also focuses on cyanobacteria and their toxins, of selected freshwater systems, and interactions between aquatic macrophytes and phytoplankton (cyanobacteria).

The study confirms the presence of toxin producing cyanobacteria in selected freshwater bodies (both natural & man-made) in Myanmar (**I**, **II**, **IV**). The common toxins in freshwater systems in Myanmar are MCs and CYNs. The high biomass of phytoplankton and the dominance of cyanobacteria with two toxin-producing taxa (*R. raciborskii* and *Microcystis*) provide evidence for a degraded state of these water bodies. Lakes and reservoirs are an important source for drinking water and irrigation in Myanmar, therefore continuous monitoring of cyanobacterial blooms and their toxins should be considered as a main activity in the integrated water resources management plan of the country (environmental and health risk assessment plan of the water bodies). Additionally, potentially harmful effects of using the water without pretreatment by humans and animals should also be considered.

This study fills knowledge gaps on the current distributions of *Chara* species in lakes and reservoirs in Myanmar (**III**), a subject that has not been addressed since the 1930s. *Chara* species are globally recognized as sensitive species to eutrophication.

In paper **IV**, we discuss the importance of aquatic macrophytes as a stabilizing factor in a large and shallow eutrophic lake at medium altitude in the tropics. This study is the first detailed whole-lake study of the interactions between phytoplankton and aquatic macrophytes and their role in shaping the Lake ecosystem in Myanmar.

Maintaining or promoting aquatic macrophyte communities can be an additional and sustainable way for mitigating water quality degradation. Our study shows that submerged macrophytes are the most important group for maintaining a clear water state in a tropical shallow Lake (Inlay Lake) in Myanmar (**IV**). We hope that this study contributes to the understanding of the importance of aquatic macrophytes as a stabilizing factor in lakes in tropical areas. With this

knowledge, we believe biological management using aquatic macrophyte can be an effective measure to control cyanobacterial blooms.

We identified two states of freshwater systems in Myanmar: 1) eutrophic water bodies (having high cyanobacteria biomass) with limited aquatic macrophyte growth and 2) clear water systems with high phytoplankton diversity and low phytoplankton biomass (especially cyanobacteria biomass) and dominated by aquatic macrophytes. However, natural and anthropogenic influences may change the second state (i.e. clear water systems with low phytoplankton biomass and dominated by aquatic macrophytes) to a more eutrophic system. The increased practice of harvesting or physical removal of aquatic macrophytes in Myanmar's freshwater bodies (like Inlay Lake) may shift a clear water state with dense macrophyte vegetation to a turbid water state dominated by phytoplankton, especially cyanobacteria.

Allelopathy has been suggested by several authors to be responsible for observed phytoplankton patterns in vegetated, shallow lakes. To achieve a better understanding, detailed studies (experiments) on the allelopathic effect of specific aquatic macrophytes on phytoplankton communities should be carried out in Myanmar's freshwater systems.

The success of the Myanmar National Water Framework depends on knowledge about different ecosystems, with different biodiversity and stability drivers. The examination and maintenance of biodiversity and water quality in other lakes and reservoirs in Myanmar should be given more attention. Knowledge about aquatic macrophyte growth and understanding the mechanisms behind community shifts is of considerable importance for management and for the establishment of good ecological status for lakes and reservoirs. The results in my PhD study can be used as the basis for the ecological assessment of freshwater bodies by using phytoplankton and aquatic macrophytes as biological indicators.

Based on the findings from my PhD research, I give the following recommendations:

- Increased effort in ecology and management of aquatic macrophytes is critically needed for providing better outcomes for freshwaters (such as water for drinking, growing crops, manufacturing, energy, transport, etc.)

- More attention should be given to the examination and maintenance of biodiversity and water quality in other lakes and reservoirs in Myanmar
- An integrated water management plan should consider possible environmental and social impacts during the early phases of the planning process (including the wetland ecosystem, lake aquatic ecosystem, and livelihoods of people who reside in the watershed areas)
- Controlling urban runoff with treatment should be prioritized
- All untreated drains from residential areas should be diverted or treated
- Biological management should include control of cyanobacterial blooms
- Preventing excessive removal (especially in Inlay Lake) or planting of macrophytes (in Yezin Dam) should be considered as a measure to help control the growth of cyanobacteria and other phytoplankton

# 6 References

AGRAWAL, M. K., GHOSH, S. K., BAGCHI, D., WECKESSER, J., ERHARD, M. & BAGCHI, S. N. 2006. Occurrence of microcystin-containing toxic water blooms in Central India. *Journal of microbiology and biotechnology,* 16**,** 212-218.

AKAISHI, F., SATAKE, M., OTAKI, M. & TOMINAGA, N. 2006. Surface water quality and information about the environment surrounding Inle Lake in Myanmar. *Limnology,* 7**,** 57-62.

AKCAALAN, R., KÖKER, L., OĞUZ, A., SPOOF, L., MERILUOTO, J. & ALBAY, M. 2014. First report of cylindrospermopsin production by two cyanobacteria (*Dolichospermum mendotae* and *Chrysosporum ovalisporum*) in Lake Iznik, Turkey. *Toxins,* 6**,** 3173-3186.

AKHURST, D. J., JONES, G. B., CLARK, M. & REICHELT-BRUSHETT, A. 2017. Effects of fish and macrophytes on phytoplankton and zooplankton community structure in a subtropical freshwater reservoir. *Limnologica,* 62**,** 5-18.

ALBERTONI, E. F., PALMA-SILVA, C., TRINDADE, C. R. T. & FURLANETTO, L. M. 2014. Field evidence of the influence of aquatic macrophytes on water quality in a shallow eutrophic lake over a 13-year period. *Acta Limnologica Brasiliensia,* 26**,** 176-185.

ALI, S.S., IJAZ, N., AMAN, N., NASIR, A., ANJUM, L., & RANDHAWA, I.A., 2017. Clinical waste management practices in District Faisalabad. Earth Sciences Pakistan, 1(2), 4–6.

ALLEN, D.J., SMITH, K.G., DARWALL, W.R.T., 2012. The Status and Distribution of Freshwater Biodiversity in Indo-Burma. IUCN, Cambridge, UK and Gland, Switzerland x+158pp+4pp cover.

ANSARI, A. A., SINGH, G. S., LANZA, G. R. & RAST, W. eds. 2010. *Eutrophication: causes, consequences and control* (Vol. 1), Springer Science & Business Media.

ANTUNES, J. T., LEÃO, P. N. & VASCONCELOS, V. M. 2015. *Cylindrospermopsis raciborskii*: review of the distribution, phylogeography, and ecophysiology of a global invasive species. *Frontiers in Microbiology,* 6**,** 473.

ARÁOZ, R., NGHIÊM, H.-O., RIPPKA, R., PALIBRODA, N., DE MARSAC, N. T. & HERDMAN, M. 2005. Neurotoxins in axenic oscillatorian cyanobacteria: coexistence of anatoxin-a and homoanatoxin-a determined by ligand-binding assay and GC/MS. *Microbiology,* 151**,** 1263-1273.

ASAEDA, T., SENAVIRATHNA, M. D. H. J., KANEKO, Y. & RASHID, M. H. 2014. Effect of calcium and magnesium on the growth and calcite encrustation of *Chara fibrosa*. *Aquatic botany,* 113**,** 100-106.

AUNG, A. T. O., VAN HUYLENBROECK, G. & SPEELMAN, S. 2017. Differential impacts of an irrigation project: Case study of the Swar Dam Project in Yedashe, Bago region of Myanmar. *Journal of Development and Agricultural Economics,* 9**,** 178-189.

AYALA, R., ACOSTA, F., MOOIJ, W. M., REJAS, D. & VAN DAMME, P. A. 2007. Management of Laguna Alalay: a case study of lake restoration in Andean valleys in Bolivia. *Aquatic ecology,* 41**,** 621-630.

BAKER, P. D. & HUMPAGE, A. R. 1994. Toxicity Associated with Commonly Occurring Cyanobacteria in Surface Waters of the Murray-Darling Basin, Australia 3. *Australian Journal of Marine and Freshwater Research,* 45**,** 773-786.

BALLOT, A., CERASINO, L., HOSTYEVA, V. & CIRES, S. 2016. Variability in the sxt Gene Clusters of PSP Toxin Producing *Aphanizomenon gracile* Strains from Norway, Spain, Germany and North America. *Plos One,* 11.

BALLOT, A., FASTNER, J. & WIEDNER, C. 2010a. Paralytic shellfish poisoning toxin-producing cyanobacterium Aphanizomenon gracile in northeast Germany. *Applied and environmental microbiology,* 76**,** 1173-1180.

BALLOT, A., FASTNER, J., LENTZ, M. & WIEDNER, C. 2010b. First report of anatoxin-a-producing cyanobacterium Aphanizomenon issatschenkoi in northeastern Germany. *Toxicon,* 56**,** 964-971.

BALLOT, A., KRIENITZ, L., KOTUT, K., WIEGAND, C. & PFLUGMACHER, S. 2005. Cyanobacteria and cyanobacterial toxins in the alkaline crater lakes Sonachi and Simbi, Kenya. *Harmful algae,* 4**,** 139-150.

BALLOT, A., KRIENITZ, L., KOTUT, K., WIEGAND, C., METCALF, J. S., CODD, G. A. & PFLUGMACHER, S. 2004. Cyanobacteria and cyanobacterial toxins in three alkaline Rift Valley lakes of Kenya—Lakes Bogoria, Nakuru and Elmenteita. *Journal of Plankton Research,* 26**,** 925-935.

BALLOT, A., MJELDE, M. & SWE, T. 2017. Integrated Water Resources Management in Myanmar. Assessing ecological status in Inlay Lake. Preliminary report. *NIVA-rapport*. L.NR. 7301-2018

BALLOT, A., SANDVIK, M., RUNDBERGET, T., BOTHA, C. J. & MILES, C. O. 2014. Diversity of cyanobacteria and cyanotoxins in Hartbeespoort Dam, South Africa. *Marine and Freshwater Research,* 65.

BANKER, R., CARMELI, S., HADAS, O., TELTSCH, B., PORAT, R. & SUKENIK, A. 1997. Identification of Cylindrospermopsin in *Aphanizomenon ovalisporum* (Cyanophyceae) Isolated from Lake Kinneret, Israel 1. *Journal of Phycology,* 33**,** 613-616.

BANKER, R., TELTSCH, B., SUKENIK, A. & CARMELI, S. 2000. 7-Epicylindrospermopsin, a Toxic Minor Metabolite of the Cyanobacterium *Aphanizomenon ovalisporum* from Lake Kinneret, Israel. *Journal of natural products,* 63**,** 387-389.

BARCELOS E RAMOS, J., SCHULZ, K. G., VOSS, M., NARCISO, Á., MÜLLER, M. N., REIS, F. V., CACHÃO, M. & AZEVEDO, E. B. 2017. Nutrient-specific responses of a phytoplankton community: a case study of the North Atlantic Gyre, Azores. *Journal of Plankton Research,* 39**,** 744-761.

BEATTIE, K. A., KAYA, K., SANO, T. & CODD, G. A. 1998. Three dehydrobutyrine-containing microcystins from Nostoc. *Phytochemistry,* 47**,** 1289-1292.

BELL, S. & CODD, G. 1994. Cyanobacterial toxins and human health. *Reviews in Medical Microbiology,* 5**,** 256-264.

BERGER, C., COUTÉ, A., BA, N. G. & GUGGER, M. 2005. Cyanobacterial taxa of the senegal river system (northern Senegal, west africa). *Algological Studies,* 117**,** 147-176.

BERNARD, C., BALLOT, A., THOMAZEAU, S., MALOUFI, S., FUREY, A., MANKIEWICZ-BOCZEK, J., PAWLIK-SKOWROŃSKA, B., CAPELLI, C. & SALMASO, N. 2017. Appendix 2: Cyanobacteria associated with the production of cyanotoxins. *Handbook of cyanobacterial monitoring and cyanotoxin analysis***,** 501-525.

BERTONI, R. 2011. Limnology of rivers and lakes. *Institute of Ecosystem Study/Encyclopedia of life support systems/UNESCO-EOLSS*.

BHAT, N. A., WANGANEO, A. & RAINA, R. 2015. Variability in water quality and phytoplankton community during dry and wet periods in the Tropical Wetland, Bhopal, India. *Journal of Ecosystem & Ecography,* 5**,** 1.

BLÁHA, L., BABICA, P. & MARŠÁLEK, B. 2009. Toxins produced in cyanobacterial water blooms-toxicity and risks. *Interdisciplinary toxicology,* 2**,** 36-41.

BORGES, H., BRANCO, L., MARTINS, M., LIMA, C., BARBOSA, P., LIRA, G., BITTENCOURT-OLIVEIRA, M. & MOLICA, R. 2015. Cyanotoxin production and phylogeny of benthic cyanobacterial strains isolated from the northeast of Brazil. *Harmful Algae,* 43**,** 46-57.

BORICS, G., ABONYI, A., KRASZNAI, E., VÁRBÍRÓ, G., GRIGORSZKY, I., SZABÓ, S., DEÁK, C. & TÓTHMÉRÉSZ, B. 2011. Small-scale patchiness of the phytoplankton in a lentic oxbow. *Journal of Plankton Research,* 33**,** 973-981.

BOUAÏCHA, N., MILES, C. O., BEACH, D. G., LABIDI, Z., DJABRI, A., BENAYACHE, N. Y. & NGUYEN-QUANG, T. 2019. Structural diversity, characterization and toxicology of microcystins. *Toxins,* 11**,** 714.

BREIER, R.E., LALESCU, C.C., WAAS, D., WILCZEK, M., MAZZA, M.G., 2018. Emergence of phytoplankton patchiness at small scales in mild turbulence. Proc. Natl. Acad. Sci. U. S. A. 115, 12112–12117.

BRETTUM, P. & ANDERSEN, T. 2005. The use of phytoplankton as indicators of water quality. *Norwegian Institute for Water Research SNO Report***,** 33.

BRIENT, L., LENGRONNE, M., BORMANS, M. & FASTNER, J. 2009. First occurrence of cylindrospermopsin in freshwater in France. *Environmental Toxicology: An International Journal,* 24**,** 415-420.

BRIX, H. 1997. Do macrophytes play a role in constructed treatment wetlands? *Water science and technology,* 35**,** 11-17.

BRUNO, M., PLOUX, O., METCALF, J. S., MEJEAN, A., PAWLIK-SKOWROŃSKA, B. & FUREY, A. 2017. Anatoxin-a, homoanatoxin-a, and natural analogues. *Handbook of cyanobacterial monitoring and cyanotoxin analysis***,** 138-147.

BYTH, S. 1980. Palm Island mystery disease. *Medical Journal of Australia,* 2**,** 40-42.

CADEL-SIX, S., PEYRAUD-THOMAS, C., BRIENT, L., DE MARSAC, N. T., RIPPKA, R. & MEJEAN, A. 2007. Different genotypes of anatoxin-producing cyanobacteria coexist in the Tarn River, France. *Applied and environmental microbiology,* 73**,** 7605-7614.

CANFIELD JR., D.E., PHLIPS, E., DUARTE, C.M., 1989. Factors influencing the abundance of blue-green algae in Florida lakes. Can. J. Fish. Aquat. Sci. 46, 1232–1237.

CARMICHAEL, W. W. 1988. Naming of cyclic heptapeptide toxins of cyanobacteria. *Toxicon,* 26**,** 971-972.

CARMICHAEL, W. W. 1992. Cyanobacteria Secondary Metabolites - The Cyanotoxins. *Journal of Applied Bacteriology,* 72**,** 445-459.

CARMICHAEL, W. W. 1994. Toxins of cyanobacteria. *Scientific American,* 270**,** 64-70.

CARMICHAEL, W. W. 1997. The cyanotoxins. *Advances in botanical research,* 27**,** 211-256.

CARMICHAEL, W. W., AZEVEDO, S., AN, J. S., MOLICA, R., JOCHIMSEN, E. M., LAU, S., RINEHART, K. L., SHAW, G. R. & EAGLESHAM, G. K. 2001. Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. *Environmental health perspectives,* 109**,** 663-668.

CARMICHAEL, W. W., BIGGS, D. F. & PETERSON, M. A. 1979. Pharmacology of anatoxin-a, produced by the freshwater cyanophyte Anabaena flos-aquae NRC-44-1. *Toxicon,* 17**,** 229-236.

CARMICHAEL, W., ESCHEDOR, J., PATTERSON, G. & MOORE, R. 1988. Toxicity and partial structure of a hepatotoxic peptide produced by the cyanobacterium Nodularia spumigena Mertens emend. L575 from New Zealand. *Applied and Environmental Microbiology,* 54**,** 2257-2263.

CASERO, M. C., BALLOT, A., AGHA, R., QUESADA, A. & CIRÉS, S. 2014. Characterization of saxitoxin production and release and phylogeny of sxt genes in paralytic shellfish poisoning toxin-producing Aphanizomenon gracile. *Harmful algae,* 37**,** 28-37.

CASTENHOLZ, R. W., WILMOTTE, A., HERDMAN, M., RIPPKA, R., WATERBURY, J. B., ITEMAN, I. & HOFFMANN, L. 2001. Phylum BX. cyanobacteria. *Bergey's manual® of systematic bacteriology.* Springer.

CERASINO, L., MERILUOTO, J., BLÁHA, L., CARMELI, S., KALOUDIS, T. & MAZUR-MARZEC, H. 2017. SOP 6. Extraction of of Cyanobacterial Monitoring and Cyanotoxin Analysis. *in: Meriluoto, J., Spoof, L., Codd, G.A. (Eds.), Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis. Wiley***,** 350-353.

CHANG, X., EIGEMANN, F. & HILT, S. 2012. Do macrophytes support harmful cyanobacteria? Interactions with a green alga reverse the inhibiting effects of macrophyte allelochemicals on *Microcystis aeruginosa*. *Harmful Algae,* 19**,** 76-84.

CHONUDOMKUL, D., YONGMANITCHAI, W., THEERAGOOL, G., KAWACHI, M., KASAI, F., KAYA, K. & WATANABE, M. M. 2004. Morphology, genetic diversity, temperature tolerance and toxicity of *Cylindrospermopsis raciborskii* (Nostocales, Cyanobacteria) strains from Thailand and Japan. *FEMS Microbiology Ecology,* 48**,** 345-355.

CHORUS, I. & BARTRAM, J. 1999. *Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management*, CRC Press.

CHORUS, I. & BARTRAM, J. 2005. Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management.

CHORUS, I. & WELKER, M. 2021. *Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management*, Taylor & Francis.

CHORUS, I. 2020. Cyanobacterial toxins: Anatoxin-a: Background document for development of WHO Guidelines for Drinking-water Quality and Guidelines for Safe Recreational Water Environments. 2019. *World Health Organization. https://www. who. int/water_sanitation_health/waterquality/guidelines/chemicals/anatoxin-a-gdwq-bd-for-review-20191122. pdf Accessed on March,* 6.

CHORUS, I., Ed. 2012. *Cyanotoxins: occurrence, causes, consequences*, Springer Science & Business Media, Berlin, 357.

CHRISTOFFERSEN, K. 1996. Ecological implications of cyanobacterial toxins in aquatic food webs. *Phycologia* 35**,** 42-50.

CIRÉS, S., WÖRMER, L., BALLOT, A., AGHA, R., WIEDNER, C., VELÁZQUEZ, D., CASERO, M. C. & QUESADA, A. 2014. Phylogeography of cylindrospermopsin and paralytic shellfish toxin-producing Nostocales cyanobacteria from Mediterranean Europe (Spain). *Applied and Environmental Microbiology,* 80**,** 1359-1370.

CODD, G. A., BELL, S. G., KAYA, K., WARD, C. J., BEATTIE, K. A. & METCALF, J. S. 1999. Cyanobacterial toxins, exposure routes and human health. *European Journal of Phycology,* 34**,** 405-415.

CODD, G. A., MORRISON, L. F. & METCALF, J. S. 2005. Cyanobacterial toxins: risk management for health protection. *Toxicology and applied pharmacology,* 203**,** 264-272.

COHEN, Y., GUREVITZ, M. 2006. The Cyanobacteria—Ecology, Physiology and Molecular Genetics. *In: Dworkin M., Falkow S., Rosenberg E., Schleifer KH., Stackebrandt E. (eds) The Prokaryotes. Springer, New York, NY.*

COOK, C. D. & LÜÖND, R. 1982. A revision of the genus *Nechamandra* (Hydrocharitaceae). *Aquatic Botany,* 13**,** 505-513.

COOK, C. D. 1996. *Aquatic and wetland plants of India*, Oxford University Press. Oxford, 385 pp.

COOKE, G. D., WELCH, E. B., PETERSON, S. & NICHOLS, S. A. 2016. *Restoration and management of lakes and reservoirs*, CRC press.

COX, P. A., BANACK, S. A. & MURCH, S. J. 2003. Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. *Proceedings of the National Academy of Sciences,* 100**,** 13380-13383.

CUNHA, D. G. F., BOTTINO, F. & DO CARMO CALIJURI, M. 2012. Can free-floating and emerged macrophytes influence the density and diversity of phytoplankton in subtropical reservoirs? *Lake and reservoir management,* 28**,** 255-264.

CUSICK, K. D. & SAYLER, G. S. 2013. An overview on the marine neurotoxin, saxitoxin: genetics, molecular targets, methods of detection and ecological functions. *Marine drugs,* 11**,** 991-1018.

CUVIN-ARALAR, M. L. 2005.Microcystins from the cyanobacteria *Microcystis aeruginosa* in Laguna de Bay, Philippines. *In M. L. Cuvin-Aralar, R. S. Punongbayan, A. Santos-Borja, L. V. Castillo, E. V. Manalili, & M. M. Mendoza (Eds.), Proceedings of the First National*

*Congress on Philippine Lakes* (pp. 116-122). Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA).

DALU, T., CLEGG, B. & NHIWATIWA, T. 2012. Aquatic macrophytes in a tropical African reservoir: diversity, communities and the impact of reservoir-level fluctuations. *Transactions of the Royal Society of South Africa,* 67**,** 117-125.

DAO, T.S., CRONBERG, G., NIMPTSCH, J., DO-HONG, L.-C.,WIEGAND, C. 2010. Toxic cyanobacteria from Tri An Reservoir, Vietnam. Nowa Hedwigia, 90, 433–448.

DAO, T.-S., NIMPTSCH, J. & WIEGAND, C. 2016. Dynamics of cyanobacteria and cyanobacterial toxins and their correlation with environmental parameters in Tri An Reservoir, Vietnam. *Journal of water and health,* 14**,** 699-712.

DE FIGUEIREDO, D. R., AZEITEIRO, U. M., ESTEVES, S. M., GONÇALVES, F. J. & PEREIRA, M. J. 2004. Microcystin-producing blooms—a serious global public health issue. *Ecotoxicology and environmental safety,* 59**,** 151-163.

DEVLIN, J. P., EDWARDS, O. E., GORHAM, P. R., HUNTER, N. R., PIKE, R. K. & STAVRIC, B. 1977. Anatoxin-A, A Toxic Alkaloid from Anabaena flos-aquae Nrc-44H. *Canadian Journal of Chemistry-Revue Canadienne de Chimie,* 55**,** 1367-1371.

DIAS, E., PEREIRA, P. & FRANCA, S. 2002. Production of paralytic shellfish toxins by *Aphanizomenon* sp. LMECYA 31 (cyanobacteria). *Journal of Phycology,* 38**,** 705-712.

DODDS, W. K., PERKIN, J. S. & GERKEN, J. E. 2013. Human impact on freshwater ecosystem services: a global perspective. *Environmental science & technology,* 47**,** 9061-9068.

DONG, B., QIN, B., GAO, G. & CAI, X. 2014. Submerged macrophyte communities and the controlling factors in large, shallow Lake Taihu (China): sediment distribution and water depth. *Journal of Great Lakes Research,* 40**,** 646-655.

DONG, J., GAO, Y., CHANG, M., MA, H., HAN, K., TAO, X. & LI, Y. 2018. Colony formation by the green alga *Chlorella vulgaris* in response to the competitor Ceratophyllum demersum. *Hydrobiologia,* 805**,** 177-187.

DONG, J., YANG, K., LI, S., LI, G. & SONG, L. 2014. Submerged vegetation removal promotes shift of dominant phytoplankton functional groups in a eutrophic lake. *Journal of environmental Sciences,* 26**,** 1699-1707.

ĐORĐEVIĆ, N. B., SIMIĆ, S. B. & ĆIRIĆ, A. R. 2015. First identification of the cylindrospermopsin (CYN) producting by cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju in Serbia. *Fresenius Environ. Bull,* 24**,** 3736-3742.

DOU, Y., CHEN, Y. & LI, C. 2015. Purification efficiency of different aquatic plants in eutrophic water. *Environmental Science & Technology (China),* 38**,** 223-272.

DOW, C. S. & SWOBODA, U. K. 2000. Cyanotoxins. *The ecology of Cyanobacteria.* Springer. Dordrecht. 613-632.

DOWNING, J.A., WATSON, S.B., MCCAULEY, E., 2001. Predicting Cyanobacteria dominance in lakes. Can. J. Fish. Aquat. Sci. 58, 1905–1908. https://doi.org/10.1139/cjfas-58-10- 1905.

DROBAC, D., TOKODI, N., SIMEUNOVIĆ, J., BALTIĆ, V., STANIĆ, D. & SVIRČEV, Z. 2013. Human exposure to cyanotoxins and their effects on health. *Arhiv za higijenu rada i toksikologiju,* 64**,** 305-315.

DUONG, T. T., LE, T. P. Q., DAO, T.-S., PFLUGMACHER, S., ROCHELLE-NEWALL, E., HOANG, T. K., VU, T. N., HO, C. T. & DANG, D. K. 2013. Seasonal variation of cyanobacteria and microcystins in the Nui Coc Reservoir, Northern Vietnam. *Journal of applied phycology,* 25**,**1065-1075.

EC 2000. Directive 2000/60/EC of the European Parliament and the Council of 23rd October 2000 establishing a framework for Community action in the field of water policy (OJ L 327, 22/12/2000, 1–72).

EDWARDS, C., BEATTIE, K. A., SCRIMGEOUR, C. M. & CODD, G. A. 1992. Identification of anatoxin-a in benthic cyanobacteria (blue-green algae) and in associated dog poisonings at Loch Insh, Scotland. *Toxicon,* 30**,** 1165-1175.

ELLAWALA, C., ASAEDA, T. & KAWAMURA, K. 2011. Influence of flow turbulence on *Chara fibrosa*: growth, stress, and tissue carbon content. *Journal of Freshwater Ecology,* 26**,** 507-515.

FALCONER, I. R. & HUMPAGE, A. R. 2006. Cyanobacterial (blue-green algal) toxins in water supplies: Cylindrospermopsins. *Environmental Toxicology: An International Journal,* 21**,** 299-304.

FALCONER, I. R., HARDY, S. J., HUMPAGE, A. R., FROSCIO, S. M., TOZER, G. J. & HAWKINS, P. R. 1999. Hepatic and renal toxicity of the blue–green alga (cyanobacterium) Cylindrospermopsis raciborskii in male Swiss albino mice. *Environmental Toxicology: An International Journal,* 14**,** 143-150.

FAO. 2011. AQUASTAT Country Profile- Myanmar. Food and Agriculture Oorganization of the United Nations (FAO). Rome. Italy [Online]. Available: http://www.fao.org/3/ca0401en/CA0401EN.pdf [Accessed 11 May 2020].

FERRÃO-FILHO, A. D. S. & KOZLOWSKY-SUZUKI, B. 2011. Cyanotoxins: bioaccumulation and effects on aquatic animals. *Marine drugs,* 9**,** 2729-2772.

FERREIRA, F. M., SOLER, J. M. F., FIDALGO, M. L. & FERNÁNDEZ-VILA, P. 2001. PSP toxins from *Aphanizomenon flos-aquae* (cyanobacteria) collected in the Crestuma-Lever reservoir (Douro river, northern Portugal). *Toxicon,* 39**,** 757-761.

FRIBERG, N., BONADA, N., BRADLEY, D. C., DUNBAR, M. J., EDWARDS, F. K., GREY, J., HAYES, R. B., HILDREW, A. G., LAMOUROUX, N. & TRIMMER, M. 2011. Biomonitoring of human impacts in freshwater ecosystems: the good, the bad and the ugly. *Advances in ecological research,* 44**,** 1-68.

FUNARI, E. & TESTAI, E. 2008. Human health risk assessment related to cyanotoxins exposure. *Critical reviews in toxicology,* 38**,** pp. 97-125.

FUNARI, E. & TESTAI, E. 2008. Human health risk assessment related to cyanotoxins exposure. *Critical reviews in toxicology,* 38**,** 97-125.

FURTADO, A. L. F. F., DO CARMO CALIJURI, M., LORENZI, A. S., HONDA, R. Y., GENUÁRIO, D. B. & FIORE, M. F. 2009. Morphological and molecular characterization of cyanobacteria from

a Brazilian facultative wastewater stabilization pond and evaluation of microcystin production. *Hydrobiologia,* 627**,** 195-209.

GANTAR, M., SEKAR, R. & RICHARDSON, L. L. 2009. Cyanotoxins from black band disease of corals and from other coral reef environments. *Microbial ecology,* 58**,** 856-864.

GAO, H., QIAN, X., WU, H., LI, H., PAN, H. & HAN, C. 2017. Combined effects of submerged macrophytes and aquatic animals on the restoration of a eutrophic water body—a case study of Gonghu Bay, Lake Taihu. *Ecological engineering,* 102**,** 15-23.

GHOSH, S. K., DAS, P. K. & BAGCHI, S. N. 2008. PCR-based detection of microcystin-producing cyanobacterial blooms from Central India. Indian J Exp Biol. 2008 Jan;46(1):66-70. PMID: 18697574.

GKELIS, S., RAJANIEMI, P., VARDAKA, E., MOUSTAKA-GOUNI, M., LANARAS, T. & SIVONEN, K. 2005. *Limnothrix redekei* (Van Goor) Meffert (Cyanobacteria) strains from Lake Kastoria, Greece form a separate phylogenetic group. *Microbial Ecology,* 49**,** 176-182.

GONZÁLEZ, E. J. & ROLDÁN, G. 2019. Eutrophication and phytoplankton: some generalities from lakes and reservoirs of the Americas. *Microalgae-From Physiology to Application.* IntechOpen.

GOPAL, B. 1990. Aquatic weed problesm and management in Asia. *In: Pieterse, . H. & K. J. Murphy (eds) 1990. Aquatic Weeds. University Press, Oxford.***,** 593 pp.

GREEN, J. 2011. Geographical variation in rotifers associated with *Microcystis* blooms. *Hydrobiologia,* 662**,**197-204.

GRIFFITHS, D. J. & SAKER, M. L. 2003. The Palm Island Mystery Disease 20 years on: A review of research on the cyanotoxin cylindrospermopsin. *Environmental Toxicology,* 18**,** 78-93.

GROSS, E. M. 2003. Allelopathy of aquatic autotrophs. *Critical reviews in plant sciences,* 22**,** 313-339.

GROSS, E. M., ERHARD, D. & IVÁNYI, E. 2003. Allelopathic activity of *Ceratophyllum demersum* L. and *Najas marina* ssp. intermedia (Wolfgang) Casper. *Hydrobiologia,* 506**,** 583-589.

GROSS, E. M., HILT, S., LOMBARDO, P. & MULDERIJ, G. 2007. Searching for allelopathic effects of submerged macrophytes on phytoplankton—state of the art and open questions. *Shallow Lakes in a Changing World.* Springer.

GUGGER, M., MOLICA, R., LE BERRE, B., DUFOUR, P., BERNARD, C. & HUMBERT, J. F. 2005. Genetic diversity of *Cylindrospermopsis* strains (Canobacteria) isolated from four continents. *Applied and Environmental Microbiology,* 71**,** 1097-1100.

GUO-FENG, L., CHENG-XIN, F., SHI-QUN, H., JUN, H. & PAERL, H. W. 2000. The Response of Macrophytes to Nutrients and Implications for the Control of Phytoplankton Blooms in East Taihu Lake, China. *Journal of Pollution Effects & Control***,** 1-5.

HALLER, W. T. 1990. Aquatic Weeds—The Ecology and Management of Nuisance Aquatic Vegetation–Arnold H. Pieterse and Kevin J. Murphy, editors. 1990. Oxford University Press, New York. 593 p. illus., Hardcover, ISBN: 0-19-854181-3, $135.00. *Weed Technology,* 5**,** 691-691.

HARADA, K.-I., OHTANI, I., IWAMOTO, K., SUZUKI, M., WATANABE, M. F., WATANABE, M. & TERAO, K. 1994. Isolation of cylindrospermopsin from a cyanobacterium *Umezakia natans* and its screening method. *Toxicon,* 32**,** 73-84.

HARKE, M. J., STEFFEN, M. M., GOBLER, C. J., OTTEN, T. G., WILHELM, S. W., WOOD, S. A. & PAERL, H. W. 2016. A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, *Microcystis* spp. *Harmful Algae,* 54**,** 4-20.

HARLAND, F., WOOD, S., BROADY, P., GAW, S. & WILLIAMSON, W. 2014. Polyphasic studies of cyanobacterial strains isolated from benthic freshwater mats in Canterbury, New Zealand. *New Zealand Journal of Botany,* 52**,** 116-135.

HAWKINS, P. R., CHANDRASENA, N. R., JONES, G. J., HUMPAGE, A. R. & FALCONER, I. R. 1997. Isolation and toxicity of *Cylindrospermopsis raciborskii* from an ornamental lake. *Toxicon,* 35**,** 341-346.

HELLSTEN, S., WILLBY, N., ECKE, F., MJELDE, M., PHILLIPS, G., TIERNEY, D. & POIKANE, S. 2014. Water Framework Directive intercalibration technical report northern lake macrophyte ecological assessment methods. *JRC. Report EUR 26513 EN. Ed. S. Poikane.*

HILT, S. & GROSS, E. M. 2008. Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes? *Basic and applied ecology,* 9**,** 422-432.

HILT, S. & LOMBARDO, P. 2010. Effects of macrophytes on phytoplankton: nutrient uptake versus allelopathy. *Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen,* 30**,** 1317-1320.

HILT, S., GHOBRIAL, M. G. & GROSS, E. M. 2006. In situ allelopathic potential of *Myriophyllum verticillatum* (Haloragaceae) against selected phytoplankton species 1. *Journal of Phycology,* 42**,** 1189-1198.

HILT, S., GROSS, E. M., HUPFER, M., MORSCHEID, H., MÄHLMANN, J., MELZER, A., POLTZ, J., SANDROCK, S., SCHARF, E.-M. & SCHNEIDER, S. 2006. Restoration of submerged vegetation in shallow eutrophic lakes–A guideline and state of the art in Germany. *Limnologica,* 36**,** 155-171.

HLAING, N. K. 2014. Limnological study of Meiktila Lake*,* Mandalay Division*.* PhD thesis, University of Mandalay.

HODOKI, Y., OHBAYASHI, K., KOBAYASHI, Y., TAKASU, H., OKUDA, N. & NAKANO, S.-I. 2013. Anatoxin-a-producing Raphidiopsis mediterranea Skuja var. grandis Hill is one ecotype of non-heterocytous *Cuspidothrix issatschenkoi* (Usačev) Rajaniemi et al. in Japanese lakes. *Harmful algae,* 21**,** 44-53.

HOFF-RISSETI, C., DORR, F. A., SCHAKER, P. D., PINTO, E., WERNER, V. R. & FIORE, M. F. 2013. Cylindrospermopsin and saxitoxin synthetase genes in *Cylindrospermopsis raciborskii* strains from Brazilian freshwater. *PLoS One,* 8**,** e74238.

HUBER, C. S. 1972. The crystal structure and absolute configuration of 2, 9-diacetyl-9-azabicyclo [4, 2, 1] non-2, 3-ene. *Acta Crystallographica Section B: Structural Crystallography and Crystal Chemistry,* 28**,** 2577-2582.

HUBER-PESTALOZZI, G. 1983. *Das Phytoplankton des süsswassers: Chlorophyceae [Grünalgen]. Ordnung Volvocales*, Schweizerbart.

HUISMAN, J. & HULOT, F. D. 2005. Population dynamics of harmful cyanobacteria. *Harmful cyanobacteria.* Springer.

HUMPAGE, A. R., FENECH, M., THOMAS, P. & FALCONER, I. R. 2000. Micronucleus induction and chromosome loss in transformed human white cells indicate clastogenic and aneugenic action of the cyanobacterial toxin, cylindrospermopsin. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis,* 472**,** 155-161.

HUMPAGE, A., ROSITANO, J., BRETAG, A., BROWN, R., BAKER, P., NICHOLSON, B. & STEFFENSEN, D. 1994. Paralytic shellfish poisons from Australian cyanobacterial blooms. *Marine and Freshwater Research,* 45**,** 761-771.

IARC MONOGRAPH 94 2010. *Cyanobacterial Peptide Toxins: This Publication Represents the Views and Expert Opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which Met in Lyon, 14-21 June 2006*, International Agency for Research on Cancer- IARC.

IKAWA, M., WEGENER, K., FOXALL, T. L. & SASNER JR, J. J. 1982. Comparison of the toxins of the blue-green alga *Aphanizomenon flos-aquae* with the Gonyaulax toxins. *Toxicon,* 20**,** 747-752.

JASSER, I. 1995. The influence of macrophytes on a phytoplankton community in experimental conditions. *Hydrobiologia,* 306**,** 21-32.

JEPPESEN, E., SONDERGAARD, M. & CHRISTOFFERSEN, K. 1998. The structuring role of submerged macrophytes in lakes Springer Verlag. *Berlin, Germany*.

JEPPESEN, E., SØNDERGAARD, M., SØNDERGAARD, M. & CHRISTOFFERSEN, K. 2012. The structuring role of submerged macrophytes in lakes, Springer Science & Business Media.

JIA, X., LUO, W., WU, X., WEI, H., WANG, B., PHYOE, W. & WANG, F. 2017. Historical record of nutrients inputs into the Xin'an Reservoir and its potential environmental implication. *Environmental Science and Pollution Research,* 24**,** 20330-20341.

JIANG, H., ZHAO, D., ZHAO, H., CAI, Y., XU, D., ZHOU, C., LENG, X. & XIE, D. 2015. Density-Dependent Interactions Between *Hydrilla verticillata* (LF) Royle and Phytoplankton: A Mesocosm Experiment. *CLEAN–Soil, Air, Water,* 43**,** 1623-1632.

JOCHIMSEN, E. M., CARMICHAEL, W. W., AN, J., CARDO, D. M., COOKSON, S. T., HOLMES, C. E., ANTUNES, M. B., DE MELO FILHO, D. A., LYRA, T. M. & BARRETO, V. S. T. 1998. Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil. *New England Journal of Medicine,* 338**,** 873-878.

KALOUDIS, T., ZERVOU, S.-K., TSIMELI, K., TRIANTIS, T. M., FOTIOU, T. & HISKIA, A. 2013. Determination of microcystins and nodularin (cyanobacterial toxins) in water by LC–MS/MS. Monitoring of Lake Marathonas, a water reservoir of Athens, Greece. *Journal of hazardous materials,* 263**,** 105-115.

KAO, C. 1993. Paralytic shellfish poisoning. *Algal toxins in seafood and drinking water,* 75**,** 86.

KARIM, Z., QURESHI, B. A. & GHOURI, I. 2013. Spatial analysis of human health risk associated with trihalomethanes in drinking water: A case study of Karachi, Pakistan. *Journal of Chemistry,* 2013.

KE, Z.-X., XIE, P. & GUO, L.-G. 2009. Impacts of two biomanipulation fishes stocked in a large pen on the plankton abundance and water quality during a period of phytoplankton seasonal succession. *ecological engineering,* 35**,** 1610-1618.

KHAUNG, T., IWAI, C. B. & CHUASAVATHI, T. 2021. Water Quality Monitoring in Inle Lake, Myanmar from the Floating Garden Activity. *Malaysian Journal of Fundamental and Applied Sciences,* 17**,** 593-608.

KLAPPER, H. 1991. *Control of eutrophication in inland waters*, Ellis Horwood Ltd.

KNISELY, K. & GELLER, W. 1986. Selective feeding of four zooplankton species on natural lake phytoplankton. *Oecologia,* 69**,** 86-94.

KOLADA, A., HELLSTEN, S., SØNDERGAARD, M., MJELDE, M., DUDLEY, B., VAN GEEST, G., GOLDSMITH, B., DAVIDSON, T., BENNION, H. & NÕGES, P. 2012. WISER Deliverable D3. 2-3: Report on the most suitable lake macrophyte based assessment methods for impacts of eutrophication and water level fluctuations. *Technical Report,***,** p.114.

KOMÁREK, J. & ANAGNOSTIDIS, K. 1986. Modern approach to the classification system of cyanophytes. II: Chroococcales. *Archiv für Hydrobiologie. Supplementband. Monographische Beiträge,* 73**,** pp. 157-226.

KOMÁREK, J. & ANAGNOSTIDIS, K., 1999. Cyanoprokaryota 1. Chroococcales. – 19/1. In: Ettl, H., G¨artner, G., Heynig, H., Mollenhauer, D. (Eds.), Süsswasserflora von Mitteleuropa Gustav Fischer. Jena-Stuttgart-Lübeck-Ulm, p. 548.

KOMÁREK, J. 2013. Cyanoprokaryota. 3. Heterocytous Genera. Süsswasserflora von Mitteleuropa, 19/3. Berlin–Heidelberg.

KÓTAI, J. 1972. Instructions for preparation of modified nutrient solution Z8 for algae. Publication B-11/69. *Norwegian Institute for Water Research, Oslo, Norway.*

KRIENITZ, L., BALLOT, A., KOTUT, K., WIEGAND, C., PÜTZ, S., METCALF, J. S., CODD, G. A. & STEPHAN, P. 2003. Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. *FEMS microbiology ecology,* 43**,** 141-148.

KUBICKOVA, B., BABICA, P., HILSCHEROVÁ, K. & ŠINDLEROVÁ, L. 2019. Effects of cyanobacterial toxins on the human gastrointestinal tract and the mucosal innate immune system. *Environmental Sciences Europe,* 31**,** 1-27.

KUIPER, J. J., VERHOFSTAD, M. J., LOUWERS, E. L., BAKKER, E. S., BREDERVELD, R. J., VAN GERVEN, L. P., JANSSEN, A. B., DE KLEIN, J. J. & MOOIJ, W. M. 2017. Mowing submerged macrophytes in shallow lakes with alternative stable states: battling the good guys? *Environmental management,* 59**,** 619-634.

KUIPER-GOODMAN, T., FALCONER, I. & FITZGERALD, J. 1999. Human Health Aspects in Chorus, I and Bartram J (Eds.). *Toxic cyanobacterias in water: a guide to their public health consequences, monitoring and management. F&FN Spon, London***,** pp. 113-153.

KUMAR, S., STECHER, G. & TAMURA, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution,* 33**,** 1870-1874.

KUMAR, S., STECHER, G., LI, M., KNYAZ, C. & TAMURA, K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular biology and evolution,* 35**,** 1547.

KURMAYER, R., SIVONEN, K., WILMOTTE, A. & SALMASO, N. 2017. *Molecular tools for the detection and quantification of toxigenic cyanobacteria*, John Wiley & Sons.

LACOUL, P. & FREEDMAN, B. 2006. Environmental influences on aquatic plants in freshwater ecosystems. *Environmental Reviews,* 14**,** 89-136.

LAKSHMANA RAO, P., BHATTACHARYA, R., GUPTA, N., PARIDA, M., BHASKAR, A. & DUBEY, R. 2002. Involvement of caspase and reactive oxygen species in cyanobacterial toxin anatoxin-a-induced cytotoxicity and apoptosis in rat thymocytes and Vero cells. *Archives of toxicology,* 76**,** 227-235.

LA-ONGSRI, W., TRISONTHI, C. & BALSLEV, H. 2009. Management and use of Nelumbo nucifera Gaertn. in Thai wetlands. *Wetlands ecology and management,* 17**,** 279-289.

LEDREUX, A., THOMAZEAU, S., CATHERINE, A., DUVAL, C., YÉPRÉMIAN, C., MARIE, A. & BERNARD, C. 2010. Evidence for saxitoxins production by the cyanobacterium *Aphanizomenon gracile* in a French recreational water body. *Harmful Algae,* 10**,** 88-97.

LEFEBVRE, K. A., BILL, B. D., ERICKSON, A., BAUGH, K. A., O'ROURKE, L., COSTA, P. R., NANCE, S. & TRAINER, V. L. 2008. Characterization of intracellular and extracellular saxitoxin levels in both field and cultured Alexandrium spp. samples from Sequim Bay, Washington. *Marine Drugs,* 6**,** 103-116.

LEI, L., PENG, L., HUANG, X. & HAN, B. P. 2014. Occurrence and dominance of *Cylindrospermopsis raciborskii* and dissolved cylindrospermopsin in urban reservoirs used for drinking water supply, South China. *Environ Monit Assess,* 186**,** 3079-90.

LEU, E., KRIEGER-LISZKAY, A., GOUSSIAS, C. & GROSS, E. M. 2002. Polyphenolic allelochemicals from the aquatic angiosperm *Myriophyllum spicatum* inhibit photosystem II. *Plant physiology,* 130**,** 2011-2018.

LEWIS, W. M. 2000. Basis for the protection and management of tropical lakes. *Lakes & Reservoirs: Research & Management,* 5**,** 35-48.

LI, R., CARMICHAEL, W. W. & PEREIRA, P. 2003. Mophological and 16S rRNA gene evidence for reclassification of the paralytic shellfish toxin producing *Aphanizomenon flos-aquae* lmecya 31 as *Aphanizomenon issatschenkoi* (Cyanophyceae) 1. *Journal of Phycology,* 39**,** 814-818.

LI, R., CARMICHAEL, W. W., BRITTAIN, S., EAGLESHAM, G. K., SHAW, G. R., LIU, Y. & WATANABE, M. M. 2001. First report of the cyanotoxins cylindrospermopsin and deoxycylindrospermopsin from *Raphidiopsis curvata* (Cyanobacteria). *Journal of Phycology,* 37**,** 1121-1126.

LI, R., CARMICHAEL, W. W., LIU, Y. & WATANABE, M. M. 2000. Taxonomic re-evaluation of *Aphanizomenon flos-aquae* NH-5 based on morphology and 16S rRNA gene sequences. *Hydrobiologia,* 438**,** 99-105.

LINDSEY, R., SCOTT, M. & SIMMON, R. 2010. What are phytoplankton. *NASA's Earth Observatory. Available on http://earthobservatory. nasa. gov/Librar y/phytoplankton*.

LIU, G., KE, M., FAN, X., ZHANG, M., ZHU, Y., LU, T., SUN, L. & QIAN, H. 2018. Reproductive and endocrine-disrupting toxicity of *Microcystis aeruginosa* in female zebrafish. *Chemosphere,* 192**,** 289-296.

LIU, Q., SUN, B., HUO, Y., LIU, M., SHI, J., JIANG, T., ZHANG, Q., TANG, C., BI, H. & HE, P. 2018. Nutrient bioextraction and microalgae growth inhibition using submerged macrophyte Myriophyllum spicatum in a low salinity area of East China Sea. *Marine pollution bulletin,* 127**,** 67-72.

LIU, Y., CHEN, W., LI, D., SHEN, Y., LIU, Y. & SONG, L. 2006. Analysis of paralytic shellfish toxins in *Aphanizomenon* DC-1 from Lake Dianchi, China. *Environmental Toxicology: An International Journal,* 21**,** 289-295.

LOMBARDO, P., MJELDE, M., KÄLLQVIST, T. & BRETTUM, P. 2013. Seasonal and scale-dependent variability in nutrient-and allelopathy-mediated macrophyte–phytoplankton interactions. *Knowledge and Management of Aquatic Ecosystems***,** 10.

LUUKKAINEN, R., SIVONEN, K., NAMIKOSHI, M., FÄRDIG, M., RINEHART, K. & NIEMELÄ, S. 1993. Isolation and identification of eight microcystins from thirteen Oscillatoria agardhii strains and structure of a new microcystin. *Applied and Environmental Microbiology,* 59**,** p. 2204.

LYCHE-SOLHEIM, A., FELD, C. K., BIRK, S., PHILLIPS, G., CARVALHO, L., MORABITO, G., MISCHKE, U., WILLBY, N., SØNDERGAARD, M. & HELLSTEN, S. 2013. Ecological status assessment of European lakes: a comparison of metrics for phytoplankton, macrophytes, benthic invertebrates and fish. *Hydrobiologia,* 704**,** 57-74.

MÄEMETS, H. & FREIBERG, L. Coverage and depth limit of macrophytes as tools for classification of lakes. Proceedings of the Estonian Academy of Sciences, Biology, Ecology, 2007. 124-140.

MAHAKHANT, A., SANO, T., RATANACHOT, P., TONG-A-RAM, T., SRIVASTAVA, V. C., WATANABE, M. M. & KAYA, K. 1998. Detection of microcystins from cyanobacterial water blooms in Thailand freshwater. *Phycological Research,* 46**,** 25-29.

MAHMOOD, N. A. & CARMICHAEL, W. W. 1986a. Paralytic shellfish poisons produced by the freshwater cyanobacterium *Aphanizomenon flos-aquae* NH-5. *Toxicon,* 24**,** 175-186.

MARTIN, A. B., COOKE, G. D. & CARLSON, R. E. 1993. Lake sediments as potential sources of trihalomethane precursors. *Water Research,* 27**,** 1725-1729.

MAZMOUZ, R., CHAPUIS-HUGON, F., MANN, S., PICHON, V., MÉJEAN, A. & PLOUX, O. 2010. Biosynthesis of cylindrospermopsin and 7-epicylindrospermopsin in Oscillatoria sp. strain PCC 6506: identification of the cyr gene cluster and toxin analysis. *Applied and environmental microbiology,* 76**,** 4943-4949.

MCGREGOR, G. B., SENDALL, B. C., HUNT, L. T. & EAGLESHAM, G. K. 2011. Report of the cyanotoxins cylindrospermopsin and deoxy-cylindrospermopsin from Raphidiopsis mediterranea Skuja (Cyanobacteria/Nostocales). *Harmful Algae,* 10**,** 402-410.

MEERHOFF, M., MAZZEO, N., MOSS, B. & RODRÍGUEZ-GALLEGO, L. 2003. The structuring role of free-floating versus submerged plants in a subtropical shallow lake. *Aquatic Ecology,* 37**,** 377-391.

MEIJER, M. L., E. JEPPESEN, E. VAN DONK, B. MOSS, M. SCHEFFER, E. LAMMENS, E. VAN NES, J. A. VAN BERKUM, G. J. DE JONG, B. A. FAAFENG & J. P. JENSEN, 1994. Long-term responses to fishstock reduction in small shallow lakes: interpretation of five-year results of four biomanipulation cases in The Netherlands and Denmark. Hydrobiologia 275/276: 457–466.

MEJEAN, A., MANN, S., MALDINEY, T., VASSILIADIS, G., LEQUIN, O. & PLOUX, O. 2009. Evidence that Biosynthesis of the Neurotoxic Alkaloids Anatoxin-a and Homoanatoxin-a in the Cyanobacterium Oscillatoria PCC 6506 Occurs on a Modular Polyketide Synthase Initiated by L-Proline. *Journal of the American Chemical Society,* 131**,** 7512-+.

MELZER, A. 1999. Aquatic macrophytes as tools for lake management. In: *Harper D.M., Brierley B., Ferguson A.J.D., Phillips G. (eds) The Ecological Bases for Lake and Reservoir Management*. Developments in Hydrobiology, vol 136. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-3282-6_17

MEZ, K., BEATTIE, K., CODD, G., HANSELMANN, K., HAUSER, B., NAEGELI, H. & PREISIG, H. 1997. Identification of a microcystin in benthic cyanobacteria linked to cattle deaths on alpine pastures in Switzerland. *European Journal of Phycology,* 32**,** 111-117.

MILES, C. O., SANDVIK, M., NONGA, H. E., RUNDBERGET, T., WILKINS, A. L., RISE, F. & BALLOT, A. 2012. Thiol derivatization for LC-MS identification of microcystins in complex matrices. *Environmental science & technology,* 46**,** 8937-8944.

MITCHELL, S. F., HAMILTON, D., MACGIBBON, W. S., NAYAR, P. B.-K. & REYNOLDS, R. 1988. Interrelations between phytoplankton, submerged macrophytes, black swans (*Cygnus atratus*) and zooplankton in a shallow New Zealand lake. *Internationale Revue der gesamten Hydrobiologie und Hydrographie,* 73**,** 145-170.

MJELDE, M. & FAAFENG, B. 1997. *Ceratophyllum demersum* hampers phytoplankton development in some small Norwegian lakes over a wide range of phosphorus concentrations and geographical latitude. *Freshwater Biology,* 37**,** 355-365.

MJELDE, M., BALLOT, A. & SWE, T. 2018. Conservation of biodiversity and improved management of protected areas in Myanmar. Aquatic macrophytes and phytoplankton in Indawgyi Lake. *NIVA-rapport*.

MJELDE, M., BALLOT, A., SWE, T., ERIKSEN, T. E., NESHEIM, I. & AUNG, T. T. 2017. Integrated Water Resources Management in Myanmar. Water usage and introduction to water quality criteria for lakes and rivers in Myanmar. Preliminary report. *NIVA-report SNO 7253-2018*.

MJELDE, M., HELLSTEN, S. & ECKE, F. 2013. A water level drawdown index for aquatic macrophytes in Nordic lakes. *Hydrobiologia,* 704**,** 141-151.

MOHAMED, Z. A. 2017. Macrophytes-cyanobacteria allelopathic interactions and their implications for water resources management—A review. *Limnologica,* 63**,** 122-132.

MOHAMED, Z. A., EL-SHAROUNY, H. M. & ALI, W. S. 2006. Microcystin production in benthic mats of cyanobacteria in the Nile River and irrigation canals, Egypt. *Toxicon,* 47**,** 584-590.

MOSS, B., M. BEKLIOGLU, L. CARVALHO, S. KILINC, S. MCGOWAN & D. STEPHEN, 1997. Vertically challenged limnology; contrasts between deep and shallow lakes. Hydrobiologia 342/343: 257–267.

MOWE, M. A. D., MITROVIC, S. M., LIM, R. P., FUREY, A. & YEO, D. C. J. 2015. Tropical cyanobacterial blooms: a review of prevalence, problem taxa, toxins and influencing environmental factors. *Journal of Limnology,* 73.

MYERS, J. L., SEKAR, R. & RICHARDSON, L. L. 2007. Molecular detection and ecological significance of the cyanobacterial genera Geitlerinema and Leptolyngbya in black band disease of corals. *Applied and Environmental Microbiology,* 73**,** 5173-5182.

NAMIKOSHI, M., MURAKAMI, T., WATANABE, M. F., ODA, T., YAMADA, J., TSUJIMURA, S., NAGAI, H. & OISHI, S. 2003. Simultaneous production of homoanatoxin-a, antitoxin-a, and a new non-toxic 4-hydroxyhomoanatoxin-a by the cyanobacterium *Raphidiopsis mediterranea* Skuja. *Toxicon,* 42**,** 533-538.

NAW, M.W.D., SAN, D.P., AUNG, Y., 2020. Occurrence of *Microcystis* Toxic Blooms in Indawgyi Lake, Kachin State. University of Mandalay Res. J. 11, 1–9.

NEGRI, A. P., JONES, G. J., BLACKBURN, S. I., OSHIMA, Y. & ONODERA, H. 1997. Effect of culture and bloom development and of sample storage on paralytic shellfish poisons in the cyanobacterium *Anabaena circinalis*. *Journal of Phycology,* 33**,** 26-35.

NESHEIM, I., WATHNE, B. M., NI, B. & TUN, Z. L. 2016. Myanmar: Pilot introducing the national water framework directive. *Water Solutions,* 1**,** 18-28.

NGUYEN, T. T. L., HOANG, T. H., NGUYEN, T. K. & DUONG, T. T. 2017. The occurrence of toxic cyanobacterium *Cylindrospermopsis raciborskii* and its toxin cylindrospermopsin in the Huong River, Thua Thien Hue province, Vietnam. *Environ Monit Assess,* 189**,** 490.

NIXDORF B., REKTINS A., MISCHKE U. (2008) Standards and Thresholds of the EU Water Framework Directive (WFD) — Phytoplankton and Lakes. In: *Schmidt M., Glasson J., Emmelin L., Helbron H. (eds) Standards and Thresholds for Impact Assessment*. Environmental Protection in the European Union, vol 3. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-31141-6_24

NOGUEIRA, I. C., PEREIRA, P., DIAS, E., PFLUGMACHER, S., WIEGAND, C., FRANCA, S. & VASCONCELOS, V. M. 2004. Accumulation of paralytic shellfish toxins (PST) from the cyanobacterium *Aphanizomenon issatschenkoi* by the cladoceran Daphnia magna. *Toxicon,* 44**,** 773-780.

NORRIS, R. L., EAGLESHAM, G. K., PIERENS, G., SHAW, G. R., SMITH, M. J., CHISWELL, R. K., SEAWRIGHT, A. A. & MOORE, M. R. 1999. Deoxycylindrospermopsin, an analog of

cylindrospermopsin from Cylindrospermopsis raciborskii. *Environmental Toxicology: An International Journal,* 14**,** 163-165.

OH, H.-M., LEE, S. J., KIM, J.-H., KIM, H.-S. & YOON, B.-D. 2001. Seasonal variation and indirect monitoring of microcystin concentrations in Daechung Reservoir, Korea. *Applied and Environmental Microbiology,* 67**,** 1484-1489

OHTANI, I., MOORE, R. E. & RUNNEGAR, M. T. 1992. Cylindrospermopsin: a potent hepatotoxin from the blue-green alga Cylindrospermopsis raciborskii. *Journal of the American Chemical Society,* 114**,** 7941-7942.

OKSANEN, I., JOKELA, J., FEWER, D. P., WAHLSTEN, M., RIKKINEN, J. & SIVONEN, K. 2004. Discovery of rare and highly toxic microcystins from lichen-associated cyanobacterium Nostoc sp. strain IO-102-I. *Applied and Environmental Microbiology,* 70**,** 5756-5763.

OKSANEN, J., BLANCHET, F., FRIENDLY, M., KINDT, R., LEGENDRE, P., MCGLINN, D., MINCHIN, P., O'HARA, R., SIMPSON, G. & SOLYMOS, P. 2020. H. WAGNER H. 2018. vegan: Community Ecology Package. R package version 2.5-3.

OLIVER, R. L. & GANF, G. G. 2000. Freshwater blooms. In *The ecology of cyanobacteria* (pp. 149-194). Springer, Dordrecht.

ONDIBA, R., OMONDI, R., MUSA, S., NYAKEYA, K., OYOO-OKOTH, E. & ABWAO, J. 2018b. Environmental constraints on macrophyte distribution and diversity in a tropical endorheic freshwater lake (Lake Baringo, Kenya). *International Journal of Fisheries and Aquatic Studies (IJFAS)* 6(3): **,** 6(3): 251-259.

OSHIMA, Y., BLACKBURN, S. & HALLEGRAEFF, G. 1993. Comparative study on paralytic shellfish toxin profiles of the dinoflagellate Gymnodinium catenatum from three different countries. *Marine Biology,* 116**,** 471-476.

OSSWALD, J., RELLÁN, S., GAGO-MARTINEZ, A. & VASCONCELOS, V. 2009. Production of anatoxin-a by cyanobacterial strains isolated from Portuguese fresh water systems. *Ecotoxicology,* 18**,** 1110-1115.

PAERL, H. W. & OTTEN, T. G. 2013. Harmful cyanobacterial blooms: causes, consequences, and controls. *Microbial ecology,* 65**,** 995-1010.

PAL, B. P. 1932. Burmese Charophyta. *Botanical Journal of the Linnean Society,* 49**,** 47-92.

PAN, Y., GUO, S., LI, Y., YIN, W., QI, P., SHI, J., HU, L., LI, B., BI, S. & ZHU, J. 2018. Effects of water level increase on phytoplankton assemblages in a drinking water reservoir. *Water,* 10**,** 256.

PARK, H. D., KIM, B., KIM, E. & OKINO, T. 1998. Hepatotoxic microcystins and neurotoxic anatoxin-a in cyanobacterial blooms from Korean lakes. *Environmental Toxicology and Water Quality: An International Journal,* 13**,** 225-234.

PARK, H. D., WATANABE, M. F., HARADA, K. I., NAGAI, H., SUZUKI, M., WATANABE, M. & HAYASHI, H. 1993. Hepatotoxin (microcystin) and neurotoxin (anatoxin-a) contained in natural blooms and strains of cyanobacteria from Japanese freshwaters. *Natural toxins,* 1**,** 353-360.

PENNING, W. E., DUDLEY, B., MJELDE, M., HELLSTEN, S., HANGANU, J., KOLADA, A., VAN DEN BERG, M., POIKANE, S., PHILLIPS, G. & WILLBY, N. 2008a. Using aquatic macrophyte community indices to define the ecological status of European lakes. *Aquatic ecology,* 42**,** 253-264.

PENNING, W. E., MJELDE, M., DUDLEY, B., HELLSTEN, S., HANGANU, J., KOLADA, A., VAN DEN BERG, M., POIKANE, S., PHILLIPS, G. & WILLBY, N. 2008b. Classifying aquatic macrophytes as indicators of eutrophication in European lakes. *Aquatic ecology,* 42**,** 237-251.

PEREIRA, P., LI, R., CARMICHAEL, W., DIAS, E. & FRANCA, S. 2004. Taxonomy and production of paralytic shellfish toxins by the freshwater cyanobacterium Aphanizomenon gracile LMECYA40. *European Journal of Phycology,* 39**,** 361-368.

PEREIRA, P., ONODERA, H., ANDRINOLO, D. O., FRANCA, S., ARAÚJO, F., LAGOS, N. & OSHIMA, Y. 2000. Paralytic shellfish toxins in the freshwater cyanobacterium *Aphanizomenon flos-aquae*, isolated from Montargil reservoir, Portugal. *Toxicon,* 38**,** 1689-1702.

PERETYATKO, A., SYMOENS, J.-J. & TRIEST, L. 2007. Impact of macrophytes on phytoplankton in eutrophic peri-urban ponds, implications for pond management and restoration. *Belgian Journal of Botany***,** 83-99.

PHILLIPS, G., EMINSON, D. & MOSS, B. 1978. A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. *Aquatic botany,* 4**,** 103-126.

PHILLIPS, G., LYCHE-SOLHEIM, A., SKJELBRED, B. MISCHKE, U., DRAKARE, S., FREE, G., JÄRVINEN, M., de HOYOS, C., MORABITO, G., POIKANE, S., CARVALHO, L. 2013 A phytoplankton trophic index to assess the status of lakes for the Water Framework Directive. Hydrobiologia, 704 (1). 75-95. https://doi.org/10.1007/ s10750-012-1390-8

PHILLIPS, G., WILLBY, N. & MOSS, B. 2016. Submerged macrophyte decline in shallow lakes: What have we learnt in the last forty years? *Aquatic Botany,* 135**,** 37-45.

POIKANE, S., PORTIELJE, R., DENYS, L., ELFERTS, D., KELLY, M., KOLADA, A., MÄEMETS, H., PHILLIPS, G., SØNDERGAARD, M. & WILLBY, N. 2018. Macrophyte assessment in European lakes: Diverse approaches but convergent views of 'good'ecological status. *Ecological indicators,* 94**,** 185-197.

POMATI, F., KELLMANN, R., CAVALIERI, R., BURNS, B. P. & NEILAN, B. A. 2006. Comparative gene expression of PSP-toxin producing and non-toxic Anabaena circinalis strains. *Environment international,* 32**,** 743-748.

POMATI, F., SACCHI, S., ROSSETTI, C., GIOVANNARDI, S., ONODERA, H., OSHIMA, Y. & NEILAN, B. A. 2000. The freshwater cyanobacterium *Planktothrix* sp. FP1: molecular identification and detection of paralytic shellfish poisoning toxins. *Journal of Phycology,* 36**,** 553-562.

POMATI, F., SHURIN, J. B., ANDERSEN, K. H., TELLENBACH, C. & BARTON, A. D. 2020. Interacting temperature, nutrients and zooplankton grazing control phytoplankton size-abundance relationships in eight Swiss lakes. *Frontiers in microbiology***,** 3155

POROJAN, C., ABBAS, F., MOWE, M. A., LEHANE, M., MITROVIC, S. M., LIM, R. P., YEO, D. C. & FUREY, A. 2020. Survey of microcystins in Singapore's reservoirs using liquid

chromatography–tandem mass spectrometry (LC-MS/MS). *Marine and Freshwater Research,* 71**,** 659-672.

PRADHAN, N., HABIB, H., VENKATAPPA, M., EBBERS, T., DUBOZ, R. & SHIPIN, O. 2015. Framework tool for a rapid cumulative effects assessment: case of a prominent wetland in Myanmar. *Environmental Monitoring and Assessment,* 187 (6)**,** 1-18.

PREUßEL, K., STÜKEN, A., WIEDNER, C., CHORUS, I. & FASTNER, J. 2006. First report on cylindrospermopsin producing *Aphanizomenon flos-aquae* (Cyanobacteria) isolated from two German lakes. *Toxicon,* 47**,** 156-162.

PRINSEP, M. R., CAPLAN, F. R., MOORE, R. E., PATTERSON, G. M. L., HONKANEN, R. E. & BOYNTON, A. L. 1992. Microcystin-LA from A Blue-Green-Alga Belonging to the Stigonematales. *Phytochemistry,* 31**,** 1247-1248.

QUEVEDO-CASTRO, A., LOPEZ, J. L., RANGEL-PERAZA, J. G., BANDALA, E. & BUSTOS-TERRONES, Y. 2019. Study of the Water Quality of a Tropical Reservoir. *Environments,* 6**,** pp.1-7.

R CORE TEAM. 2020. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2014. *Available online: www. r-project. org (accessed on 14 Febuary 2019)*.

RAPALA, J., SIVONEN, K., LUUKKAINEN, R. & NIEMELÄ, S. I. 1993. Anatoxin-a concentration in *Anabaena* and *Aphanizomenon* under different environmental conditions and comparison of growth by toxic and non-toxic *Anabaena*-strains—a laboratory study. *Journal of applied phycology,* 5**,** pp.581-591.

RCoreTeam, 2020. R: a Language and Environment for Statistical Computing. URL. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

REYNOLDS, C. & WALSBY, A. 1975. Water-blooms. *Biological reviews,* 50**,** pp.437-481.

REYNOLDS, C. S. 1997. *Vegetation processes in the pelagic: a model for ecosystem theory*, Ecology Institute Oldendorf.

REYNOLDS, C. S., HUSZAR, V., KRUK, C., NASELLI-FLORES, L. & MELO, S. 2002. Towards a functional classification of the freshwater phytoplankton. *Journal of plankton research,* 24**,** pp.417-428.

RICHARDSON, L. L., SEKAR, R., MYERS, J. L., GANTAR, M., VOSS, J. D., KACZMARSKY, L., REMILY, E. R., BOYER, G. L. & ZIMBA, P. V. 2007. The presence of the cyanobacterial toxin microcystin in black band disease of corals. *FEMS microbiology letters,* 272**,** pp.182-187.

RINEHART, K. L., NAMIKOSHI, M. & CHOI, B. W. 1994. Structure and biosynthesis of toxins from blue-green algae (cyanobacteria). *Journal of applied phycology,* 6**,** 159-176.

ROSÉN, G. 1981. Phytoplankton indicators and their relations to certain chemical and physical factors. *Limnologica,* 13 (2), pp.263-290.

ROY-LACHAPELLE, A., SOLLIEC, M., BOUCHARD, M. F. & SAUVÉ, S. 2017. Detection of cyanotoxins in algae dietary supplements. *Toxins,* 9**,** 76. doi: 10.3390/toxins9030076. PMID: 28245621; PMCID: PMC5371831.

SAHOO, G., FORREST, A., SCHLADOW, S., REUTER, J., COATS, R. & DETTINGER, M. 2016. Climate change impacts on lake thermal dynamics and ecosystem vulnerabilities. *Limnology and Oceanography,* 61**,** pp496-507.

SAKER, M. L. & NEILAN, B. A. 2001. Varied diazotrophies, morphologies, and toxicities of genetically similar isolates of *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) from northern Australia. *Applied and Environmental Microbiology,* 67**,** 1839-1845.

SAKER, M.L., & GRIFFITHS, D.J., 2000. The effect of temperature on growth and cylindrospermopsin content of seven isolates of *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) from water bodies in northern Australia. Phycologia 39, 349–354.

SALAS, H. J. & MARTINO, P. 1991. A simplified phosphorus trophic state model for warm-water tropical lakes. *Water research,* 25**,** 341-350.

SALUJA, R. & GARG, J. 2017a. Macrophyte species composition and structure along littoral region in relation to limnological variables of a tropical wetland ecosystem. *Chemistry and Ecology,* 33**,** 499-515.

SÁNCHEZ-BARACALDO, P. & CARDONA, T. 2020. On the origin of oxygenic photosynthesis and Cyanobacteria. *New Phytologist,* 225**,** 1440-1446.

SANT'ANNA, C. L. 1991. Two new taxa of Anabaena and other Nostocaceae (Cyanophyceae) from the State of São Paulo, southeastern Brazil. *Algological Studies/Archiv für Hydrobiologie, Supplement Volumes***,** 527-545.

SAYER, C. D., DAVIDSON, T. A. & JONES, J. I. 2010. Seasonal dynamics of macrophytes and phytoplankton in shallow lakes: a eutrophication-driven pathway from plants to plankton? *Freshwater Biology,* 55**,** 500-513.

SCHANTZ, E. J., MOLD, J. D., STANGER, D. W., SHAVEL, J., RIEL, F. J., BOWDEN, J. P., LYNCH, J. M., WYLER, R. S., RIEGEL, B. & SOMMER, H. 1957. Paralytic shellfish poison. VI. A procedure for the isolation and purification of the poison from toxic clam and mussel tissues. *Journal of the American Chemical Society,* 79**,** 5230-5235.

SCHEFFER, M. & JEPPESEN, E. 1998. Alternative stable states. In *The structuring role of submerged macrophytes in lakes* (pp. 397-406). Springer, New York, NY.

SCHEFFER, M., BAKEMA, A. H. & WORTELBOER, F. G. 1993a. MEGAPLANT: a simulation model of the dynamics of submerged plants. *Aquatic Botany,* 45**,** 341-356.

SCHEFFER, M., HOSPER, S. H., MEIJER, M. L., MOSS, B. & JEPPESEN, E. 1993b. Alternative equilibria in shallow lakes. *Trends in ecology & evolution,* 8**,** 275-279.

SCHEMBRI, M. A., NEILAN, B. A. & SAINT, C. P. 2001. Identification of genes implicated in toxin production in the cyanobacterium *Cylindrospermopsis raciborskii*. *Environmental Toxicology: An International Journal,* 16**,** 413-421.

SCHNEIDER, B., CUNHA, E. R., MARCHESE, M. & THOMAZ, S. M. 2018. Associations between macrophyte life forms and environmental and morphometric factors in a large sub-tropical floodplain. *Frontiers in plant science,* 9**,** 195.

SCHNEIDER, S. C., NOWAK, P., VON AMMON, U. & BALLOT, A. 2016. Species differentiation in the genus Chara (Charophyceae): considerable phenotypic plasticity occurs within homogenous genetic groups. *European Journal of Phycology,* 51 (3)**,** 282-293. doi:10.1080/09670262.2016.1147085.

SCHNEIDER, S.C., CARA, M., ERIKSEN, T.E., GORESKA, B.B., IMERI, A., KUPE, L., LOKOSKA, T., PATCEVA, S., TRAJANOVSKA, S., TRAJANOVSKI, S., TALEVSKA, M., SARAFILOSKA, E.V., 2014. Eutrophication impacts littoral biota in Lake Ohrid while water phosphorus concentrations are low. Limnologica 44, 90–97. https://doi.org/10.1016/j. limno.2013.09.002.

SEIFERT, M., MCGREGOR, G., EAGLESHAM, G., WICKRAMASINGHE, W. & SHAW, G. 2007. First evidence for the production of cylindrospermopsin and deoxy-cylindrospermopsin by the freshwater benthic cyanobacterium, Lyngbya wollei (Farlow ex Gomont) Speziale and Dyck. *Harmful algae,* 6**,** 73-80.

SELWOOD, A. I., HOLLAND, P. T., WOOD, S. A., SMITH, K. F. & MCNABB, P. S. 2007. Production of anatoxin-a and a novel biosynthetic precursor by the cyanobacterium Aphanizomenon issatschenkoi. *Environmental science & technology,* 41**,** 506-510.

SHAMS, S., CAPELLI, C., CERASINO, L., BALLOT, A., DIETRICH, D. R., SIVONEN, K. & SALMASO, N. 2015. Anatoxin-a producing *Tychonema* (Cyanobacteria) in European waterbodies. *Water research,* 69**,** 68-79

SHARMA, A., GUPTA, M. K. & SINGHAL, P. K. 1996. Toxic effects of leachate of water hyacinth decay on the growth of *Scenedesmus obliquus* (Chlorophyta). *Water Research,* 30**,** 2281-2286.

SHIMIZU, Y. Chemistry and Mechanism of Action. 2000. Food Sceince and Technology-New Yourk-Marcel Dekker-, 151-172.

SINANG, S. C., POH, K. B., SHAMSUDIN, S. & SINDEN, A. 2015. Preliminary assessment of cyanobacteria diversity and toxic potential in ten freshwater lakes in Selangor, Malaysia. *Bulletin of environmental contamination and toxicology,* 95**,** 542-547.

SIU YEON TAN, ZHU LI & CHENG, S. 2017. Ecological interaction between submerged macrophytes and zoobenthos. *J. Earth Sci. Environ. Stud,* 2**,** 173-182.

SIVONEN, K. & JONES, G. 1999. Cyanobacterial toxins. *Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management,* 1**,** 43-112.

SIVONEN, K. 1996. Cyanobacterial toxins and toxin production. *Phycologia,* 35**,** 12-24.

SIVONEN, K., CARMICHAEL, W., NAMIKOSHI, M., RINEHART, K., DAHLEM, A. & NIEMELÄ, S. 1990b. Isolation and characterization of hepatotoxic microcystin homologs from the filamentous freshwater cyanobacterium Nostoc sp. strain 152. *Applied and Environmental Microbiology,* 56**,** 2650-2657.

SIVONEN, K., HIMBERG, K., LUUKKAINEN, R., NIEMELÄ, S., POON, G. & CODD, G. 1989a. Preliminary characterization of neurotoxic cyanobacteria blooms and strains from Finland. *Toxicity Assessment,* 4**,** 339-352.

SMITH, F. M., WOOD, S. A., VAN GINKEL, R., BROADY, P. A. & GAW, S. 2011. First report of saxitoxin production by a species of the freshwater benthic cyanobacterium, Scytonema Agardh. *Toxicon,* 57**,** 566-573.

SMITH, F. M., WOOD, S. A., WILKS, T., KELLY, D., BROADY, P. A., WILLIAMSON, W. & GAW, S. 2012. Survey of Scytonema (Cyanobacteria) and associated saxitoxins in the littoral zone of recreational lakes in Canterbury, New Zealand. *Phycologia,* 51**,** 542-551.

SOMDEE, T., KAEWSAN, T. & SOMDEE, A. 2013. Monitoring toxic cyanobacteria and cyanotoxins (microcystins and cylindrospermopsins) in four recreational reservoirs (Khon Kaen, Thailand). *Environ Monit Assess,* 185**,** 9521-9.

SØNDERGAARD, M. & JEPPESEN, E. 2007. Anthropogenic impacts on lake and stream ecosystems, and approaches to restoration. Wiley Online Library.

SOTO-LIEBE, K., MÉNDEZ, M. A., FUENZALIDA, L., KROCK, B., CEMBELLA, A. & VÁSQUEZ, M. 2012. PSP toxin release from the cyanobacterium Raphidiopsis brookii D9 (Nostocales) can be induced by sodium and potassium ions. *Toxicon,* 60**,** 1324-1334.

SRIVASTAVA, J., GUPTA, A. & CHANDRA, H. 2008. Managing water quality with aquatic macrophytes. *Reviews in Environmental Science and Bio/Technology,* 7**,** 255-266.

STEPHEN, D., B. MOSS & G. PHILLIPS, 1998. The relative importance of top-down and bottom-up control in a shallow macrophytedominated lake. Freshwat. Biol. 39: 699–713.

STRAILE, D., 2015. Zooplankton biomass dynamics in oligotrophic versus eutrophic conditions: A test of the PEG model. Freshwater Biology, 60(1), 174–183.

SU, M. & JASSBY, A. D. 2000. Inle: a large Myanmar lake in transition. *Lakes & Reservoirs: Research & Management,* 5**,** 49-54.

ŠVANYS, A., EIGEMANN, F., GROSSART, H.-P. & HILT, S. 2016. Microcystins do not necessarily lower the sensitivity of *Microcystis aeruginosa* to tannic acid. *FEMS microbiology letters,* 363.

SWE, L. L. W. 2019. Overview of Water Pollution in Myanmar. A briefing Note. A regional fellowship program. *© 2019 Parliamentary Institute of Cambodia (PIC)*.

TESTAI, E., BURATTI, F. M., FUNARI, E., MANGANELLI, M., VICHI, S., ARNICH, N., BIRÉ, R., FESSARD, V. & SIALEHAAMOA, A. 2016. Review and analysis of occurrence, exposure and toxicity of cyanobacteria toxins in food. *EFSA Supporting Publications,* 13**,** 998E.

TESTÉ, V., BRIAND, J.-F., NICHOLSON, B. C. & PUISEUX-DAO, S. 2002. Comparison of changes in toxicity during growth of *Anabaena circinalis* (cyanobacteria) determined by mouse neuroblastoma bioassay and HPLC. *Journal of applied phycology,* 14**,** 399-407.

THAN, M. M. 2007. Community activities contribution to water environment conservation of Inle Lake. *Union Of Myanmar Ministry Of Agriculture And Irrigation, Irrigation Department. Myanmar*.

THIN, M. M., SACCHI, E. & SETTI, M. 2016. Hydrological processes at Inle Lake (Southern Shan State, Myanmar) inferred from hydrochemical, mineralogical and isotopic data. *Isotopes in environmental and health studies,* 52**,** 455-467.

THOMAZ, S., ESTEVES, F., MURPHY, K., DOS SANTOS, A., CALIMAN, A. & GUARIENTO, R. 2008. Aquatic macrophytes in the tropics: ecology of populations and communities, impacts of invasions and human use. *Tropical biology and conservation management,* 4**,** 27-60.

TIMMS, R. & MOSS, B. 1984. Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing, in the presence of zooplanktivorous fish, in a shallow wetland ecosystem. *Limnology and Oceanography,* 29**,** 472-486.

TRIEST, L., 1988. A revision of the genus *Najas* L. (Najadaceae) in the Old World (Najadaceae). Koninklijke Academie voor Overzeese Wetenschappen. Klass voor Natuur-en Geneeskundige Wetenschappen. Verhandelingen in-8. Nieuwe Reeks, Boek 22, afl. I, Brussel, p. 172 + plates.

TRUNG, B., DAO, T.-S., FAASSEN, E. & LÜRLING, M. 2018. Cyanobacterial blooms and microcystins in Southern Vietnam. *Toxins,* 10**,** 471

USUP, G., KULIS, D. M. & ANDERSON, D. M. 1994. Growth and toxin production of the toxic dinoflagellate *Pyrodinium bahamense* var. *compressum* in laboratory cultures. *Natural toxins,* 2**,** 254-262.

UTERMÖHL, H. 1958. Zur vervollkommnung der quantitativen phytoplankton-methodik: Mit 1 Tabelle und 15 abbildungen im Text und auf 1 Tafel. *Internationale Vereinigung für theoretische und angewandte Limnologie: Mitteilungen,* 9**,** 1-38.

VAIDYA, B. 1967. Study of some environmental factors affecting the occurrence of charophytes in western India. *Hydrobiologia,* 29**,** 256-262.

VAN APELDOORN, M. E., VAN EGMOND, H. P., SPEIJERS, G. J. & BAKKER, G. J. 2007. Toxins of cyanobacteria. *Molecular nutrition & food research,* 51**,** 7-60.

VAN DONK, E. & VAN DE BUND, W. J. 2002. Impact of submerged macrophytes including charophytes on phyto-and zooplankton communities: allelopathy versus other mechanisms. *Aquatic botany,* 72**,** 261-274.

VAN DONK, E., GULATI, R.D., IEDEMA, A., MEULEMANS, J.T., 1993. Macrophyte-related shifts in the nitrogen and phosphorus contents of the different trophic levels in a biomanipulated shallow lake. Hydrobiologia 251, 19–26. https://doi.org/10.1007/ bf00007160.

VANDERLEY, R. F., GER, K. A., BECKER, V., BEZERRA, M. G. T. & PANOSSO, R. 2021. Abiotic factors driving cyanobacterial biomass and composition under perennial bloom conditions in tropical latitudes. *Hydrobiologia,* 848**,** 943-960.

VESTERGAARD, O. & SAND-JENSEN, K. 2000. Alkalinity and trophic state regulate aquatic plant distribution in Danish lakes. *Aquatic Botany,* 67**,** 85-107.

VÉZIE, C., RAPALA, J., VAITOMAA, J., SEITSONEN, J. & SIVONEN, K. 2002. Effect of nitrogen and phosphorus on growth of toxic and nontoxic Microcystis strains and on intracellular microcystin concentrations. *Microbial ecology***,** 443-454.

WATANABE, M. F., TSUJIMURA, S., OISHI, S., NIKI, T. & NAMIKOSHI, M. 2003. Isolation and identification of homoanatoxin-a from a toxic strain of the cyanobacterium *Raphidiopsis mediterranea* Skuja isolated from Lake Biwa, Japan. *Phycologia,* 42**,** 364-369.

WELKER, M., CHORUS, I., FASTNER, J., KHAN, S., HAQUE, M. M., ISLAM, S. & KHAN, N. H. 2005. *Microcystins* (cyanobacterial toxins) in surface waters of rural Bangladesh: Pilot study. *Journal of Water and Health,* 3**,** 325-337

WFD 2000. The EU water framework directive-integrated river basin managment for Europe-directive 2000/60/EC. *[accessed 2020 Jul 03].* https://ec.europa.eu/environment/water/water-framework/index_en.html.

WHO 1999. *Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management,* E & FN Spon An imprint of Routledge, CRC Press.

WHO 2019. Cyanobacterial toxins: anatoxin-a and analogues. World Health Organization.

WHO 2020a. Cyanobacterial toxins: microcystins. World Health Organization.

WHO 2020b. Cyanobacterial toxins: cylindrospermopsis. World Health Organization.

WIEGLEB, G. 1990. The importance of stem anatomical characters for the systematics of the genus Potamogeton L. *Flora,* 184**,** 197-208.

WIEGLET, G. & KAPLAN, Z. 1998. An account of the species of *Potamogeton L.* (Potamogetonaceae). *Folia Geobotanica,* 33 (3)**,** 241-316. https://doi.org/10.1007/BF03216205

WIESE, M., D'AGOSTINO, P. M., MIHALI, T. K., MOFFITT, M. C. & NEILAN, B. A. 2010. Neurotoxic alkaloids: saxitoxin and its analogs. *Marine drugs,* 8**,** 2185-2211.

WILLIAMS, A. E., 1999. Effects of coarse fish in shallow lake ecosystems: an ecological and sociological appraisal. Ph.D. Thesis, University of Liverpool. Winfield, I. J., G. Peirson, M. Cryer & C. R. Townsend, 1983. The behavioural basis of prey selection by underyearling bream (Abramis brama (L.)) and roach (Rutilus rutilus (L.)). Freshwat. Biol. 13: 139–149

WIMMER, K. M., STRANGMAN, W. K. & WRIGHT, J. L. 2014. 7-Deoxy-desulfo-cylindrospermopsin and 7-deoxy-desulfo-12-acetylcylindrospermopsin: Two new cylindrospermopsin analogs isolated from a Thai strain of Cylindrospermopsis raciborskii. *Harmful Algae,* 37**,** 203-206.

WOJCIECHOWSKI, J., BURDA, T. M., SCHEER, M. B., COSTA, E. A. D. D. & FERNANDES, L. F. 2018. Potential effects of mechanically removing macrophytes on the phytoplankton community of a subtropical reservoir. *Acta Botanica Brasilica,* 32**,** 588-594.

WOOD, R.D. & IMAHORI, K., 1965. A Revision of the Characeae: Monograph of the Characeae. Part I & II. Verlag J. Cramer, Weinhein, Germany.

WOOD, S. A., RASMUSSEN, J. P., HOLLAND, P. T., CAMPBELL, R. & CROWE, A. L. 2007a. FIRST REPORT OF THE CYANOTOXIN ANATOXIN-A FROM APHANIZOMENON ISSATSCHENKOI (CYANOBACTERIA) 1. *Journal of Phycology,* 43**,** 356-365.

WU, T., WANG, Z., NIU, C., ZHANG, Y., LI, B. & LI, P. 2015. The effect of intense hydrodynamic disturbance on chromophoric dissolved organic matter in a shallow eutrophic lake. Journal of Freshwater Ecology, 30 (1), 143–156.

WU, X., PAN, J., REN, W., YANG, J. & LUO, L. 2021. The effects of water depth on the growth of two submerged macrophytes in an in situ experiment. *Journal of Freshwater Ecology,* 36**,** 271-284.

XIE, Y., REN, B. & LI, F. 2009. Increased nutrient supply facilitates acclimation to high-water level in the marsh plant *Deyeuxia angustifolia*: the response of root morphology. *Aquatic Botany,* 91**,** 1-5.

YASIN, H.; USMAN, M.; RASHID, H.; NASIR, A., and RANDHAWA, I.A., 2017b. Alternative approaches for solid waste management: A case study in Faisalabad Pakistan. Earth Sciences Pakistan, 1(2), 7–9.

YE, B., CHU, Z., WU, A., HOU, Z. & WANG, S. 2018. Optimum water depth ranges of dominant submersed macrophytes in a natural freshwater lake. *PLoS One,* 13**,** e0193176.

YU, J., LIU. Z., HE. H., ZHEN. W., GUAN. B., CHEN. F., LI. K., ZHONG. P., TEIXEIRA-DE MELLO. F., JEPPESEN, E. 2016. Submerged macrophytes facilitate dominance of omnivorous fish in a subtropical shallow lake: implications for lake restoration. Hydrobiologia. DOI 10.1007/s10750-016-2717-7

YU, L. & YU, D. 2009. Responses of the threatened aquatic plant *Ottelia alismoides* to water level fluctuations. *Fundamental and applied limnology,* 174**,** 295-300.

YUASA, T., MANGO, G., SHIBATA, K. & MATSUMARU, R. 2019. Current Water Quality of Inle Lake in Myanmar; A Potential Threat to the Lake Environment. The Second International Conference of International Alliance for Sustainable Urbanization and Regeneration (IASUR) 2009.

YUNES, J. S., DE LA ROCHA, S., GIROLDO, D., SILVEIRA, S. B. D., COMIN, R., BICHO, M. D. S., MELCHER, S. S., SANT'ANNA, C. L. & VIEIRA, A. A. H. 2009. Release of carbohydrates and proteins by a subtropical strain of *Raphidiopsis brookii* (Cyanobacteria) able to produce saxitoxin at three nitrate concentrtions. *Journal of phycology,* 45**,** 585-591.

ZANCHETT, G. & OLIVEIRA-FILHO, E. C. 2013. Cyanobacteria and cyanotoxins: from impacts on aquatic ecosystems and human health to anticarcinogenic effects. *Toxins,* 5**,** 1896-1917.

ŽEGURA, B., ŠTRASER, A. & FILIPIČ, M. 2011. Genotoxicity and potential carcinogenicity of cyanobacterial toxins–a review. *Mutation Research/Reviews in Mutation Research,* 727**,** 16-41.

ZENG, L., HE, F., ZHANG, Y., LIU, B., DAI, Z., ZHOU, Q. & WU, Z. 2017. How Submerged Macrophyte Restoration Promotes a Shift of Phytoplankton Community in a Shallow Subtropical Lake? Polish Journal of Environmental Studies, 26(3), 1363-1373. https://doi.org/10.15244/pjoes/68228.

ZHANG, N. & ZANG, S. 2015. Characteristics of phytoplankton distribution for assessment of water quality in the Zhalong Wetland, China. *International journal of environmental science and technology,* 12**,** 3657-3664.

ZHANG, X., GUO, K., LU, C., AWAIS, R. M., JIA, Y., ZHONG, L., LIU, P., DONG, R., LIU, D. & ZENG, W. 2020. Effects of origin and water depth on morphology and reproductive modes of the submerged plant Vallisneria natans. *Global Ecology and Conservation,* 24**,** e01330.

ZHOU, N., HU, W., DENG, J., ZHU, J., XU, W. & LIU, X. 2017. The effects of water depth on the growth and reproduction of Potamogeton crispus in an in situ experiment. *Journal of Plant Ecology,* 10**,** 546-558.

ZHOU, Y., ZHOU, X., HAN, R., XU, X., WANG, G., LIU, X., BI, F. & FENG, D. 2017. Reproduction capacity of *Potamogeton crispus* fragments and its role in water purification and algae inhibition in eutrophic lakes. *Science of the Total Environment,* 580**,** 1421-1428.

ZHU, H., LIU, X. & CHENG, S. 2021. Phytoplankton community structure and water quality assessment in an ecological restoration area of Baiyangdian Lake, China. *International Journal of Environmental Science and Technology,* 18**,** 1529-1536.

ZHU, J., LIU, B., WANG, J., GAO, Y. & WU, Z. 2010. Study on the mechanism of allelopathic influence on cyanobacteria and chlorophytes by submerged macrophyte (*Myriophyllum spicatum*) and its secretion. *Aquatic Toxicology,* 98**,** 196-203.

ZHU, L., WU, Y., SONG, L. & GAN, N. 2014. Ecological dynamics of toxic *Microcystis* spp. and microcystin-degrading bacteria in Dianchi Lake, China. *Applied and environmental microbiology,* 80**,** 1874-1881.

## Paper 1

Ballot, A., Swe, T., Mjelde, M., Cerasino, L., Hostyeva, V. & Miles, C. O. (2020). Cylindrospermopsin- and Deoxycylindrospermopsin-Producing *Raphidiopsis raciborskii* and Microcystin-Producing *Microcystis* spp. in Meiktila Lake, Myanmar. *Toxins,* 12, 232.

*toxins*

MDPI

*Article*

# Cylindrospermopsin- and Deoxycylindrospermopsin-Producing *Raphidiopsis raciborskii* and Microcystin-Producing *Microcystis* spp. in Meiktila Lake, Myanmar

**Andreas Ballot [1,*], Thida Swe [1,2,3], Marit Mjelde [1], Leonardo Cerasino [4], Vladyslava Hostyeva [1] and Christopher O. Miles [5]**

1 Norwegian Institute for Water Research, Gaustadalléen 21, N-0349 Oslo, Norway; thida.swe@niva.no (T.S.); marit.mjelde@niva.no (M.M.); vladyslava.hostyeva@niva.no (V.H.)
2 Forest Research Institute, 15013 Yezin, Myanmar
3 Department of Natural Sciences and Environmental Health, University of South- Eastern Norway, Gullbringvegen 36, N-3800 Bø, Norway
4 Department of Sustainable Agro-ecosystem and Bioresources, Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38010 San Michele all'Adige, Italy; leonardo.cerasino@fmach.it
5 National Research Council, 1411 Oxford Street, Halifax, NS B3H 3Z1, Canada; christopher.miles@nrc-cnrc.gc.ca
* Correspondence: andreas.ballot@niva.no

Received: 12 March 2020; Accepted: 3 April 2020; Published: 7 April 2020


**Abstract:** Meiktila Lake is a shallow reservoir located close to Meiktila city in central Myanmar. Its water is used for irrigation, domestic purposes and drinking water. No detailed study of the presence of cyanobacteria and their potential toxin production has been conducted so far. To ascertain the cyanobacterial composition and presence of cyanobacterial toxins in Meiktila Lake, water samples were collected in March and November 2017 and investigated for physico-chemical and biological parameters. Phytoplankton composition and biomass determination revealed that most of the samples were dominated by the cyanobacterium *Raphidiopsis raciborskii*. In a polyphasic approach, seven isolated cyanobacterial strains were classified morphologically and phylogenetically as *R. raciborskii*, and *Microcystis* spp. and tested for microcystins (MCs), cylindrospermopsins (CYNs), saxitoxins and anatoxins by enzyme-linked immunosorbent assay (ELISA) and liquid chromatography–mass spectrometry (LC–MS). ELISA and LC–MS analyses confirmed CYNs in three of the five *Raphidiopsis* strains between 1.8 and 9.8 μg $mg^{-1}$ fresh weight. Both *Microcystis* strains produced MCs, one strain 52 congeners and the other strain 20 congeners, including 22 previously unreported variants. Due to the presence of CYN- and MC-producing cyanobacteria, harmful effects on humans, domestic and wild animals cannot be excluded in Meiktila Lake.

**Keywords:** Meiktila Lake; *Raphidiopsis*; *Microcystis*; cylindrospermopsin; deoxycylindrospermopsin; microcystin

**Key Contribution:** This study confirmed the production of CYN and deoxyCYN by *Raphidiopsis raciborskii* strains and numerous MCs by *Microcystis* strains isolated from Meiktila Lake in Myanmar. The MCs included many novel congeners demonstrated by LC–MS and chemical derivatization methods. Among these were the rarely reported L-Glu and L-dihydrotyrosine-containing congeners. This is the first finding of toxin-producing cyanobacteria in a Myanmar waterbody.

## 1. Introduction

Many lakes and reservoirs worldwide are affected by periodic cyanobacterial dominance or even cyanobacterial blooms. Such mass developments of cyanobacteria are typical for eutrophic conditions and are often induced by nutrient enrichment caused by increased agricultural, urban and industrial activities and are also expected to increase due to regional and global climate change [1]. Various cyanobacterial species forming such blooms are potential producers of hepatotoxic or neurotoxic compounds and their presence is often associated with animal poisonings and a threat to human health [2].

Myanmar is characterized by the presence of several natural lakes and numerous man-made reservoirs. Meiktila Lake is one of the numerous reservoirs in Myanmar and was built in ancient times, dating from an unknown period [3] but most likely in the reign of King Narapathisithu (1173–1210) [4]. Today the lake is divided by a dam into a northern and a southern part (Figure 1) [5]. Meiktila Lake is exposed to sedimentation due to deforestation in the catchment and especially the northern part has been partially filled with sediment over a period of more than 100 years [6]. The priority use of water from Meiktila Lake is drinking water, water for domestic purposes and for irrigation, although the lake is also polluted with domestic waste water, street runoff and solid waste [6,7].

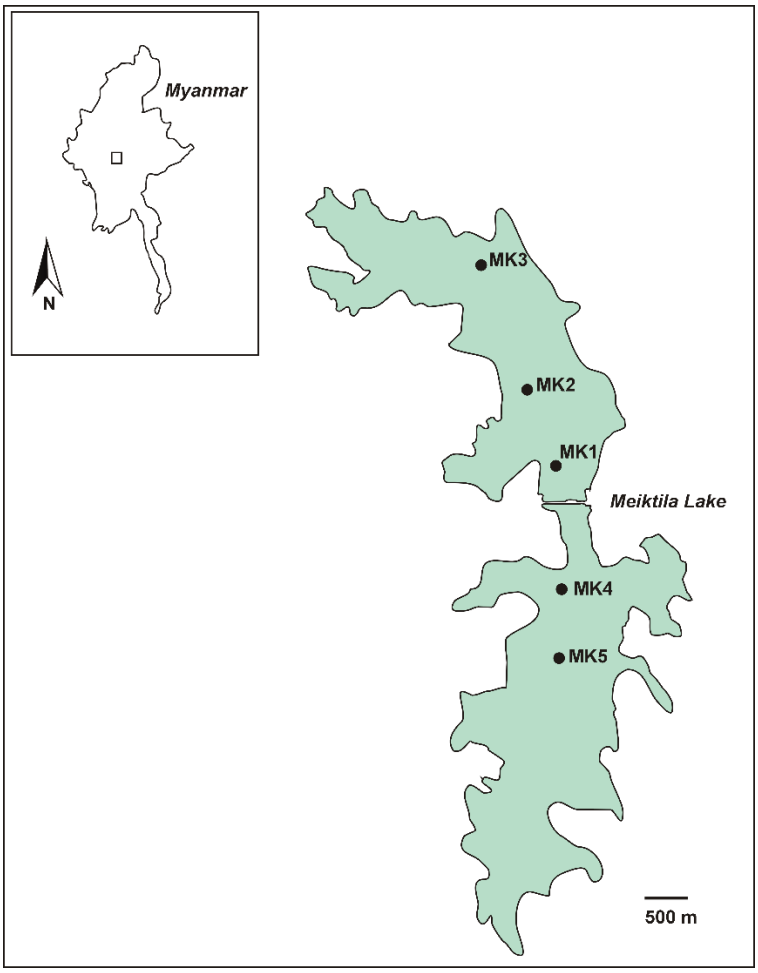



**Figure 1.** Map of Meiktila Lake. The map shows the locations of water sampling (Stations MK1-MK5). The location of Meiktila Lake in Myanmar is shown in the inset.

Only limited information is available about the limnological characteristics of Meiktila Lake and other freshwater habitats in Myanmar. In 1995, a study of the algal flora of Meiktila Lake was reported [4]. A recent study described the investigation of physical parameters, macrophyte and phytoplankton

composition in the period 2011–2014 in Meiktila Lake [5]. Twenty taxa of aquatic macrophytes including helophytes have been documented in Meiktila Lake [5]. Several heterocytous cyanobacterial taxa, e.g., *Anabaena*, *Anabaenopsis* and *Calothrix* and a few nonheterocytous cyanobacterial taxa e.g., *Aphanocapsa*, *Chroococcus*, *Microcystis*, *Arthrospira* and *Oscillatoria* have been reported but not further investigated [4,5]. Neither study mentioned the presence of the cyanobacterium *Raphidiopsis raciborskii* (formerly *Cylindrospermopsis raciborskii*) (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno nor the presence of the microcystin (MC) and cylindropsermopsin (CYN) groups of cyanobacterial toxins, which are documented from many lakes in Asia [8–10].

We suspect that the number of cyanobacterial species documented to date in Meiktila Lake was underestimated and that various toxin-producing cyanobacteria were present in the cyanobacterial community. There is clearly a lack of information about cyanobacteria and the production of cyanobacterial toxins in Meiktila Lake and the recently described potentially toxic cyanobacterium *Microcystis* is most likely not the only potential toxin-producing cyanobacterium. The ongoing pollution of the lake suggests the potential for occurrence of more frequent severe cyanobacterial blooms, which would have a negative impact on the use of the lake for drinking water and domestic purposes by the residents. This study aimed therefore to investigate the presence of cyanobacteria and their potential toxins in Meiktila Lake, applying modern analytical methods in a polyphasic approach to elucidate in detail the cyanobacterial composition, phylogeny and toxin production and toxin profiles.

## 2. Results

### 2.1. Physico-Chemical Parameters

At both sampling dates in March and November 2017, all sampling points in Meiktila Lake were characterized by water temperatures of 26.2–28.0 °C, pH of 8.5–9.3 and conductivities of 580–729 µS $cm^{-1}$. Secchi depth was between 0.8 m (at sampling point MK1) and 1.8 m (at MK3). Total phosphorus and total nitrogen concentrations were 12–23 and 360–570 µg $L^{-1}$, respectively.

### 2.2. Phytoplankton Community

At all sampling stations, and at both sampling dates, cyanobacteria were the dominant group in the phytoplankton in both parts of Meiktila lake together with diatoms (Bacillariophyceae) Cryptophyceae, Chlorophyceae and Euglenophyceae (Table 1). The most dominant cyanobacterium was *R. raciborskii*, which comprised biomasses between 0.2 and 1.9 mg $L^{-1}$ fresh weight (FW), or 27%–91% of the cyanobacterial biomass at the sampling points MK1, MK2, MK4 and MK5. Other cyanobacteria present in the samples belonged to the genera *Aphanocapsa*, *Aphanothece*, *Chroococcus*, *Merismopedia*, *Limnothrix*, *Microcystis*, *Planktolyngbya*, *Planktothrix*, *Sphaerospermopsis* and *Synechococcus*. They together comprised biomasses between 0.06 and 1.2 mg $L^{-1}$ cyanobacterial wet weight. At MK3, the biomass of *R. raciborskii* at both sampling dates (0.02–0.08 mg $L^{-1}$) was lower than at the other sampling points MK1, MK2, MK4 and MK5 (0.20–1.94 mg $L^{-1}$) (data not shown). The *Microcystis* biomass was lower than the *Raphidiopsis* biomass at all sampling points and sampling dates and ranged from 0.003 to 0.16 mg $L^{-1}$ (data not shown).

**Table 1.** Biomass (mg $L^{-1}$ FW) of phytoplankton groups at sampling points MK1–MK5 in Meiktila Lake in March and November of 2017.

| Sampling Point | MK1 | MK 1 | MK 2 | MK 2 | MK 3 | MK 3 | MK 4 | MK 4 | MK 5 | MK 5 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Sampling Date** **Phytoplankton Group** | **Mar** | **Nov** | **Mar** | **Nov** | **Mar** | **Nov** | **Mar** | **Nov** | **Mar** | **Nov** |
| Bacillariophyceae | 0.525 | 0.149 | 1.316 | 0.101 | 0.035 | 0.301 | 0.083 | 0.064 | 0.088 | 0.059 |
| Chlorophyceae | 0.143 | 0.055 | 0.182 | 0.146 | 0.102 | 0.045 | 0.101 | 0.102 | 0.107 | 0.165 |
| Chrysophyceae | 0.011 | 0 | 0.002 | 0 | 0.045 | 0.021 | 0.006 | 0.007 | 0 | 0.004 |
| Conjugatophyceae | 0.099 | 0.006 | 0.141 | 0 | 0.013 | 0 | 0.008 | 0.017 | 0.045 | 0 |
| Cryptophyceae | 0.023 | 0.132 | 0.007 | 0.170 | 0.155 | 0.299 | 0.127 | 0.275 | 0.106 | 0.138 |
| Cyanobacteria | 1.063 | 1.965 | 1.640 | 1.050 | 0.606 | 0.078 | 1.064 | 1.990 | 0.751 | 2.397 |
| Dinophyceae | 0.186 | 0.005 | 0.222 | 0 | 0.285 | 0 | 0.084 | 0.006 | 0.078 | 0 |
| Euglenophyceae | 0.255 | 0.029 | 0.159 | 0.005 | 0.014 | 0.050 | 0.009 | 0.002 | 0.053 | 0.021 |
| Eustigmatophyceae | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.002 | 0 | 0 |
| Klebsormidiophyceae | 0 | 0 | 0 | 0 | 0 | 0.023 | 0 | 0 | 0.002 | 0 |
| Prymnesiophyceae | 0 | 0.002 | 0.000 | 0.002 | 0.003 | 0.004 | 0.002 | 0.003 | 0.008 | 0.007 |
| Trebouxiophyceae | 0 | 0 | 0.007 | 0 | 0 | 0.005 | 0.005 | 0 | 0 | 0.001 |
| Xanthophyceae | 0 | 0 | 0.008 | 0 | 0.005 | 0 | 0.003 | 0.001 | 0 | 0 |
| Total | 2.306 | 2.344 | 3.684 | 1.474 | 1.264 | 0.827 | 1.493 | 2.467 | 1.238 | 2.790 |

### 2.3. Morphological and Phylogenetic Characterization

Seven potentially toxin-producing cyanobacterial strains were isolated from Meiktila Lake (Table 2). Based on morphological features, e.g., presence and form of colonies or filaments, vegetative cells and heterocytes, five of the isolated cyanobacterial strains were identified as *R. raciborskii* and two strains as *M. aeruginosa* and *M. novacekii*, respectively (Figure 2). However, Harke et al. [11] suggested all *Microcystis* warrant placement into the same species complex. Therefore, we use "*Microcystis*" instead of species names in the following parts of the manuscript.

**Table 2.** Strains isolated from Meiktila Lake, strain codes and European Nucleotide Archive (ENA) accession numbers.

| Species | Strain | Accession nr. 16S rRNA Gene |
|---|---|---|
| *Raphidiopsis* | | |
| *R. raciborskii* | AB2017/05 | LR590626 |
| *R. raciborskii* | AB2017/09 | LR590627 |
| *R. raciborskii* | AB2017/12 | LR590628 |
| *R. raciborskii* | AB2017/13 | LR590629 |
| *R. raciborskii* | AB2017/16 | LR746263 |
| *Microcystis* | | |
| *Microcystis* | AB2017/14 | LR590630 |
| *Microcystis* | AB2017/15 | LR590631 |

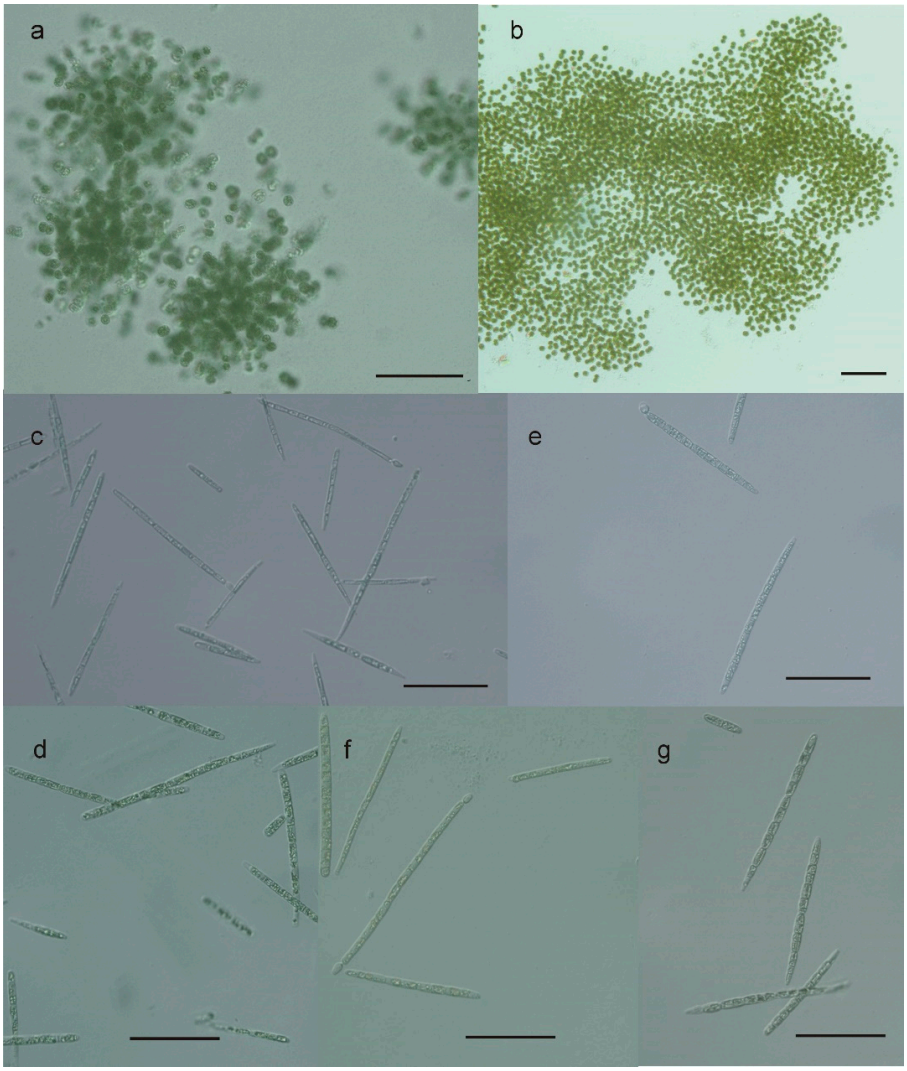



**Figure 2.** Micrographs of cyanobacteria investigated in this study. (**a**) *Microcystis novacekii* (AB2017/14); (**b**) *Microcystis aeruginosa* (AB2017/15); (**c**) *Raphidiopsis raciborskii* (AB2017/05); (**d**) *Raphidiopsis raciborskii* (AB2017/09); (**e**) *Raphidiopsis raciborskii* (AB2017/12); (**f**) *Raphidiopsis raciborskii* (AB2017/13); (**g**) *Raphidiopsis raciborskii* (AB2017/16). Scale bars indicate 50 µm.

The *Raphidiopsis* strains were mostly characterized by straight tapered filaments. The filament length and width varied between 8.8–90 × 1.9–5.8 µm. Heterocytes were observed in some filaments of all isolated strains. Akinetes were not observed in any of the investigated strains. As in the cultured strains, only a few of the filaments possessed heterocytes in the environmental samples. The two *Microcystis* strains were characterized by cell diameters ranging from 4.2 to 6.6 µm (strain AB2017/15) and from 3.7 to 5.8 µm (strain AB2017/14) (data not shown).

The morphological determination of the isolated strains was supported by phylogenetic analyses (Figure 3; Figure 4). Phylogenetic relationships of the investigated strains are presented in the maximum-likelihood (ML) tree of the 16S rRNA gene of *Cylindrospermopsis*/*Raphidiopsis* (Figure 3) and a separate ML tree of the *Microcystis* 16S rRNA gene (Figure 4). In the ML tree in Figure 3, the *Raphidiopsis* strains from Meiktila Lake grouped together with 16S rRNA gene sequences derived from *Cylindrospermopsis* and *Raphidiopsis* strains from Asia, Europe, Africa, Australia and North America (cluster I). The CYN-producing and nonCYN-producing *Raphidiopsis* strains from Meiktila Lake could not be distinguished phylogenetically using 16S rRNA gene and had similar 16 rRNA gene sequences (Figure 3). In cluster II, strains from North and South America, (USA, Mexico, Brazil), North

Africa (Tunisia), Southwest Europe (Spain) and New Zealand, grouped together. Both *Microcystis* strains from Meiktila Lake possessed similar 16S rRNA gene sequences and clustered together with 16S rRNA gene sequences of *Microcystis* from Europe, Asia, Africa and South America (Figure 4).

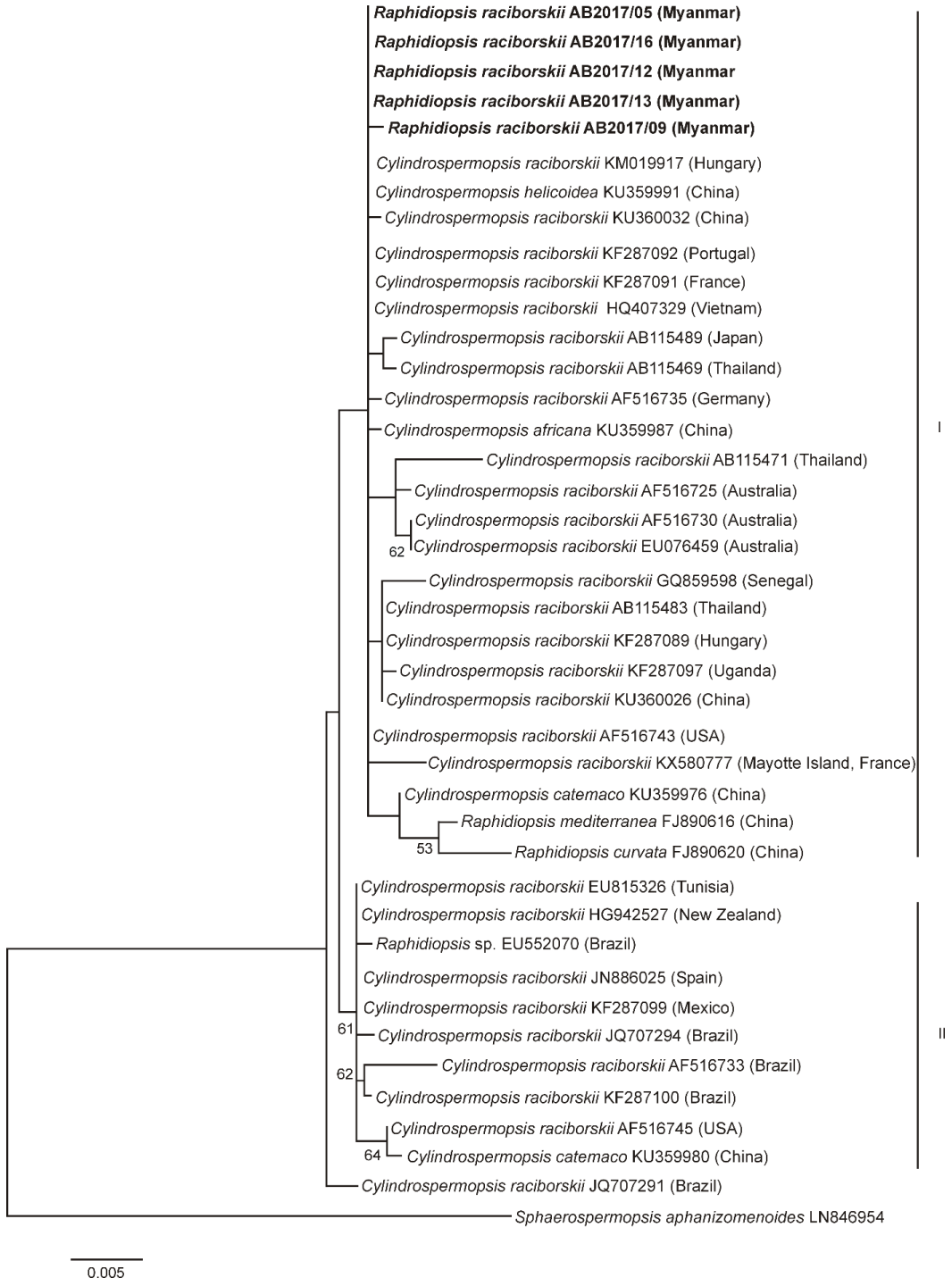



**Figure 3.** ML tree based on partial 16S rRNA gene sequences of 40 *Raphidiopsis*/*Cylindrospermospis* strains. Outgroup = *Sphaerospermopsis aphanizomenoides* (LN846954). Cluster I includes *Cylindrospermopsis* and *Raphidiopsis* strains from Asia, Europe, Africa, Australia and North America, cluster II includes strains from North and South America (USA, Mexico, Brazil), North Africa (Tunisia), Southwest Europe (Spain) and New Zealand. Strains from this study are marked in bold. Bootstrap values above 50 are included. The scale bar indicates 0.5% sequence divergence.

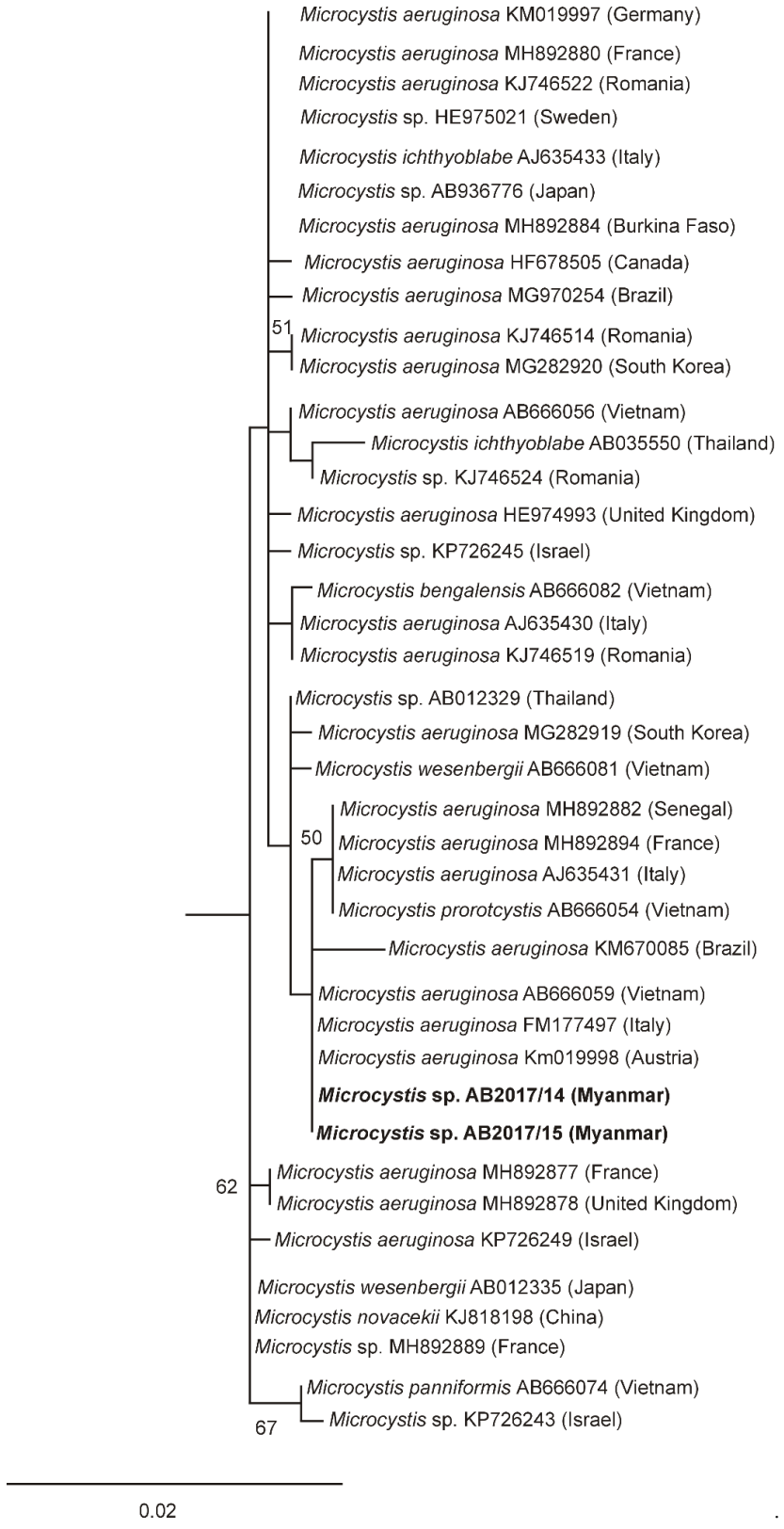



**Figure 4.** ML tree based on partial 16S rRNA gene sequences of 40 *Microcystis* strains. Outgroup = *Chroococcus subviolaceus* (MF072353). Strains from this study are marked in bold. Bootstrap values above 50 are included. The scale bar indicates 2% sequence divergence.

### 2.4. Identification of Cyanobacterial Toxins and Toxin-Producing Strains

Three of the five investigated *Raphidiopsis* strains produced CYNs in variable amounts by either enzyme-linked immunosorbent assay (ELISA) or liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Table 3). Concentrations of CYNs were 1.8–4.3 μg $mg^{-1}$ FW by ELISA. Using LC–MS/MS, CYN concentrations of 1.7–2.5 μg $mg^{-1}$ FW and deoxyCYN from 1.3 to 7.3 μg $mg^{-1}$ FW were detected. In the three CYN-producing strains, deoxyCYN comprised 43%–75% of the total CYNs.

**Table 3.** Concentrations (μg $mg^{-1}$ FW) of CYNs by ELISA and of CYN and deoxyCYN by LC–MS/MS in cultured *R. raciborskii* strains isolated from Meiktila Lake*.

| Strain | ELISA | LC-MS/MS | | | |
|---|---|---|---|---|---|
| | CYNs | CYN | deoxyCYN | CYN (%) | deoxyCYN (%) |
| AB2017/09 | 2.18 | - | - | 38 | 62 |
| AB2017/05 | n.d. | n.d. | n.d. | n.d. | n.d. |
| AB2017/16 | 1.84 | 1.65 | 1.25 | 57 | 43 |
| AB2017/12 | n.d. | n.d. | n.d. | n.d. | n.d. |
| AB2017/13 | 4.31 | 2.46 | 7.29 | 25 | 75 |

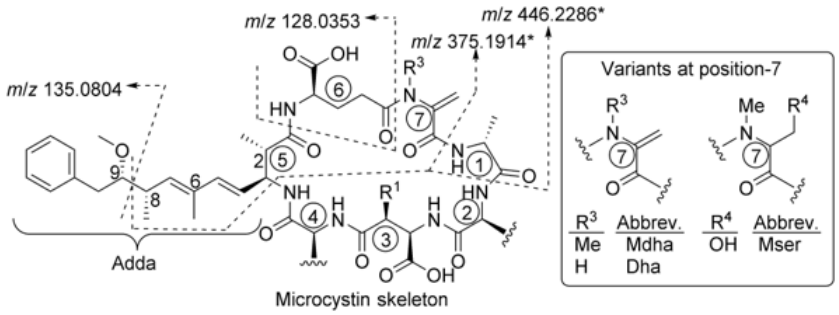
*- = biomass not determined; n.d. = not detected; FW = fresh weight; percentages are of total CYNs by LC–MS/MS.

All investigated *Raphidiopsis* strains tested negative for saxitoxins (STXs), anatoxins (ATXs) and MCs by ELISA. Both *Microcystis* strains tested negative for CYNs, STXs and ATXs by ELISA but were identified as MC-producers by ELISA and their MC profiles were therefore investigated by high resolution LC–MS/MS (LC–HRMS/MS).

Underivatized samples were analysed by LC-HRMS/MS in positive and negative ionisation modes as previously described [12,13] and then after reaction with mercaptoethanol (targeting $Mdha^7$/$Dha^7$ moieties in MCs) [14] and Oxone/DMSO (targets sulfide groups in methionine and Cys/GSH conjugates of MCs) [15]. Results of these analyses are summarised in Figure 5, Table 4 and Table S1. Peaks from putative MCs were identified by their reaction with mercaptoethanol, production of characteristic product ions in data-dependent and/or data-independent acquisition (DDA and/or DIA) LC-MS/MS screens, and possessing plausible potential elemental formulae based on both positive and negative mode full scan HRMS. These peaks were then targeted by LC-HRMS/MS at suitable collision energies to obtain structurally informative HRMS/MS spectra to assist with identification, compared by LC–HRMS with samples containing some of the putative MCs, and subjected to selective oxidation to detect the presence of sulfide moieties that could be present in some of the MCs.

Peaks were only considered to be MCs if they: 1, showed apparent pseudo-molecular ions appropriate for a MC in both positive and negative ionisation modes; 2, showed one or more of the characteristic MC fragments shown in Figure 5; 3, displayed appropriate chemical reactivity for the putative structure, and; 4, displayed retention times ($t_R$) and charge states ($z$) appropriate to the putative structure (e.g., based on the apparent number of polar and charged residues, such as Arg). MCs were considered "confirmed" (**1**, **3**, **13**, **14**, **17**, **18**, **21**, **25**, **26** and **41**) if they behaved identically in all respects to the standards (Table 4). Structures were considered "probable" if they behaved identically in all respects to a compound already identified with high probability in an available sample (**2**, **4**, **8**, **11**, **12**, **20**, **28**, **29**, **36**, **45** and **50**). For compounds for which standards or appropriate samples were not available, these were regarded as "probable" if, in addition to displaying the appropriate physical and chemical characteristics (Table 4 and Table S1), they also displayed interpretable MS/MS spectra that were clearly consistent with the proposed structure by comparison with related compounds (**5**–**7**, **9**, **10**, **15**, **16**, **24**, **27**, **30**–**32**, **37**–**40**, **42**–**44**, **46**–**49**, **51**, **53** and **54**). Compounds were considered tentative if there was limited MS/MS spectral evidence (**23**) or if the evidence was ambiguous (e.g., several isomers were present that showed indistinguishable MS/MS spectra, i.e., **52**, **55** and **56**). Compounds designated "unidentified" were definitively identified as MCs, but the spectral data was insufficient

to identify them (**19**, **22** and **33**–**35**). All compounds listed in Table 4 as containing Mdha[7] and which gave adequate signal-to-noise in their MS/MS spectra in positive mode, showed product ions at *m*/*z* 135.0804, 375.1914 and 446.2286, indicative of the presence of Adda[5]–D-Glu[6]–Mdha–D-Ala[1], while in those listed as containing Mser[7] or Dha[7] the latter two product ions were heavier, or lighter, by a mass corresponding to $H_2O$ or $CH_2$, respectively (all with $\Delta m < 5$ ppm), and the presence of these units is implicit in the discussion of the structural elucidation in Section 3.




| | | ② | ④ | ⑦ | $R^1$ | $[M-H]^-$ *m/z* | | | ② | ④ | ⑦ | $R^1$ | $[M-H]^-$ *m/z* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | [D-Asp[3]]MC-RR | Arg | Arg | Mdha | H | 1022.5429 | **29** | MC-RAbu | Arg | Abu | Mdha | Me | 965.5102 |
| **2** | [Dha[7]]MC-RR | Arg | Arg | Dha | Me | 1022.5429 | **30** | MC-(H2)YG | (H2)Tyr | Gly | Mdha | Me | 946.4568 |
| **3** | MC-RR | Arg | Arg | Mdha | Me | 1036.5585 | **31** | MC-RM | Arg | Met | Mdha | Me | 1011.4979 |
| **4** | [D-Asp[3]]MC-ER | Glu | Arg | Mdha | H | 995.4844 | **32** | MC-(H2)YA | (H2)Tyr | Ala | Mdha | Me | 960.4724 |
| **5** | MC-LR-Cys conjugate | Leu | Arg | Mlan | Me | 1114.5612 | **36** | [D-Asp[3]]MC-EE | Glu | Glu | Mdha | H | 968.4259 |
| **6** | MC-(H2)YR | (H2)Tyr | Arg | Mdha | Me | 1045.5364 | **37** | MC-EE | Glu | Glu | Mdha | Me | 982.4415 |
| **7** | [D-Asp[3]]MC-(H2)YR | (H2)Tyr | Arg | Mdha | H | 1031.5208 | **38** | [D-Asp[3]]MC-LE | Leu | Glu | Mdha | H | 952.4673 |
| **8** | [D-Asp[3]]MC-(H4)YR | (H4)Tyr | Arg | Mdha | H | 1033.5364 | **39** | [D-Asp[3]]MC-LA | Leu | Ala | Mdha | H | 894.4618 |
| **9** | MC-ER | Glu | Arg | Mdha | Me | 1009.5000 | **40** | MC-LE | Leu | Glu | Mdha | Me | 966.4830 |
| **10** | [D-Asp[3]]MC-YR | Tyr | Arg | Mdha | H | 1029.5051 | **41** | MC-LA | Leu | Ala | Mdha | Me | 908.4775 |
| **11** | MC-(H4)YR | (H4)Tyr | Arg | Mdha | Me | 1047.5521 | **42** | MC-HilA | Hil | Ala | Mdha | Me | 922.4931 |
| **12** | [Mser[7]]MC-LR | Leu | Arg | Mser | Me | 1011.5521 | **43** | MC-WA | Trp | Ala | Mdha | Me | 981.4727 |
| **13** | [D-Asp[3]]MC-LR | Leu | Arg | Mdha | H | 979.5258 | **44** | MC-FA | Phe | Ala | Mdha | Me | 942.4618 |
| **14** | MC-YR | Tyr | Arg | Mdha | Me | 1029.5051 | **45** | MC-LAbu | Leu | Abu | Mdha | Me | 922.4931 |
| **15** | MC-MR | Met | Arg | Mdha | Me | 1011.4979 | **46** | MC-HilAbu | Hil | Abu | Mdha | Me | 936.5088 |
| **16** | MC-Y(OMe)R | Tyr(OMe) | Arg | Mdha | Me | 1073.5313 | **47** | MC-WAbu | Trp | Abu | Mdha | Me | 995.4884 |
| **17** | MC-LR | Leu | Arg | Mdha | Me | 993.5415 | **48** | MC-FAbu | Phe | Abu | Mdha | Me | 956.4775 |
| **18** | [Dha[7]]MC-LR | Leu | Arg | Dha | Me | 979.5258 | **49** | MC-LV | Leu | Val | Mdha | Me | 936.5088 |
| **20** | MC-KynR | Kyn | Arg | Mdha | Me | 1070.5317 | **50** | MC-LL | Leu | Leu | Mdha | Me | 950.5244 |
| **21** | MC-HilR | Hil | Arg | Mdha | Me | 1007.5571 | **51** | MC-FV | Phe | Val | Mdha | Me | 970.4931 |
| **23** | MC-OiaR | Oia | Arg | Mdha | Me | 1082.5317 | **52** | *iso*-MC-LL | Leu | Leu | Mdha | Me | 950.5244 |
| **24** | [Asp[3]]MC-RE | Arg | Glu | Mdha | H | 995.4844 | **53** | MC-WV | Trp | Val | Mdha | Me | 1009.5040 |
| **25** | MC-FR | Phe | Arg | Mdha | Me | 1027.5258 | **54** | MC-WL | Trp | Leu | Mdha | Me | 1023.5197 |
| **26** | MC-WR | Trp | Arg | Mdha | Me | 1066.5367 | **55** | *iso*-MC-FV | Phe | Val | Mdha | Me | 970.4931 |
| **27** | MC-RE | Arg | Glu | Mdha | Me | 1009.5000 | **56** | *iso*-MC-WL | Trp | Leu | Mdha | Me | 1023.5197 |
| **28** | MC-RA | Arg | Ala | Mdha | Me | 951.4945 | | | | | | | |

**Figure 5.** Structures and exact negative ionisation *m*/*z* of microcystins (MCs) identified in cultures AB2017/14 and AB2017/15 in this study, showing characteristic product ions at *m*/*z* 135.0804 (positive) and 128.0353 (negative) LC–MS/MS spectra (see Table 4 and Table S1). The origins of additional positive mode product ions containing Mdha[7] ($R^3$ = Me) at *m*/*z* 375.1914 and 446.2286 are also shown. Note that the corresponding product ions containing Dha[7] ($R^3$ = H) have *m*/*z* 361.1758 and 432.2129, and *m*/*z* 393.2020 and 464.2391 for Mser[7] ($R^4$ = OH). A full version of this table including positive ionisation data is shown in the Supporting Information (Table S1). Abbreviations: Abu, aminobutyric acid; Dha, dehydroalanine; (H2)Tyr, dihydrotyrosine; (H4)Tyr, 4,5,6,7-tetrahydrotyrosine; Kyn, kynurenine; Mdha, *N*-methyldehydroalanine; Mlan, *N*-methyllanthionine; Mser, *N*-methylserine; Oia, oxindolylalanine; Tyr(OMe), methoxytyrosine.

**Table 4.** Identities of microcystins detected by LC-HRMS/MS analysis in *Microcystis* strains AB2017/14 and /15 isolated from Meiktila Lake, their retention times ($t_R$), concentrations, relative abundances (%) and observed *m*/*z* values in negative ionisation mode[a].

| | *m*/*z* | Compound Name | Confidence | $t_R$ (min) | Concentration[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | AB2017/14 | | AB2017/15 | |
| | | | | | µg g$^{-1}$ | % | µg g$^{-1}$ | % |
| **1** | 1022.5443 | [D-Asp$^3$]MC-RR[c] | Confirmed | 4.18 | 2.4 | 0.21 | 1002.4 | 7.21 |
| **2** | 1022.5454 | [Dha$^7$]MC-RR[c] | Probable | 4.53 | 0.1 | 0.01 | 31.8 | 0.23 |
| **3** | 1036.5597 | MC-RR[c] | Confirmed | 4.55 | 19.4 | 1.74 | 1458.0 | 10.49 |
| **4** | 995.4858 | [D-Asp$^3$]MC-ER[c] | Probable | 6.14 | ND | - | 119.6 | 0.86 |
| **5** | 1114.5657 | MC-LR–Cys[d] | Probable | 6.16 | 0.1 | 0.01 | ND | - |
| **6** | 1045.5378 | MC-(H2)YR[c] | Probable | 6.18 | 32.4 | 2.90 | ND | - |
| **7** | 1031.5224 | [D-Asp$^3$]MC-(H2)YR[c] | Probable | 6.20 | 32.9 | 2.95 | ND | - |
| **8** | 1033.5381 | [D-Asp$^3$]MC-(H4)YR[c] | Probable | 6.27 | 0.6 | 0.05 | 148.1 | 1.07 |
| **9** | 1009.5015 | MC-ER[c] | Probable | 6.55 | 0.6 | 0.05 | 37.8 | 0.27 |
| **10** | 1029.5072 | [D-Asp$^3$]MC-YR[c] | Probable | 6.59 | ND | - | 144.1 | 1.04 |
| **11** | 1047.5540 | MC-(H4)YR[c] | Probable | 6.64 | 6.4 | 0.57 | 310.8 | 2.24 |
| **12** | 1011.5530 | [Mser$^7$]MC-LR | Probable | 6.80 | 0.4 | 0.04 | 13.3 | 0.10 |
| **13** | 979.5273 | [D-Asp$^3$]MC-LR[c] | Confirmed | 6.89 | 40.8 | 3.65 | 1870.3 | 13.45 |
| **14** | 1043.5224 | MC-YR[c] | Confirmed | 6.99 | 9.1 | 0.81 | 317.4 | 2.28 |
| **15** | 1011.4999 | MC-MR[c,d,e] | Probable | 6.99 | 3.6 | 0.32 | ND | - |
| **16** | 1073.5329 | MC-Y(OMe)R[c] | Probable | 7.07 | 7.2 | 0.64 | ND | - |
| **17** | 993.5435 | MC-LR[c] | Confirmed | 7.12 | 183.0 | 16.38 | 3332.5 | 23.97 |
| **18** | 979.5289 | [Dha$^7$]MC-LR[c] | Confirmed | 7.13 | 0.8 | 0.07 | 37.4 | 0.27 |
| **19** | 1080.5170 | Unidentified MC[c] | Unidentified | 7.15 | 59.0 | 5.28 | ND | - |
| **20** | 1070.5332 | MC-KynR[c] | Probable | 7.35 | 4.5 | 0.40 | ND | - |
| **21** | 1007.5582 | MC-HilR[c] | Confirmed | 7.38 | 41.8 | 3.74 | 17.9 | 0.13 |
| **22** | 1080.5169 | Unidentified[c] | Unidentified | 7.38 | 59.6 | 5.34 | ND | - |
| **23** | 1082.5311 | MC-OiaR[c] | Tentative | 7.40 | 102.4 | 9.17 | ND | - |
| **24** | 995.4857 | [D-Asp$^3$]MC-RE[c] | Probable | 7.43 | ND | - | 110.1 | 0.79 |
| **25** | 1027.5269 | MC-FR[c] | Confirmed | 7.48 | 255.2 | 22.85 | ND | - |
| **26** | 1066.5380 | MC-WR[c] | Confirmed | 7.64 | 0.7 | 0.06 | ND | - |
| **27** | 1009.5015 | MC-RE[c] | Probable | 7.79 | 3.8 | 0.34 | 1281.0 | 9.21 |
| **28** | 951.4955 | MC-RA[c] | Probable | 8.24 | 13 | 1.16 | ND | - |
| **29** | 965.5116 | MC-RAbu[c] | Probable | 8.83 | 7.1 | 0.64 | ND | - |
| **30** | 946.4577 | MC-(H2)YG[c] | Probable | 9.06 | 5.4 | 0.48 | ND | - |
| **31** | 1011.4966 | MC-RM[c,d,e] | Probable | 9.23 | 0.2 | 0.02 | ND | - |
| **32** | 960.4738 | MC-(H2)YA[c] | Probable | 9.34 | 10.1 | 0.90 | ND | - |
| **33** | 953.4783 | Unidentified MC[c] | Unidentified | 9.50 | 12 | 1.07 | ND | - |
| **34** | 967.4942 | Unidentified MC[c] | Unidentified | 9.78 | 2.3 | 0.21 | ND | - |
| **35** | 984.4735 | Unidentified MC[c] | Unidentified | 9.96 | 3.7 | 0.33 | ND | - |
| **36** | 968.4272 | [D-Asp$^3$]MC-EE[c] | Probable | 11.43 | ND | - | 403.3 | 2.90 |
| **37** | 982.4432 | MC-EE[c] | Probable | 12.58 | 0.9 | 0.08 | 1533.5 | 11.03 |
| **38** | 952.4690 | [D-Asp$^3$]MC-LE[c] | Probable | 12.82 | 1.2 | 0.11 | 358.8 | 2.58 |
| **39** | 894.4631 | [D-Asp$^3$]MC-LA[c] | Probable | 13.32 | 6.5 | 0.58 | ND | - |
| **40** | 966.4849 | MC-LE[c] | Probable | 13.69 | 12.9 | 1.15 | 1376.1 | 9.90 |
| **41** | 908.4788 | MC-LA[c] | Confirmed | 14.87 | 44.8 | 4.01 | ND | - |
| **42** | 922.4942 | MC-HilA[c] | Probable | 15.29 | 5.9 | 0.53 | ND | - |
| **43** | 981.4737 | MC-WA[c] | Probable | 15.88 | 4.4 | 0.39 | ND | - |
| **44** | 942.4629 | MC-FA[c] | Probable | 15.95 | 42.3 | 3.79 | ND | - |
| **45** | 922.4943 | MC-LAbu[c] | Probable | 16.09 | 16.6 | 1.49 | ND | - |
| **46** | 936.5110 | MC-HilAbu[c] | Probable | 16.55 | 2.2 | 0.20 | ND | - |
| **47** | 995.4898 | MC-WAbu[c] | Probable | 17.20 | 13.4 | 1.20 | ND | - |
| **48** | 956.4784 | MC-FAbu[c] | Probable | 17.39 | 26.4 | 2.36 | ND | - |
| **49** | 936.5106 | MC-LV[c] | Probable | 17.62 | 3.6 | 0.32 | ND | - |
| **50** | 950.5254 | MC-LL[c] | Probable | 17.93 | 3.9 | 0.35 | ND | - |

**Table 4.** *Cont.*

| | *m/z* | Compound Name | Confidence | $t_R$ (min) | Concentration[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | AB2017/14 | | AB2017/15 | |
| | | | | | μg $g^{-1}$ | % | μg $g^{-1}$ | % |
| **51** | 970.4944 | MC-FV[c] | Probable | 18.40 | 0.7 | 0.06 | ND | - |
| **52** | 950.5262 | *iso*-MC-LL[c] | Tentative | 18.49 | 1.2 | 0.11 | ND | - |
| **53** | 1009.5041 | MC-WV[c] | Probable | 18.54 | 2.9 | 0.26 | ND | - |
| **54** | 1023.5210 | MC-WL[c] | Probable | 18.74 | 2.8 | 0.25 | ND | - |
| **55** | 970.4942 | *iso*-MC-FV[c] | Tentative | 18.79 | 2.1 | 0.19 | ND | - |
| **56** | 1023.5216 | *iso*-MC-WL[c] | Tentative | 19.16 | 1.3 | 0.12 | ND | - |

[a] A comprehensive version of this table, including positive and negative ionisation MS data, reactivity towards thiols and mild oxidising agents, number of rings plus double-bond equivalents (RDBE) and presence of characteristic ions observed in positive and negative ionisation MS/MS spectra, is in the Supporting Information (Table S1) together with LC–HRMS/MS spectra (Figures S1–S59). [b] Concentration expressed per weight of biomass (FW) and as a percentage of total microcystins detected in each culture); ND = not detected; [c] Reacted with mercaptoethanol; [d] Oxidised by Oxone/DMSO; [e] HRMS/MS spectrum of oxidation product obtained.

In culture AB2017/14, 52 microcystin congeners were detected by LC–HRMS, with a total concentration of 1100 μg $g^{-1}$ FW. Twenty-one of these were unidentified or previously unreported variants. In culture AB2017/15, 20 microcystin variants (of which six were previously unreported) were detected, with a total concentration of 14000 μg $g^{-1}$ FW. The microcystin variants and the concentrations found in each strain are shown in Table 4 and Table S1.

## 3. Discussion

This study clearly demonstrates for the first time the presence of CYN- and deoxyCYN-producing *R. raciborskii* and MC-producing *Microcystis* in the phytoplankton community of Meiktila Lake in Myanmar. The relatively high biomass of *R. raciborskii*, up to 1.9 mg $L^{-1}$ in the phytoplankton community of Meiktila Lake, is expected to cause elevated concentrations of CYNs in the lake water. The results suggest a higher risk for humans and animals to be affected by CYNs than by MCs, although this could be affected by variations in the biomass of CYN-producing *R. raciborskii* versus MC-producing *Microcystis*. Variations in cyanobacterial bloom composition and toxin production are influenced by abiotic factors such as nutrients, temperature and light and by biotic factors such as grazing, parasitism and predation [16,17]. The distribution of CYN/deoxyCYN-producing and nonproducing *Raphidiopsis* strains in Meiktila Lake is likely to vary over time, and dominance by a *Raphidiopsis* strain such as AB2017/13 would lead to CYN and deoxyCYN concentrations up to 20 μg $L^{-1}$ for the highest *Raphidiopsis* biomasses measured in this study. It is therefore expected that CYN/ deoxyCYN concentrations in the lake water will at times exceed the guideline value for CYN in drinking water of 1 μg $L^{-1}$ [18]. The tolerable daily intake value of 0.03 μg $kg^{-1}$ for a person of 70 kg body weight would be exceeded after the intake of slightly more than 100 mL of lake water if CYN and deoxyCYN are similarly toxic. However, the toxicity of deoxyCYN to humans is not yet clear. According to Norris et al. [19], deoxyCYN does not contribute significantly to the toxicity of *R. raciborskii*. In contrast, cell viability assays showed that deoxyCYN was only slightly less toxic than CYN and most likely operates by similar toxicological mechanisms [20]. The potential risk of deoxyCYN for humans needs therefore to be clarified [20]. The use of Meiktila Lake water for drinking water, irrigation, domestic purposes or animal consumption is complicated by the fact that an unknown proportion of CYNs can be extracellular and is therefore not eliminated by filtration. Lake water contaminated by CYN and other toxins like MC-LR can lead to morphological and physiological changes and potential loss of productivity by agricultural plants, and bioaccumulation of cyanotoxins in the tissues of edible terrestrial plants in a concentration-dependent manner has been reported [21].

Griffith and Saker [22] have shown that in stationary phase of cultures, more than 50% of CYN can be extracellular. In environmental samples, the same authors found that extracellular CYN could exceed 90%. Boiling in water does not significantly degrade CYN within 15 min [23]. The removal

of extracellular CYN/deoxyCYN therefore needs other methods, like the use of activated carbon, membrane filtration or chemical inactivation (Ultraviolet (UV), or oxidants) [24]. The presence of *Raphidiopsis* strains in Meiktila Lake that do not produce CYN makes it likely that CYN concentrations in the lake will vary considerably depending on the ratio of the two chemotypes in the phytoplankton community. As Meiktila Lake water is used for domestic purposes (drinking water, irrigation, washing of clothes, and personal hygiene), regular monitoring of cyanobacterial biomass and CYNs is recommended.

*R. raciborskii* has not been described from Myanmar water bodies and was not observed in a phytoplankton community study conducted in Meiktila Lake from 2011 to 2012 [5]. *Raphidiopsis* (and *Cylindrospermopsis*) spp., however, have been described from various other Southeast Asian freshwater habitats [25–27] and other water habitats worldwide [8]. *R. raciborskii* is only known to produce CYNs in Australia and the Asian countries China, Japan, Vietnam and Thailand and to produce STXs in Brazil [26,28–32]. The prime radiation centre of *R. raciborskii* is thought to be in Africa, with a second radiation centre in Australia [8]. Our 16S rRNA gene analysis confirms the close relationship of the *Raphidiopsis* strains from Meiktila Lake to other *Raphidiopsis* strains from Asia, Europe and Australia. Our 16S rRNA gene tree also clearly supports the suggested movement of *Raphidiopsis* from the American continents to Southwest Europe and North Africa and probably further to Greece and China, as has been described [33,34]. The close relationship of *Raphidiopsis* strains from Australia, Asia and Europe does not, however, explain why CYN- and deoxyCYN-producing strains have only been found in Asia and Australia but not in Europe, Africa or the Americas. Parts of, or the whole, CYN gene cluster could have been lost during the spread from Asia westwards, or only nonCYN-producing strains may have spread to Europe. Our finding of nontoxic *Raphidiopsis* strains in Meiktila Lake supports the latter hypothesis.

Both *Microcystis* strains AB 2017/14 and AB2017/15 isolated from Meiktila Lake are confirmed microcystin producers and are closely related to *Microcystis* strains from Africa, Europe and Asia based on 16S rRNA gene phylogeny. Both strains had identical 16S rRNA gene sequences but were clearly distinguished chemically by their MC congener profiles. Fifty-six microcystin variants were found in *Microcystis* strains AB2017/14 and AB2017/15 isolated from Meiktila Lake (Figure 6).

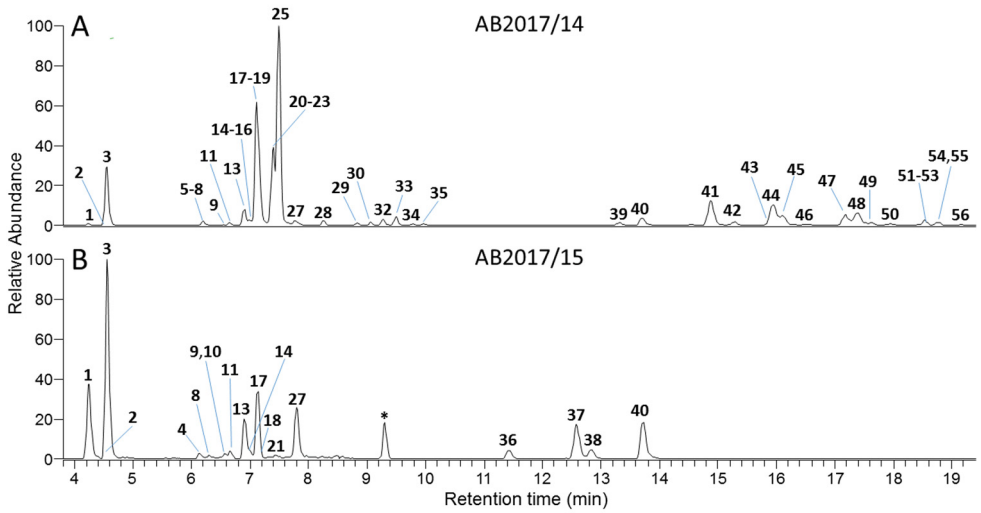



**Figure 6.** LC–HRMS full scan extracted ion chromatograms (3.8–19.4 min, positive ionization mode) of extracts from (**A**) *Microcystis* culture AB2017/14 and (**B**) *Microcystis* culture AB2017/15. Chromatograms were produced by extracting at *m*/*z* (± 5 ppm) for all MCs listed in Table 4 (see Table S1 for positive ionisation *m*/*z* values). Note that some of the smaller peaks are not labelled on the chromatograms, and the peak marked with an asterisk is not from a MC.

In order to reliably estimate the quantities of the MCs in the extracts by LC-HRMS, it was necessary to characterize, and if possible, identify all of them. The reason for this is that the response in LCMS can be expected to vary from congener-to-congener, primarily due to variations in the number of easily ionisable amino acid residues, especially Arg, present in the MC's structure. Only the identification of previously unreported MCs (see Bouaïcha et al. [35]) in the cultures is discussed further (i.e., **7**, **9**, **19**, **22**, **24**, **27**, **30**–**35**, **37**, **38**, **40**, **42**, **46**, **51**–**53**, **55** and **56**) but spectra of all compounds for which adequate MS/MS spectra were obtained are available in the Supporting Information.

Three of the compounds were sulfide-containing variants (**5**, **15**, and **31**) which reacted when the extract was oxidised with Oxone/DMSO (Table 4). The first two have been reported in cultures and blooms [15,36], and their characteristics were fully consistent with those reported for **5** and **15** here, and in the case of **15** its oxidation product (MC-M(O)R) showed characteristic product ions including neutral loss of $CH_4OS$ and displayed an MS/MS spectrum (Figures S8, S11 and S14) identical to that reported previously for MC-M(O)R [15]. The third of sulfide-containing MC was identified as MC-RM (**31**) based on its physical and chemical properties (Table 4), which were essentially identical to those of **15** except for its longer $t_R$ and that its MS/MS spectrum closely paralleled that of MC-RA (**28**) and displayed product ions characteristic of an MC with one Arg at position-2 rather than at the more common position-4 (Figures S8, S14 and S15). For example, fragments at *m/z* 440.2263 ($C_{18}H_{30}O_6N_7^+$, $\Delta m$ = 2.2 ppm, from Mdha$^7$–D-Ala$^1$–Arg$^2$–D-Masp$^3$) and 731.3716 ($C_{34}H_{51}O_{10}N_8^+$, $\Delta m$ = −0.9 ppm, from Adda$^5$–D-Glu$^6$–Mdha$^7$–D-Ala$^1$–Arg$^2$–D-Masp$^3$), together with the complete absence of a product ion at *m/z* 599.3552 (from Arg$^4$–Adda$^5$–D-Glu$^6$), confirmed Arg at position-2 and Met at position-4 (see Okello et al. [37] for assigned product ions from MC-YR) of **31**.

Eight of the compounds (**4**, **9**, **24**, **27**, **36**–**38** and **40**) showed characteristics of MCs containing one or more Glu residues at position-2 or -4. Two of these (**9** and **27**) had formulae consistent with MC-RE or MC-ER (Table 4). Compound **9** gave product ions (Figures S2 and S5–S7) typical of a MC with Arg at position-4, including *m/z* 599.3536 ($C_{31}H_{47}O_6N_6^+$, $\Delta m$ = −2.6 ppm, from Arg$^4$–Adda$^5$–D-Glu$^6$), 284.1238 ($C_{12}H_{18}O_5N_3^+$, $\Delta m$ = −1.1 ppm, from Mdha$^7$–D-Ala$^1$–Glu$^2$), and 286.1497 ($C_{11}H_{20}O_4N_5^+$, $\Delta m$ = −4.3 ppm, from D-Masp$^3$–Arg$^4$), indicating **9** to be MC-ER. The MS/MS spectrum of **27** (Figures S2 and S15) included product ions at *m/z* 440.2248 ($C_{18}H_{30}O_6N_7^+$, $\Delta m$ = −0.9 ppm, from Mdha$^7$–D-Ala$^1$–Arg$^2$–D-Masp$^3$), and 731.3678 ($C_{34}H_{51}O_{10}N_8^+$, $\Delta m$ = −6.1 ppm, from Adda$^5$–D-Glu$^6$–Mdha$^7$–D-Ala$^1$–Arg$^2$–D-Masp$^3$) which, together with the complete absence of a product ion at *m/z* 599.3552, confirmed Arg at position-2 and Glu at position-4 (see Okello et al. [37]) for assigned product ions from MC-RY), showing **27** to be MC-RE. The characteristics of **37** (Table 4) were consistent with MC-EE. In addition, **37** gave product ions (Figures S27–S33) including *m/z* 276.1189 ($C_{10}H_{18}O_6N_3^+$, $\Delta m$ = −0.4 ppm, from D-Masp$^3$–Glu$^4$), 405.1605 ($C_{15}H_{25}O_9N_4^+$, $\Delta m$ = −2.9. ppm, from Glu$^2$–D-Masp$^3$–Glu$^4$) and 575.2703 ($C_{28}H_{39}O_9N_4^+$, $\Delta m$ = −1.5 ppm, from Adda$^5$–D-Glu$^6$–Mdha$^7$–D-Ala$^1$–Glu$^2$) that confirmed **37** as MC-EE. Compound **40** was identified as MC-LE based on the characteristics presented in Table 4, as well as product ions (Figures S27–S33) observed in its MS/MS spectra, including *m/z* 460.2397 ($C_{19}H_{34}N_5O_8^+$, $\Delta m$ = −1.2, from D-Ala$^1$–Leu$^2$-D-Masp$^3$–Glu$^4$) and 397.2073 ($C_{18}H_{29}N_4O_6^+$, $\Delta m$ = −2.2, from Mdha$^7$–DAla$^1$–Leu$^2$-D-Masp$^3$), confirmed its identity as MC-LE (**40**). Earlier-eluting desmethylated D-variants of **9**, **27**, **37** and **40** were similarly identified as the corresponding D-Asp$^3$-congeners [D-Asp$^3$]MC-ER (**4**), [D-Asp$^3$]MC-RE (**24**), [D-Asp$^3$]MC-EE (**36**) and [D-Asp$^3$]MC-LE (**38**) based on analysis of their MS/MS spectra (Figures S21–S27) and characteristics presented in Table 4. Furthermore, the **4** and **36** in this sample coeluted with, and gave identical product ion spectra to, [D-Asp$^3$]MC-ER (**4**) and [D-Asp$^3$]MC-EE (**36**) identified [12] in an extract of a culture of *Planktothrix prolifica* NIVA-CYA544.

Twenty-one conventional late-eluting nonArg-containing MCs (**30**, **32** and **36**–**56**) were detected. Of these, the identities of four that contained Glu$^2$ or Glu$^4$ (**36**–**38** and **40**) were discussed above. The remaining previously unreported nonArg MCs were **30**, **32**, **42**, **46**, **51**–**53**, **55** and **56**. Compound **42** had the same characteristics as MC-LAbu (**45**) (Table 4), however, its MS/MS spectrum (Figures S34–S36) was consistent with MC-HilA. In particular, product ions at *m/z* 573.3270 ($C_{30}H_{45}N_4O_7^+$,

$\Delta m$ = −2.3 ppm, from Adda$^5$–D-Glu$^6$–Mdha$^7$–D-Ala$^1$–Hil$^2$ minus $C_9H_{10}O$ (cf *m/z* 559.3126 for **41** and **45**)) and 411.2231 ($C_{19}H_{31}N_4O_6^+$, $\Delta m$ = −1.6 ppm, from Mdha$^7$–D-Ala$^1$–Hil$^2$-D-Masp$^3$ (cf *m/z* 397.2082 for **41** and **45**)) as well as a range of other ions indicated the identity as MC-HilA (**42**), although the actual connectivity of the carbons in the amino acid side-chain at position-2 cannot be determined by mass spectrometry. A related compound (**46**) had characteristics (Table 4) and gave product ions (Figures S40–S42) that were consistent with MC-HilAbu. Product ions included *m/z* 573.3260 ($C_{30}H_{45}N_4O_7^+$, $\Delta m$ = −3.9, from Adda$^5$–D-Glu$^6$–Mdha$^7$–D-Ala$^1$–Hil$^2$ minus $C_9H_{10}O$), 232.1291 ($C_9H_{18}N_3O_4^+$, $\Delta m$ = −0.4, from D-Masp$^3$–Abu$^4$) and 430.2651 ($C_{19}H_{36}N_5O_6^+$, $\Delta m$ = −2.1, from D-Ala$^1$–Hil$^2$-D-Masp$^3$–Abu$^4$ (cf *m/z* 402.2347 for **41** and 416.2504 **42**). This data unambiguously shows the presence of an extra $CH_2$ group in both amino acid-2 and -4 in **46**, relative to MC-LA (**41**) and is consistent with MC-HilAbu (**46**). Compound **51** displayed characteristics consistent with MC-FV (Table 4), as well as product ions (Figures S49–S51) at *m/z* 246.1456 ($C_{10}H_{20}N_3O_4^+$, $\Delta m$ = −0.4, from D-Masp$^3$–Val$^4$), 593.2960 ($C_{32}H_{41}N_4O_7^+$, $\Delta m$ = −1.6, from Adda$^5$–D-Glu$^6$–Mdha$^7$–D-Ala$^1$–Phe$^2$ minus $C_9H_{10}O$ (cf. 559.3126 for **41**)) and 464.2494 ($C_{22}H_{34}N_5O_6^+$, $\Delta m$ = −3.1, from D-Ala$^1$–Phe$^2$ –D-Masp$^3$–Val$^4$ (cf. 402.2347 for **41**)). This establishes an extra $C_5H_2$ and 4 RDBE in amino acid-2 and $C_2H_4$ in amino acid-4, relative to MC-LA (**41**), consistent with MC-FV (**51**). Compound **53** had characteristics consistent with MC-WV (Table 4). This was supported by its MS/MS spectra (Figures S49–S51), which included product ions at *m/z* 246.1456 ($C_{10}H_{20}N_3O_4^+$, $\Delta m$ = 2.9, from D-Masp$^3$–Val$^4$), 632.3063 ($C_{34}H_{42}N_5O_7^+$, $\Delta m$ = −2.5, from Adda$^5$–D-Glu$^6$–Mdha$^7$–D-Ala$^1$–Trp$^2$ minus $C_9H_{10}O$ (cf. 559.3126 for **41**)) and 503.2605 ($C_{24}H_{35}N_6O_6^+$, $\Delta m$ = −2.6, from D-Ala$^1$–Trp$^2$–D-Masp$^3$–Val$^4$ (cf. 402.2347 for **41**)). This indicates the presence of an extra $C_5HN$ and 6 RDBE in amino acid-2 and $C_2H_4$ in amino acid-4, relative to MC-LA (**41**), consistent with MC-WV (53). Later-eluting isomers of **50**, **51** and **54** were also present (i.e., **52**, **55** and **56**), with identical characteristics (Table 5) and product ion spectra (Figures S52–S56). These compounds all contain branching amino acids at the variable position-2 (nominally Leu for **52** and **56**) or -4 (Val for **55**), and most likely the isomers present result from changes to this branching (e.g., Ile or 2-aminohexanoic acid at position-2, and 2-aminopentanoic acid or isovaline at position-4).

**Table 5.** Sampling points and sampling depth in Meiktila Lake for chemical and biological measurements.

| Sampling Point | Water Depth (m) | Depth of Integrated Sample (m) | Geographical Position |
|---|---|---|---|
| MK1 | 3.3 | 0–1 | N 20° 52′ 59.196, E 95° 51′ 12.204 |
| MK2 | 2 | 0–1 | N 20° 53′ 21.48, E 95° 51′ 2.124 |
| MK3 | 2.5 | 0–1 | N 20° 54′ 16.38, E 95° 50′ 40.092 |
| MK4 | 4.4 | 0–2 | N 20° 52′ 21.468, E 95° 51′ 12.528 |
| MK5 | 7.1 | 0–3 | N 20° 51′ 58.752, E 95° 51′ 18.936 |

In addition, **30** and **32** differed from each other by $CH_2$ and had characteristics consistent with MC-(H2)YA (**32**) and MC-(H2)YG (**30**), respectively (Table 4). These fragmented somewhat differently from typical Arg-free MCs such as MC-LA (**41**) (Figure S17). Both compounds showed weak product ions at *m/z* 155.0815 and 580.3017, indicating that **30** and **32** both contained Adda$^5$–D-Glu$^6$–Mdha$^7$–D-Ala$^1$. However, both **30** and **32** also gave product ions at *m/z* 320.1605, 611.3075 and 745.3807 (cf. 268.1650, 559.3117 and 693.3854 in MC-LA (**41**)), indicating the presence of an extra $C_3O$ and 3 RDBE at amino acid-2 relative to **41**, consistent with the presence of the unusual amino acid L-dihydrotyrosine ((H2)Y) at position-2. Compounds **30** and **32** gave product ions as *m/z* 449.2033 and 449.2015 ($C_{21}H_{29}N_4O_7^+$, $\Delta$ 0.5 and −3.6 ppm, respectively, from Mdha$^7$–D-Ala$^1$–(H2)Tyr$^2$–D-Masp$^3$; cf. *m/z* 397.2082 for **41**, from Mdha$^7$–D-Ala$^1$–Leu$^2$–D-Masp$^3$). Thus, the difference in mass (14.0157, i.e., $CH_2$) between **30** and **32** lies not in residue-3 (D-Masp$^3$ vs D-Asp$^3$) as might be expected but in residue-4. Thus, **32** is identified as MC-(H2)YA, and **30** as MC-(H2)YG, which appears to be the first MC so far reported [35] with Gly at position-4.

Compounds **6** and **7** coeluted but differed by a mass corresponding to $CH_2$, with **6** having the same accurate mass, MS/MS spectrum (Figure S3) and retention time as MC-(H2)YR (**6**) identified in a sample from a recent study [13]. The MS of **7** was consistent with [D-Asp$^3$]MC-(H2)YR, and the MS/MS spectra of **6** and **7** were very similar. The MS/MS spectrum of **7** included product ions (Figure S4) at *m/z* 155.0813 ($\Delta m$ = −1.6 ppm, from Mdha$^7$–D-Ala$^1$) and 599.3525 ($C_{31}H_{47}O_6N_6^+$, $\Delta m$ = −4.5 ppm, from Arg$^4$–Adda$^5$–D-Glu$^6$), 120.0806 ($C_8H_{10}N^+$, $\Delta m$ = −1.6 ppm, from (H2)Tyr), and 320.1611 ($C_{16}H_{22}O_4N_3^+$, $\Delta m$ = 2.0 ppm, from Mdha$^7$–D-Ala$^1$–(H2)Tyr$^2$), 272.1353 ($C_{10}H_{18}O_4N_5^+$, $\Delta m$ = 2.0 ppm, from D-Asp$^3$–Arg$^4$) and 714.3802 ($C_{35}H_{52}O_9N_7^+$, $\Delta m$ = −2.7 ppm, from D-Asp$^3$–Arg$^4$–Adda$^5$–D-Glu$^6$), showing that **7** is [D-Asp$^3$]MC-(H2)YR.

Five MCs were present (**19**, **22** and **33**–**35**) whose structures could not be identified from their characteristics (Table 4 and Table S1) or product ion spectra (Figures S12 and S18–S20).Compounds **33** and **34** gave apparent *m/z* values that did not correspond to known or plausible MC variants and differed from each other by a mass corresponding to $CH_2$. They did not contain Arg but contained one extra nitrogen atom, 5 extra RDBE and, more surprisingly, one less oxygen atom than MC-LA (**41**). Compound **33** gave product ions at *m/z* 375.1911 ($C_{20}H_{27}O_5N_2^+$, $\Delta m$ = −0.9 ppm, from Adda$^5$–D-Glu$^6$–Mdha$^7$ minus $C_9H_{10}O$), 446.2284 ($C_{23}H_{32}N_3O_6^+$, $\Delta m$ = −0.4 ppm, from Adda$^5$–D-Glu$^6$–Mdha$^7$–D-Ala$^1$ minus $C_9H_{10}O$) and 580.2986 ($C_{32}H_{42}N_3O_7^+$, $\Delta m$ = −5.4 ppm, from Adda$^5$–D-Glu$^6$–Mdha$^7$–D-Ala$^1$), however, fragments containing amino acids 2–4 were either shifted or absent. Although the data appear to be consistent with analogues containing Orn and Phe at positions 2 and 4 with an amide linkage to the neighbouring D-Asp$^3$/Masp$^3$ residue, further data are required for even a tentative structural assignment. The apparent pseudomolecular ion isotope envelopes of **33** and **34** (Figure S60) displayed unusual patterns that suggest that these compounds may be reacting during ionisation, possibly including dehydration, further complicating mass spectral analysis.

As with **30** and **32**, compound **35** showed product ions at *m/z* 213.0866, 320.1583, 375.1909, 446.2279, 449.2013, 509.2680 and 611.3040, consistent with the presence of Adda$^5$–D-Glu$^6$–Mdha$^7$–D-Ala$^1$–(H2)Tyr$^2$–D-Masp$^3$, which would require an MC with an amino acid side-chain at position-4 possessing an unprecedented $C_3H_3$ and 2 RDBE. This could possibly be due to the presence of a larger fragile amino acid at this position that undergoes ready elimination during MS, and the full structure of **35** remains undetermined.

Compounds **19** and **22** had identical product ion spectra including ions at *m/z* 135.0803, 213.0866, 269.1235, 375.1907, 446.2281, 599.3533, 640.2807 and 710.3886, identical to those from MC-LR, indicating the presence of D-Masp$^3$–Arg$^4$–Adda$^5$–D-Glu$^6$–Mdha$^7$–D-Ala$^1$ and that these compounds differed from MC-LR (**17**) and MC-WR (**26**) only in the amino acid at position-2. Product ions at 469.1815 and 486.2111 (both from X$^2$–D-Masp$^3$–Arg$^4$) and 173.0709 and 144.0455 (from X$^2$ and 173.0709−$CH_3N$) were consistent with this and indicated the presence of a side chain at position-2 containing $C_9H_6NO$ and 7 RDBE (cf. $C_9H_8N$ and 6 RDBE for the side chain of Trp in **26**), suggesting the presence of an unidentified oxidised variant of Trp present in the two isomeric MCs, **19** and **22**.

Between them, the two cultures contained 34 known and 22 previously unreported MCs of which 14 were assigned probable structures based on their chemical and mass spectrometric properties. In all cases, the number of Arg residues present in the MCs were reliably determined from their elemental compositions and charge-state, even for congeners for which definitive structures could not be established. Quantitation of the individual congeners was then performed from negative (Table 4) and positive mode full scan chromatograms, which gave essentially identical results, relative to 3-point calibration curves of the appropriate MC reference materials (RMs) containing no, one or two Arg residues ([D-Leu$^1$]MC-LY [38], MC-LR (**17**) and MC-RR (**3**), respectively). AB2017/14 contained 52 MCs, of which only 31 had been previously reported, and the newly reported MCs constituted nearly 20% of the total identified MC content. AB2017/15 contained fewer MCs (20) of which only six had not been reported previously, but these constituted nearly 34% of the total MC content in this culture. Notable amongst the newly reported MCs are those containing Glu at positions 2 and 4 (**4**, **9**, **24**, **27**, **36**–**38**, and **40**), which constituted 1.7% of the MCs in AB2017/14 and 37.5% of the MCs in AB2017/15. MCs

of this type have only been reported previously from two sources [12,39]. AB2017/14 also contained a number of congeners that appeared to be derived from MC-WR (**19**, **20**, **22**, **23** and **26**) that together constituted 20.2% of its MC content, and an unusually high number (25) of nonArg-containing MCs (**30**, **32**–**35** and **37**–**56**) together constituting 20.5% of the total MC content. These results underscore both the diversity of MCs that may be present in a single sample and the potential difficulty of reliably quantitating the total MCs using traditional targeted LC–MS/MS methods. These factors may contribute to the reported apparent overestimation of MC levels when using less-targeted methods such as ELISA and PP2A inhibition, relative to highly congener-targeted LC–MS/MS approaches [40].

Although both strains produce a variety of MC variants, the risk of harmful effects caused by microcystins is likely to be low due to the low *Microcystis* biomasses observed in Meiktila Lake during this study. An increase in *Microcystis* biomass cannot, however, be excluded due to the pollution from various sources, e.g., waste water and street runoff [6]. This will most likely lead to an increase in microcystin concentrations in the lake, with potential harmful effects on humans, domestic and wild animals using the untreated lake water. The toxicity of microcystins found in the strains isolated from Meiktila Lake varies from highly toxic variants like MC-LR or MC-LA to less toxic variants such as MC-RR [41]. The toxicity of most of the MC variants found in this study has not yet been described, which makes a full risk assessment difficult. The production of a high number of MC variants (up to 47) has also been shown for two other *Microcystis* strains isolated from South African Haartbeestpoort Dam and Japanese Lake Kasumigaura [42,43].

Shallow lakes like Meiktila Lake are often characterized by competition between macrophytes and phytoplankton. High nutrient loading and phytoplankton growth lead to turbid water conditions and prevent the growth of macrophytes [44]. In Meiktila Lake the present turbid conditions are explained by deforestation and erosion in the catchment area, pollution with domestic waste water, street runoff and solid waste [6]. The relatively strong growth of *R. raciborskii* most likely is an additional reason for the observed turbidity at sampling points MK1, MK2, MK4 and MK5 in Meiktila Lake. The *Potamogeton* belt which separates MK2 and MK3 seems to act as a kind of filter or barrier because the Secchi depth at MK3 (1.8 m) was considerably higher than at MK1 (0.8 m). According to Van Donk and Van de Bund [45], macrophytes significantly modify the composition of the phytoplankton community and lead to a decrease in its abundance and biomass. It has been shown that certain macrophyte species exhibit allelopathic activity against certain phytoplankton species [46]. The obvious decrease of *Raphidiopsis* biomass from MK2 to MK3 may be therefore attributable to allelopathic effects of the macrophyte species in the belt. Allelopathy has been suggested by several authors to be responsible for observed phytoplankton patterns in whole-lake studies of vegetated, shallow lakes, but evidence for or against allelopathy has not been provided [47–49].

## 4. Conclusions

In conclusion, this is the first report of *R. raciborskii* and *Microcystis* in Meiktila Lake, Myanmar. Three of five *Raphidiopsis* isolates produced CYN and deoxyCYN, like other *Raphidiopsis/Cylindrospermopsis* isolates described from other Asian countries and Australia. Both *Microcystis* strains isolated from Meiktila Lake produced at least 56 MC variants (52 for AB2017/14 and 20 for AB2017/15), including 22 previously undescribed congeners. Harmful effects on humans and animals using Meiktila Lake as a water source cannot therefore be excluded.

## 5. Materials and Methods

### *5.1. Study Area, Measurements and Sampling*

Meiktila Lake is a shallow reservoir, located close to Meiktila city in central Myanmar in the Mandalay region at an altitude of 230 m (Figure 1). During the rainy season, from April/May to October/November, it receives water from Mondaing Dam, located ca. 15 km west of Meiktila at an altitude of 245 m. Depending on the season, Meiktila Lake covers an area of around 54 km$^2$ with

a maximum water depth of 10 m. It is divided by a dam into a northern and a southern lake [5]. Five sampling points were selected, three in the northern part of Meiktila Lake and two in the southern part (Figure 1, Table 5).

Sampling points 2 and 3 in the Northern Part of Meiktila Lake were separated by a broad *Potamogeton* belt. At all five sampling points in March and November 2017, in situ measurements of water temperature, pH, conductivity and dissolved oxygen were conducted, and integrated water samples were taken (1 m steps up to max. 3 m water depth) for the analysis of chemical parameters (ammonium, nitrate, total nitrogen, soluble reactive phosphorous, total phosphorous, Ca, phytoplankton composition and biomass and for the isolation of cyanobacterial strains. For quantitative phytoplankton analysis, a 50 mL subsample was removed from a sample taken from integrated samples and preserved with Lugol's solution and a concentrated net sample (mesh size 20 µm) was taken and preserved by addition of formaldehyde (4% final concentration) for qualitative analysis. A 50 mL water sample for isolation of cyanobacteria was taken at each sampling point and kept in a cool shady place and gently shaken twice per day before further treatment in Norway.

### 5.2. Phytoplankton Analysis

The Lugol-fixed phytoplankton samples were counted in sedimentation chambers (Hydro-Bios Apparatebau GmbH Kiel, Germany) using an inverted microscope (Leica DMi8; Ortomedic, Oslo, Norway) according to Utermöhl [50]. Phytoplankton biomass was calculated by geometrical approximations using the computerized counting programme Opticount (SequentiX - Digital DNA Processing, Klein Raden, Germany). The specific density of phytoplankton cells was calculated as 1 g $cm^{-3}$.

### 5.3. Isolation of Strains and Morphological Characterization

Using a microcapillary, single colonies of *Microcystis* and filaments of *Raphidiopsis* were isolated. They were washed five times and placed in wells on microtiter plates containing 300 µL Z8 medium [51]. After successful growth, the samples were placed in 50 mL Erlenmeyer flasks containing 20 mL Z8 medium and maintained at 22 °C. Strains were classified based on morphological traits [52,53]. Morphological examination was conducted using a Leica DM2500 light microscope, Leica DFC450 camera and Leica Application Suite software (LAS) (Leica, Oslo, Norway). The morphological identification was based on the following criteria: (i) size of vegetative cells and heterocytes and (ii) nature and shape of filaments or colonies. Length and width of 50–250 vegetative cells or filaments and of 20–50 heterocytes were measured. Akinetes were not detected in the samples. All strains used in this study (Table 2) are maintained at the Norwegian Institute for Water Research, Oslo, Norway.

### 5.4. Genomic DNA Extraction, PCR Amplification and Sequencing

Genomic DNA was extracted according to Ballot et al. [54]. All PCRs were performed on a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Oslo, Norway) using the iProof High-Fidelity PCR Kit (Bio-Rad Laboratories, Oslo, Norway). The 16S rRNA gene of the isolated strains from Meiktila Lake was amplified using the primers as described by Ballot et al. [54]. PCR products were visualized by 1% agarose gel electrophoresis with GelRed staining (GelRed Nucleic Acid Gel Stain, Biotium, Fremont, CA, USA) and UV illumination.

Amplified 16S rRNA gene products were purified through Qiaquick PCR purification columns (Qiagen, Hilden, Germany). Sequencing of the purified 16S rRNA gene products was performed using the same primers as for PCR and intermediate primers as described in Ballot et al. [54]. For each PCR product, both strands were sequenced on an ABI 3730 Avant genetic analyser using the BigDye terminator V.3.1 cycle sequencing kit (Applied Biosystems, Thermo Fisher Scientific Oslo, Norway) according to the manufacturer's instructions.

### *5.5. Phylogenetic Analysis*

Sequences of the 16S rRNA gene of the cyanobacterial strains were analysed using the Seqassem software package (version 07/2008) and the Align MS Windows-based manual sequence alignment editor (version 03/2007) (SequentiX - Digital DNA Processing, Klein Raden, Germany). Segments with highly variable and ambiguous regions and gaps making proper alignment impossible were excluded from the analyses.

A 16S rRNA gene set containing 1135 positions was used in the phylogenetic tree for *Cylindrospermopsis/Raphidiopsis*. *Sphaerospermopsis aphanizomenoides* (LN846954) was employed as the outgroup, five *Raphidiopsis* strains from Meiktila Lake and 35 additional *Cylindrospermopsis/Raphidiopsis* sequences derived from GenBank were included in the analyses. A set containing 1426 positions was used for the *Microcystis* 16S rRNA gene analysis. *Chroococcus subviolaceus* (MF072353) was employed as the outgroup, two strains from Meiktila Lake and 38 additional *Microcystis* sequences derived from GenBank were included in the analysis. Phylogenetic trees for 16S rRNA genes were constructed using the ML algorithm in Mega v. 7 [55]. In the ML analyses, evolutionary substitution models were evaluated using Mega v. 7 [55]. The HKY+G+I evolutionary model was found to be the best-fitting evolutionary model for the Nostocales 16S rRNA gene tree and T92+G+I for the *Microcystis* 16S rRNA gene tree. ML analyses of both trees were performed with 1000 bootstrap replicates using Mega v. 7 [55]. The sequence data were submitted to the European Nucleotide Archive (ENA) under the accession numbers listed in Table 2.

### *5.6. Toxin Analysis*

#### 5.6.1. Material

LC–MS/MS utilised standards of ATX-a (Tocris Bioscience, Bristol, UK), homoATX-a (Novakits, Nantes, France) and CYN (Vinci Biochem, Vinci, Italy) and certified reference materials (CRMs) of STX, dcSTX, NeoSTX, GTX1, GTX4, GTX5 and C1 and C2 toxins (National Research Council of Canada, Halifax, NS, Canada (NRC)). LC–HRMS utilised CRMs of MC-RR (**3**), MC-LR (**17**) and [Dha$^{7}$]MC-LR (**18**) (NRC) and an RM of [D-Leu$^{1}$]MC-LY [38]. Additional RMs of [D-Asp$^{3}$]MC-RR (**1**), D-Asp$^{3}$]MC-LR (**13**), MC-YR (**14**), MC-HilR (**21**) containing traces of MC-FR (**25**), MC-WR (**26**) and MC-LA (**41**) were prepared at NRC from commercial samples (Enzo Life Sciences, Farmingdale, NY, USA), and extracts containing an array of other identified MCs were available from previous work [12,13,15]. Standards for the Adda-ELISA and for the CYN, ATX and STX ELISAs were as provided with the kits (Abraxis LLC, Warminister, PA, USA).

#### 5.6.2. ELISA for MCs, CYNs, ATXs and STXs

Fresh culture material of two *Microcystis* and five *Raphidiopsis* strains was frozen and thawed three times. The *R. raciborskii* strains were tested for CYNs using the Abraxis Cylindrospermopsin ELISA kit (Abraxis LLC, Warminister, PA, USA) following the manufacturer's instructions. The test is a direct competitive ELISA that detects cylindrospermopsin but also recognizes deoxycylindrospermopsin and 7-*epi*-cylindrospermopsin. The ELISA results do not distinguish between dissolved and cell-bound toxins. Both *Microcystis* strains were tested for microcystins using the Abraxis Microcystins/Nodularins (ADDA) ELISA kits (Abraxis LLC, Warminister, PA, USA). The test is an indirect competitive ELISA designed to detect Adda, (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid), based on specific recognition of the Adda moiety [56]. ADDA is a nonprotein amino acid and is the most common side chain at position-5 in microcystins (Figure 5).

All strains were also tested for saxitoxins and anatoxin-a using the Abraxis Saxitoxins (PSP) and Abraxis Anatoxin (VFDF) ELISA kits (Abraxis LLC, Warminister, PA, USA). The saxitoxin ELISA is a direct competitive ELISA that detects saxitoxin based on specific antibody recognition but also recognizes other saxitoxins (e.g., dcSTX, GTXs, lyngbyatoxin, NeoSTX) to varying degrees according to the manufacturer's instructions. The test for anatoxin-a is a direct competitive ELISA that detects

anatoxin-a based on specific antibody recognition but also recognizes homoanatoxin according to the manufacturer's instructions The colour reaction of all ELISA tests was evaluated at 450 nm on a Perkin Elmer1420 Multilabel counter Victor3 (Perkin Elmer, Waltham, MA, USA), and concentrations were evaluated by manual analysis of the absorbance data as recommended by the vendor.

#### 5.6.3. Microcystin Analysis by LC–HRMS

Fresh culture material of both *Microcystis* strains was prepared for LC–HRMS by freeze-thawing (3 times), diluting with an equal volume of MeOH and filtering (0.22 μm) [57]). LC–HRMS/MS analysis was performed on a Q Exactive-HF Orbitrap mass spectrometer equipped with a HESI-II heated electrospray ionization interface (ThermoFisher Scientific, Waltham, MA, USA) using an Agilent 1200 LC system including a binary pump, autosampler and column oven (Agilent, Santa Clara, CA, USA). Analyses were performed with SymmetryShield 3.5 μm C18 column (100 × 2.1 mm; Waters, Milford, MA, USA) held at 40 °C with mobile phases A and B of $H_2O$ and $CH_3CN$, respectively, each of which contained formic acid (0.1% v/v). Gradient elution (0.3 mL $min^{-1}$) was from 20% to 90% B over 18 min, then to 100% B over 0.1 min and a hold at 100% B (2.9 min), then returned to 20% B over 0.1 min with a hold at 20% B (3.9 min) to equilibrate the column (total run time 25 min). Injection volume was typically 1–5 μL.

The MS was operated in positive ion mode and calibrated from *m*/*z* 74 to 1622. The spray voltage was 3.7 kV, the capillary temperature was 350 °C and the sheath and auxiliary gas flow rates were 25 and 8 units, respectively, with MS data acquired from 2 to 20 min. Mass spectral data were collected using a combined full scan (FS) and data independent acquisition (DIA) method. FS data was collected from *m*/*z* 500 to 1400 using the 60,000-resolution setting, an AGC target of $1 \times 10^6$ and a max IT of 100 ms. DIA data was collected using the 15,000 resolution setting, an AGC target of $2 \times 10^5$, maxIT set to "auto" and a stepped collision energy of 30, 60 and 80 eV. Precursor isolation windows were 62 *m*/*z* wide with centering at *m*/*z* 530, 590, 650, 710, 770, 830, 890, 950, 1010, 1070, 1130, 1190, 1250, 1310 and 1370. Mass spectral data were also collected using a combined full scan (FS) and top-10 data-dependent acquisition (DDA) method. Data was acquired as for DIA but with an exclusion list generated from a blank injection and an inclusion list (both at ± 5 ppm) from a publicly available database of MC *m*/*z* values [58], except that maxIT was set to 100 ms and dynamic exclusion 5.0 s and "if idle pick others" were selected. Putative MCs detected using the above FS/DIA method were further probed in a targeted manner using the parallel reaction monitoring scan (PRM) mode with a 0.7 *m*/*z* precursor isolation window, typically using the 30,000-resolution setting, an AGC target of $5 \times 10^5$ and a max IT of 400 ms. Typical collision energies were: stepped CE at 30 and 35 eV for MCs with no Arg; stepped CE at 60, 65 and 70 eV for MCs with one Arg; and CE at 65 eV for $[M+H]^+$ and stepped CE at 20, 25 and 30 eV for $[M+2H]^{2+}$ of MCs with two Arg groups. Full scan chromatograms were obtained in MS-SIM mode as for DIA but with resolution 120,000 and max IT 300 ms.

In negative mode, the mass spectrometer was calibrated from *m*/*z* 69 to 1780 and the spray voltage was −3.7 kV, while the capillary temperature, sheath and auxiliary gas flow rates were the same as for positive mode. Mass spectrometry data were collected in FS/DIA scan mode as above using a scan range of *m*/*z* 750–1400, a resolution setting of 60,000, AGC target of $1 \times 10^6$ and a max IT of 100 ms. For DIA, MS/MS data was collected from *m*/*z* 93 to 1400 using a resolution setting of 15,000, AGC target of $2 \times 10^5$, max IT set to "auto" and stepped collision energy 65 and 100 eV. Isolation windows were 45 *m*/*z* wide and centered at *m*/*z* 772, 815, 858, 902, 945, 988, 1032, 1075, 1118, 1162, 1205, 1248, 1294, 1335 and 1378. Mass spectral data were also collected using a combined full scan (FS) and top-10 data dependent acquisition (DDA) method. Data were acquired as for DIA but with an exclusion list generated from a blank injection and an inclusion list from a publicly available list (both at ± 5 ppm) of MC *m*/*z* values [58], except that maxIT was set to 100 ms and dynamic exclusion 5.0 s and "if idle pick others" were selected. Full scan chromatograms were obtained over a scan range *m*/*z* 750–1400 at a resolution setting of 120,000 using an AGC target of $1 \times 10^6$ and a max IT of 300 ms.

Thiol derivatizations were performed by addition of $(NH_4)_2CO_3$ (0.1 M, 200 µL) to the filtered extract (200 µL), with 200 µL transferred to two LC-MS vials. To one vial was added 1 µL of a 1:1 mixture of mercaptoethanol and $d_4$-mercaptoethanol (Sigma–Aldrich, St. Louis, MO, USA), while 1 µL of water was added to the other vial as a control. Oxidations were performed by addition of DMSO (5 µL) and Oxone (10 mg/mL in water; 25 µL) to 50 µL of extract [15]. Samples and reactions were placed in the sample tray (held at 15 °C) for analysis, and the reactions were monitored periodically until completion and then analysed.

#### 5.6.4. CYN, deoxyCYN, ATXs and STXs Analysis by LC-MS/MS

Extraction was performed on freeze-dried cultures (40 mL), according to the protocol in [59]. In brief, dry material was treated with 6 mL of 50% methanol and sonicated (Omniruptor4000 probe sonicator, Omni-Inc., Kennesaw, MA, USA) for 10 min in pulsed mode (50%) using 160 W power. An aliquot of the solution was then filtered on Phenex RC syringe filters (0.2 µm; Phenomenex, Castel Maggiore, Italy) and analyzed by LC–MS/MS.

LC-MS/MS analysis were performed using a Waters Acquity UPLC system (Waters, Milford, MA, USA) coupled to a SCIEX 4000 QTRAP mass spectrometer (AB Sciex Pte. Ltd., Singapore). Chromatographic separation of analytes was performed using a HILIC column (Ascentis Express OH5, 2.7 µm, 50 × 2.1 mm; Merck Life Science S.r.l., Milan, Italy), while MS detection was performed using positive electrospray ionization using scheduled Multiple Reaction Monitoring. Details of the experimental set up are as described by Cerasino et al. [60], and the method was suitable for the detection and quantification of the following toxins: ATX-a, homoATX-a, CYN, STX, dcSTX, NeoSTX, GTX1, GTX4, GTX5 and C1 and C2 [60]. Quantification limits were 0.2–200 µg $L^{-1}$. Other toxic alkaloids not available as pure standards were also screened but only for tentative analysis (hydroxy- and epoxy- and homo-ATXs, deoxyCYN, dcNeoSTX, GTX2/3, dcGTX2/3 and C3 and C4 toxins) using equivalent detection settings to their most similar analogs.

**Supplementary Materials:** The following are available online at http://www.mdpi.com/2072-6651/12/4/232/s1, Figures S1–S59: LC-HRMS/MS spectra of **1**, **3**, **4**, **6**, **7**, **9**, **11**–**15**, **17**, **19**–**25**, and **27**–**56**, Figure S60: LC-HRMS spectra of unidentified microcystins **33**–**35,** Table S1: Identities of microcystins detected by LC-HRMS/MS analysis in *M. aeruginosa* strains AB2017/14 and /15, their retention times ($t_R$), concentrations, elemental compositions, observed *m/z* values in positive and negative ionisation modes, whether they matched retention times in reference samples and whether characteristic microcystin product ions were observed.

**Author Contributions:** Conceptualization, A.B.; methodology, A.B., C.O.M., L.C., V.H.; validation, A.B., C.O.M., L.C.; investigation, A.B., C.O.M., L.C., M.M., T.S.; data curation, A.B., C.O.M., L.C.; writing—original draft preparation, A.B., T.S., C.O.M.; writing—review and editing, A.B., C.O.M., M.M., L.C., T.S., V.H.; supervision, A.B.; project administration A.B. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was supported by the Project "Integrated Water Resources Management (IWRM) – Institutional Building and Training in Myanmar (funded by the Norwegian Ministry of Foreign Affairs and the Royal Norwegian Embassy in Myanmar).

**Acknowledgments:** We thank K. Thomas for preparation of RMs of [D-Asp$^3$]MC-RR (**1**), D-Asp$^3$]MC-LR (**13**), MC-YR (**14**), MC-HilR (**21**) containing traces of MC-FR (**25**), MC-WR (**26**) and MC-LA (**41**) at National Research Council, Halifax, Canada in partnership with the Norwegian Veterinary Institute (Oslo, Norway).

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

## References

1. Paerl, H.W.; Huisman, J. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. *Environ. Microbiol. Rep.* **2009**, *1*, 27–37. [CrossRef] [PubMed]
2. Sivonen, K. Cyanobacterial toxins. In *Encyclopedia of Microbiology*; Schaechter, M., Ed.; Elsevier: Oxford, UK, 2009; pp. 290–307.
3. Harvey, G.E. *History of Burma from the Earliest Times to 10 March 1824: The Beginning of the English Conquest, 1*; Longmans, Green and Company: London, UK, 1925.

4. Nyunt, K.K. Algal flora of Meiktila Lake and its surrounding area. Master´s Thesis, Mandalay University, Mandalay, Myanmar, 1995.
5. Hlaing, N.K. Limnological study of Meiktila Lake, Mandalay Division. Ph.D. Thesis, University of Mandalay, Mandalay, Myanmar, 2014.
6. Klink, T.; de Jongh, I.; Leushuis, M.; Bijlmakers, L.; Speets, R.; Brinkman, L. *Scoping Study Meiktila Lake Area IWRM*; Royal HaskoningDHV, Arcadis, Rebel Group, Dutch Water Authorities: Den Haag, The Netherlands, 2015; pp. 1–50.
7. Aung, T.T.; Zin, T. *Zooplankton community in Meiktila Lake, Meiktila, central Myanmar*; Universities Research Journal; The Government of The Republic of the Union of Myanmar Ministry of Education: Yangon, Myanmar, 2015; Volume 7, pp. 1–11.
8. Antunes, J.T.; Leao, P.N.; Vasconcelos, V.M. *Cylindrospermopsis raciborskii*: Review of the distribution, phylogeography, and ecophysiology of a global invasive species. *Front. Microbiol.* **2015**, *6*, 13. [CrossRef] [PubMed]
9. Burford, M.A.; Beardall, J.; Willis, A.; Orr, P.T.; Magalhaes, V.F.; Rangel, L.M.; Azevedo, S.; Neilan, B.A. Understanding the winning strategies used by the bloom-forming cyanobacterium *Cylindrospermopsis raciborskii*. *Harmful Algae* **2016**, *54*, 44–53. [CrossRef] [PubMed]
10. Mowe, M.A.D.; Mitrovic, S.M.; Lim, R.P.; Furey, A.; Yeo, D.C.J. Tropical cyanobacterial blooms: A review of prevalence, problem taxa, toxins and influencing environmental factors. *J. Limnol.* **2015**, *74*, 205–224. [CrossRef]
11. Harke, M.J.; Steffen, M.M.; Gobler, C.J.; Otten, T.G.; Wilhelm, S.W.; Wood, S.A.; Paerl, H.W. A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, *Microcystis* spp. *Harmful Algae* **2016**, *54*, 4–20. [CrossRef]
12. Mallia, V.; Uhlig, S.; Rafuse, C.; Meija, J.; Miles, C.O. Novel microcystins from *Planktothrix prolifica* NIVA-CYA 544 identified by LC-MS/MS, functional group derivatization and $^{15}$N-labeling. *Mar. Drugs* **2019**, *17*, 643. [CrossRef]
13. Yilmaz, M.; Foss, A.J.; Miles, C.O.; Özen, M.; Demir, N.; Balci, M.; Beach, D.G. Comprehensive multi-technique approach reveals the high diversity of microcystins in field collections and an associated isolate of *Microcystis aeruginosa* from a Turkish lake. *Toxicon* **2019**, *167*, 87–100. [CrossRef]
14. Miles, C.O.; Sandvik, M.; Haande, S.; Nonga, H.; Ballot, A. LC-MS analysis with thiol derivatization to differentiate [Dhb$^7$]- from [Mdha$^7$]-microcystins: analysis of cyanobacterial blooms, *Planktothrix* cultures and European crayfish from Lake Steinsfjorden, Norway. *Environ. Sci. Technol.* **2013**, *47*, 4080–4087. [CrossRef]
15. Miles, C.O.; Melanson, J.E.; Ballot, A. Sulfide oxidations for LC-MS analysis of methionine-containing microcystins in *Dolichospermum flos-aquae* NIVA-CYA 656. *Environ. Sci. Technol.* **2014**, *48*, 13307–13315. [CrossRef]
16. Lürling, M.; Van Oosterhout, F.; Faassen, E. Eutrophication and warming boost cyanobacterial biomass and microcystins. *Toxins* **2017**, *9*, 64. [CrossRef]
17. Guedes, I.A.; Rachid, C.T.C.C.; Rangel, L.M.; Silva, L.H.S.; Bisch, P.M.; Azevedo, S.M.F.O.; Pacheco, A.B.F. Close link between harmful cyanobacterial dominance and associated bacterioplankton in a tropical eutrophic reservoir. *Front. Microbiol.* **2018**, *9*, 13. [CrossRef] [PubMed]
18. Humpage, A.R.; Falconer, I.R. Oral toxicity of the cyanobacterial toxin cylindrospermopsin in male Swiss albino mice: determination of no observed adverse effect level for deriving a drinking water guideline value. *Environ. Toxicol.* **2003**, *18*, 94–103. [CrossRef] [PubMed]
19. Norris, R.L.; Eaglesham, G.K.; Pierens, G.; Shaw, G.R.; Smith, M.J.; Chiswell, R.K.; Seawright, A.A.; Moore, M.R. Deoxycylindrospermopsin, an analog of cylindrospermopsin from *Cylindrospermopsis raciborskii*. *Environ. Toxicol.* **1999**, *14*, 163–165. [CrossRef]
20. Neumann, C.; Bain, P.; Shaw, G. Studies of the comparative in vitro toxicology of the cyanobacterial metabolite deoxycylindrospermopsin. *J. Toxicol. Environ. Health, Part A* **2007**, *70*, 1679–1686. [CrossRef]
21. Machado, J.; Campos, A.; Vasconcelos, V.; Freitas, M. Effects of microcystin-LR and cylindrospermopsin on plant–soil systems: a review of their relevance for agricultural plant quality and public health. *Environ. Res.* **2017**, *153*, 191–204. [CrossRef]
22. Griffiths, D.J.; Saker, M.L. The Palm Island mystery disease 20 years on: A review of research on the cyanotoxin cylindrospermopsin. *Environ. Toxicol.* **2003**, *18*, 78–93. [CrossRef]

23. Chiswell, R.K.; Shaw, G.R.; Eaglesham, G.; Smith, M.J.; Norris, R.L.; Seawright, A.A.; Moore, M.R. Stability of cylindrospermopsin, the toxin from the cyanobacterium, *Cylindrospermopsis raciborskii*: Effect of pH, temperature, and sunlight on decomposition. *Environ. Toxicol.* **1999**, *14*, 155–161. [CrossRef]
24. Environmental Protection Agency. *Cyanobacteria and Cyanotoxins: Information for Drinking Water Systems*; United States Environmental Protection Agency: Washington, DC, USA, 2014; pp. 1–11.
25. Li, R.H.; Carmichael, W.W.; Brittain, S.; Eaglesham, G.K.; Shaw, G.R.; Liu, Y.D.; Watanabe, M.M. First report of the cyanotoxins cylindrospermopsin and deoxycylindrospermopsin from *Raphidiopsis curvata* (cyanobacteria). *J. Phycol.* **2001**, *37*, 1121–1126. [CrossRef]
26. Nguyen, T.T.L.; Hoang, T.H.; Nguyen, T.K.; Duong, T.T. The occurrence of toxic cyanobacterium *Cylindrospermopsis raciborskii* and its toxin cylindrospermopsin in the Huong River, Thua Thien Hue province, Vietnam. *Environ. Monit. Assess.* **2017**, *189*, 11. [CrossRef]
27. Dao, T.S.; Cronberg, G.; Nimptsch, J.; Do-Hong, L.-C.; Wiegand, C. Toxic cyanobacteria from Tri An Reservoir, Vietnam. *Nowa Hedwigia* **2010**, *90*, 433–448. [CrossRef]
28. Hawkins, P.R.; Chandrasena, N.R.; Jones, G.J.; Humpage, A.R.; Falconer, I.R. Isolation and toxicity of *Cylindrospermopsis raciborskii* from an ornamental lake. *Toxicon* **1997**, *35*, 341–346. [CrossRef]
29. Saker, M.L.; Neilan, B.A. Varied diazotrophies, morphologies, and toxicities of genetically similar isolates of *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) from northern Australia. *Appl. Environ. Microbiol.* **2001**, *67*, 1839–1845. [CrossRef] [PubMed]
30. Li, R.; Carmichael, W.W.; Brittain, S.; Eaglesham, G.K.; Shaw, G.R.; Mahakhant, A.; Noparatnaraporn, N.; Yongmanitchai, W.; Kaya, K.; Watanabe, M.M. Isolation and identification of the cyanotoxin cylindrospermopsin and deoxy-cylindrospermopsin from a Thailand strain of *Cylindrospermopsis raciborskii* (Cyanobacteria). *Toxicon* **2001**, *39*, 973–980. [CrossRef]
31. Chonudomkul, D.; Yongmanitchai, W.; Theeragool, G.; Kawachi, M.; Kasai, F.; Kaya, K.; Watanabe, M.M. Morphology, genetic diversity, temperature tolerance and toxicity of *Cylindrospermopsis raciborskii* (Nostocales, Cyanobacteria) strains from Thailand and Japan. *FEMS Microbiol. Ecol.* **2004**, *48*, 345–355. [CrossRef] [PubMed]
32. Zarenezhad, S.; Sano, T.; Watanabe, M.M.; Kawachi, M. Evidence of the existence of a toxic form of *Cylindrospermopsis raciborskii* (Nostocales, Cyanobacteria) in Japan. *Phycol. Res.* **2012**, *60*, 98–104. [CrossRef]
33. Cirés, S.; Wörmer, L.; Ballot, A.; Agha, R.; Wiedner, C.; Velazquez, D.; Casero, M.C.; Quesada, A. Phylogeography of cylindrospermopsin and paralytic shellfish toxin-producing Nostocales cyanobacteria from Mediterranean Europe (Spain). *Appl. Environ. Microbiol.* **2014**, *80*, 1359–1370. [CrossRef]
34. Panou, M.; Zervou, S.K.; Kaloudis, T.; Hiskia, A.; Gkelis, S. A Greek *Cylindrospermopsis raciborskii* strain: missing link in tropic invader's phylogeography tale. *Harmful Algae* **2018**, *80*, 96–106. [CrossRef]
35. Bouaïcha, N.; Miles, C.O.; Beach, D.G.; Labidi, Z.; Djabri, A.; Benayache, N.Y.; Nguyen-Quang, T. Structural diversity, characterization and toxicology of microcystins. *Toxins* **2019**, *11*, 714. [CrossRef]
36. Foss, A.J.; Miles, C.O.; Samdal, I.A.; Lovberg, K.E.; Wilkins, A.L.; Rise, F.; Jaabaek, J.A.H.; McGowan, P.C.; Aubel, M.T. Analysis of free and metabolized microcystins in samples following a bird mortality event. *Harmful Algae* **2018**, *80*, 117–129. [CrossRef]
37. Okello, W.; Portmann, C.; Erhard, M.; Gademann, K.; Kurmayer, R. Occurrence of microcystin-producing cyanobacteria in Ugandan freshwater habitats. *Environ. Toxicol.* **2010**, *25*, 367–380. [CrossRef]
38. LeBlanc, P.; Merkley, N.; Thomas, K.; Lewis, N.I.; Békri, K.; Renaud, S.L.; Pick, F.R.; McCarron, P.; Miles, C.O.; Quilliam, M.A. Isolation and characterization of [D-Leu$^1$]microcystin-LY from *Microcystis aeruginosa* CPCC-464. *Toxins* **2020**, *12*, 77. [CrossRef] [PubMed]
39. Namikoshi, M.; Yuan, M.; Sivonen, K.; Carmichael, W.W.; Rinehart, K.L.; Rouhiainen, L.; Sun, F.; Brittain, S.; Otsuki, A. Seven new microcystins possessing two L-glutamic acid units, isolated from *Anabaena* sp. strain 186. *Chem. Res. Toxicol.* **1998**, *11*, 143–149. [CrossRef] [PubMed]
40. Foss, A.J.; Aubel, M.T. Using the MMPB technique to confirm microcystin concentrations in water measured by ELISA and HPLC (UV, MS, MS/MS). *Toxicon* **2015**, *104*, 91–101. [CrossRef] [PubMed]
41. Rinehart, K.L.; Namikoshi, M.; Choi, B.W. Structure and biosynthesis of toxins from blue–green algae (cyanobacteria). *J. Appl. Phycol.* **1994**, *6*, 159–176. [CrossRef]
42. Ballot, A.; Sandvik, M.; Rundberget, T.; Botha, C.J.; Miles, C.O. Diversity of cyanobacteria and cyanotoxins in Hartbeespoort Dam, South Africa. *Mar. Freshwat. Res.* **2014**, *65*, 175–189. [CrossRef]

43. Fewer, D.P.; Rouhiainen, L.; Jokela, J.; Wahlsten, M.; Laakso, K.; Wang, H.; Sivonen, K. Recurrent adenylation domain replacement in the microcystin synthetase gene cluster. *BMC Evol. Biol.* **2007**, *7*, 183. [CrossRef]
44. Scheffer, M.; Carpenter, S.; Foley, J.A.; Folke, C.; Walker, B. Catastrophic shifts in ecosystems. *Nature* **2001**, *413*, 591–596. [CrossRef]
45. van Donk, E.; van de Bund, W.J. Impact of submerged macrophytes including charophytes on phyto- and zooplankton communities: allelopathy versus other mechanisms. *Aquat. Bot.* **2002**, *72*, 261–274. [CrossRef]
46. Gao, Y.N.; Dong, J.; Fu, Q.Q.; Wang, Y.P.; Chen, C.; Li, J.H.; Li, R.; Zhou, C.J. Allelopathic effects of submerged macrophytes on phytoplankton. *Allelopathy J.* **2017**, *40*, 1–22.
47. Mjelde, M.; Faafeng, B.A. *Ceratophyllum demersum* hampers phytoplankton development in some small Norwegian lakes over a wide range of phosphorus concentrations and geographical latitude. *Freshwat. Biol.* **1997**, *37*, 355–365. [CrossRef]
48. Blindow, I.; Hargeby, A.; Andersson, G. Seasonal changes of mechanisms maintaining clear water in a shallow lake with abundant *Chara* vegetation. *Aquat. Bot.* **2002**, *72*, 315–334. [CrossRef]
49. Lombardo, P. Applicability of littoral food-web biomanipulation for lake management purposes: snails, macrophytes, and water transparency in northeast Ohio shallow lakes. *Lake Reserv. Manage.* **2005**, *21*, 186–202. [CrossRef]
50. Utermöhl, H. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt. Int. Ver. Theor. Angew. Limnol.* **1958**, *9*, 1–38. [CrossRef]
51. Kotai, J. *Instructions for Preparation of Modified Nutrient Solution Z8 for Algae.—Publication B-11/69*; Norwegian Institute for Water Research: Oslo, Norway, 1972.
52. Komárek, J.; Anagnostidis, K. Cyanoprokaryota 1. Chroococcales.—19/1. In *Süsswasserflora von Mitteleuropa*; Ettl, H., Gärtner, G., Heynig, H., Mollenhauer, D., Eds.; Gustav Fischer: Jena-Stuttgart-Lübeck-Ulm, Germany, 1999; p. 548.
53. Komárek, J. Cyanoprokaryota. 3. Heterocytous genera. In *Süsswasserflora von Mitteleuropa/Freshwater flora of Central Europe*; Büdel, B., Krienitz, L., Gärtner, G., Schagerl, M., Eds.; Springer Spektrum: Berlin-Heidelberg, Germany, 2013; p. 1130.
54. Ballot, A.; Cerasino, L.; Hostyeva, V.; Cirés, S. Variability in the sxt gene clusters of PSP toxin producing *Aphanizomenon gracile* strains from Norway, Spain, Germany and North America. *PLoS One* **2016**, *11*, e0167552. [CrossRef] [PubMed]
55. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874. [CrossRef] [PubMed]
56. Fischer, W.J.; Garthwaite, I.; Miles, C.O.; Ross, K.M.; Aggen, J.B.; Chamberlin, A.R.; Towers, N.R.; Dietrich, D.R. Congener-independent immunoassay for microcystins and nodularins. *Environ. Sci. Technol.* **2001**, *35*, 4849–4856. [CrossRef] [PubMed]
57. Miles, C.O.; Sandvik, M.; Nonga, H.E.; Rundberget, T.; Wilkins, A.L.; Rise, F.; Ballot, A. Thiol derivatization for LC-MS identification of microcystins in complex matrices. *Environ. Sci. Technol.* **2012**, *46*, 8937–8944. [CrossRef]
58. Miles, C.O.; Stirling, D. Toxin mass list, version 15b. Available online: https://www.researchgate.net/publication/324039408_Toxinmasslist_COM_v15b (accessed on 11 October 2018).
59. Cerasino, L.; Meriluoto, J.; Bláha, L.; Carmeli, S.; Kaloudis, T.; Mazur-Marzec, A. SOP 6. Extraction of cyanotoxins from cyanobacterial biomass. In *Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis*; Meriluoto, J., Spoof, L., Codd, G.A., Eds.; Wiley: Chichester, UK, 2017; pp. 350–353.
60. Cerasino, L.; Capelli, C.; Salmaso, N. A comparative study of the metabolic profiles of common nuisance cyanobacteria in southern perialpine lakes. *Adv. Oceanogr. Limnol.* **2017**, *8*, 22–32. [CrossRef]


## Paper 2

Swe, T., Miles, C. O., Cerasino, L., Mjelde M., Kleiven, S. & Ballot, A., (2021). *Microcystis aeruginosa, Raphidiopsis raciborskii* and *Dolichospermum smithii,* toxin producing and non-toxigenic cyanobacteria in Yezin Dam, Myanmar. *Limnologica*, 90, 125901.

Limnologica 90 (2021) 125901

Contents lists available at ScienceDirect

Limnologica

journal homepage: www.elsevier.com/locate/limno

# *Microcystis, Raphidiopsis raciborskii and Dolichospermum smithii*, toxin producing and non-toxigenic cyanobacteria in Yezin Dam, Myanmar

Thida Swe [a,b,c], Christopher O. Miles [d], Leonardo Cerasino [e], Marit Mjelde [a], Synne Kleiven [c], Andreas Ballot [a,*]

[a] Norwegian Institute for Water Research, Gaustadalléen 21, N-0349, Oslo, Norway
[b] Forest Research Institute, 15013, Yezin, Myanmar
[c] Department of Natural Sciences and Environmental Health, University of South-Eastern Norway, Campus Bø, N-3833, Bø, Norway
[d] Biotoxin Metrology, National Research Council, 1411 Oxford Street, Halifax, NS B3H 3Z1, Canada
[e] Department of Sustainable Agro-ecosystem and Bioresources, Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38010, San Michele all'Adige, Italy

ARTICLE INFO

*Keywords:*
Yezin Dam
Myanmar
*Dolichospermum*
*Raphidiopsis*
*Microcystis*
Cylindrospermopsin
Deoxycylindrospermopsin
Microcystin

ABSTRACT

Yezin Dam is a man-made reservoir located close to Yezin village in Myanmar. Its water is used for irrigation, domestic purposes and as drinking water for many urban communities in the watershed area. In recent years, increased pollution due to the concurrent development around the dam has led to water quality deterioration. No detailed study on the distribution of cyanobacteria and toxin production has been conducted so far. In order to provide insight into the extent of cyanobacteria and cyanotoxins in the dam, water samples were collected once in January 2014 for the isolation of cyanobacterial strains and eight times between March 2017 and June 2018 for the investigation of physical, chemical and biological parameters. A total of 99 phytoplankton taxa belonging to 50 genera were recorded from Yezin Dam. Microscopic examination showed that a *Dolichospermum* sp. was the dominant cyanobacterium followed by small numbers of *Microcystis*, and *Raphidiopsis raciborskii* in all samples throughout the sampling period. 15 isolated cyanobacterial strains were classified morphologically and phylogenetically as *Dolichospermum smithii*, *R. raciborskii* and *Microcystis* and tested for microcystins (MCs), cylindrospermopsins (CYNs), saxitoxins (STXs) and anatoxins (ATXs) by liquid chromatography-tandem mass spectrometry (LC–MS/MS) and enzyme-linked immunosorbent assay (ELISA). The toxin analysis of all isolated *Dolichospermum* strains by ELISA and LC–MS did not indicate the presence of ATXs, STXs, CYNs nor MCs. Four of the five isolated *Raphidiopsis* strains produced CYN and deoxyCYN. One of the isolated *Microcystis* strains (AB2017/08) from Yezin Dam produced 22 MC congeners. Concentrations of 0.12 μg $L^{-1}$ CYNs and 0.34 μg $L^{-1}$ MCs were also found in an environmental sample from Yezin Dam by ELISA. The potential therefore exists for the use of untreated water from Yezin Dam to cause harmful effects on humans, domestic and wild animals.

## 1. Introduction

Like other countries in Asia, Myanmar's economy is based on agriculture. Myanmar has made vast investments in the construction of irrigation dams throughout the country to enhance the availability of water for irrigation during the dry season to increase agricultural production and socioeconomic development (Oo et al., 2017). In Myanmar, around 240 dams have been constructed so far (Oo et al., 2017). Most of these dams, especially those near urban areas, are subject to quick deterioration due to increasing population growth and anthropogenic activities. Sewage discharge and agricultural runoff are the main causes of eutrophication, and harmful algal and cyanobacterial blooms in these water bodies lead to deterioration in water quality and ecosystem health.

Cyanobacterial blooms are often accompanied by the production of cyanobacterial toxins and have been recognized to cause serious chronic human and acute animal health problems and even mortalities (Carmichael, 2001; Hilborn and Beasley, 2015; Paerl and Huisman, 2009). The knowledge about cyanobacteria and their toxins in lakes and reservoirs in Myanmar is, however, poor. Only one recent study has described in detail the presence of cyanobacteria and cyanobacterial toxins, in Meiktila Lake close to the city of Meiktila in Myanmar (Ballot

* Corresponding author.
*E-mail address:* andreas.ballot@niva.no (A. Ballot).

https://doi.org/10.1016/j.limno.2021.125901
Received 3 November 2020; Received in revised form 4 June 2021; Accepted 1 July 2021
Available online 12 July 2021

et al., 2020). It showed that cylindrospermopsin (CYN)- and deoxy-cylindrospermopsin (deoxyCYN)-producing *Raphidiopsis raciborskii* (formerly *Cylindrospermopsis raciborskii*) (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno and microcystin-producing *Microcystis aeruginosa* (Kützing) Kützing and *Microcystis novacekii* (Komárek) Compère are common cyanobacteria in Meiktila Lake (Ballot et al., 2020).

Yezin Dam, which is located near Yezin village in the Zeyar Thiri Township, Nay Pyi Taw, is a man-made earth embankment reservoir. It was completed in 1974 with a total capacity of 0.074 $km^3$ (FAO, 2020). Its main uses are for irrigation and to protect against flooding from the Sinthay River and Yezin Stream (Than et al., 2019). The communities living near the dam use some of the water for domestic purposes and drinking without treatment. The consumption of water from Yezin Dam has been increasing, with increasing development activities and expanding urban areas in the watershed area. Forest degradation and annual forest fires in the Yezin watershed area have gradually increased the rate of sedimentation in the dam since 1975 (Win et al., 2003). Landsat data showed that the water surface area of Yezin Dam decreased by 74 % between 2011 and 2016 due to reduced rainfall in the catchment area and increased domestic water usage (Than et al., 2019). Anthropogenic activities like fishing, bathing, washing clothes with laundry detergents in the dam, poor sanitation and wastewater treatment systems in the watershed area further negatively affect the water quality of the dam.

This study was initiated after we noticed increased algal and cyanobacterial growth in the dam in 2014. We suspected that the presence of cyanobacteria could counteract the societal need for good water quality and could pose serious health problems to the people who directly use the water from the dam. The knowledge about cyanobacteria and cyanotoxins in reservoirs in many tropical countries is still poor. This study aimed therefore to add new information by

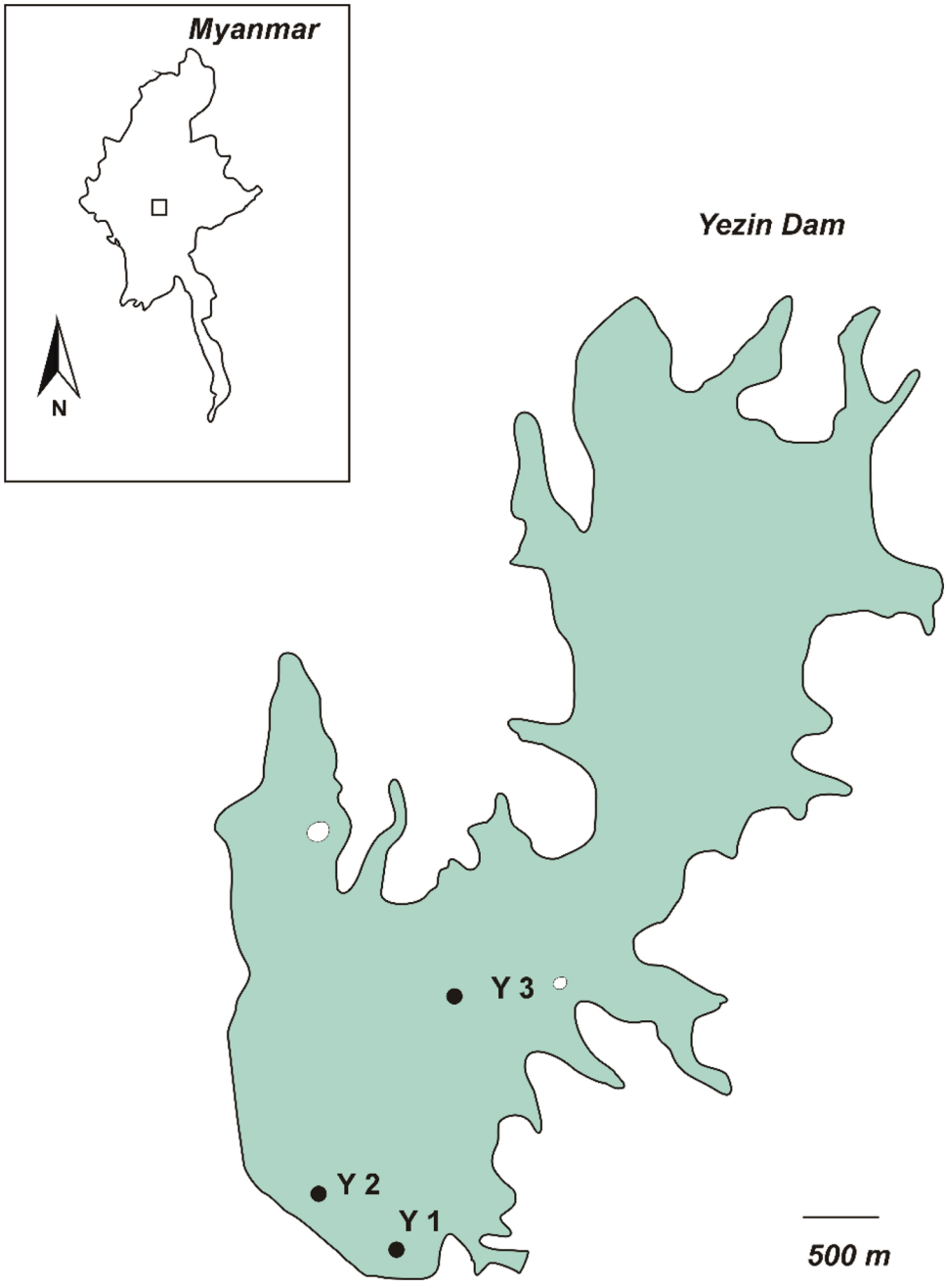



**Fig. 1.** Map of Yezin Dam showing the locations of water sampling (Stations Y1–Y3). The location of Yezin Dam in Myanmar is shown in the inset.

investigating the presence of cyanobacteria and cyanobacterial toxins in Yezin Dam and in cyanobacterial strains isolated from the dam as well as to elucidate in detail the cyanobacterial composition, phylogeny, toxin production and toxin profiles which were investigated in water and cyanobacterial strains isolated from the dam.

## 2. Material and methods

### 2.1. Study area, sampling methods and analyses

Yezin Dam is a man-made reservoir located in the Zeyar Thiri Township, Nay Pyi Taw and situated between Latitudes 19 50′ 36″ N and 19 53′ 08″ N and Longitudes 96 16′ 00″ E and 96 17′ 52″ E, at 133 m above sea level. The dam has been operational since 1976, with a total water storage capacity of 0.074 $km^3$ (FAO, 2020). When filled, it has a surface area of approximately 9.3 $km^2$ (Than et al., 2019).

Three sampling points were selected in the southern part of the dam: near the shore, near the dam, and in the center (Fig. 1). Water sampling could not be performed in the northern part as it is the security area of a military zone. Water samples were collected once in January 2014 for the isolation of cyanobacterial strains, 8 times between March 2017 and June 2018 for phytoplankton analyses and isolation of cyanobacterial strains and once in February 2020 to test for anatoxins, cylindrospermopsins, microcystins and saxitoxins using enzyme-linked immunosorbent assay (ELISA).

Water transparency was measured with a Secchi disk. The water temperature, pH, conductivity and oxygen content were measured in situ with a Hach, HQd Portable Meter (Hach, Loveland, CO, USA). For water chemistry analyses, integrated water samples (1 m steps) were collected from the trophogenic zone (two times the Secchi depth) using a Limnos water sampler (Limnos, Komorów, Poland). Water samples were preserved with 4 M $H_2SO_4$ for nutrient analysis (to 1 % final concentration) and not preserved for analysis of turbidity, total alkalinity, color, calcium and other cations. These samples were analysed at the Water Quality Laboratory in the Forest Research Institute, Yezin, Myanmar.

Subsamples (50 mL) were taken from the integrated samples for quantitative phytoplankton analysis and preserved with acidic Lugol's solution. For qualitative phytoplankton analysis, a concentrated net sample (mesh size 20 μm) was collected and preserved by addition of formaldehyde (to 4 % final concentration). All samples for quantitative and qualitative analysis were stored in the dark until they were analysed. Water samples (50 mL) were taken at each sampling point for isolation of cyanobacteria and kept in a cool shady place and gently shaken twice per day until processing at the Norwegian Institute for Water Research (NIVA) in Norway.

The Lugol-fixed samples were analyzed for phytoplankton composition and biomass using Utermöhl sedimentation chambers (Utermöhl, 1958) and an inverted microscope (Olympus Optical Co-Ltd Japan Model CK2, Olympus, Tokyo, Japan). For cyanobacterial taxa, length and width of 100 vegetative cells, heterocytes and akinetes were measured for morphological characterization. Phytoplankton biomass was calculated by geometrical approximations using the computerized counting program Opticount (SequentiX - Digital DNA Processing, Klein Raden, Germany). The specific density of phytoplankton cells was calculated as 1 g $cm^{-3}$. The taxa were identified to species or genus level using selected identification keys (e.g. Komárek, 2013; Komárek and Anagnostidis, 1999).

### 2.2. Isolation of strains and morphological characterization

Single colonies of *Microcystis* and single filaments of *Dolichospermum* sp. and *Raphidiopsis* sp. were isolated using a microcapillary. They were washed five times and then placed in wells of microtiter plates containing 300 μL Z8 medium (Kotai, 1972). After successful growth, they were placed in 50 mL Erlenmeyer flasks which contained 20 mL Z8 medium and maintained at 22 °C and a photon flux density of 80 mol of photons $m^{-2}$ $s^{-1}$. Classification of the strains was based on morphological traits (Komárek, 2013; Komárek and Anagnostidis, 1999).

The morphology of the isolated strains was identified with a Leica DM2500 light microscope, Leica DFC 450 camera and Leica Application Suite software (LAS) (Leica, Oslo, Norway) based on the: (i) size of vegetative cells and heterocytes, and; (ii) nature and shape of filaments or colonies. The Length and width of 50–250 vegetative cells and of 20–50 heterocytes were measured. All isolated strains used for this study are kept at NIVA.

### 2.3. Genomic DNA extraction, PCR amplification and sequencing

Genomic DNA of all isolated strains was extracted according to Ballot et al. (2016). All PCRs were performed on a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Oslo, Norway) using the iProof High-Fidelity PCR Kit (Bio-Rad Laboratories, Oslo, Norway). The 16S rRNA gene of the isolated strains from Yezin Dam was amplified using the primers as described by Ballot et al. (2016) (Table S1). PCR products were visualized by 1 % agarose gel electrophoresis with GelRed staining (GelRed Nucleic Acid Gel Stain, Biotium, Fremont, CA, USA) and UV illumination. The amplified PCR products were purified with Qiagen PCR purifications columns (Qiagen, Hilden, Germany) in accordance with the manufacturer's protocol. Sequencing was performed on both strands of each PCR product, with the same primers used for PCR and intermediate primers as used in Ballot et al. (2016), on an ABI 3730 Avant genetic analyser using the BigDye terminator V.3.1 cycle sequencing kit (Applied Biosystems, Thermo Fisher Scientific, Oslo, Norway) following the manufacturer's instructions.

### 2.4. Phylogenetic analysis

16S rRNA gene sequences of *Dolichospermum, Raphidiopsis* and *Microcystis* strains were analyzed using the Seqassem software package (version 07/2008) and the Align MS Windows-based manual sequence alignment editor (version 03/2007) (SequentiX - Digital DNA Processing, Klein Raden, Germany).

A 16S rRNA gene set containing 1136 positions was used for the calculation of the *Anabaena/Dolichospermum* phylogenetic tree. Eight *Dolichospermum* sequences from Yezin Dam and 23 additional *Dolichospermum* sequences derived from GenBank were included in the analysis and *Cuspidothrix issatschenkoi* (FN689797) was employed as the outgroup. A 16S rRNA gene set of 1136 positions was used for the calculation of the *Raphidiopsis/Cylindrospermopsis* phylogenetic tree. Five sequences from *Raphidiopsis* strains isolated from Yezin Dam, and 37 additional *Cylindropsermopsis/Raphidiopsis* sequences derived from GenBank were included in the analyses and *Sphaerospermopsis aphanizomenoides* (LN846954) was employed as the outgroup. A set containing 1427 positions was used for the *Microcystis* 16S rRNA gene analysis. *Chroococcus subviolaceus* (MF072353) was employed as the outgroup. Two sequences from *Microcystis* strains isolated from Yezin Dam, and 42 additional *Microcystis* sequences derived from GenBank were included in the analysis.

Phylogenetic trees for 16S rRNA gene sequences were constructed using the maximum likelihood (ML) algorithm in Mega versionX with 1000 bootstrap replicates (Kumar et al., 2018). Evolutionary substitution models were evaluated using Mega version X (Kumar et al., 2018). The HKY + G + I evolutionary model was found to be the best-fitting evolutionary model for the *Anabaena/Dolichospermum* 16S rRNA gene tree, K2+G + I for the *Cylindrospermopsis/Raphidiopsis* 16S rRNA gene tree and T92+G + I for the *Microcystis* 16S rRNA gene tree. The data were submitted to the European Nucleotide Archive (ENA) under the accession numbers listed in Table 1. 16S rRNA gene sequence similarities were calculated separately for the *Dolichospermum*, *Microcystis* and *Raphidiopsis* sequences and are depicted in Table S2.

**Table 1**
Cyanobacterial strains isolated from Yezin Dam, strain codes and European Nucleotide Archive (ENA) accession numbers.

| Taxon | Strain | ENA accession nr. 16S rRNA gene |
|---|---|---|
| *Dolichospermum* | | |
| *D. smithii* | AB2014/01 | LR794159 |
| *D. smithii* | AB2014/02 | LR794160 |
| *D. smithii* | AB2014/03 | LR794161 |
| *D. smithii* | AB2014/04 | LR794162 |
| *D. smithii* | AB2014/05 | LR794163 |
| *D. smithii* | AB2014/06 | LR794164 |
| *D. smithii* | AB2017/17 | LR794165 |
| *D. smithii* | AB2017/18 | LR794166 |
| *Raphidiopsis* | | |
| *R. raciborskii* | AB2017/03 | LR794167 |
| *R. raciborskii* | AB2017/04 | LR794168 |
| R. *raciborskii* | AB2017/06 | LR794169 |
| *R. raciborskii* | AB2017/19 | LR794170 |
| *R. raciborskii* | AB2017/20 | LR794171 |
| *Microcystis* | | |
| *Microcystis* | AB2017/08 | LR794172 |
| *Microcystis* | AB2017/10 | LR794173 |

### 2.5. Toxin analysis

#### 2.5.1. Materials

The standards used for LC–MS analyses were: anatoxin-a (ATX) (Tocris Bioscience, Bristol, UK), homoATX (Novakits, Nantes, France), cylindrospermopsin (CYN) (Vinci Biochem, Vinci, Italy); certified reference materials (CRMs) of saxitoxin (STX), decarbamoylSTX (dcSTX), NeoSTX, gonyautoxins (GTX1, GTX4, GTX5), *N*-sulfocarbamoylgonyautoxins (C1 and C2 toxins), MC-RR, MC-LR (**10**) and [Dha$^7$]MC-LR (**11**) (National Research Council of Canada, Halifax, Canada); reference materials (RMs) of [Leu$^1$]MC-LY (LeBlanc et al., 2020), and of [D-Asp$^3$]MC-RR, [D-Asp$^3$]MC-LR (**8**), MC-YR, MC-HilR (**12**) having traces of MC-FR, MC-WR, and MC-LA prepared from commercial samples (Enzo Life Sciences, Farmingdale, NY, USA) produced at NRC Canada, and; extracts containing an array of other identified MCs were available from previous work (Ballot et al., 2020; Mallia et al., 2019; Miles et al., 2014; Yilmaz et al., 2019). Standards for the Adda-ELISA and for the CYN, ATX and STX ELISAs were provided in the kits (Abraxis LLC, Warminister, PA, USA).

#### 2.5.2. ELISA for MCs, CYNs, ATXs and STXs

Fresh cultures of *Dolichospermum*, *Microcystis*, and *Raphidiopsis* strains isolated from Yezin Dam were frozen and thawed three times. The *Raphidiopsis* strains were tested for CYNs using the Abraxis Cylindrospermopsin ELISA kit following the manufacturer's instructions. The test is a direct competitive ELISA that detects CYN but also recognizes deoxyCYN and 7-*epi*-CYN to varying degrees. The *Microcystis* strains were tested for MCs using the Abraxis Microcystins/Nodularins (ADDA) ELISA kit. The test is an indirect competitive ELISA designed to detect Adda, (3*S*-amino-9*S*-methoxy-2*S*,6,8*S*-trimethyl-10-phenyldeca-4*E*,6*E*-dienoic acid), based on specific recognition of the Adda moiety (Fischer et al., 2001). ADDA is a non-protein amino acid and forms a side chain in the microcystin molecule that is present in about 80 % of reported microcystin variants (Bouaïcha et al., 2019). The Adda-ELISA is reported to have very low sensitivity for MCs containing modified Adda variants (Foss et al., 2020).

All strains were also tested for saxitoxins and anatoxin-a analogues using the Abraxis Saxitoxins (PSP) and Abraxis Anatoxin (VFDF) ELISA kits. The saxitoxin ELISA is a direct competitive ELISA that detects STX based on specific antibody recognition but also recognizes other saxitoxins (e.g., dcSTX, GTXs, lyngbyatoxin, and NeoSTX) to varying degrees. The test for ATX-a is a direct competitive ELISA that detects anatoxin-a based on specific antibody recognition and but also recognizes homoATX. In addition, a water sample taken from Yezin Dam in February 2020 was tested for the presence of STXs and ATXs, CYNs, and MCs with the above-mentioned ELISA kits. The colour reaction of all ELISA tests was evaluated at 450 nm on a Perkin Elmer 1420 Multilabel counter Victor3 (Perkin Elmer, Waltham, MA, USA) (strain samples) or a Multiskan FC Microplate Photometer (Thermo Fisher Scientific, Waltham, MA, USA) (field samples).

#### 2.5.3. Microcystin analysis by LC–HRMS

Fresh culture material of both *Microcystis* strains was prepared for LC–HRMS by freeze-thawing (3 times), diluting with an equal volume of MeOH, and filtering (0.22 μm) (Miles et al., 2012). LC–HRMS/MS analysis was performed on a Q Exactive-HF Orbitrap mass spectrometer equipped with a HESI-II heated electrospray ionization interface (ThermoFisher Scientific, Waltham, MA, USA), using an Agilent 1200 LC system including a binary pump, autosampler and column oven (Agilent, Santa Clara, CA, USA), and a SymmetryShield 3.5 μm C18 column (100 × 2.1 mm; Waters, Milford, MA, USA) as described by Ballot et al. (2020). Extracts were derivatized with a 1:1 mixture of mercaptoethanol and $d_4$-mercaptoethanol (Sigma–Aldrich, St. Louis, MO, USA), or oxidized with DMSO/Oxone, as described by Ballot et al. (2020) and analyzed by LC-HRMS.

#### 2.5.4. CYNs, ATXs and STXs analysis by LC–MS/MS

Cyanobacterial toxins were extracted from freeze-dried cultures (40 mL), with 6 mL of 50 % methanol and sonicated (Omniruptor4000 probe sonicator, Omni-Inc., Kennesaw, MA, USA) for 10 min in pulsed mode (50 %) using 160 W power (Cerasino et al., 2017b). An aliquot of the solution was then filtered on Phenex RC syringe filters (0.2 m; Phenomenex, Castel Maggiore, Italy) and analyzed by LC–MS/MS using a Waters Acquity UPLC system (Waters, Milford, MA, USA) coupled to a SCIEX 4000 QTRAP mass spectrometer (AB Sciex Pte. Ltd., Singapore). Chromatographic separation of analytes was performed using a HILIC column (Ascentis Express OH5, 2.7 μm, 50 × 2.1 mm; Merck Life Science S.r.l., Milan, Italy), while MS detection was performed using positive electrospray ionization using scheduled Multiple Reaction Monitoring (Cerasino et al., 2017a). The method was suitable for the detection and quantification of the following toxins: ATX, CYN, STX, dcSTX, NeoSTX, GTX1, GTX4, GTX5 and C1 and C2 (Cerasino et al., 2017a). Quantification limits were 0.2–200 μg $L^{-1}$. Other toxic alkaloids not available as pure standards were screened only for tentative analysis (hydroxy-, epoxy-, and homo-ATXs, deoxyCYN, dcNeoSTX, GTX2/3, dcGTX2/3 and C3 and C4 toxins) using equivalent detection settings to their most similar analogs.

## 3. Results

### 3.1. Physical and chemical parameters

The pH varied between 7.2–8.7, the water temperature between 27.4 °C and 32.5 °C and the conductivity between 77 μS $cm^{-1}$ and 87 μS $cm^{-1}$. The Secchi depth varied between 1 and 1.5 m. The total phosphorous (TP) concentrations ranged from 20 to 57 μg $L^{-1}$ (see Table S3 for further physical and chemical data).

### 3.2. Phytoplankton community

During the study period, 99 phytoplankton taxa belonging to 50 genera and 10 classes were identified in water samples from Yezin Dam. Of those genera, 12 belonged to Chlorophyceae, 11 to Cyanobacteria, 8 to Trebouxiophyceae, 6 to Zygnematophyceae, 4 to Bacillariophyceae, 3 each to Dinophyceae and Euglenophyceae, and 1 genus to each of Synurophyceae, Cryptophyceae and Chrysophyceae.

The phytoplankton community in Yezin Dam showed a patchy distribution at all three sampling sites, with different groups dominating at different sites and time (Fig. 2). In general, cyanobacteria were the

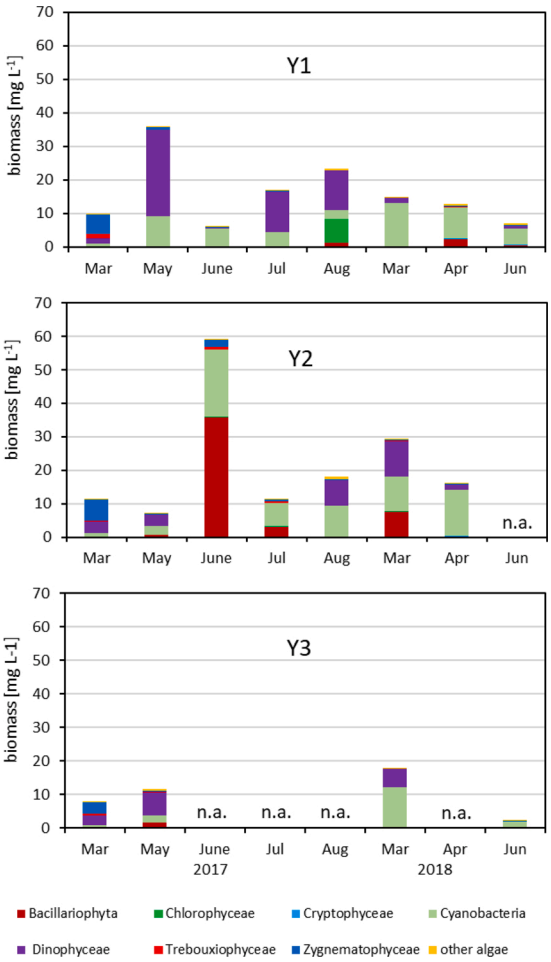



**Fig. 2.** Biomass (mg $L^{-1}$ FW) of phytoplankton groups at all three sampling points in Yezin Dam (March 2017–June 2018). n.a. = not analysed.

dominant phytoplankton organisms, with highest biomasses, recorded at site Y2 in June 2017 and April 2018 and at site Y1 in March 2018, comprising between 34 % and 86 % of the total phytoplankton biomass (Fig. 2, Table S4). Dinophyceae was the most dominant class of algae in May, July and August 2017 at sampling point Y1, while diatoms (Bacillariophyceae) were dominant at sampling point Y2 in June 2017. (Fig. 2, Table S4)

Among the cyanobacteria, *Dolichospermum* was the dominating taxon at all sampling stations throughout the sampling period, followed by *Oscillatoria* spp., *Limnothrix* spp. and *Microcystis*. The *R. raciborskii* biomass was lower than the *Dolichospermum* or *Microcystis* biomasses at all sampling points and sampling dates (Fig. 3, Table S5).

### 3.3. Morphological and phylogenetic characterization

Fifteen potentially toxin-producing cyanobacterial strains were isolated from Yezin Dam (Table 1). According to their morphological features (e.g., presence and form of vegetative cells, heterocytes and akinetes), eight of the isolated strains were morphologically classified as *D. smithii*, five as *R. raciborskii*, and two as *M. aeruginosa*. As genetic methods do not support the morphologically based assignment of *Microcystis* spp., Harke et al. (2016) suggested all

*Microcystis* spp. warrant placement into the same species complex. In the following parts of the manuscript, we use therefore "Microcystis" instead of species names.

The morphological determinations of the isolated strains were confirmed by genetic methods. Phylogenetic relationships of the investigated strains are presented in the maximum likelihood (ML) trees of

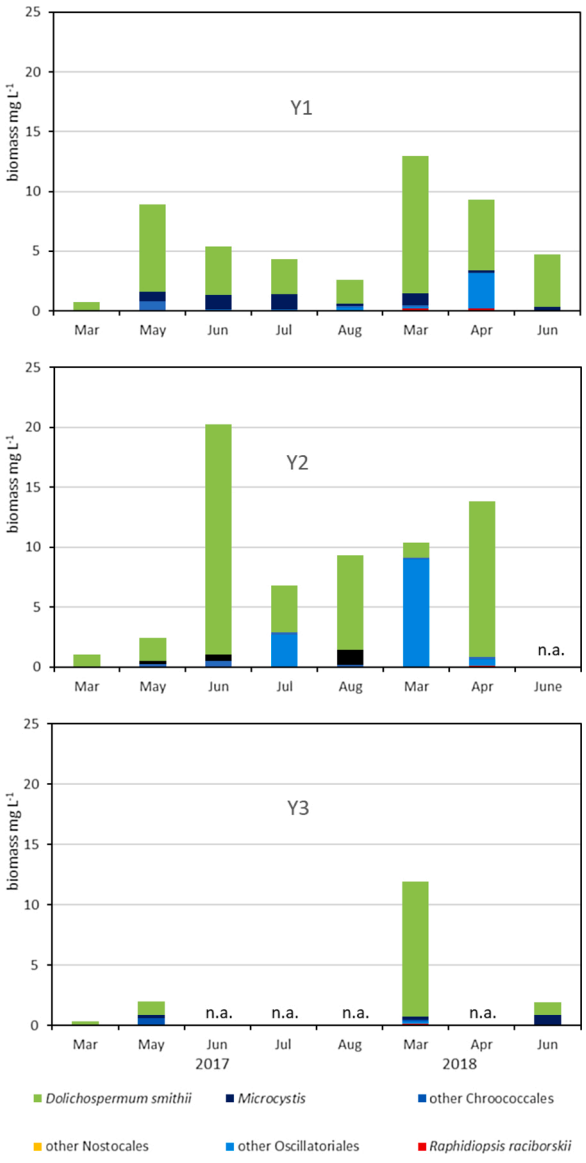



**Fig. 3.** Biomass (mg $L^{-1}$ FW) of the cyanobacterial taxa at all three sampling points in Yezin Dam (from March 2017–June 2018). n.a. = not analysed.

the 16S rRNA gene for *Dolichospermum* (Fig. S1), *Raphidiopsis* (Fig. S2), and *Microcystis* (Fig. S3).

In the ML tree in Fig. S1, all isolated *Dolichospermum* strains from Yezin Dam were grouped in the same subcluster and were assigned to *D. smithii*. The phylogenetic analyses of the 16S rRNA gene revealed that they were most similar to other *D./A. smithii*, but also to *D. spiroides, D. mucosum, D. ucrainicum, D. viguieri, D. circinale* and *D. pseudocompactum* sequences, described from the Czech Republic and Japan. In the *Raphdiopsis* ML tree, the strains from Yezin Dam grouped together with 16S rRNA gene sequences of *Cylindrospermopsis* and *Raphidiopsis* strains from Asia, Europe, Australia and North America. *Raphidiopsis* strains isolated from Meiktila Lake in Myanmar were also found in the same cluster (Fig. S2).

Both *Microcystis* strains from Yezin Dam clustered together with 16S rRNA gene sequences of *Microcystis* from Europe, Asia and Africa (Fig. S3). They differed slightly in their 16S rRNA sequences (99.72 % similarity).

### 3.4. Identification of cyanobacterial toxins

All investigated *D. smithii* strains from Yezin Dam were negative for MCs, CYNs, STXs, and ATXs by ELISA. All five *R. raciborskii* strains from Yezin Dam were also negative for STXs, ATXs and MCs by ELISA, but four of the strains were shown to produce CYNs either by ELISA or LC–MS/MS (Table 2).

Both *Microcystis* strains AB2017/08 and AB2017/10 were negative for CYNs, STXs and ATXs by ELISA, but strain AB2017/08 was identified as MC-producer by ELISA. In culture, 22 microcystin congeners were detected in this strain by LC–HRMS/MS, with a total concentration of 1160 μg $g^{-1}$ FW. Six of the congeners were unidentified or previously unreported variants. The microcystin variants and their concentrations are shown in Tables 3 and S6. In the environmental sample taken from Yezin Dam in February 2020, 0.12 μg $L^{-1}$ CYNs and 0.34 μg $L^{-1}$ MCs were measured by ELISA, but no ATXs and STXs were detected.

## 4. Discussion

The water of Yezin Dam is used for domestic purposes and drinking water by the local people. TP concentrations between 20 and 57 μg $L^{-1}$ demonstrate that Yezin Dam is in a mesotrophic to eutrophic status (Salas and Martino, 1991) and explain the high phytoplankton and cyanobacterial biomasses. Rigosi et al. (2015) have investigated the probability for hazardous cyanobacterial blooms based on water temperature and TP by using Bayesian networks in multiple lake systems. They showed that at temperatures above 24 °C and TP values between 20 and 100 μg $L^{-1}$, which are typical conditions for Yezin Dam, the probability for hazardous cyanobacterial blooms is almost 50 %. Different cyanobacterial taxa have diverse responses to varying nitrogen vs. phosphorus enrichment and cannot be treated as a single group when considering the effects of nutrient loading on the composition of the phytoplankton community (Dolman et al., 2012). Beside the presence or absence of toxin producing species, the amount of toxins is also dependent on the relation of toxic versus non-toxic strains and can therefore not be predicted by biomass of a certain taxon only. The phytoplankton data show clearly that there is a spatial and temporal variation in the distribution of the dominant phytoplankton groups in Yezin Dam. Patchiness on small and bigger scales has been described for phytoplankton distribution in lake and ocean environments, especially for species that have good buoyancy-regulating mechanisms like cyanobacteria or dinoflagellates (Borics et al., 2011; Breier et al., 2018). These spatial and temporal variations make it difficult to relate nutrient values to the biomass of phytoplankton groups.

The low occurrence of aquatic macrophytes and high nutrient loading in Yezin Dam may be the reason for the high algal and cyanobacterial biomasses observed. Only a few aquatic macrophytes, *Hydrilla verticillata* and *Chara fibrosa* are found in the dam due to the excessive removal of aquatic macrophytes since the 1990s (Mjelde et al., 2021; personal communication with local population) and water level regulations. Aquatic macrophytes can through allelopathic effects and competition for nutrients prevent the growth of planktonic algae and potentially toxic cyanobacteria and help to maintain a clear water state (Wojciechowski et al., 2018; Cheng et al., 2017; Hilt and Gross, 2008). Biological management can therefore be a suitable measure to control cyanobacterial blooms, and planting of macrophytes should be considered as a measure to help control the growth of cyanobacteria and other phytoplankton in Yezin Dam.

During the study period *D. smithii* was the dominant cyanobacterium in the phytoplankton of Yezin Dam but no cyanobacterial toxins of the MC, STX, ATX or CYN groups were produced by the isolated *D. smithii* strains. Similarly, no strains of *D. smithii* from other locations worldwide have yet been reported to produce cyanobacterial toxins. *D. smithii* is neither part of the phytoplankton in Meiktila Lake, nor has it been described from other water bodies in Myanmar (Ballot et al., 2020). *D. smithii* is reported of mesotrophic to slightly eutrophic ponds, reservoirs and lakes in temperate areas (Komárek, 2013). Although *D. smithii* is not seen as a tropical species, we assigned the strains isolated from Yezin Dam to this taxon because the morphological characteristics fitted best to *D. smithii,* which is also supported by the phylogenetic analyses. Although the *Dolichospermum* strains isolated from Yezin Dam are grouped in a separate subcluster in the phylogenetic tree in Fig. S1, they are part of a cluster including species of *D. smithii* but also *D. viguieri*, *D. mucosa*, *D. spiroides*, *D. circinale* and *D. ucrainicum*. This demonstrates the difficulty to assign *Dolichospermum* sp. from Yezin Dam to a particular species based on publicly available genetic data only, because reliable use of phylogenetic data from public databases (e.g., NCBI, ENA) depends strongly on their unambiguity. Reports of *D. smithii* from tropical Brazil and Senegal support our assignment (Berger et al., 2005;

**Table 2**
Concentrations (μg $mg^{-1}$ FW) of CYNs (ELISA), and of CYN and deoxyCYN (LC–MS/MS) in *R. raciborskii* strains isolated from Yezin Dam, Myanmar.

| Strain | ELISA | LC-MS/MS | | | |
|---|---|---|---|---|---|
| | CYNs | CYN | deoxyCYN | CYN % | deoxyCYN % |
| AB2017/03 | 0.89 | 0.17 | 0.11 | 61 | 39 |
| AB2017/04 | 3.50 | 0.2 | 0.11 | 65 | 35 |
| AB2017/06 | n.d. | n.d. | n.d | n.d. | n.d. |
| AB2017/19 | 3.18 | 0.75 | 1.84 | 29 | 71 |
| AB2017/20 | 1.86 | 1.03 | 2.19 | 32 | 68 |

Percentages are % of total CYNs measured by LC–MS/MS. n.d. = not detected.

**Table 3**
Microcystins detected by LC-HRMS/MS analysis in *Microcystis* strain AB2017/08 isolated from Yezin Dam, their retention times ($t_R$), concentrations (μg $g^{-1}$ FW), relative abundances (%), and observed *m/z* values in negative ionisation mode.[a]

| | *m/z* | Compound name | Confidence | $t_R$ (min) | Concentration[b] AB2017/08 μg $g^{-1}$ | % |
|---|---|---|---|---|---|---|
| 1 | 1011.5521[c] | Unidentified MC | Unidentified | 5.10 | 1.3 | 0.11 |
| 2 | 1011.5521 | Unidentified MC | Unidentified | 5.85 | 22.4 | 1.93 |
| 3 | 1130.5562 | MC-LR-Cys(O) | Tentative | 6.14 | 0.1 | 0.01 |
| 4 | 1114.5612 | MC-LR-Cys | Probable | 6.16 | 10.3 | 0.89 |
| 5 | 997.5364 | [D-Asp$^3$,Mser$^7$] MC-LR | Tentative | 6.70 | 1.3 | 0.11 |
| 6 | 1011.5521 | [Mser$^7$]MC-LR | Probable | 6.81 | 9.5 | 0.82 |
| 7 | 997.5364 | [D-Asp$^3$,Mser$^7$] MC-LR | Tentative | 6.88 | 1.4 | 0.12 |
| 8 | 979.5258[c] | [D-Asp$^3$]MC-LR | Confirmed | 6.90 | 181.9 | 15.68 |
| 9 | 1011.5521[c] | Unidentified | Unidentified | 7.06 | 7.8 | 0.67 |
| 10 | 993.5415[c] | MC-LR | Confirmed | 7.12 | 891.7 | 76.89 |
| 11 | 979.5258[c] | [Dha$^7$]MC-LR | Confirmed | 7.13 | 9.6 | 0.82 |
| 12 | 1007.5571[c] | MC-HilR | Confirmed | 7.39 | 9.5 | 0.82 |
| 13 | 980.5099[c] | Unidentified | Unidentified | 11.26 | 0.5 | 0.04 |
| 14 | 994.5255[c] | Unidentified | Unidentified | 12.20 | 0.8 | 0.06 |
| 15 | 952.5037[c] | Unidentified | Unidentified | 13.39 | 0.2 | 0.01 |
| 16 | 986.4881[c] | [D-Asp$^3$]MC-LY | Tentative | 14.62 | 0.3 | 0.02 |
| 17 | 1000.5037[c] | MC-LY | Tentative | 15.14 | 1.4 | 0.12 |
| 18 | 1009.5082[c] | [D-Asp$^3$]MC-LW | Tentative | 16.41 | 1.7 | 0.14 |
| 19 | 970.4931[c] | [D-Asp$^3$]MC-LF | Tentative | 16.95 | 0.5 | 0.05 |
| 20 | 1023.5197[c] | MC-LW | Tentative | 17.00 | 3.7 | 0.32 |
| 21 | 984.5088[c] | MC-LF | Tentative | 17.61 | 3.8 | 0.33 |
| 22 | 950.5244[c] | MC-LL | Probable | 18.03 | 0.2 | 0.01 |

[a] A comprehensive version of this Table, including positive and negative ionisation MS data, reactivity towards thiols, proposed formulae, mass error, number of rings plus double-bond equivalents (RDBE), and presence of characteristic ions observed in positive and negative ionisation MS/MS spectra, is in the Supporting Information (Table S1) together with LC–HRMS/MS spectra (Figs. S4–S7). [b] Concentration is expressed per weight of biomass (FW) and as a percentage of the total microcystins detected in each culture; [c] Reacted with mercaptoethanol (1 equivalent).

Sant'Anna, 1991).

ELISA analyses of a water sample from February 2020 confirm the presence of both CYNs and MCs in Yezin Dam. Our study demonstrated clearly that CYN- and deoxyCYN-production can be related to the presence of *R. raciborskii* and MCs production to the presence of *Microcystis*. The biomass of both taxa has been relatively low during the study period. This fact and the co-existence of non-toxin-producing and toxin-producing *Microcystis* and *R. raciborskii* strains can explain the relatively low toxin concentrations in February 2020.

In a recent study, CYNs-producing *R. raciborskii* was also found in Meiktila Lake with up to ten times higher biomasses than in Yezin Dam (Ballot et al., 2020). The *Raphidiopsis* strains from Yezin Dam produce CYN and deoxyCYN is in the same or slightly lower range as found for *R. raciborskii* strains from Meiktila Lake (2.9–9.8 μg $mg^{-1}$ FW) (Ballot et al., 2020). Australian *Raphidiopsis* strains are described to produce up to 3.5 μg $mg^{-1}$ FW of CYNs, but these values comprise intracellular CYNs only (Saker and Griffiths, 2000).

Production of CYNs by *R. raciborskii* has also been reported from lakes in China, Japan, Thailand and Vietnam (Chonudomkul et al., 2004; Hawkins et al., 1997; Nguyen et al., 2017). Brazilian, *Raphidiopsis* strains are known to produce saxitoxins but not CYNs (Hoff-Risseti et al., 2013). Although *R. raciborskii* is also widely distributed in Europe, only one study by Đorđević et al. (2015) has related findings of CYN in Serbian Lake Aleksandrovac to the presence of *R. raciborski.* This was not confirmed by genetic and chemical investigations of isolated strains.

*Microcystis* is widespread in many Asian countries (Harke et al., 2016), but has been reported from only a few water bodies in Myanmar (Ballot et al., 2020; Green, 2011; Naw et al., 2020). Both *Microcystis* strains from Yezin Dam differ slightly in their 16S sequences, which also differ slightly from those of the two *Microcystis* strains AB2017/14 and AB2017/15 isolated from Meiktila Lake (Ballot et al., 2020). The four *Microcystis* strains from Yezin Dam and Meiktila Lake cluster together with strains from Asia, Europe and South America.

Only one of the two *Microcystis* strains isolated from Yezin Dam was found to produce MCs. With 1160 μg $g^{-1}$ FW the MC concentration is in the same range as described for a *Microcystis* strain from South African Hartbeespoort Dam (Ballot et al., 2014). Higher MC concentrations in *Microcystis* strains up to 5.8 μg $mg^{-1}$ dry weight are reported by Vézie et al. (2002). The coexistence of MC-producing and non-producing *Microcystis* strains has also been reported from Hartbeespoort Dam, where only one of 16 isolated strains produced MCs (Ballot et al., 2014). The MC profile of strain AB2017/08 from Yezin Dam differs considerably from those of the strains from Hartbeespoort Dam and Meiktila Lake (Ballot et al., 2014, 2020). Twenty-two MC congeners were produced by AB2017/08, MC-LR and [D-$Asp^3$]MC-LR were the dominant MC congeners, comprising 76.9 % and 15.7 %, respectively, of the total MCs detected. Of the six unidentified MCs (Table 3), the accurate masses of **1**, **2** and **9** correspond to MC-LR + $H_2O$ but had different retention times to [Mser7]MC-LR (**6**). However, due to the lack of MS/MS spectra with adequate signal-to-noise for these minor compounds, they must all be regarded as "unidentified". One interesting feature is that although the majority of the MCs (entries 5–21 have similar relative intensities in both positive and negative ionization modes), the four earliest-eluting compounds were significantly relatively more intense in negative mode (Fig. 4). This may be due to the presence of extra phenolic or carboxylic acid groups, with the latter appearing to be the case for **4** and **3** (the Cys-adduct of MC-LR, and its sulfoxide). With 52 MC congeners, *Microcystis* strain AB2017/14 from Meiktila Lake produces a considerably higher number of MCs, but MC-LR and [D-$Asp^3$]MC-LR together make up only 20 % of the total MC concentration in that strain (Ballot et al., 2020).

The concentrations of CYNs (0.12 μg $L^{-1}$) and MCs (0.34 μg $L^{-1}$) measured in Yezin Dam were well below the provisional short-term drinking-water guideline value (GV) of 3 μg $L^{-1}$ for CYNs and 12 μg

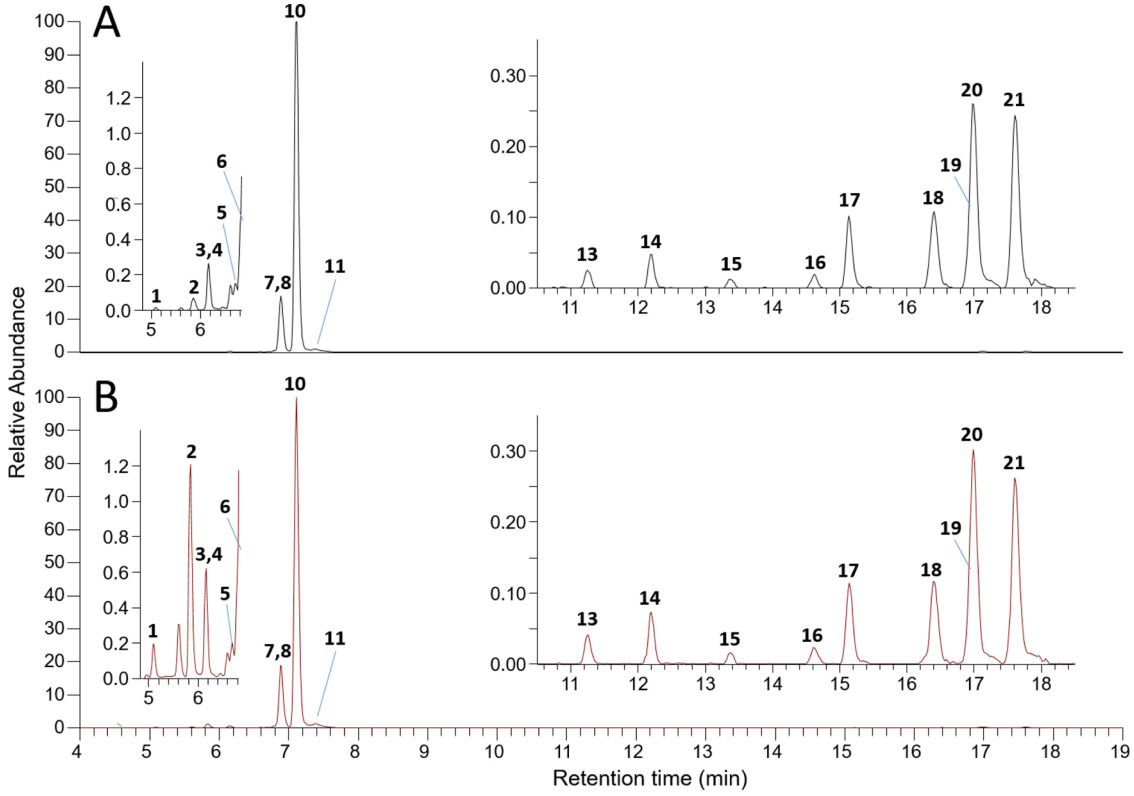



**Fig. 4.** LC–HRMS FS chromatograms, extracted (±5 ppm) for the *m/z* values in Table 3, from analysis of microcystins in an extract from a culture of *Microcystis* strain AB2017/08 isolated from Yezin Dam: A, in positive ionization mode, and; B, in negative ionization mode. The two insets in each chromatogram show vertical expansions that display the minor components. Peaks are labelled with the compound numbers in Table 3, and some of the very minor peaks are not labelled. The intensity scales of both chromatograms are scaled relative to the most intense peak (MC-LR (**10**)).

$L^{-1}$ for MCs and well below the provisional lifetime drinking-water GV of 0.7 μg $L^{-1}$ for CYN and 1 μg $L^{-1}$ for MC-LR (WHO, 2020a, 2020b). The provisional lifetime drinking water GVs would be exceeded after the daily intake of more than 6 L of water (CYNs) or 3 L of Water (MCs). In the event of a bloom of toxic *Raphidiopsis* or *Microcystis* strains, a much lower intake of water would be enough to exceed the above mentioned GVs. However, this assumes also that CYN and deoxyCYN or the MC variants found have similar toxicities. Norris et al. (1999) and Li et al. (2001) did not find any significant contribution of deoxyCYN to the total toxicity of *R. raciborskii*. In contrast, cell viability assays showed the toxicity of deoxyCYN to be only slightly lower than that of CYN and that deoxyCYN and CYN act by same mechanism of toxicity (Neumann et al., 2007) which is also supported by WHO (2020a). The GV values for MC are based on the toxicity of MC-LR. MCs in water samples are often measured as MC-LR equivalents. However, the toxicity has only been investigated for some of the 257 MC variants known today and can differ considerably from that of MC-LR (Bouaïcha et al., 2019). It is to be expected that the relative abundance of the different cyanobacterial taxa in Yezin Dam will vary over time, and a future shift from the dominance of non-toxic *Dolichospermum* to dominance of CYN-producing *Raphidiopsis* or MC-producing *Microcystis* cannot be excluded. An increase in their biomasses would lead to increased concentrations of CYNs and MCs in the water.

## 5. Conclusions

In conclusion, the present study investigates for the first time the occurrence of cyanobacteria and cyanotoxins in Yezin Dam, Myanmar. Although all *Dolichospermum* strains isolated from Yezin Dam were negative for production of cyanotoxins, one of the *Microcystis* strains produced MCs, and four of the five isolated *Raphidiopsis* strains produced CYN and deoxyCYN.

Aside from their toxins being relevant to human health, cyanobacteria have the potential to cause substantial ecological impacts on aquatic food webs. As the dam is an important source for drinking water and irrigation, the monitoring of cyanobacterial blooms and their toxins should be considered as an important basis for the integrated water resource management plan of the country (environmental and health risk assessment plan of the water bodies).

## Authors statement

Thida Swe: Conceptualization, Methodology, Visualization, Investigation, Writing - original draft, Writing - review & editing.

Christopher O. Miles: Methodology, Visualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing.

Leonardo Cerasino Methodology, Formal analysis, Writing - original draft, Writing - review & editing.

Marit Mjelde: Conceptualization, Writing - original draft, Writing - review & editing, Supervision.

Synne Kleiven: Investigation, Writing - review & editing.

Andreas Ballot: Conceptualization, Methodology, Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing, Supervision.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

We thank Moe Myint Thu, Khant Ko Hlaing and Thida Cho for help during field work. This research was supported by the Project “Integrated Water Resources Management (IWRM) –Institutional Building and Training in Myanmar (funded by the Norwegian Ministry of Foreign Affairs and the Royal Norwegian Embassy in Myanmar).

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.limno.2021.125901.

## References

Ballot, A., Sandvik, M., Rundberget, T., Botha, C.J., Miles, C.O., 2014. Diversity of cyanobacteria and cyanotoxins in Hartbeespoort Dam, South Africa. Mar. Freshwat. Res. 65, 175–189.

Ballot, A., Cerasino, L., Hostyeva, V., Cirés, S., 2016. Variability in the sxt gene clusters of PSP toxin producing *Aphanizomenon gracile* strains from Norway, Spain, Germany and North America. PLoS One 11, e0167552.

Ballot, A., Swe, T., Mjelde, M., Cerasino, L., Hostyeva, V., Miles, C.O., 2020. Cylindrospermopsin- and deoxycylindrospermopsin-producing *Raphidiopsis raciborskii* and microcystin-producing *Microcystis* spp. in Meiktila Lake, Myanmar. Toxins 12, 232.

Berger, C., Coute, A., Gansoumana, N., Gugger, M., 2005. Cyanobacterial taxa of the Senegal River system (northern Senegal, West Africa). Algol. Stud. 117, 147–176.

Borics, G., Abonyi, A., Krasznai, E., Várbíró, G., Grigorszky, I., Szabó, S., Déak, C., Tóthmérész, B., 2011. Small-scale patchiness of the phytoplankton in a lentic oxbow. J. Plankt. Res. 33, 973–981.

Bouaïcha, N., Miles, C.O., Beach, D.G., Labidi, Z., Djabri, A., Benayache, N.Y., Nguyen-Quang, T., 2019. Structural diversity, characterization and toxicology of microcystins. Toxins 11, 714.

Breier, R.E., Lalescu, C.C., Waas, D., Wilczek, M., Mazza, M.G., 2018. Emergence of phytoplankton patchiness at small scales in mild turbulence. Proc. Natl. Acad. Sci. U. S. A. 115, 12112–12117.

Carmichael, W.W., 2001. Health effects of toxin-producing cyanobacteria: "The CyanoHABs". Hum. Ecol. Risk Assess. 7, 1393–1407.

Cerasino, L., Capelli, C., Salmaso, N., 2017a. A comparative study of the metabolic profiles of common nuisance cyanobacteria in southern perialpine lakes. Adv. Oceanogr. Limnol. 8, 22–32.

Cerasino, L., Meriluoto, J., Bláha, L., Carmeli, S., Kaloudis, T., Mazur-Marzec, A., 2017b. SOP 6. Extraction of cyanotoxins from cyanobacterial biomass. In: Meriluoto, J., Spoof, L., Codd, G.A. (Eds.), Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis. Wiley, pp. 350–353.

Cheng, L., Cao, X., Yu, X., 2017. Mechanism of growth inhibition effect of aquatic plants on water cyanobacteria. Insights Biol. Med. 1, 1–9.

Chonudomkul, D., Yongmanitchai, W., Theeragool, G., Kawachi, M., Kasai, F., Kaya, K., Watanabe, M.M., 2004. Morphology, genetic diversity, temperature tolerance and toxicity of *Cylindrospermopsis raciborskii* (Nostocales, Cyanobacteria) strains from Thailand and Japan. FEMS Microbiol. Ecol. 48, 345–355.

Đorđević, N.B., Simić, S.B., Ćirić, A.R., 2015. First identification of the cylindrospermopsin (CYN)-producing cyanobacterium *Cylindrospermopsis raciborkii* (Woloszyńska) Seenayya & Subba Raju in Serbia. Fresenius Environ. Bull. 24, 3736–3742.

Dolman, A.M., Rücker, J., Pick, F.R., Fastner, J., Rohrlack, T., Mischke, U., Wiedner, C., 2012. Cyanobacteria and cyanotoxins: the influence of nitrogen versus phosphorus. PLoS One 11, e38757.

FAO, 2020. AQUASTAT - FAO’s Global Information System on Water and Agriculture. Water Resources, Myanmar accessed on 11 May 2020. http://www.fao.org/nr/water/aquastat/countries_regions/Profile_segments/MMR-WR_esp.stm.

Fischer, W.J., Garthwaite, I., Miles, C.O., Ross, K.M., Aggen, J.B., Chamberlin, A.R., Towers, N.R., Dietrich, D.R., 2001. Congener-independent immunoassay for microcystins and nodularins. Environ. Sci. Technol. 35, 4849–4856.

Foss, A.J., Miles, C.O., Wilkins, A.L., Rise, F., Trovik, K.W., Cieslik, K., Aubel, M.T., 2020. Analysis of total microcystins and nodularins by oxidative cleavage of their ADMAdda, DMAdda, and Adda moieties. Anal. Chim. Acta: X 6, 100060.

Green, J., 2011. Geographical variation in rotifers associated with *Microcystis* blooms. Hydrobiologia 662, 197–204.

Harke, M.J., Steffen, M.M., Gobler, C.J., Otten, T.G., Wilhelm, S.W., Wood, S.A., Paerl, H. W., 2016. A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, *Microcystis* spp. Harmful Algae 54, 4–20.

Hawkins, P.R., Chandrasena, N.R., Jones, G.J., Humpage, A.R., Falconer, I.R., 1997. Isolation and toxicity of *Cylindrospermopsis raciborskii* from an ornamental lake. Toxicon 35, 341–346.

Hilborn, E.D., Beasley, V.R., 2015. One health and cyanobacteria in freshwater systems: animal illnesses and deaths are sentinel events for human health risks. Toxins 7, 1374–1395.

Hilt, S., Gross, E.M., 2008. Can allelopathically active submerged macrophytes stabilise clear-water states in shallow lakes? Basic Appl. Ecol. 9, 422–432.

Hoff-Risseti, C., Dorr, F.A., Schaker, P.D.C., Pinto, E., Werner, V.R., Fiore, M.F., 2013. Cylindrospermopsin and saxitoxin synthetase genes in *Cylindrospermopsis raciborskii* strains from Brazilian freshwater. PLoS One 8, 14.

Komárek, J., 2013. Cyanoprokaryota. 3. Heterocytous genera. In: Büdel, B., Krienitz, L., Gärtner, G., Schagerl, M. (Eds.), Süsswasserflora von Mitteleuropa/Freshwater flora of Central Europe. Springer Spektrum Berlin, Heidelberg, p. 1130.

Komárek, J., Anagnostidis, K., 1999. Cyanoprokaryota 1. Chroococcales. – 19/1. In: Ettl, H., Gärtner, G., Heynig, H., Mollenhauer, D. (Eds.), Süsswasserflora von Mitteleuropa Gustav Fischer. Jena-Stuttgart-Lübeck-Ulm, p. 548.

Kotai, J., 1972. Instructions for Preparation of Modified Nutrient Solution Z8 for Algae. - Publication B-11/69. Norwegian Institute for Water Research, Blindern, Oslo, Norway.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549.

LeBlanc, P., Merkley, N., Thomas, K., Lewis, N.I., Békri, K., Renaud, S.L., Pick, F.R., McCarron, P., Miles, C.O., Quilliam, M.A., 2020. Isolation and characterization of [D-Leu$^{1}$]microcystin-LY from *Microcystis aeruginosa* CPCC-464. Toxins 12, 77.

Li, R.H., Carmichael, W.W., Brittain, S., Eaglesham, G.K., Shaw, G.R., Liu, Y.D., Watanabe, M.M., 2001. First report of the cyanotoxins cylindrospermopsin and deoxycylindrospermopsin from *Raphidiopsis curvata* (cyanobacteria). J. Phycol. 37, 1121–1126.

Mallia, V., Uhlig, S., Rafuse, C., Meija, J., Miles, C.O., 2019. Novel microcystins from *Planktothrix prolifica* NIVA-CYA 544 identified by LC-MS/MS, functional group derivatization and $^{15}$N-labeling. Mar. Drugs 17, 22.

Miles, C.O., Sandvik, M., Nonga, H.E., Rundberget, T., Wilkins, A.L., Rise, F., Ballot, A., 2012. Thiol derivatization for LC-MS identification of microcystins in complex matrices. Environ. Sci. Technol. 46, 8937–8944.

Miles, C.O., Melanson, J.E., Ballot, A., 2014. Sulfide oxidations for LC-MS analysis of methionine-containing microcystins in *Dolichospermum flos-aquae* NIVA-CYA 656. Environ. Sci. Technol. 48, 13307–13315.

Mjelde, M., Swe, T., Langangen, A., Ballot, A., 2021. A contribution to the knowledge of Charophytes in Myanmar; morphological and genetic identification and ecology notes. Bot. Lett. 168, 102–109.

Naw, M.W.D., San, D.P., Aung, Y., 2020. Occurrence of Microcystis Toxic Blooms in Indawgyi Lake, Kachin State. University of Mandalay Res. J. 11, 1–9.

Neumann, C., Bain, P., Shaw, G., 2007. Studies of the comparative in vitro toxicology of the cyanobacterial metabolite deoxycylindrospermopsin. J. Toxicol. Environ. Health, Part A 70, 1679–1686.

Nguyen, T.T.L., Hoang, T.H., Nguyen, T.K., Duong, T.T., 2017. The occurrence of toxic cyanobacterium *Cylindrospermopsis raciborskii* and its toxin cylindrospermopsin in the Huong River, Thua Thien Hue province, Vietnam. Environ. Monit. Assess. 189, 11.

Norris, R.L., Eaglesham, G.K., Pierens, G., Shaw, G.R., Smith, M.J., Chiswell, R.K., Seawright, A.A., Moore, M.R., 1999. Deoxycylindrospermopsin, an analog of cylindrospermopsin from *Cylindrospermopsis raciborskii*. Environ. Toxicol. 14, 163–165.

Oo, A.T., Van Huylenbroeck, G., Speelman, S., 2017. Differential impacts of an irrigation project: case study of the Swar Dam Project in Yedashe, Bago region of Myanmar. J. Dev. Agric. Econ. 9, 178–189.

Paerl, H.W., Huisman, J., 2009. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Environ. Microbiol. Rep. 1, 27–37.

Rigosi, A., Hanson, P., Hamilton, D.P., Hipsey, M., Rusak, J.A., Bois, J., Sparber, K., Chorus, I., Watkinson, A.J., Qin, B., Kim, B., Brookes, J.D., 2015. Determining the probability of cyanobacterial blooms: the application of Bayesian networks in multiple lake systems. Ecol. Appl. 25, 186–199.

Saker, M.L., Griffiths, D.J., 2000. The effect of temperature on growth and cylindrospermopsin content of seven isolates of *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) from water bodies in northern Australia. Phycologia 39, 349–354.

Salas, H.J., Martino, P., 1991. A simplified phosphorus trophic state model for warm-water tropical lakes. Water Res. 25, 341–350.

Sant'Anna, C.L., 1991. Two new taxa of *Anabaena* and other Nostocaceae (Cyanophyceae) from the State of São Paulo, southeastern Brazil. Algol. Stud./Arch. Hydrobiol., Suppl. 64, 527–545.

Than, M.M., Yee, K.M., Lint, K., Thu, M.M., Han, M., Zin, M.M., Khaing, E.E., 2019. Water level changes in Yezin Dam, Zayarthiri township, Nay Pyi Taw, using geospatial techniques. Sci. Res. J. 7, 78–83.

Utermöhl, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt. Int. Ver. Theor. Angew. Limnol. 9, 1–38.

Vézie, C., Rapala, J., Vaitomaa, J., Seitsonen, J., Sivonen, K., 2002. Effect of nitrogen and phosphorus on growth of toxic and nontoxic Microcystis strains and on intracellular microcystin concentrations. Microb. Ecol. 43, 443–454.

WHO, 2020a. Cyanobacterial Toxins: Cylindrospermopsins. Background Document for Development of WHO Guidelines for Drinking-water Quality and Guidelines for Safe Recreational Water Environments. World Health Organization, Geneva. https://apps.who.int/iris/handle/10665/338063.

WHO, 2020b. Cyanobacterial Toxins: Microcystins. Background Document for Development of WHO Guidelines for Drinking-water Quality and Guidelines for Safe Recreational Water Environments. World Health Organization, Geneva. https://apps.who.int/iris/handle/10665/338066.

Win, B.N., Keh, K., Than, M.M., 2003. Application of RS/GIS for the assessment of Yezin Dam Watershed area. In: Annual Research Conference. Forest Research Institute, Ministry of Forestry, Myanmar accessed on 16 September 2020. https://forestdepartment.gov.mm/books/research/application-rsgis-assessment-yezin-dam-watershed-area.

Wojciechowski, J., Burda, T.M., Scheer, M.B., Costa, E.A.D.d., Fernandes, L.F., 2018. Potential effects of mechanically removing macrophytes on the phytoplankton community of a subtropical reservoir. Acta Bot. Bras. 32, 588–594.

Yilmaz, M., Foss, A.J., Miles, C.O., Özen, M., Demir, N., Balci, M., Beach, D.G., 2019. Comprehensive multi-technique approach reveals the high diversity of microcystins in field collections and an associated isolate of *Microcystis aeruginosa* from a Turkish lake. Toxicon 167, 87–100.

## Paper 3

Mjelde, M., Swe, T., Langangen, A. & Ballot, A. (2020). A contribution to the knowledge of charophytes in Myanmar; morphological and genetic identification and ecology notes. *Botany Letters*, 168:1, 102-109.

Botany Letters

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tabg21

# A contribution to the knowledge of charophytes in Myanmar; morphological and genetic identification and ecology notes

Marit Mjelde , Thida Swe , Anders Langangen & Andreas Ballot

**To cite this article:** Marit Mjelde , Thida Swe , Anders Langangen & Andreas Ballot (2020): A contribution to the knowledge of charophytes in Myanmar; morphological and genetic identification and ecology notes, Botany Letters, DOI: 10.1080/23818107.2020.1847189

**To link to this article:** https://doi.org/10.1080/23818107.2020.1847189



Published online: 21 Nov 2020.

BOTANY LETTERS
https://doi.org/10.1080/23818107.2020.1847189

Taylor & Francis
Taylor & Francis Group



# A contribution to the knowledge of charophytes in Myanmar; morphological and genetic identification and ecology notes

Marit Mjelde[a], Thida Swe[a,b,c], Anders Langangen[d] and Andreas Ballot[a]

[a]Norwegian Institute for Water Research, Oslo, Norway; [b]Forest Research Institute, Yezin, Myanmar; [c]Department of Natural Sciences and Environmental Health, University of South- Eastern Norway, Bø, Norway; [d]Oslo, Norway

**ABSTRACT**

Information on the distribution and species composition of charophytes in Myanmar is scarce. Only a few studies on charophytes in ponds were conducted in Myanmar at the end of the nineteenth and first half of the twentieth century and lake habitats were not included in these studies. To increase the knowledge, we investigated *Chara* spp. from seven Myanmar lakes and reservoirs. In a polyphasic approach using morphological traits and DNA barcoding the specimens found were classified as *Chara zeylanica* and *Chara fibrosa*. *Chara zeylanica* is the most common of the two species found in Myanmar and was observed in five lakes, while *Chara fibrosa* was only found in three lakes. *Chara zeylanica* seems to prefer calcareous lakes while *C. fibrosa* was found in both highly and moderate alkaline lakes. Both species were recorded in low-impacted lakes only, with total phosphorous (TP) concentrations below 20 µg $L^{-1}$. Increased human impact on freshwater habitats must therefore be considered as a factor reducing *Chara* biodiversity in Myanmar.

**ARTICLE HISTORY**
Received 3 September 2020
Accepted 3 November 2020

**KEYWORDS**
Myanmar; *Chara zeylanica*; *Chara fibrosa*; DNA barcoding; *matK*; *rbcl*

## Introduction

Charophytes (Charales, Charophyceae) are macroscopic green algae, included among the submerged macrophyte vegetation in fresh and brackish waters. They have a world-wide distribution and comprise six genera (Wood 1965), of which *Chara* and *Nitella* are the most species rich. In total, 678 charophyte species are identified (Guiry and Guiry 2020).

Most *Chara* species utilize bicarbonates ($HCO_3$) as a carbon source for photosynthesis and are limited to calcareous water bodies. They seem to have higher light demands than the vascular plants (Blindow 1992) and are vulnerable to eutrophication (e.g. Penning et al. 2008). Hence, the preferable habitat for the *Chara* species are oligotrophic-slightly mesotrophic calcareous lakes or ponds. In the Water Framework Directive (WFD 2000) charophytes are recognized as sensitive species and the occurrence of *Chara* in a lake is considered as indicative of low trophy and high ecological status (e.g. Penning et al. 2008). However, due to anthropogenic stressors, e.g. water abstraction and eutrophication, freshwater habitats with charophyte vegetation are decreasing and hence, red listed in Asia as well as in Europe (Allen et al. 2012; Janssen et al. 2016). Correspondingly, the distribution of many *Chara* taxa is decreasing and they are considered as threatened in several countries (Blaženčić et al. 2006; Henriksen and Hilmo 2015).

Several taxonomy studies concerning charophytes have been conducted, with most of the recent studies including molecular analyses (e.g. Borges and Necchi 2017). Charophytes are widespread in the temperate zone, but are also known in several countries in West and South Asia, including India (Groves 1924; Pal et al. 1962; Subramanian 2002), Pakistan (Faridi 1955; Langangen and Leghari 2001), Bangladesh (Naz et al. 2011; Naz and Diba 2012), Saudi Arabia (Khoja and Hussain 1990; Hussain et al. 1996), Iran (Ahmadi et al. 2012; Ghaemmaghami et al. 2012) and China (Ling et al. 2000). From Afghanistan, only old scattered information is available (Braun and Nordstedt 1882; Vilhelm 1928; Corillion 1957).

Studies of charophytes in Myanmar are very scarce, including the comprehensive study published in 1932 by B.P. Pal: "Burmese Charophyta". In that study, 24 charophyte species were reported from Myanmar including twelve *Chara*-species. In our study the species are updated to be in accordance with Guiry and Guiry (2020) and the number of species are reduced to nine. In case of changed status, the names given by Pal (1932) are in brackets.

*Chara wallichii* A. Braun, *C. corallina* Klein ex Willdenow, *C. nuda* B.S. Pal, *C. hydropithus* Reichenbach, *C. fibrosa* f. *erythrogyna* (Griffith) R.D. Wood. (*C. erythrogyna* (Griffith) R.D. Wood, *C. fibrosa* var. *burmanica* (Pal) van Raam. (*C. burmanica* Pal), *C. fibrosa* Agardh ex Bruzelius (*C. flaccida* A.Br., *C. gymnopitus* A.Br.), *C. grovesii* Pal, *C. handae* Pal, *C. setosa* Klein ex Willdenow (*C. brachypus* A. Br.), *C. zeylandica* Klein ex Willdenow.

**CONTACT** Andreas Ballot andreas.ballot@niva.no Norwegian Institute for Water Research, Oslo 0349, Norway



Published online 21 Nov 2020

The distribution areas included paddy-fields, ponds, drains and marshy areas, and similar habitats, but no lakes were mentioned in the study by Pal (1932). Little attention has been paid to charophyte studies in Myanmar after that study, and no molecular analyses have been conducted on charophytes from Myanmar. As in the rest of the world, freshwater habitats in Myanmar are increasingly impacted by anthropogenic stressors, and today the status of species richness and diversity of *Chara* in Myanmar is therefore highly uncertain.

The goals of this study are 1) to contribute to the knowledge about *Chara* species distribution in lakes in Myanmar, 2) to identify *Chara* species, using a polyphasic approach including both traditional morphological methods and DNA barcoding, and 3) to indicate some ecological demands for the detected *Chara* species. As far as we know, our study is the first study concerning charophytes in the lakes of Myanmar.

## Materials and methods

The studied Chara-lakes are part of an ongoing biological investigation of lakes and reservoirs in Myanmar, which for now includes 17 water bodies (Table 1). The overall goal of the project, which ends in 2024, is to improve the knowledge on the biological parameters, including charophytes, as a basis for the development of a biological monitoring system in Myanmar. *Chara* species are recorded in seven of these waterbodies, Inlay Lake, Meiktila Lakes (north and south), Yezin Dam, Ngalaik Dam, Kyet Mauk Taung, and Wethtigan Lake.

### Studied lakes with Chara vegetation

Inlay Lake is a shallow lake (average depth around 3–3.5 m) located in Nyaung Shwe township, Taunggyi district, Southern Shan State. It is the second-largest natural lake in Myanmar (lake surface ca. 116 km$^2$, however with decreasing open areas) and located 884 m above sea level. It is a clear, very calcareous lake (Table 1) (Ballot et al. 2018). Most of the lake area is covered by a luxurious and diverse community of submerged macrophytes. *Chara* is a common taxon in the macrophyte community, especially in the northern part of the lake.

Meiktila Lake is a shallow reservoir, divided into two separate basins, and located close to Meiktila city in the Mandalay region in central Myanmar. The lake basins are located at an altitude of 230 m and cover an area of around 9 km$^2$ with a maximum water depth of 10 m. The lake water is moderate alkaline and slightly turbid (Table 1), with the southern basin more turbid than the northern basin. The *Chara* vegetation was common in both basins, whilst the northern basin had in addition extensive stands of the floating leaved *Nelumbo nucifera*.

Yezin Dam is a reservoir used for irrigation and drinking water. It is located in the Zeyar Thiri township, Nay Pyi Taw, Mandalay region at 128 m above sea level and covers an area of around 6.5 km$^2$. The maximum water depth is more than 10 m. The reservoir is a low-moderate alkaline and turbid lake (Table 1) with large water level fluctuations. At the time of investigation, the coverage of aquatic macrophytes was very low, and only a few specimens of *Chara* were found.

**Table 1.** Lakes in Myanmar, visited in 2014–2019, including characteristic physico-chemical data and coverage of *Chara* spp. The data for each lake include average values from all visited years and sites. *Chara* coverage; 1 = seldom, 2 = common, 3 = large stands. Lake area can vary considerable between wet and dry season. Lake areas measurements are based on Google Earth where satellite photo dates can vary from region to region.

| Lake | State/Region/ Division | Latitude | Longitude | Altitude m | Lake area km$^2$ | Calcium mg L$^{-1}$ | TOC mg L$^{-1}$ | Tot P µg L$^{-1}$ | Tot N µg L$^{-1}$ | *Chara*-coverage |
|---|---|---|---|---|---|---|---|---|---|---|
| Inlay Lake | Shan State | 20,563,046 | 96,918,640 | 884 | 116* | 45 | 5.0 | 15.4 | 448 | 3 |
| Sakar Inn | Shan State | 20,169,411 | 96,932,716 | 884 | 3 | 40 | 3.9 | 7 | 430 | - |
| Pekon Lake | Shan State | 19,879,100 | 97,032623 | 884 | 134 | 45 | 5.5 | 9 | 440 | - |
| Indawgyi Lake | Kachin State | 25,116,667 | 96,316,667 | 170 | 123 | 10 | 2.3 | 20 | 532 | - |
| Meiktila Lake, North | Mandalay Region | 20,886,560 | 95,852,966 | 230 | 4.8 | 19 | 4.9 | 21 | 428 | 2 |
| Meiktila Lake, South | Mandalay Region | 20,863,464 | 95,854,511 | 230 | 4.3 | 14 | 4.1 | 15 | 415 | 3 |
| Yezin Dam | Mandalay Region | 19,855,852 | 96,276,798 | 128 | 6.4 | 8 | 5.1 | 15 | 402 | 1 |
| Nga Laik Dam | Mandalay Region | 19,861,665 | 96,005058 | 163 | 5.5 | 22 | - | 20 | 510 | 2 |
| Moeyingyi Reservoir | Bago Region | 17,570,721 | 96,596,947 | 10 | 15 | 2 | 16.4 | 103 | 702 | - |
| Taung Taman Lake, North | Mandalay Region | 21,900,833 | 96,060556 | 61 | 3 | 45 | 13 | 520 | 2900 | - |
| Kantawgyi Lake, South | Mandalay Region | 21,936,389 | 96,065833 | 66 | 1.8 | 18 | 3.5 | 35 | 520 | - |
| Pyu Kan Lake | Mandalay Region | 21,768,056 | 95,891,111 | 102 | 2 | 18 | 2.2 | 45 | 500 | - |
| Khu Le Inn | Mandalay Region | 22,592,222 | 95,980,000 | 76 | 2.5 | 7 | 3.8 | 31 | 560 | - |
| Sunye In Tank | Mandalay Region | 21,679,722 | 96,230,000 | 91 | 4 | 21 | 6.1 | 26 | 490 | - |
| Pauk In | Mandalay Region | 21,326,944 | 95,048056 | 55 | 0.15 | 24 | 10.3 | 190 | 2000 | - |
| Kyetmauk Taung Dam | Mandalay Region | 20,812,222 | 95,250,833 | 279 | 4.5 | 40 | 5.1 | 19 | 1900 | 2 |
| Wethtigan Lake | Magwe Division | 20,575,833 | 94,641,111 | 66 | 1.7 | 44 | 3.5 | 8 | 530 | 3 |

*: Inlay lake area is reduced. Today open water area is measured to 46 km$^2$.

Ngalaik Dam is a medium-large irrigation reservoir, located in a boreal area in Ottara Thiri Township, Nay Pyi Taw, Mandalay region. The reservoir has a surface area of around 5.5 km$^2$, is situated 163 m above sea level, and is a calcareous, clear water lake. The aquatic vegetation consists of a moderate coverage of a few submerged species, including charophytes.

Kyet Mauk Taung is a medium-large reservoir and is mainly used for irrigation (Pa 1983). It is located in a boreal area in the Kyaukpadaung Township, Mandalay region. The reservoir is situated 279 m above sea level, close to Popa National Park, and covers an area of about 7.3 km$^2$. The reservoir is a calcareous and slightly humic water body.

Wethtigan (Whattae) Lake is included in the Wethtigan Wildlife Sanctuary, situated in Salin Township, Magway Region. The lake is situated 66 m above sea level and has a surface area of around 1.7 km$^2$. It is a clear, calcareous lake (Table 1) with luxurious growth of both floating leaved vegetation (mainly *Nelumbo nucifera*) and submerged vegetation, dominated by *Chara*.

Integrated water samples were taken (1 m steps up to max. 3 m water depth) for the analysis of chemical parameters (ammonium, nitrate, total nitrogen (TN), soluble reactive phosphorous, total phosphorous (TP), Ca, turbidity and total organic carbon (TOC)). The *Chara* specimens were collected from a boat, using an aquascope and a rake. In total, 11 *Chara* samples were analysed from 7 lakes. All specimens were preserved as herbarium samples directly after collection, for later morphological and molecular analysis.

## Morphological analysis

The species were determined following the nomenclature in Wood (1965).

## DNA- barcoding

Genomic DNA from *Chara* material was isolated after Schneider et al. (2016). PCR for the *matK* gene and the *rbcl* gene was performed on a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Oslo, Norway) using the iProof High-Fidelity PCR Kit (Bio-Rad Laboratories, Oslo, Norway). Amplification of the *matK* gene region was conducted using the primers F-Chara (agaatgagcttaaacaaggat) and R-Chara (acgatttgaacatccactataata) and for the *rbcl* gene using the primer rbclaf (atgtcaccacaaacagagactaaagc) and rbclar (gtaaaatcaagtccaccrcg). The following cycling protocol was used for *matk* and *rbcl*: one cycle of 5 min at 94°C, and then 35 cycles each consisting of 10 s at 94°C, 20 s at 62°C, and 20 s at 72°C, followed by a final elongation step of 72°C for 5 min. PCR products were visualized by 1.5% agarose gel electrophoresis with GelRed staining (GelRed Nucleic Acid Gel Stain, Biotium, Fremont, CA, USA) and UV illumination. For sequencing the same primers and for *matk* additionally the intermediate primers charaintF (gatggctattcaagcagga), charaintR (ctaccgataagttcgtcct), charaBt2F (datatggcaacaycaaaagac) and charaBT2R (atacagaccatgcagcytt) were used. For each PCR product, both strands were sequenced on an ABI 3730 Avant genetic analyser using the BigDye terminator V.3.1 cycle sequencing kit (Applied Biosystems, Thermo Fisher Scientific Oslo, Norway) according to the manufacturer's instructions.

The sequence data were deposited at the National Center for Biotechnology Information (NCBI) under the accession numbers given in Table 2.

## Phylogenetic analyses

Sequences were analysed and aligned using Seqassem (version 04/2008) and Align (version 03/2007) MS Windows-based manual sequence alignment editor (SequentiX – DigitalDNA Processing, Klein Raden Germany) to obtain DNA sequence alignments, which were then corrected manually. A *matK* set containing 11 *Chara* samples from Myanmar (Table 2) and 24 other *Chara* sequences, and 953 nucleotide positions were used for the phylogenetic analysis. *Nitellopsis obtusa* (AY170447) was used as an outgroup taxon in the *matK* phylogenetic tree. For the *rbcl* phylogenetic tree, seven samples from Myanmar and 32 other *Chara* sequences were used and 567 Nucleotide positions. The datasets were analysed using maximum likelihood (ML), maximum parsimony (MP) and distance (neighbour-joining (NJ)) in MEGA version x (Kumar et al. 2018). GTR+G was selected as the best-fitting evolutionary model for the *matK* gene region and T92 + G for the *rbcl* region. ML, MP, and distance analyses were performed with 1000 bootstrap replicates in MEGA version X (Kumar et al. 2018).

**Table 2.** *Chara* samples, origin, sampling date and NCBI accession numbers.

| ID | Lake | date | *matK* | *rbcl* |
|---|---|---|---|---|
| MMYA-1 | Inlay Lake | 05.11.2014 | MT739758 | MT739769 |
| MMYA-2 | Inlay Lake | 05.11.2014 | MT739759 | MT739774 |
| MY-32 | Yezin Dam | 23.05.2017 | MT739768 | - |
| MY-33 | Yezin Dam | 23.05.2017 | MT739760 | - |
| MY-45 | Meiktila North | 16.11.2017 | MT739767 | - |
| MY-34 | Meiktila South | 16.11.2017 | MT739761 | - |
| MY-35 | Ngalaik Dam | 17.11.2017 | MT739762 | MT739772 |
| MY-58 | Kyet Mauk Taung | 22.11.2019 | MT739765 | MT739773 |
| MY-59 | Kyet Mauk Taung | 22.11.2019 | MT739763 | MT739775 |
| MY-60 | Wethtigan Lake | 23.11.2019 | MT739766 | MT739771 |
| MY-61 | Wethtigan Lake | 23.11.2019 | MT739764 | MT739770 |

- = not analysed

## Results

### Morphological determination

The collected specimens include two charophyte species: *Chara zeylanica* Klein ex Willdenow and *Chara fibrosa* Agardh ex Bruzelius. (*C. fibrosa* was first determined as *C. flaccida* A. Braun, based on the yellow/brown colour of the oospore).

*Chara zeylanica* was found in Inlay lake, Meiktila Lake south, Ngalaik Dam, Kyet Mauk Taung Reservoir, and Wethtigan Lake. *C. fibrosa* was found in Yezin Dam, Kyet Mauk Taung Reservoir, and Wethtigan Lake (Table 3).

### Description of Chara zeylanica collected in Myanmar

15–40 cm high. Axis 0.35–0.8 mm in diameter. Internodes up to 10 cm long, but most commonly less than 4 cm. Slightly encrusted with lime. Stem cortex regularly triplostichous, isostichous (The examined specimens, all collected in November have stems strongly overgrown with diatoms, and therefore difficult to see). Spine-cells papillous to short, acute, from papillous up to 0.4 mm long, solitary. Stipulodes in two rows (Diplostephanous), upper row with cells to 0.5 mm long and lower row to 0.4 mm long, acute. 8–12 branchlets in a whorl, 20–50 mm long, 0.5x to 1.0x times the length of the internodes. 8–11 segments on each branchlet, first segment *ecorticate* (Gymnopodous). End segments 2–4, ecorticate. *End-cell* acute.

### Description of Chara fibrosa collected in Myanmar

Specimens 5–25 cm high, commonly lower to 15 cm. Axis diameter 0.4–0.6 mm. Internodes up to 3 cm long. Slightly to moderate encrusted with lime. Cortex diplostichous, tylacanthous to isostichous. Spine-cells scattered, solitary 0.2 mm to 0.6 mm long, acute. Stipulodes one row (Haplostephanous), 0.8 mm –2.0 mm long. Branchlets 8–10 in each whorl, up to 15 mm long and 0.25 to 1 times the length of internodes. All branchlets ecorticate and with 3–5 segments. Bract-cells verticillate up to 0.8 mm long. In two of three localities the plants were richly fertile. Oogonia 0.7 mm long, 0.3–0.5 mm wide. Oospore up to 0.5 mm long and 0.35 mm wide, yellow to brown. Antheridia up to 0.3 mm in diameter. The species are further described by Langangen and Leghari (2001) and Langangen (2015).

### Phylogenetic analyses

In the matk phylogenetic tree, seven *Chara* samples, morphologically determined as *C. zeylanica*, from the Myanmar lakes Inlay Lake, Meiktila Lake, Nga Laik Dam, Kyat Mauk Taung Reservoir, and Wethtigan Lake, formed a monophyletic cluster together with the Brazilian *Chara* species; *C. hydropithys, C. guairensis* R. Bicudo, *C. rusbyana* M. Howe, *C. haitensis* Turpin and *C. braunii* var. *schweinitzii* (A.Braun) Zaneveld (Figure 1). The whole cluster was supported by a bootstrap value of 100%. *C. hydropithys* was the closest related *Chara* species to *Chara zeylanica* from Myanmar, which was supported by a bootstrap value of 57%. Although the *Chara* sample MY-45 was not investigated morphologically its matk sequence was 100% identical with those from the other *C. zeylanica* samples. It was therefore also assigned to *C. zeylanica*.

Four *Chara* specimens from Yezin Dam, Wethtigan Lake and Kyat Mauk Taung Reservoir were determined as *Chara fibrosa*. They clustered together with *C. braunii* Gmelin from Lake Kasumigaura (Japan), which was supported by a bootstrap value of 100%. In the phylogenetic tree based on partial rbcl gene of *Chara* spp. only five *Chara zeylanica* from Myanmar were included. They grouped in a separate subcluster but clearly clustered together with other *C. zeylanica* from Japan, USA (California), New Caledonia and New Zealand (Figure 2). The rbcl phylogenetic tree therefore confirmed the assignment of the *Chara* samples MMYA-1, MMYA-2, MY-34, MY-35, MY-45, MY-59, and MY-61 to *C. zeylanica*. Only two of the four *C. fibrosa* from Myanmar were analysed for the rbcl gene and grouped in a subcluster. They clustered together with *C. fibrosa* from Japan and *C. braunii* from Japan, Hawaii (USA) and New Zealand (Figure 2).

### Ecological notes

*Chara zeylanica* is the most common of the two species found in Myanmar and was observed in five lakes.

Table 3. Comments to the collected *Chara* specimens from Myanmar.

| No. | Lake | Comments | Specimens quality |
|---|---|---|---|
| ***Chara zeylanica*** | | | |
| MMYA-1,2 | Inlay Lake | - | Poorly developed and preserved |
| MY-34 | Meiktila North | Broken specimens but have been long. Special locality. | Poorly developed |
| MY-35 | Ngalaik Dam | Typical specimens. Richly fertile with ripe, black oospores. | Well developed |
| MY-45 | Meiktila South | - | - |
| MY-59 | Kyet Mauk Taung | Richly fertile with ripe, black oospores. | Well developed |
| MY-61 | Wethtigan Lake | With very long internodes. Sterile. | Well developed |
| ***Chara fibrosa*** | | | |
| MY-32,33 | Yezin | Small specimens. Fertile | Well developed |
| MY-58 | Kyet Mauk Taung | Richly fertile specimens | Well developed |
| MY-60 | Wethtigan Lake | Sterile specimens | Well developed |

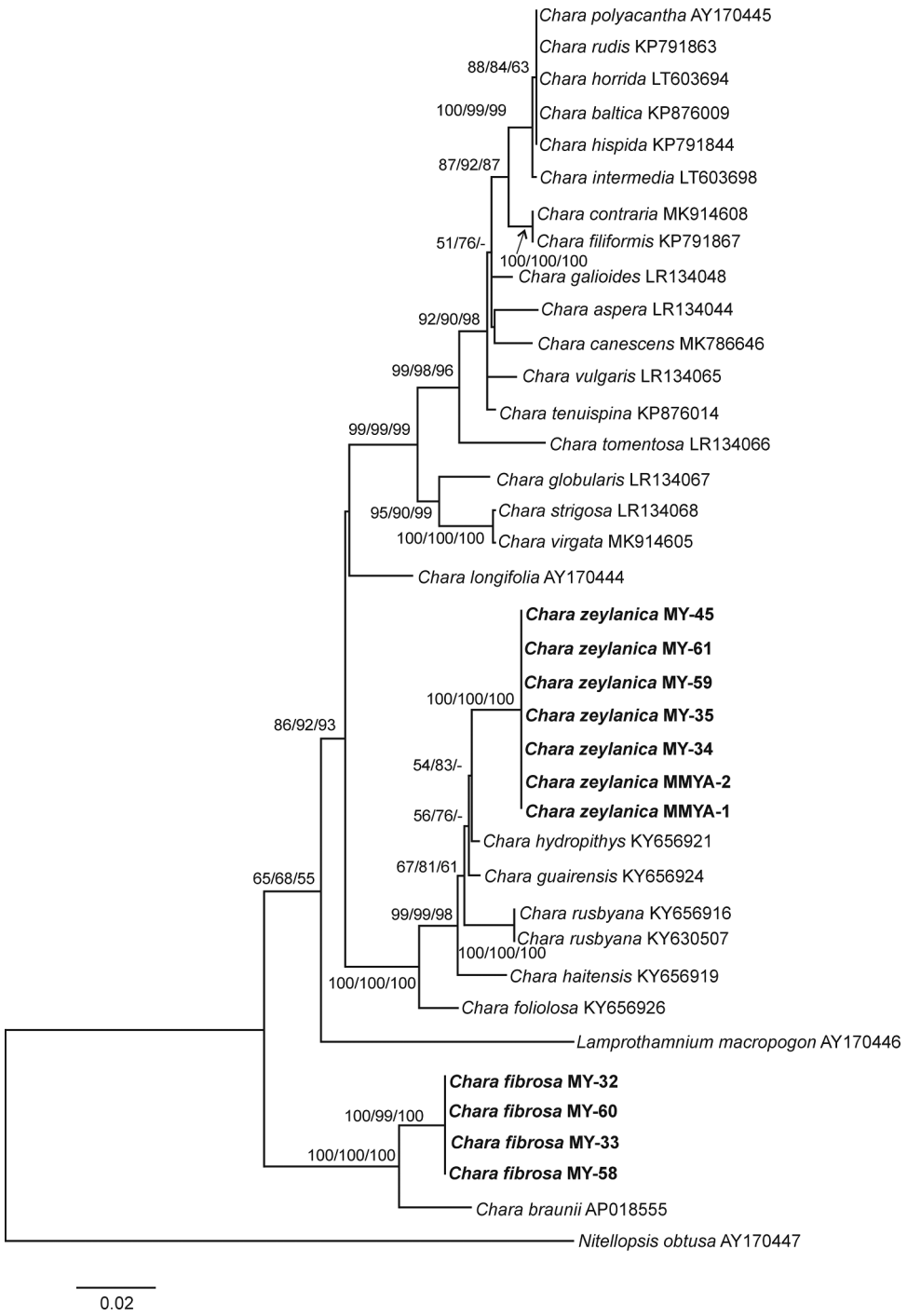



**Figure 1.** Maximum likelihood tree of the *matK* gene of *Chara* spp. Bootstrap values (ML/MP/NJ) above 50 are included. Strains from this study are marked in bold. The scale bar indicates 2 % sequence divergence.

It is present in different habitat types; from the medium-altitude and calcium-rich natural Inlay Lake in Shan state to lowland lakes in the dry zone area close to Mandalay. The species is growing in Ngalaik Dam and Kyet Mauk Taung Reservoir, but it was not recorded in the heavily regulated Yezin Dam.

*Chara fibrosa* had a sparser distribution and was only found in three lakes in the dry zone. In Yezin Dam only a few small specimens were recorded, while it was more common in Kyet Mauk Taung reservoir and in the natural Wethtigan Lake, where it grew in between mass stands of *C. zeylanica*. Both Kyet Mauk Taung Reservoir and the natural Wethtigan Lake are calcareous lakes with calcium levels around 40 mg $L^{-1}$, while Yezin Dam is characterised by more moderate calcium concentrations of around 8 mg $L^{-1}$.

Both species were recorded in low-impacted lakes only, with TP concentrations below 20 µg $L^{-1}$.

## Discussion

Two *Chara* species, *C. zeylanica* and *C. fibrosa* were recorded in our lake survey in 2014–2019. The morphological determination of both species has been

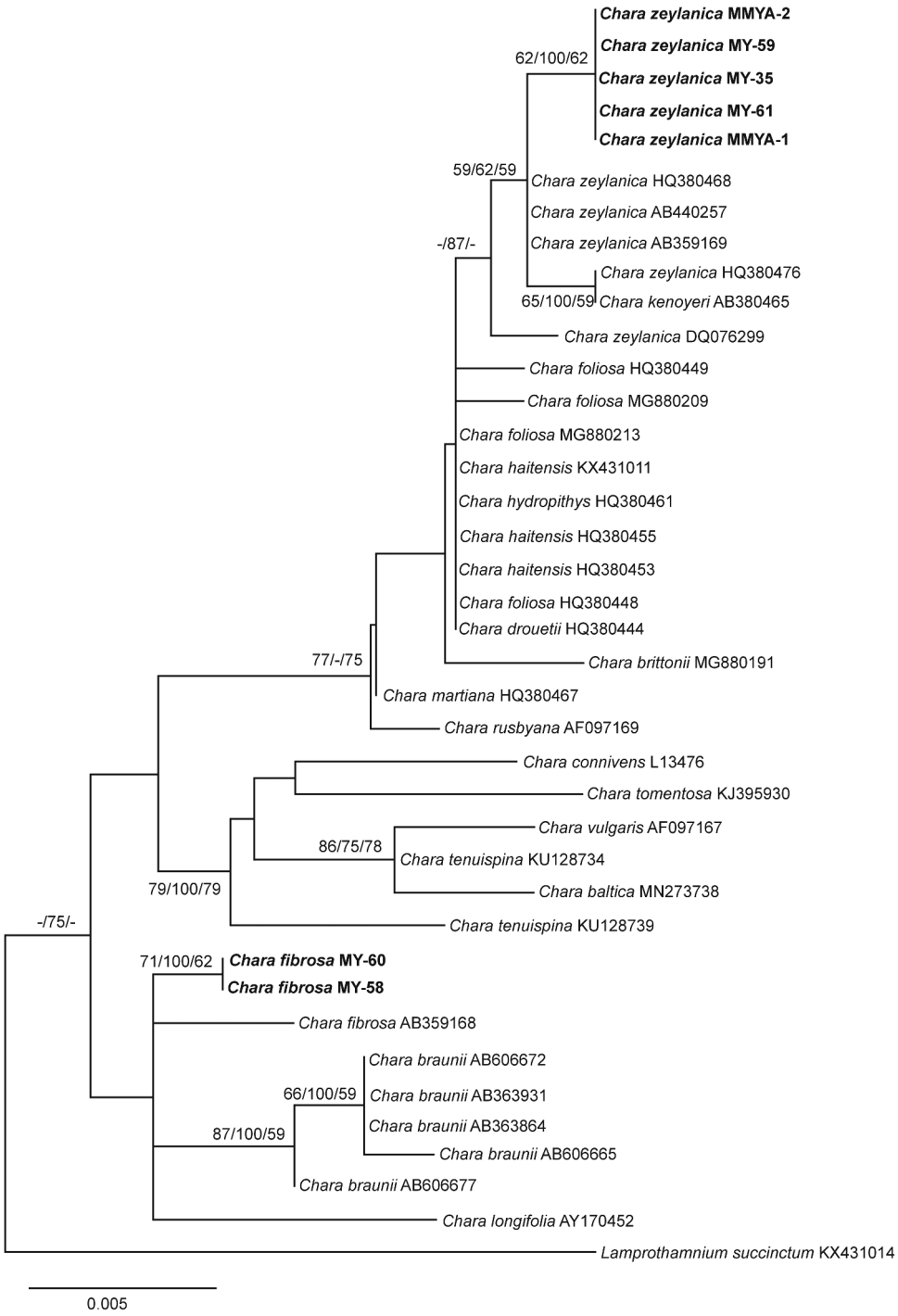



**Figure 2.** Maximum likelihood tree of the *rbcl* gene of *Chara* spp. Bootstrap values (ML/MP/NJ) above 50 are included. Strains from this study are marked in bold. The scale bar indicates 0.5 % sequence divergence.

supported by phylogenetic studies using matk and rbcl genes. Although the *Chara* sample MY-45 was not determined morphologically, the phylogenetic analysis confirms its assignment to *C. zeylanica*.

In the 1920s and the beginning of the 1930s, 12 *Chara* species were recognised in Myanmar (Pal 1932). However, four of these species are today recognised as variants or synonyms to *C. fibrosa*, i.e. *C. erythrogyna, C. burmanica, C. flaccida* and *C. gymnopitys* (Guiry and Guiry 2020).

Both *C. zeylanica* and *C. fibrosa* are commonly distributed species, especially *C. zeylanica*, which is common in tropical and sub-tropical areas in southern parts of Africa, Asia, Southern Australia, Oceania (Hawaii), and Central- and South-America, but also in North-America (Wood 1967). *Chara fibrosa* is common in Africa, South-Asia and Australia (Wood and Imahori 1959). Both species have a wide distribution and are not considered as red-listed *Chara*-species.

The study by Pal (1932) on charophytes was very comprehensive and included several localities in different regions and areas in Myanmar. These include the large delta area around Yangon, the dry zone area around Mandalay, the intermediate zone between

Naypyitaw and Yangon, the large Shan Plateau in the east, and the extreme south-eastern part. The study focused on small freshwater habitats like paddy-fields, ponds, drains and marshy areas.

Our study is less comprehensive than the study conducted by Pal (1932). However, it includes only lakes, a habitat which was not investigated by Pal (1932). We have so far visited 17 lakes, situated in different regions, Mandalay region, Magwe region, Bago region, Shan State, and Kachin state, i.e. covering the same areas as in Pal (1932), except from the extreme southeast area. *Chara* species were recorded in seven of the visited lakes.

There may be several reasons why we have recognised so few species compared to the 1930s:

- different survey seasons
- different habitat preferences
- increased impacts on habitats
- taxonomic changes since the 1930s

Pal (1932) found that the best season for the charophyte flourishment in Myanmar was between the months of August and March. In our study all lakes were surveyed twice, February–March and November, which covers the best season for charophyte growth indicated by Pal (1932).

In contrast to the study of Pal (1932) our study focuses on lakes only. Surveys conducted in different habitats can be a reason for the discrepancy between the two observations. His surveys were conducted in small habitats, like paddy-fields and ponds. It can be argued that these habitats are the preferable habitats for charophytes. In other countries, most *Chara* species including *C. zeylanica* and *C. fibrosa*, inhabit different types of water bodies, including lakes (Siong and Asaeda 2006; Penning et al. 2008). However, the existence of *Chara* species with special preferences for ditches and ponds cannot be excluded.

In our study, *C. fibrosa* appears in both high and moderate alkaline lakes. In the high alkaline lakes, they grow with larger specimens and more vigorous stands. This agrees with Asaeda et al. (2014) who indicated 40–80 mg Ca $L^{-1}$ as the optimum range for this species, and is similar to the results from Vaidya (1967). The smaller *C. fibrosa* specimens observed in Yezin Dam can be the result of stress caused by water level regulations (Ellawala et al. 2011). *C. zeylanica* also seems to prefer calcareous lakes. All investigated lakes in our study except for one had calcium concentrations >19 mg $L^{-1}$, which is also in agreement with Vaidya (1967). We have recorded both species only in low-impacted lakes, with TP concentrations below 20 µg $L^{-1}$. This is supported by several European studies (e.g. Blindow 1992), with *Chara* recognised as a species sensitive to eutrophication (Penning et al. 2008).

Increased human impact on freshwater habitats must be considered as a factor reducing *Chara* biodiversity in Myanmar as elsewhere. The destruction of small freshwater habitats, increased urbanization and enhanced agricultural activities, followed by increased eutrophication and reduced light conditions, are already mentioned by Pal (1932). These activities are today considered as the main impacts on freshwater habitats and recognised as reasons for decreased occurrence of *Chara* vegetation in Asia as well as in Europe (Allen et al. 2012; Janssen et al. 2016).

The "taxonomic development" during the last 90 years has also caused a considerable change in the assignment of *Chara* species to certain taxa.

In addition to "taxonomic development", surveys in different habitats may be a reason for the discrepancy between the two surveys. However, we believe that increased human impact on freshwater habitats must be considered as a factor reducing *Chara* biodiversity, in Myanmar as elsewhere.

## Acknowledgments

We thank May Phoo (Watershed Management Division - Forest department - Ministry of Natural Resources and Environmental Conservation, Myanmar) for support during field work.

## Disclosure statement

The authors declare that there is no conflict of interest.

## Funding

This research was supported by the Project "Integrated Water Resources Management (IWRM) –Institutional Building and Training in Myanmar" (funded by the Norwegian Ministry of Foreign Affairs and the Royal Norwegian Embassy in Myanmar.).

## Author contributions

Marit Mjelde: study design, field work, manuscript writing.
Thida Swe: field work, manuscript writing
Anders Langangen: morphological studies, manuscript writing,
Andreas Ballot: study design, field work, genetic and phylogenetic analyses, manuscript writing.

## References

Ahmadi, A, H Riahi, M Sheidai, J Van Raam. 2012. Some charophytes (Characeae, Charophyta) from central and western of Iran including *Chara kohrangiana* species nova. Cryptogamie Algologie. 33(4):359–390. doi:10.7872/crya.v33.iss4.2012.359.

Allen, DJ, KG Smith, WRT Darwall. 2012. The status and distribution of freshwater biodiversity in Indo-Burma. Cambridge, UK and Gland, Switzerland: IUCN; x +158pp+4pp cover.

Asaeda, T, M Senavirathna, Y Kaneko, MH Rashid. 2014. Effect of calcium and magnesium on the growth and calcite encrustation of *Chara fibrosa*. Aquat Bot. 113:100–106. doi:10.1016/j.aquabot.2013.11.002.

Ballot, A, M Mjelde, T Swe 2018. Integrated water resources management in Myanmar. Assessing ecological status in Inlay Lake. NIVA Report No.: 7301–2018. Norwegian Institute for Water Research, Oslo, Norway.

Blaženčić, J, B Stevanović, Z Blaženčić, V Stevanović. 2006. Red data list of charophytes in the Balkans. Biodivers Conserv. 15(11):3445–3457. doi:10.1007/s10531-005-2008-5.

Blindow, I. 1992. Decline of charophytes during eutrophication - comparison with angiosperms. Freshwater Biol. 28 (1):9–14. doi:10.1111/j.1365-2427.1992.tb00557.x.

Borges, FR, O Jr Necchi. 2017. Taxonomy and phylogeny of *Chara* (Charophyceae, Characeae) from Brazil with emphasis on the midwest and southeast regions. Phytotaxa. 302(2):101–121. doi:10.11646/phytotaxa.302.2.1.

Braun, A, O Nordstedt. 1882. Fragmente einer Monographie der Characeen. Nach den hinterlassenen Manuscripten A. Brauns's herausgegeben von Dr. O. Nordstedt. Abh K Akad Wiss Berlin. Verlag der Königlichen Akademie der Wissenschaften, Berlin, pp. 1–211.

Corillion, R. 1957. Les Charophycées de France et d'Europe occidentale. Trav Lab Fac Sc Angers. 11–12:1–259.

Ellawala, C, T Asaeda, K Kawamura. 2011. Influence of flow turbulence on *Chara fibrosa*: growth, stress, and tissue carbon content. J Freshwater Ecol. 26(4):507–515.

Faridi, M. 1955. A contribution to the Charales of West Pakistan. Biologia. 1(1):70–81.

Ghaemmaghami, SS, S Afsharzadeh, GR Balali. 2012. Characterization of genetic diversity in three *Chara* L. species (Characeae) in Iran using RAPD-PCR technique. Iran J Bot. 18(2):319–326.

Groves, J. 1924. Notes on Indian charophyta. Bot J Linn Soc. 46(310):359–376. doi:10.1111/j.1095-8339.1924.tb00493.x.

Guiry, M, G Guiry 2020. AlgaeBase. World-wide electronic publication. Galway: National University of Ireland. [accessed 2020 Jun 30]. https://www.algaebase.org.

Henriksen, S, O Hilmo. 2015. The 2015 Norwegian red list for species. Norway: Norwegian Biodiversity Information Centre.

Hussain, MI, TM Khoja, M Guerlesquin. 1996. Chemistry, ecology and seasonal succession of charophytes in the Al-Kharj Irrigation Canal, Saudi Arabia. Hydrobiologia. 333 (2):129–137. doi:10.1007/BF00017575.

Janssen, JAM, JS Rodwell, M García Criado, S Gubbay, T Haynes, A Nieto, N Sanders, F Landucci, J Loidi, A Ssymank, T Tahvanainen, M Valderrabano, A Acosta, M Aronsson, G Arts, F Attorre, E Bergmeier, RJ Bijlsma, F Bioret, C Biță-Nicolae, I Biurrun, M Calix, J Capelo, A Čarni, M Chytrý, J Dengler, P Dimopoulos, F Essl, H Gardfjell, D Gigante, G Giusso del Galdo, M Hájek, F Jansen, J Jansen, J Kapfer, A Mickolajczak, JA Molina, Z Molnár, D Paternoster, A Piernik, B Poulin, B Renaux, JHJ Schaminée, K Šumberová, H Toivonen, T Tonteri, I Tsiripidis, R Tzonev, M Valachovič.. 2016. European red list of habitats: part 2. Terrestrial and freshwater habitats. European Union. Luxembourg: Publications Office of the European Union. doi: 10.2779/091372

Khoja, TM, MI Hussain. 1990. Preliminary studies on the distribution of charophytes in Saudi-Arabia. Cryptogamie Algologie. 11(3):187–196.

Kumar, S, G Stecher, M Li, C Knyaz, K Tamura. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 35(6):1547–1549. doi:10.1093/molbev/msy096.

Langangen, A. 2015. Some finds of charophytes from East-Africa (Zambia, Tanzania, Kenya and Somalia). Acta Musei Nationalis Pragae Ser B Hist Nat. 71(3–4):239–248.

Langangen, A, SM Leghari. 2001. Some charophytes (Charales) from Pakistan. Stud Bot Hung. 32:63–85.

Ling, YJ, SL Xie, A Langangen. 2000. Charales of China. Nowa Hedwigia. 71(1–2):69–94.

Naz, S, N Diba. 2012. Some morphological observations of charophytes (Characeae) from Bangladesh. J Life Earth Sci. 7:71–77. doi:10.3329/jles.v7i0.20124.

Naz, S, N Diba, H Schubert. 2011. Monograph on charophytes of Bangladesh: taxonomy, diversity, distribution, charophyta, Northern Bangladesh. Saarbrücken (Germany): VDM Publishing Dr. Müller.

Pa, UY 1983. Economic aspects of the Kyet Mauk Taung irrigation project in Central Burma [Master Thesis]. Canberra (Australia): Australian National University.

Pal, B. 1932. Burmese charophyta. Bot J Linn Soc. 49 (327):47–92.

Pal, B, B Kundu, V Sunderlingam, G Venkataraman. 1962. Charophyta. New Delhi: ICAR Monograph on Algae.

Penning, WE, M Mjelde, B Dudley, S Hellsten, J Hanganu, A Kolada, M van den Berg, S Poikane, G Phillips, N Willby, F Ecke. 2008. Classifying aquatic macrophytes as indicators of eutrophication in European lakes. Aquat Ecol. 42(2):237–251. doi:10.1007/s10452-008-9182-y.

Schneider, SC, P Nowak, U von Ammon, A Ballot. 2016. Species differentiation in the genus Chara (Charophyceae): considerable phenotypic plasticity occurs within homogenous genetic groups. Eur J Phycol. 51(3):282–293. doi:10.1080/09670262.2016.1147085.

Siong, K, T Asaeda. 2006. Does calcite encrustation in *Chara* provide a phosphorus nutrient sink? J Environ Qual. 35 (2):490–494. doi:10.2134/jeq2005.0276.

Subramanian, D. 2002. Monograph on Indian charophyta. Dehra Dun (India): Bishen Singh Mahendra Pal Singh.

Vaidya, B. 1967. Study of some environmental factors affecting the occurrence of charophytes in western India. Hydrobiologia. 29(1–2):256–262.

Vilhelm, J. 1928. Characeae Europae orientalis et Asiae ex herbario instituti cryptogamici horti botanici Reipublicae rossicae (ante Petropolitani). Prir Fak Karlovy Univ. 80:1–24.

WFD. 2000. The EU water framework directive - integrated river basin management for Europe - directive 2000/60/EC. [accessed 2020 Jul 03]. https://ec.europa.eu/environment/water/water-framework/index_en.html.

Wood, RD. 1965. Monograph of the characeae. In R D Wood, K Imahori, editors. A revision of the characeae (Vol. 1). Weinhein (Germany): Verlag J. Cramer; p. 1–904.

Wood, RD, K Imahori. 1959. Geographical distribution of characeae. Bull Torrey Bot. 86(3):172–183. doi:10.2307/2482517.

Wood, RD. 1967. Charophytes of North America. West Kingston (Rhode Island): Stella's Printing; p. 72.

## Paper 4

Swe, T., Lombardo, P., Ballot, A., Thrane Jan-Erik, Sample, J. Eriksen, T. E., & Mjelde, M., (2021). The importance of aquatic macrophytes in a eutrophic tropical shallow lake. *Limnologica*, 90, 125910.

Limnologica 90 (2021) 125910

Contents lists available at ScienceDirect

Limnologica

journal homepage: www.elsevier.com/locate/limno

# The importance of aquatic macrophytes in a eutrophic tropical shallow lake

Thida Swe [a,b,c], Paola Lombardo [d], Andreas Ballot [a], Jan-Erik Thrane [a], James Sample [a], Tor Erik Eriksen [a], Marit Mjelde [a,*]

[a] Norwegian Institute for Water Research, Gaustadalléen 21, N-0349, Oslo, Norway
[b] Forest Research Institute, 15013, Yezin, Myanmar
[c] Department of Natural Sciences and Environmental Health, University of South- Eastern Norway, Gullbringvegen 36, N-3800, Bø, Norway
[d] Limno Consulting, via Bedollo 303, I-00124, Rome, Italy

ARTICLE INFO

*Keywords:*
Lake ecology
Biodiversity
Biotic interactions
Water quality
Lake management

ABSTRACT

Inlay Lake is the second largest natural lake in Myanmar. Located in Shan State, in the eastern part of the country, it is a known biodiversity hotspot. The lake is negatively affected by an increasing local human population and rapid growth in both agriculture and tourism. In recent decades, several studies have listed faunistic and floristic groups in Inlay Lake, but there is still a general lack of knowledge about the aquatic macrophyte and phytoplankton community composition and abundance, and their interactions. To fill this knowledge gap, field surveys of biological and physical and chemical parameters were carried out in the period 2014–2017. They show that Inlay Lake is a shallow, clear water and calcareous lake, with nutrient concentrations indicating mesotrophic-eutrophic conditions. However, close to the shore, nutrient concentrations are generally higher, reflecting pollution from inflowing rivers, shoreline villages and floating gardens. Both the richness and abundance of aquatic macrophytes in Inlay Lake were high, with several species forming extensive stands in most of the lake over the whole survey period. Total phytoplankton and cyanobacterial biomass were low, but cyanobacteria included toxin-producing strains of *Microcystis*, suggesting that cyanobacterial and total phytoplankton biomass need to be kept low to avoid potentially harmful cyanobacterial blooms. Submerged macrophyte abundance and phytoplankton biomass were inversely correlated in the heavily vegetated northern lake area. Our survey suggests a great importance of the submerged macrophytes to the general water quality and the clear water state in Inlay Lake. Maintaining high macrophyte abundances should therefore be a goal in management strategies, both for Inlay Lake and other lakes in Myanmar. It is highly desirable to include macrophytes and phytoplankton in the lake monitoring in Myanmar.

## 1. Introduction

Submerged macrophytes play an important role in the structuring and functioning of aquatic freshwater systems (e.g. Carpenter and Lodge, 1986; Jeppesen et al., 1998; Timms and Moss, 1984; James and Barko, 1994; Vermaat et al., 2000; Burks et al., 2002) and are especially important for in-lake nutrient cycling and for stabilizing a clear water state in nutrient rich lakes (Phillips et al., 1978; Scheffer et al., 1993). In shallow lakes, submerged macrophytes can suppress algal growth and enhance water clarity through a number of mechanisms, including nutrient competition (Mjelde and Faafeng, 1997; Phillips et al., 1978) or release of allelopathic substances toxic to algae (Gross et al., 2007). Consequently, shallow lakes with macrophyte cover are more resistant to increasing nutrient load than lakes without or with limited macrophyte cover. Based on this, Scheffer et al. (1993) suggested two alternative stable states in temperate eutrophic lakes: a clear water state abundant in submerged macrophytes and a turbid and phytoplankton-dominated state. In addition, high macrophyte diversity seems to play a role in preventing the shift to phytoplankton dominance (Sayer et al., 2010).

Studies about excessive aquatic macrophyte growth and control have long been an issue everywhere, including in Asia (Pieterse and Murphy, 1990). However, while knowledge about the dynamics between physical and chemical water quality and biological communities in temperate lakes is large (e.g., Lyche-Solheim et al., 2013; Phillips et al., 2016), it is more limited for tropical lakes. The scattered studies, however, show that both physical and chemical conditions and biological interactions in tropical lakes differ from temperate lakes (Lewis, 2000; Meerhoff et al.,

* Corresponding author.
*E-mail address:* marit.mjelde@niva.no (M. Mjelde).

https://doi.org/10.1016/j.limno.2021.125910
Received 18 January 2021; Received in revised form 22 July 2021; Accepted 22 July 2021
Available online 8 August 2021

2003; Jeppesen et al., 2005), and it is not obvious that knowledge and conclusions from temperate areas can be transferred to tropical lakes.

A turbid state dominated by phytoplankton is considered undesirable, while water dominated by submerged macrophytes is regarded as better quality (EC, 2000). Knowledge about aquatic macrophyte growth and the mechanisms behind community shifts is therefore of considerable importance for management and for the establishment of good ecological status for lakes and reservoirs.

Large natural lakes are scarce in Myanmar and therefore given special attention under the Myanmar National Water Framework Directive (NWRC, 2014) (see Ballot et al., 2018; Nesheim et al., 2016). Phytoplankton and aquatic macrophytes are considered among the most important bioindicators for ecological status evaluation in lakes (EC, 2000). The use of these parameters is therefore desirable in monitoring and management strategies for Myanmar lakes. However, to develop appropriate tools it is necessary to increase the general knowledge about freshwater ecology in the country, including interactions between macrophytes and phytoplankton.

Recently, a few studies including phytoplankton and aquatic macrophyte composition and abundance have been conducted in some lakes and reservoirs in Myanmar (Ballot et al., 2020; Mjelde et al., 2021; Swe et al., 2021; Mjelde and Ballot, 2016; Mjelde et al., 2018). However, there is a general lack of knowledge about macrophyte–phytoplankton interactions in waterbodies in Myanmar, as well as in other Asian countries and in tropical and equatorial areas in general.

Inlay Lake is the second largest natural lake in Myanmar and is a known biodiversity hotspot. However, to date, only lists of macrophytes exist for Inlay Lake (Allen et al., 2012); there are no datasets describing macrophyte abundance, or investigations of phytoplankton communities and the interactions between the macrophytes and phytoplankton.

To our knowledge, the present work is the first detailed whole-lake study about the interactions between phytoplankton and aquatic macrophytes in Myanmar and their role in shaping the Inlay Lake ecosystem.

The aim of this study is to assess the richness and composition of the aquatic macrophyte and phytoplankton communities in Inlay Lake in Myanmar, and to discuss the importance of aquatic macrophytes as a stabilizing factor in a large and shallow eutrophic lake in a tropical area. Our hypothesis is that the submerged macrophytes control phytoplankton biomass and composition and hence stabilize a clear water state in the lake. However, due to the large climatic variations in a tropical lake, we expect large differences in the physical, chemical and biological conditions between the rainy and dry periods, which can be important for the stability. Inlay Lake is a large lake, and correlations between macrophytes and phytoplankton may differ between areas, i.e. from north to south and from littoral to middle part of the lake.

## 2. Methods

### 2.1. Study area

Inlay Lake is characterized by a high botanical and wildlife biodiversity and was therefore established as a Wildlife Sanctuary in 1985. In 2003, Inlay Lake became an ASEAN Heritage Park and was listed as an Important Bird and Biodiversity Area (IBA) in 2004. In 2015, it was designated as Myanmar's first UNESCO Biosphere Reserve and in 2018 it became Myanmar's fifth Ramsar site. Inlay Lake is considered as one of the freshwater biodiversity hotspots in Myanmar (Lwin and Sharma, 2012). The diverse fauna and flora, the unique location of the lake and the unique lifestyles and traditions of the local human population have also made it one of the primary tourist destinations in Myanmar (Lwin and Sharma, 2012), followed by an increasing need for tourist accommodation.

Inlay Lake is a freshwater lake located at 884 m above sea level in Shan State in the eastern part of Myanmar. It is the second largest natural lake in Myanmar, with a surface area that varies between 94.4 $km^2$ (May) and 126.1 $km^2$ (November) based on data from 2015 to 2016 (Michalon et al., 2019). The largest inlets are Tham Daung in the north and Belui in southwest, while the outlet river is in the south (Fig. 1). The total catchment area is estimated to be 3800 $km^2$ (Michalon et al., 2019). This gives a ~34.5:1 catchment:lake area ratio, strongly suggesting a high influence of the land use in the catchment area on the lake ecosystem (especially high runoff and siltation; Cooke et al., 2005).

The lake is shallow, with a maximum depth of around 3.2 m and an average depth varying between 1 m in the dry season and 2.2–2.5 m in the rainy season (Michalon et al., 2019). Generally, the water level is lowest from mid-April to mid-May and rises to its highest level between mid-September and mid-October. Based on monthly data from 2014 to 2017, average water depth in the middle of the lake in April-May and in September-October is estimated to be 1.28 and 2.35 m, respectively (data from Forest Department Myanmar). The lake is regarded as warm polymictic (Akaishi et al., 2006).

Floating tomato gardens have been the most important agricultural activity in the lake region since the 1940s, and in 2018 the total area of floating gardens was estimated to be 24.5 $km^2$ (Irrigation Department, Nyaung Shwe, Myanmar). Large amounts of chemical fertilizers and pesticides are used in these areas, but management practices are changing towards more sustainable alternatives, such as using submerged macrophytes from the lake as organic fertilizers. The main species used for this purpose are *Eichhornia crassipes* (Michalon et al., 2019), *Najas indica* & *Ceratophyllum demersum*, and filamentous algae. An application of a fertilizer species lasts for 7–20 days, depending on the species, before new plants must be added to the crops (Irrigation Department, Nyaung Shwe, Myanmar). The expansion of floating gardens and the occurrence of free-floating macrophyte species has caused a decrease in open lake surface area from 65.4 $km^2$ in 1967 to 50.1 $km^2$ in 2014 (Michalon et al., 2019).

In addition to fertilizer and pesticide use by floating gardens, lake water quality is affected by a growing human population and tourist activity in the area, combined with a lack of adequate sanitation infrastructure (Htwe, 2015). A recent study on surface water quality revealed eutrophic conditions in Inlay Lake and a high level of bacterial contamination (Akaishi et al., 2006).

Deforestation and agricultural practices in the catchment have also led to erosion and siltation in the lake. Total silt discharge from all subcatchments is estimated at ≈270,000 tonnes per year, of which 62 % is deposited in deltas, 20 % in marshes and 1 % in the lake itself (Furuichi, 2008). Silt discharge is considered an important component of eutrophication (Cooke et al., 2005; Everall et al., 2018).

The climate in Myanmar is tropical and can be divided in three seasons: the dry winter or northeast monsoon season (November–February), the summer or hot (and dry) season (March – mid-May), and the rainy or southwest monsoon season (mid-May– October; Aung et al., 2017). The average annual maximum and minimum temperature for 1981–2010 at the Taunggyi weather station, 25 km north of Inlay Lake, were 25 °C (April) and 14 °C (December-January), respectively. The annual rainfall is about 1010 mm, and the precipitation is mostly confined to the rainy season (May–October). The overall predominant wind in the area is from the southeast, and the speed is generally low (less than 4−5 m/s; Aung et al., 2017).

Species lists of aquatic flora and fauna are available from earlier surveys in Inlay Lake (Allen et al., 2012). Around 30 species of aquatic macrophytes have been listed (Ito and Barfod, 2014; Lansdown, 2012; Nair, 1960), but no information about abundances exists. The fish community consists of 17 endemic and 15 widespread fish species (Kullander, 2012). The most important fish species for local people is the Inlé Carp (*Cyprinus carpio intha*), both as food source and as a cultural symbol of the ethnic Intha people (Allen et al., 2012). A high diversity of gastropods, mainly Viviparidae, Pachychilidae and Bithyniidae and bivalves from the families Unionidae, Cyrenidae and Sphaeriidae, is reported from the lake, however this information is from old studies (see references in Allen et al., 2012). The phytoplankton community has not been studied.

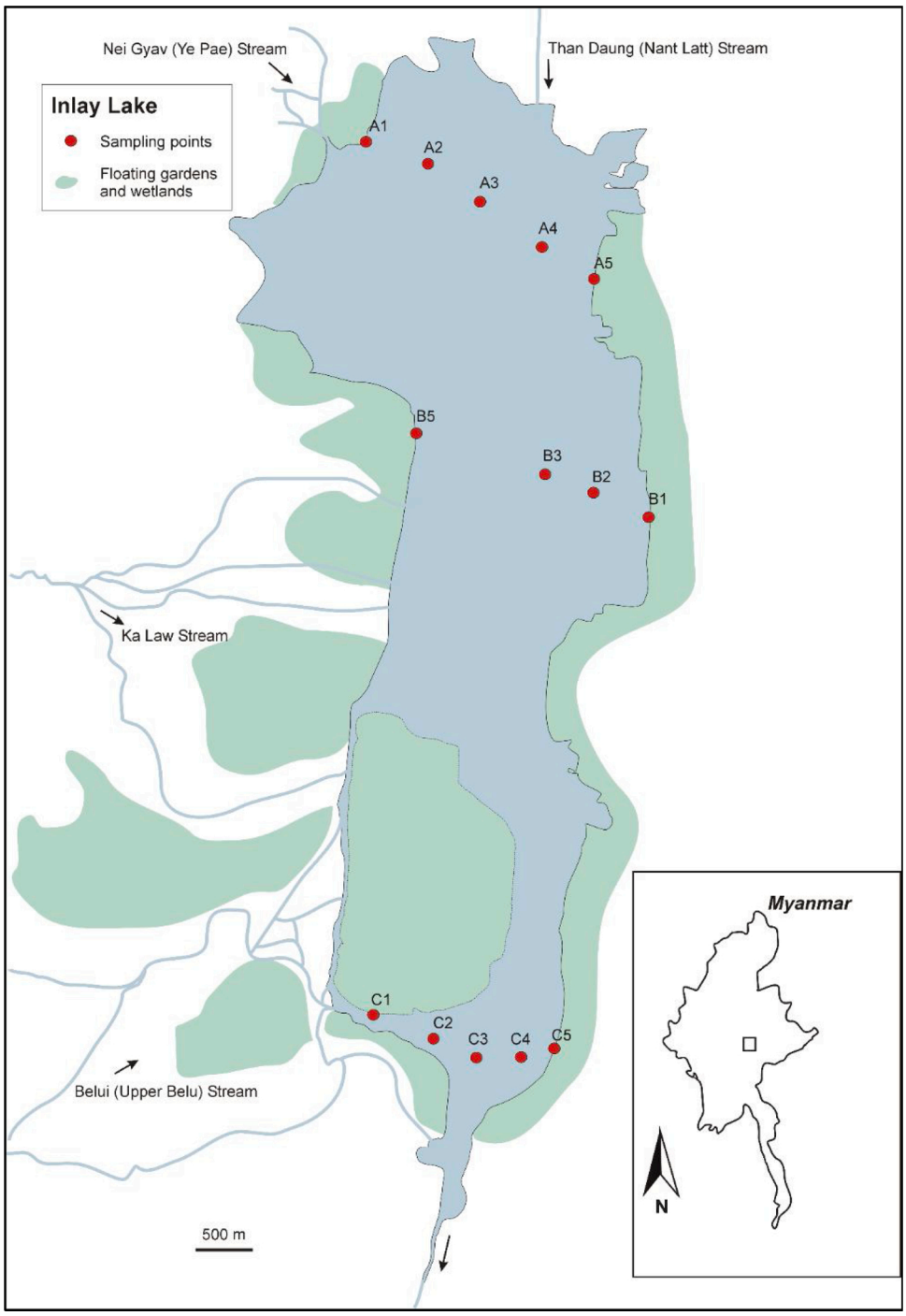



**Fig. 1.** Sampling sites in Inlay Lake in 2014–2017. Inlay Lake (square) is situated in Shan state in the eastern part of Myanmar (right).

### 2.2. Field and analysis methods

The field work was carried out in the period 2014–2017 with sampling conducted at four occasions, twice after the end of the rainy season (November 2014, 2015) and twice at the end of the cold dry winter period (February 2015 and March 2017). Physical measurements, water samples, phytoplankton and aquatic macrophytes were collected at 14 different lake sites (Fig. 1, Table 1) covering different areas, depths and habitats in the lake. The number and placement of sites captured most of the macrophyte and phytoplankton species (cf. the rarefaction curves later in the chapter) and reflected the main variations in physical, chemical and biological conditions in the lake. In March 2017, we sampled at a reduced set of open-water sites (A3, B3 and C3) and shore sites (A1, B1 and C1) in the northern, middle, and southern lake areas. Water samples from the main tributaries rivers Belui (Belu), Nei Gyar (Ye Pae) and Tham Daung (Nant Latt), and from the outlet river, were collected as part of the field work in 2014−2015. Physical and chemical data from tributaries from November 2017 are based on Eriksen et al. (2021).

Between November 29th and 30th 2017, water depth was determined using a handheld echosounder along 16 approximately east–west transects (211 depth measurements in total). The open water boundary of the lake was downloaded from OpenStreetMap (OpenStreetMap, 2018) and used to add an additional 400 points with zero depth around the lake's perimeter. The combined dataset of 611 points was then interpolated to a regular grid with 200 m resolution using Inverse

**Table 1**
Sampling localities in Inlay Lake in 2014–2017. Coordinates in decimal degrees.

| Lake area | Loc. no | Latitude | Longitude | Quality elements |
|---|---|---|---|---|
| North | A1 | 20.603837 | 96.895600 | P&W, PP, AM |
| | A2 | 20.602125 | 96.901859 | P&W, PP, AM |
| | A3 | 20.593999 | 96.913220 | P&W, PP, AM |
| | A4 | 20.589542 | 96.919611 | P&W, PP, AM |
| | A5 | 20.58426 | 96.927998 | P&W, PP, AM |
| Middle | B1 | 20.550628 | 96.935768 | P&W, PP, AM |
| | B2 | 20.553565 | 96.926082 | P&W, PP, AM |
| | B3 | 20.557946 | 96.918544 | P&W, PP, AM |
| | B5 | 20.562589 | 96.902898 | P&W, PP, AM |
| South | C1 | 20.48066 | 96.895886 | P&W, PP, AM |
| | C2 | 20.477061 | 96.901475 | P&W, PP, AM |
| | C3 | 20.473896 | 96.910144 | P&W, PP, AM |
| | C4 | 20.470792 | 96.913435 | P&W, PP, AM |
| | C5 | 20.47489 | 96.923759 | P&W, PP, AM |

P&W = physical measurements and water chemistry, PP = phytoplankton, AM = aquatic macrophytes.

Distance Weighting (Wong, 2017) to create the bathymetric map shown in Fig. 2. The Osgood Index (OI; Osgood, 1988) calculated from this gridded dataset and the lake area varies between 0.16 and 0.27, depending on variations in lake surface area. The index estimates the probability of partial or complete mixing of the lake, and the very low index values for Inlay lake indicate year-round polymixis and a high probability of internal phosphorus loading (Osgood, 1988; Mataraza and Cooke, 1997).

### 2.2.1. *Physical measurements and water samples*

Physical measurements and water samples were taken approximately 20 cm below the water surface at each locality. For the physical measurements we used a Hach, HQd Portable Meter (Hach, Loveland, CO, USA). For the chemical analyses, water samples were collected using a Ruttner sampler. One 100 mL water aliquot (preserved in the field with 4 M $H_2SO_4$ to 1 % final concentration) and one 100 mL aliquot (unpreserved) were stored at 4 °C. All samples were transported to and analysed at the ISO-certified NIVA laboratory in Norway. The physical and chemical parameters included water temperature, pH, conductivity, oxygen, colour, calcium (Ca), ammonium ($NH_4$), nitrate-nitrite

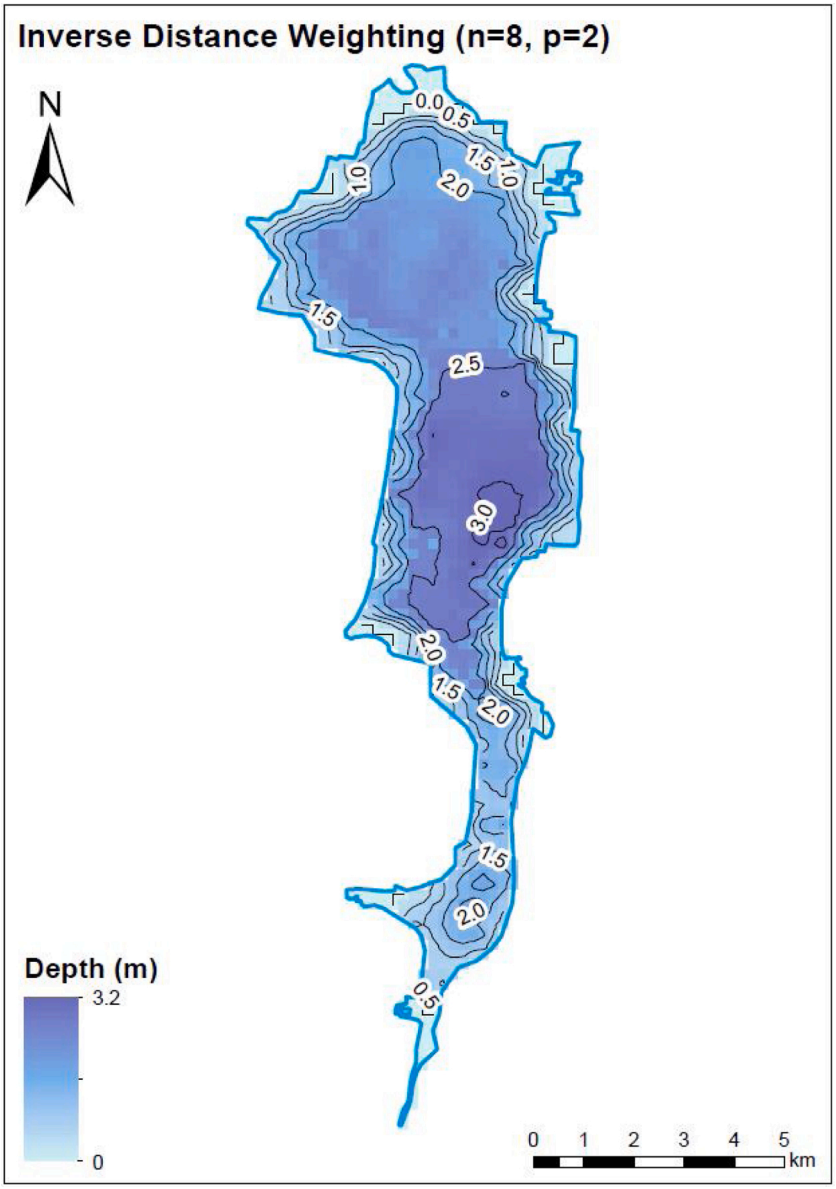



**Fig. 2.** Bathymetric map of Inlay Lake. The depth measurements were carried out 29-30 November 2017.

($NO_3$+$NO_2$), ortho-phosphate ($PO_4$), total nitrogen (TN), total phosphorus (TP), total carbon (TOC) and silicate ($SiO_2$) concentrations. All chemical analyses were carried out according to Norwegian standard methods (see Supplementary Table S1).

#### 2.2.2. Aquatic macrophytes

The surveys of aquatic macrophytes in Inlay Lake included only hydrophytes, i.e. species belonging to the submerged, floating-leaved and free-floating groups; emergent species (helophytes) were thus excluded. Hydrophytes reflect water quality much more directly than helophytes, making hydrophytes particularly important for ecological assessment in eutrophic lakes. They are therefore prioritized in assessment methods in EU WFD (e.g., Hellsten et al., 2014).

The plants were surveyed in an area of approx. 1 $m^2$ at each locality with an aqua scope and collected by dredging from the boat with a casting rake (e.g., Kolada et al., 2012). The abundances of the species were scored according to a semi-quantitative scale, where 1 = rare, 2 = scattered, 3 = common, 4 = locally dominant and 5 = dominant. Where possible, all taxa were identified to species level, using floras for the region (mainly Cook, 1996), in addition to updated or more specialised taxonomic work (e.g., La-Ongsri, 2008; Triest, 1988; Wiegleb, 1990; Wiegleb and Kaplan, 1998). Charophytes were identified based on Wood and Imahori (1965) and later verified by genetic analysis (Mjelde et al., 2021). In a few cases, identification to species could not be done with certainty, hence these taxa were identified to genus only. Sampling effort for macrophytes at species level was adequate according to a sample-based Coleman rarefaction curve with 50 runs without replication, constructed using the "EstimateS" software package (v8.20; Colwell, 2013). Ninety percent and 99 % of the 28 macrophyte species found in the 51 samples from 2014 to 2017 were found after 16 and 46 samples, respectively.

The estimated abundance of aquatic macrophytes is based on the semi-quantitative scores for all localities. We estimated the total abundance at each locality by adding the cubed five-level values for each species. The linear five-level scale commonly used for abundance estimation of aquatic macrophytes does not reflect the non-linear abundance increases, and using cubed 5-level values for total abundance is regarded as the "best possible" approximation for comparing abundances among macrophyte groups and among sites (Melzer, 1999; Schneider et al., 2018).

#### 2.2.3. Phytoplankton

Quantitative water samples were taken at all localities using a Ruttner sampler. A 50-mL aliquot was taken for quantitative phytoplankton analysis (assemblage taxonomic composition and biomass) and preserved with acidic Lugol's solution. For qualitative phytoplankton analysis (taxonomic composition), a concentrated net sample (mesh size 20 μm) was collected and preserved by addition of formaldehyde (to 4 % final concentration). All samples for quantitative and qualitative analysis were stored in the dark until they were analysed. The Lugol-fixed samples were analysed for phytoplankton composition and biomass using Utermöhl sedimentation chambers (Utermöhl, 1958) and an inverted microscope (Olympus Optical Co-Ltd Japan Model CK2, Olympus, Tokyo, Japan). Sampling effort was more than adequate according to a sample-based Coleman rarefaction curve constructed as for macrophytes, with 99 % of the 14 major phytoplankton groups detected after 2 out of the total 46 samples collected. All taxa were identified to species or genus level, using selected identification keys (e.g., Büdel et al., 1978-2015; Huber-Pestalozzi, 1969; Komárek and Anagnostidis, 1999; Komárek and Fott, 1983; Skuja, 1949). However, some taxa could only be identified to family level. In addition, water samples (50 mL) were taken at each sampling point for isolation of cyanobacteria and kept in a cool shady place and gently shaken twice per day until processing at the Norwegian Institute for Water Research (NIVA) in Norway.

Using a microcapillary, single colonies of *Microcystis* were isolated. They were washed five times and placed in wells on microtiter plates containing 300 μL Z8 medium (Kotai, 1972). After successful growth, the samples were placed in 50 mL Erlenmeyer flasks containing 20 mL Z8 medium and maintained at 22 °C. Strains were classified based on morphological traits according to Komárek and Anagnostidis (1999). Morphological characterisations were conducted using a Leica DM2500 light microscope, Leica DFC450 camera and Leica Application Suite software (LAS; Leica, Oslo, Norway). The morphological identification was based on the following criteria: (i) size of vegetative cells, and (ii) nature and shape of colonies. Length and width of 50–250 vegetative cells were measured. All strains used in this study are maintained at the Norwegian Institute for Water Research, Oslo, Norway.

To test for the production of microcystins, fresh culture material of all four Microcystis strains was frozen and thawed three times using the Eurofins Abraxis microcystin enzyme-linked immunosorbent assay (ELISA) kits (Eurofins Abraxis, Warminister, PA, USA). The test is an indirect competitive ELISA designed to detect microcystins based on specific antibody recognition. The colour reaction of the ELISA test was evaluated at 450 nm on a Perkin Elmer1420 Multilabel counter Victor3 (Perkin Elmer, Waltham, MA, USA). All strains were also tested for saxitoxin, anatoxin-a and cylindrospermopsin using the Eurofins Abraxis anatoxin, saxitoxin and cylindrospermopsin kits (Eurofins Abraxis, Warminister, PA, USA).

#### 2.2.4. Statistical analysis

To assess the relationship between environmental variables and biology, to describe the relationship between phytoplankton and macrophytes and to detect possible differences between seasons and/or lake areas we have analysed the data using multivariate, univariate and bivariate statistics.

Relationships between community composition and environmental variables were assessed using redundancy analysis (RDA). Analyses were done using the Vegan library (Oksanen et al., 2020) in R (R core team, 2020). Due to incomplete water chemistry from 2014 and a reduced sampling program in 2017, we only analysed community-environment relationships on the data from 2015. Data from November (end of the rainy season) and February (end of the dry season) in 2015 were analysed separately. Prior to analysis, all water chemical variables except pH (TP, TN, dissolved inorganic N (DIN; $NO_3$+$NO_2$), $NH_4$, $PO_4$, $SiO_2$, TOC and conductivity) were log-transformed to normalize the data. To assure that all the predictor variables were on comparable scales, all predictor variables (water chemistry, water temperature, depth, latitude and longitude) were also normalized by subtracting the mean and transformed to unit standard deviation. The RDAs of phytoplankton community composition were done using Hellinger-transformed abundances (square root of relative biovolumes) of the main phytoplankton classes. For macrophytes, we used Hellinger-transformed abundances based on species abundance (see Schneider et al., 2018).

To test which variables in the RDAs that significantly could explain variation in community composition, we did backward and forward selection using the set of predictor variables mentioned above. Only significant environmental variables ($p < 0.05$) were kept. Many of the environmental variables were correlated. Hence, if one variable was included in the model, other correlated variables would not explain much of the residual variation and therefore would likely be excluded from the model. The effect on community composition, however, might still be due to one or more of the correlated variables, even though another variable was "chosen" in the model selection. To assist interpretation of the RDAs, we therefore also analysed the environmental variables by PCA and plotted the main gradients in these variables along with the RDA-plots (see Figs. 5 & 9).

To disentangle spatial community gradients from effects from local environmental conditions (water chemistry, temperature and depth), we used variance partitioning by RDA (using function varpart() in vegan). We included the significant environmental variables as one group of

predictors and latitude/longitude as another group of predictors. The analysis then calculates the fraction of variation explained by the environmental variables, spatial variables, and variation shared (confounded) by the two groups. Finally, we tested for significance of the environmental variables given that the spatial variables were included in the model, and vice versa.

Uni- and bivariate analyses were performed in addition and in support of multivariate analyses. Univariate analyses included two-way type I ANOVAs that were run to detect differences in average abundance/biomass among lake areas (north, middle, and south) and between sampling seasons (November, corresponding to the end of the rainy summer season, and February/March, corresponding to the dry winter season). Macrophyte and phytoplankton data from the same sites were collected six months apart and thus considered sufficiently distant and independent to be applied to ANOVAs. November 2014 and 2015 and February/March 2015 and 2017 were clumped to obtain a November (end of the rainy season) and a February/March (end of the dry season) data set, respectively, to increase replication and thus ANOVA reliability. A type I ANOVA was chosen as both factors were set by the investigators. Tukey HSD (Honestly Significant Difference) *post-hoc* multiple comparisons were run after significant ANOVAs to ascertain which average values were different from which. Among the plethora of *post-hoc* multiple comparison tests, Tukey HSD tests have the advantage of not increasing the risk of committing experiment-wide type I errors, as the test power is kept at the nominal level ($\alpha = 0.05$; e.g. Quinn and Keough, 2002: 199–200). When the ANOVA factor interaction was significant, factor levels within the ANOVA (two sampling seasons and three lake areas) were considered statistically different from one another (Zar, 2009).

Linear regressions were performed for selected data sets. We performed linear regressions between macrophyte abundance and phytoplankton biomass to see if the mutual exclusion of macrophytes and phytoplankton, typical in nutrient-rich, shallow temperate lakes (e.g., Jeppesen et al., 1998), also exists in tropical Inlay Lake. For such regressions, run for total, submerged, and floating-leaved macrophytes separately and for November and February/March separately, macrophyte abundance was treated as the independent variable based on the overwhelming evidence that macrophytes drive macrophyte–phytoplankton interactions (e.g., Timms and Moss, 1984; van Donk et al., 1993; Jasser, 1995; Mjelde and Faafeng, 1997; Pelton et al., 1998; Körner and Nicklisch, 2002; Hilt and Lombardo, 2010; Lombardo et al., 2013).

Uni- and bivariate analyses were performed with Addinsoft® XLSTAT®©, with significance assumed for $p < 0.05$. The assumptions of these parametric techniques were checked by visual inspection of data and residual distributions (Zar, 2009). However, ANOVAs and regressions are robust and give reliable results provided that non-normality and/or heteroskedasticity are not extreme (Zar, 2009).

## 3. Results

### 3.1. Physical and chemical variables

Inlay Lake is a shallow, clear and calcareous lake. However, some areas close to inflowing rivers and at macrophyte and sediment removal areas had higher turbidity and TOC than the rest of the lake (pers. obs.).

Water temperature measured during sample collection (central time of the day) was slightly but significantly higher in November and in the middle lake area (Table 2). Average pH was higher in February than in November. Conductivity was different across lake area and sampling season, with a significant lake area × season interaction, probably due to the small standard errors (Table 2). The dissolved oxygen in the surface layer was at or above 100 % saturation; and tended to be lower after the rainy period (November) than after the dry period (February/March) (Table 2).

TP and TN concentrations indicated mesotrophic conditions (Table 2) and were generally higher close to the shore (Supplementary Table S1), reflecting the pollution from inflowing rivers (Table 3), shoreline villages and floating gardens, while TOC was higher in the northern lake area (Table 2, Fig. 3).

The first two axes in a principal component analysis (PCA) of physicochemical variables from all sampling times and sites, explained 52 % of the total variation in the dataset (Fig. 3). The first axis was related to total nutrient concentrations (TN, TP, $SiO_2$ and conductivity) and pH, while the second axis was related to TOC and dissolved inorganic N (DIN; $NO_3$+$NO_2$), but also $PO_4$. The ordination did not show any separation between lake regions or sampling seasons, however, there was a general trend for northern sites to have higher TN concentrations and TOC than southern sites while dissolved nutrient availability ($NO_3$+$NO_2$, $PO_4$) was slightly higher at southern lake sites.

### 3.2. Aquatic macrophytes

The lake is surrounded by helophytes, dominated by *Phragmites karka*, and floating gardens, covering approximately 35 and 65 % of the shoreline, respectively. The average maximum depth for the helophytes is estimated to be 1.4 m (measured on 23 November 2015; not correlated to median water level). Maximum depth of submerged aquatic macrophytes was estimated to be 2.8–3 m (depth measured in November 2014).

A total of 28 species of aquatic macrophytes were identified during the survey period. The species included 16 submerged (including charophytes), 7 floating-leaved and 5 free-floating species (Supplementary Table S3). In general, the highest richness (as number of taxa) was

**Table 2**
Physical measurements and water chemistry from Inlay Lake, 2014–2017, by season (November and February/March) and lake area (north, middle and south); **average ± standard error**. Incomplete datasets and statistical analysis for complete datasets is in Supplementary Table S2.

| | | | November | | | February/March | | |
|---|---|---|---|---|---|---|---|---|
| variable[1] | abbrev. | unit | north | middle | south | north | middle | south |
| water temperature | T | °C | 24.6 ± 0.5 | 25.8 ± 0.2 | 25.3 ± 0.3 | 23.3 ± 0.5 | 25.4 ± 0.3 | 24.1 ± 0.5 |
| pH | pH | pH units | 7.9 ± 0.1 | 8.1 ± 0.1 | 8.0 ± 0.2 | 8.5 ± 0.1 | 8.4 ± 0.1 | 8.3 ± 0.2 |
| conductivity | cond | µS/cm | 416.7 ± 14.3 | 363.6 ± 6.4 | 360.1 ± 4.4 | 351.4 ± 15.4 | 347.0 ± 8.5 | 349.3 ± 15.4 |
| dissolved oxygen[c] | DO | mg $O_2$/L | | 6.4 ± 0.5 | | | 8.8 ± 0.5 | |
| oxygen saturation | %DO | % | | 85.6 ± 6.8 | | | 115.4 ± 6.9 | |
| calcium[c] | Ca | mg Ca/L | | 48.6 ± 1.9 | | | 40.1 ± 4.8 | |
| total organic carbon[c] | TOC | mg C /L | 6.4 ± 0.8 | 6.0 ± 0.3 | 3.8 ± 0.7 | 5.3 ± 0.5 | 5.0 ± 0.2 | 3.8 ± 0.6 |
| total phosphorus[c] | TP | µg P/L | 14.2 ± 3.1 | 18.2 ± 2.8 | 15.0 ± 1.9 | 9.1 ± 1.2 | 28.5 ± 18.9 | 16.3 ± 3.0 |
| phosphate[c] | $PO_4$-P | µg P/L | 3.3 ± 1.1 | 3.8 ± 0.7 | 4.3 ± 0.5 | 2.6 ± 1.1 | 3.0 ± 1.1 | 5.5 ± 2.1 |
| total nitrogen[c] | TN | µg N/L | 442.1 ± 23.8 | 450.0 ± 9.5 | 429.4 ± 42.6 | 530.0 ± 56.2 | 467.2 ± 74.7 | 446.4 ± 37.5 |
| ammonium[c] | $NH_4$ | µg N/L | 53.4 ± 5.1 | 32.8 ± 3.0 | 32.8 ± 4.7 | 38.7 ± 6.5 | 35.7 ± 4.0 | 40.3 ± 4.5 |
| nitrate + nitrite[c] | $NO_x$ | µg N/L | 24.2 ± 6.3 | 5.0 ± 0.4 | 52.0 ± 22.2 | 11.6 ± 2.1 | 10.3 ± 2.2 | 95.3 ± 56.5 |

[1] Superscript c denotes concentrations.

**Table 3**
Water chemistry from the main inlet rivers Belui (upper Belu), Tham Daung (Nant Latt), Nei Gyav (Ye Pae) and Ka Law (see Fig. 1). **Single values** from different dates. Nov.17-data from Eriksen et al. (2021), from slightly different sampling sites as the other dates.

| | | | Belui (upper Belu) | | | | Tham Daung | | Nei Gyav | | Ka Law |
|---|---|---|---|---|---|---|---|---|---|---|---|
| variable | abbrev. | unit | nov.14 | febr.15 | nov.15 | nov.17 | nov.15 | nov.17 | nov.15 | nov.17 | nov.17 |
| total phosphorus | TP | µg P/L | 57 | 18 | 57 | 27 | 27 | 68 | 42 | 93 | 10 |
| phosphate | $PO_4$-P | µg P/L | 29 | 13 | 29 | 19 | 14 | 50 | 20 | 11 | 1 |
| total nitrogen | TN | µg N/L | 530 | 635 | 690 | 1390 | 620 | 1600 | 560 | 142,000 | 885 |
| ammonium | $NH_4$ | µg N/L | 19 | 19 | 30 | 13 | 14 | 215 | 60 | 75 | 31 |
| nitrate + nitrite | $NO_x$ | µg N/L | 410 | 550 | 485 | 1300 | 500 | 920 | 130 | >20,000 | 900 |

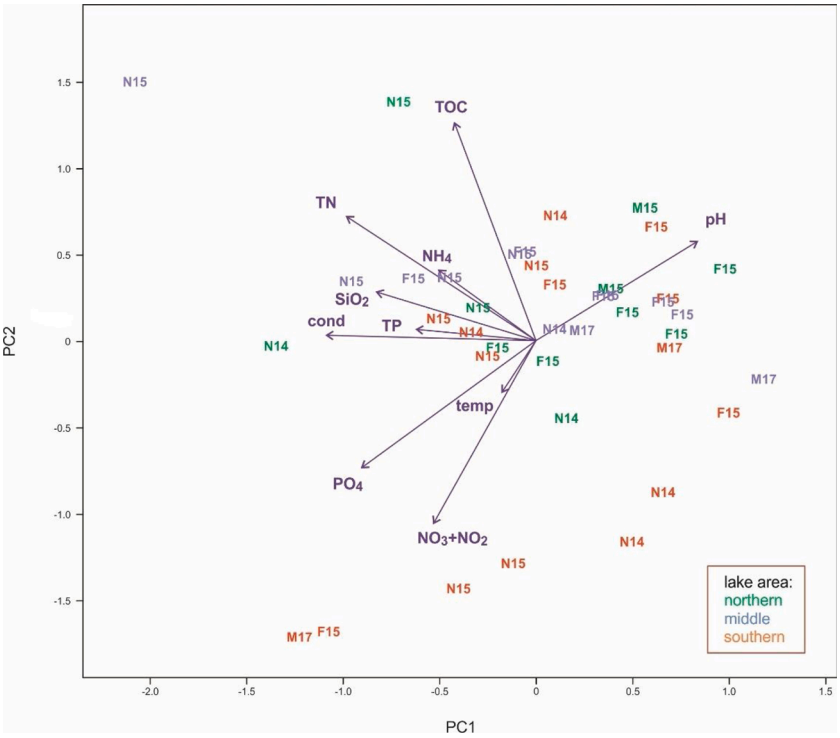



**Fig. 3.** PCA on environmental variables for all periods (2014–2017) and sites. Station coding/legend is as follows: Green = area "north"; orange: area "south"; blue: area "middle". The letter (F = February; M = March; N = November) and the number (14, 15, 17) are the month and year of sampling (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

recorded close to the shores while the central parts of the lake had the lowest richness, but macrophyte taxonomic richness in the observational plots was statistically similar across lake areas and sampling periods (two-way ANOVA for total macrophyte richness: $F_{period(1,1)} = 0.131$, $p = 0.720$; $F_{area(1,2)} = 2.721$, $p = 0.077$; $F_{interaction(1,2)} = 1.284$, $p = 0.287$; for submerged species: $F_{period(1,1)} = 0.266$, $p = 0.609$ $F_{area(1,2)} = 1.078$, $p = 0.350$; $F_{interaction(1,2)} = 0.272$, $p = 0.763$; for free-floating and floating-leaved species: $F_{period(1,1)} = 3.957$, $p = 0.053$; $F_{area(1,2)} = 0.616$, $p = 0.545$; $F_{interaction(1,2)} = 0.397$, $p = 0.675$) (Supplementary Table S3). Some protected bays close to hotels appeared to have a high taxonomic richness of floating-leaves species (pers. obs., not included in the survey).

The submerged vegetation, dominated by *Nechamandra alternifolia* and *Potamogeton lucens,* and to a lesser degree, *Ceratophyllum demersum, Myriophyllum verticillatum, Najas indica* and *Chara zeylanica,* made extensive stands in most of the lake and throughout the year, while the free-floating species, dominated by *Eichhornia crassipes*, formed small- to medium-sized moving islands (Supplementary Table S3). The floating-leaved species were very rare in the lake.

Total macrophyte and submerged species (elodeids and charophytes) abundance were lower in the middle and deepest part of the lake regardless of the sampling period (Fig. 4, Supplementary Table S4). Total abundance of floating-leaved and free-floating species (nympheids and lemnids) was lower than total submerged vegetation and remained similar across lake areas and sampling periods (Supplementary Table S4).

The community composition in November 2015 was not significantly related to any of the environmental variables (water chemistry, depth, temperature, latitude and longitude) in the RDA (data not shown). However, testing effects of latitude and longitude alone, there was a weak, but non-significant effect of latitude. A PCA on environmental variables in February 2015 revealed that the main gradient in environmental conditions (PC axis 1, Fig. 5A) was most strongly related to conductivity, $PO_4$, DIN, depth and pH (Fig. 5A). The second strongest gradient (axis 2, Fig. 5A) was related to longitude, TOC, and total phytoplankton biomass. The same analysis on the community composition in February 2015 revealed no significant effects of water chemistry, but depth and latitude were significant (Fig. 5B). Together, these variables explained 25 % of the variation in macrophyte community composition.

### 3.3. Phytoplankton

Phytoplankton taxonomic richness (as number of major groups) was

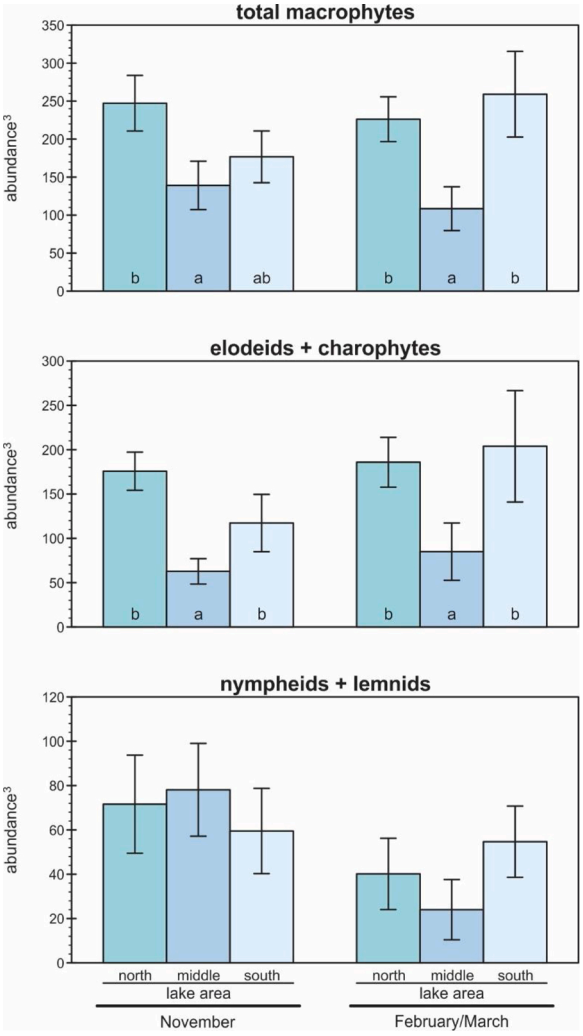



**Fig. 4.** Abundance of macrophytes (as cubed 1–5 scale) by lake area and sampling period for all periods (2014–2017). From top: total macrophytes; submerged forms (elodeids + charophytes); floating-leaved and free-floating forms (nympheids + lemnids). Abundance is expressed as cubed five-level values for each species (see Schneider et al., 2018). Average ± standard error; sample sizes: *n*north,Nov = 8, *n*middle,Nov = 8, *n*south,Nov = 10, *n*north, Feb = 7, *n*middle,Feb = 6, *n*south,Feb = 7. Different letters denote statistically different average values (in alphabetical order with a = lowest) according to multiple-comparison Tukey HSD tests after significant two-way ANOVAs; complete statistical results are in Supplementary Table S4. Please note the different *y* scales.

the same across lake areas and in both sampling periods (two-way ANOVA: period: $F_{1,40} = 0.407$, $p = 0.527$; area: $F_{2,40} = 1.508$, $p = 0.238$; period × area: $F_{2,40} = 1.382$, $p = 0.263$). At all localities, Bacillariophyceae, Cryptophyceae, Chlorophyceae, Euglenophyceae or Cyanobacteria were the dominating groups. The cyanobacterium *Microcystis* sp. was found in low amounts at almost all localities. Using ELISA, production of hepatotoxic microcystins was confirmed in four *Microcystis* cultures isolated from Inlay Lake.

In general, the phytoplankton biomass in Inlay Lake was low, with average biomasses less than 1 mg fresh weight (FW) $L^{-1}$ (Fig. 6). However, higher biomasses (more than 2 mg/L) were observed at 3–4 localities close to the shore and floating gardens (Supplementary

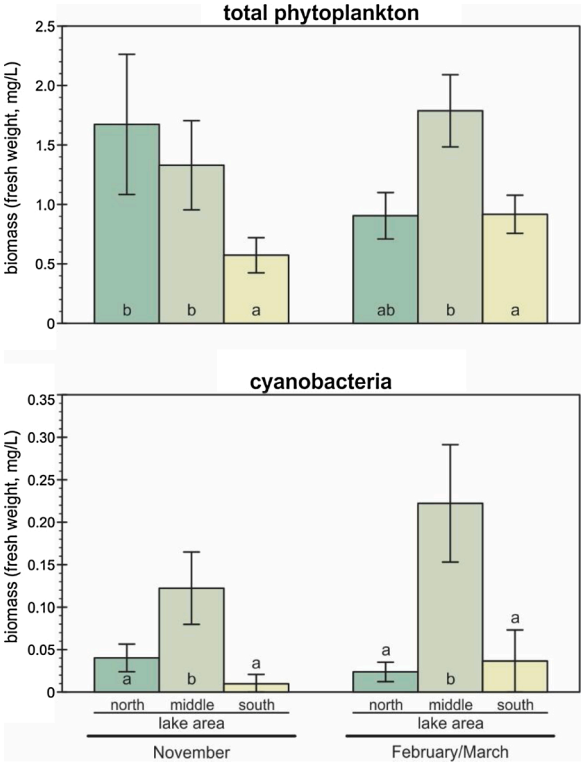



**Fig. 6.** Total phytoplankton biomass and cyanobacteria biomass (as mg $L^{-1}$ of fresh biomass) by sampling season (Nov, Feb) and lake area (northern, middle, southern). Average ± standard error; sample sizes: *n*north,Nov = 8, *n*middle, Nov = 8, *n*south,Nov = 10, *n*north,Feb = 7, *n*middle,Feb = 6, *n*south,Feb = 7. Different letters denote statistically different average values according to multiple-comparison Tukey HSD tests after significant two-way ANOVAs; complete statistical results are in Supplementary Table S4). Please note the different *y* scales.

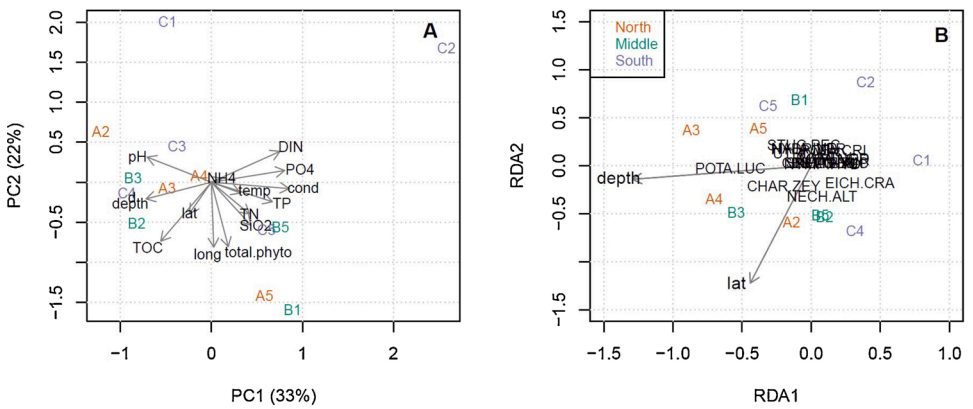



**Fig. 5.** A) PCA on environmental variables, and B) RDA on macrophyte community composition from February 2015. Only significant variables were included in the RDA plot. Station coding/legend is as follows: Orange = area "north", green = area "middle", purple = area "south". Abbreviations: TN: Total Nitrogen, TOC: Total Organic Carbon, TP: Total Phosphorous, $PO_4$: Phosphate, DIN: dissolved inorganic N ($NO_2$+$NO_3$), $SiO_2$: silicate, cond: conductivity, lat: latitude, long: longitude. Species abbreviations: see Supplementary Table S3 (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table S5).

Total phytoplankton biomass (mg of fresh weight $L^{-1}$) was relatively variable across sites. The highest total phytoplankton biomass was found in the northern area in November while in February-March the middle area had the highest biomass. The biomass was lowest in November in southern area (Fig. 6). Cyanobacteria were significantly more abundant in the middle lake area, regardless of sampling period (Fig. 6).

A PCA on the environmental variables from November 2015 revealed that the main gradient in environmental conditions (PC axis 1, Fig. 7A) was most strongly related to TOC, TN, latitude, and DIN. The second strongest gradient (axis 2) was related to temperature, DIN, $NH_4$, conductivity and TP. Two variables could significantly explain variability in phytoplankton community composition in November 2015, namely TOC and TN (Fig. 7B). Latitude and longitude did not come out as significant in the model selection or in the variation partitioning procedure. Hence, most of the explained variation in phytoplankton community composition in November 2015 was related to environmental factors, not spatial position. The fraction explained by TOC and TN was 24 % according to the adjusted $R^2$ of the RDA. There were, however, few strong relationships between relative abundances of specific phytoplankton classes and the significant variables (Fig. 7B). A PCA on environmental variables in February 2015 revealed that the main gradient in environmental conditions (PC axis 1, Fig. 7C) was most strongly related to conductivity, $PO_4$, DIN, TP and pH (Fig. 7C). The second strongest gradient (axis 2, Fig. 7C) was related to longitude, TOC, $SiO_2$ and TN. Three variables came out as significant predictors of phytoplankton community composition in February 2015: $PO_4$, $SiO_2$ and TOC (Fig. 7D). Latitude and longitude were not significant in the model selection or the variation partitioning. According to the adjusted $R^2$ of the RDA, 32 % of the variation in phytoplankton community composition was explained by environmental factors in February 2015, which was higher than in November. There were, however, no strong relationships between relative abundances of phytoplankton classes and single variables.

### 3.4. Phytoplankton vs. aquatic macrophytes

In November, total phytoplankton biomass was negatively associated with total macrophyte abundance in the northern lake area and positively associated in the middle area (Fig. 8, Supplementary Table S7). No relationship was found in February. Submerged macrophytes in the northern lake area explained 69 % of phytoplankton biomass, and their relationship was negative both in November and in February. In the

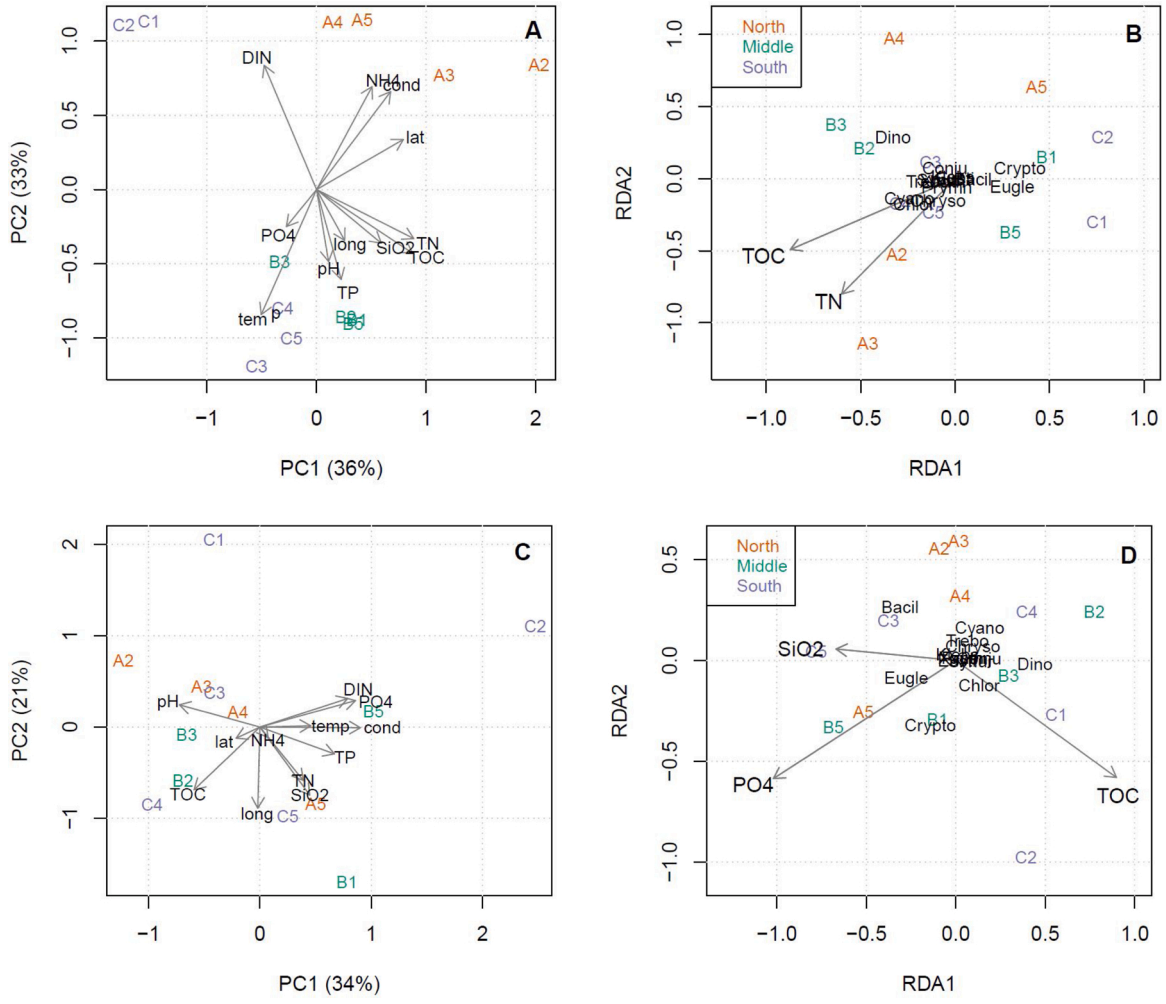



**Fig. 7.** A) PCA on environmental variables, and B) RDA on phytoplankton community composition from November 2015. C) and D) show the same plots from February 2015. Only significant variables were included in the RDA plots. Station coding/legend is as follows: Orange = area "north", green = area "middle", purple = area "south". Abbreviations: Crypt: Cryptophyceae, Xanth: Xanthphyceae, Chlor: Chlorophyceae, Klebs: Klebsormidiophyceae, Eusti: Eustigmatophyceae, Cyano: Cyanophyta, Chrys: Chrysophyceae, Prymn: Prymnesiophyceae, Conju: Conjugatophyceae, Dinop: Dinophyceae, Synur: Synurophyceae, Bacil: Bacillariophyceae, Eugle: Euglenophyceae, TN: Total Nitrogen, TOC: Total Organic Carbon, TP: Total Phosphorous, $PO_4$: Phosphate, DIN = dissolved inorganic N ($NO_2$+$NO_3$), SiO2 = silicate, cond = conductivity, lat = latitude, long = longitude (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

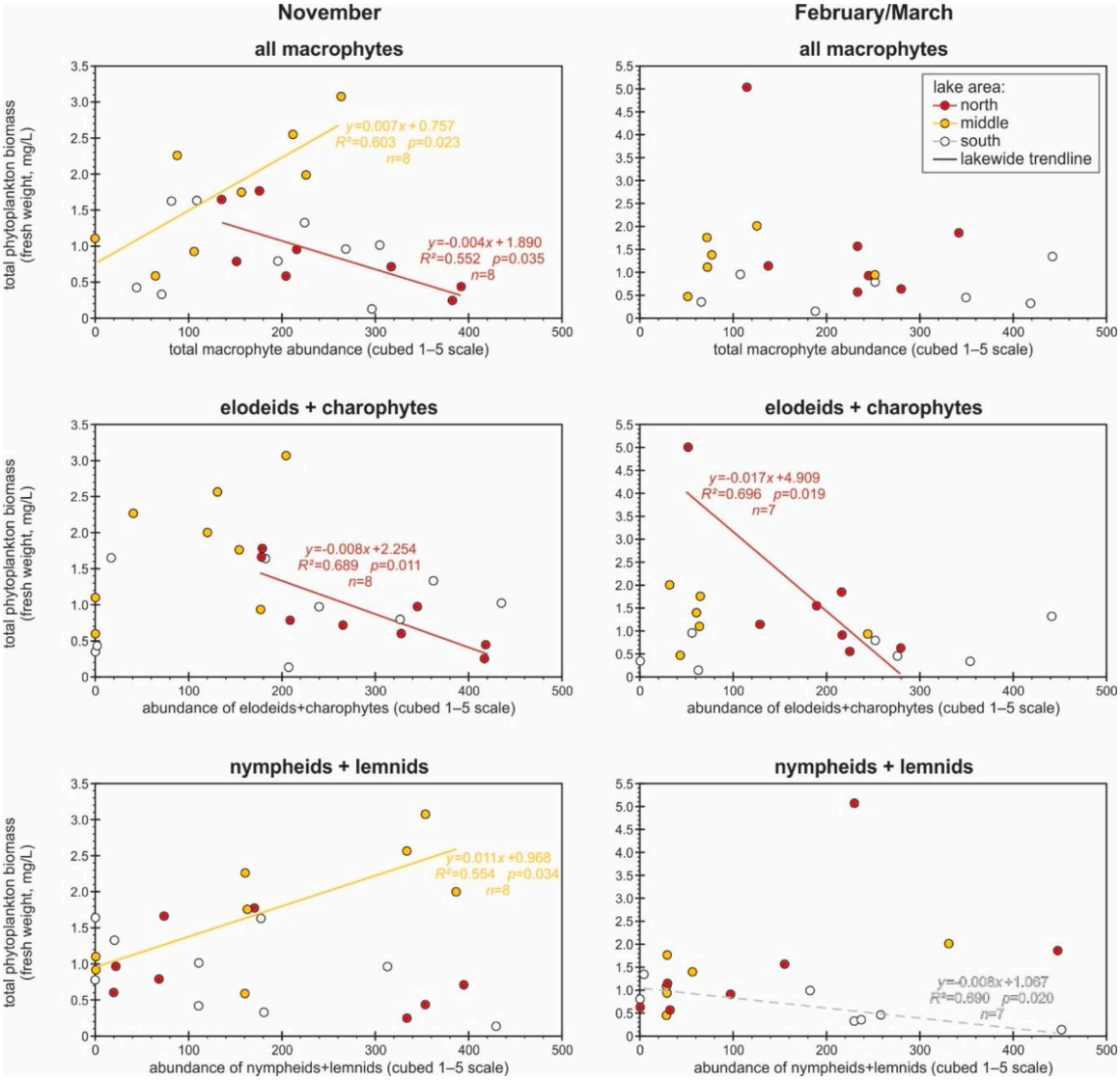



**Fig. 8.** Relationships (as linear regressions) between total, submerged (elodeids + charophytes), and floating-leaved (nympheids + lemnids) macrophyte abundance (cubed five-level scale) and total phytoplankton biomass by lake area and sampling period. Complete regression results are in Supplementary Table S7.

middle of the lake, the floating-leaved and free-floating macrophytes had a positive relationship with total phytoplankton biomass in November.

Cyanobacteria biomass was negatively correlated with total and submerged macrophyte abundance in the northern lake area in February (Supplementary Table S7).

## 4. Discussion

In contrast to earlier water chemistry data (Akaishi et al., 2006), we found relatively low nutrient concentrations in Inlay Lake. However, data from the tributaries show periodically very high nutrient input, indicating potentially eutrophic conditions. In addition, studies of benthic macroinvertebrates in the tributaries in November 2018 showed that communities in some places were dominated by organisms having high tolerances to low oxygen, which may also indicate high nutrient inputs at least in some parts of the year (Eriksen et al., 2021). The contradicting results between our survey and earlier data indicate large temporal and spatial variations in nutrient concentrations in the lake. In addition, a large amount of nutrients is bound in the rich macrophyte vegetation (Mjelde and Faafeng, 1997; Schneider et al., 2014; Van Donk et al., 1993).

The species richness of hydrophytes in Inlay Lake seems high compared to other tropical lakes (e.g., Dalu et al., 2012; Dong et al., 2014; Lacoul and Freedman, 2006; Ondiba et al., 2018; Saluja and Garg, 2017), and higher than in other lakes in Myanmar (pers. obs.). However, the species richness is only medium-high compared to the species richness in lakes of similar size and type in low-altitude temperate areas in Europe (e.g., Rørslett, 1991; Viana et al., 2014). The high tropical diversity of freshwater flora (e.g., Chambers et al., 2008) typically belongs to the wetland flora and helophytes (which were not included in our study), and not the hydrophyte flora. In addition, the massive helophyte-covered littoral zone and eutrophic water prevent growth of more pollution-sensitive submerged species in Inlay Lake.

The high aquatic macrophyte diversity and species richness is mainly due to submerged species, which constitute 53 % of the total richness. Most of the submerged species, e.g., the dominant *Nechamandra alternifolia, Potamogeton lucens*, *Ceratophyllum demersum, Myriophyllum verticillatum* and *Najas indica* are bicarbonate users (e.g., Madsen and Sandjensen, 1991), and their abundances reflect and depend on the high alkalinity (calcium concentration) in the lake (e.g., Vestergaard and Sand-Jensen, 2000). In addition, most of the submerged species in Inlay Lake tolerate high nutrient conditions and turbid waters, due to low-light tolerance, canopy forming ability, etc. (Mjelde and Faafeng,

1997; Sand-Jensen and Madsen, 1991; Tóth and Herodek, 2011). Because of their elongated and canopy-forming growth form (Frodge et al., 1990), these species occupy a large part of the water column in shallow areas in Inlay Lake. The submerged species have different flowering and fruiting periods (Panda et al., 2016) and show some seasonality in their abundance. So, due to high species richness with different functional traits and phenology, the lake maintains a continuous submerged macrophyte community throughout the year in most parts of the lake, especially in the northern and southern areas.

The dominating free-floating *Eichhornia crassipes* is an invasive South American species and was introduced to the lake as an ornamental plant probably during the early 1900s (Su and Jassby, 2000). It is today considered as the most harmful aquatic weed throughout the tropics and subtropics (Cooke et al., 2005; Gopal, 1990). The abundance of this species is low in Inlay Lake and it may be nutrient limited by submerged species and constrained by wind erosion and heavy boat traffic on the lake. *E. crassipes* is also used as a fertilizer on the floating gardens which can affect its abundance. Several of the floating-leaved species are used for ornamental purpose (*Nymphaea*) and for weaving products (mainly *Nelumbo*), which may be the reason for their very low abundance in the lake.

Reduced abundance of free-floating species in the middle lake areas from November (after rainy period) to February/March (after dry period) may be due to decreased water depth and hence increased competition with submerged species. No significant differences between sampling periods for total macrophytes or submerged macrophytes indicate stability throughout the year independent of dry or wet period. This in contrast to a eutrophic Myanmar lowland reservoir (Moeyingyi) where both richness and abundance of macrophytes were markedly reduced in the dry period (pers. obs., see also Mjelde and Ballot, 2016). No significant associations between macrophytes and physical and chemical variables indicates that the water quality in all areas is suitable for submerged macrophytes, which is important for lake resilience, and hence, for lake management. The significant associations between macrophytes and spatial factors (latitude, i.e. lake area, and depth) probably reflect the lower abundance of submerged macrophytes in the deeper central area, indicating a maximum growing depth of submerged species at approx. 3 m depth. In addition, the spatial factors probably reflect the differences in functional traits and habitat preferences, e.g., *Nechamandra alternifolia* seems to prefer the northern area while *Chara zeylanica* had highest abundance in the southern part.

As expected, we found significant associations between phytoplankton and water quality factors. However, the general low phytoplankton biomasses and the negative relationship between phytoplankton and submerged macrophytes, especially in November, most probably indicate competition with the submerged macrophytes. The higher standing crop typically allows macrophytes to outcompete and control microscopic algae development despite the latter's higher uptake rates (e.g., Körner and Nicklisch, 2002; Pelton et al., 1998). The higher biomass of some phytoplankton groups observed close to the floating gardens (longitude) can indicate higher nutrient and/or organic matter concentrations in those areas (Novarino, 2011; Rosén, 1981; Wolowski, 2011).

Although we could not quantify the cyanobacterial–total phytoplankton relationship due to overall low biomass values and a high datapoint scatter, our observations suggest that cyanobacteria biomass in Inlay Lake may be directly related to total phytoplankton biomass as it has been observed in subtropical (Canfield et al., 1989) and temperate lakes (Downing et al., 2001). Among the most common cyanobacteria species in Inlay Lake there were four toxin-producing strains of *Mycrocystis*, strongly suggesting that Inlay Lake could experience potentially harmful cyanobacterial blooms should in-lake nutrient concentrations increase to a full-blown eutrophic state. However, our data suggest that total phytoplankton and cyanobacterial biomass were inhibited by total and submerged macrophytes in the heavily vegetated northern lake area, in agreement with findings from subtropical and temperate lakes (e.g., Timms and Moss, 1984; Canfield et al., 1989; Scheffer, 1998; Downing et al., 2001). Such relationships suggest that cyanobacteria might become dominant in Inlay Lake if nutrient concentrations increase and/or loss of submerged macrophyte abundance occur in the future, as it happens in temperate lakes (e.g., Timms and Moss, 1984). Cyanobacterial dominance may be further exacerbated by global warming (e.g., Newcombe et al., 2012).

The dominating submerged species in Inlay Lake may be capable of removing and storing large quantities of nutrients from the water by foliar and root uptake, as it was observed from temperate lakes (Pelton et al., 1998; Phillips et al., 1978; Van Donk et al., 1993). Several studies demonstrate how macrophytes affect the nutrient concentrations in the water and suppress phytoplankton biomasses (e.g., Hilt and Lombardo, 2010; Scheffer et al., 1993). Based on the physical, chemical, macrophyte, and phytoplankton patterns that we have observed in Inlay Lake, we suspect a co-involvement of competitive nutrient uptake by macrophytes behind the very low phytoplankton biomasses also in Inlay Lake.

The middle and deepest part of the lake, around 3 m depth in the rainy season, has low abundance of both submerged macrophytes and the free-floating species *E. crassipes*. The relatively low macrophyte abundance in relation to water volume might prevent the macrophytes from exerting their control on phytoplankton growth. This is supported by the relatively high abundance of cyanobacteria in the middle lake area, as cyanobacteria are typically more susceptible to macrophyte allelopathy than other phytoplankton groups (Gross et al., 2003; Jasser, 1995; Lombardo et al., 2013). Therefore, we suggest that, in addition to nutrient competition, allelopathic compounds released from *Myriophyllum verticillatum*, *Ceratophyllum demersum* and *Chara* species (Gross et al., 2003, 2007; Hilt et al., 2006) may be important in inhibiting the growth of cyanobacteria. *Nechamandra alternifolia*, which dominates the submerged vegetation in the northern area, also might inhibit phytoplankton growth in general and especially cyanobacteria. The leaves in this species possess large secretory cells (Cook and Lüönd, 1982), which make the whole plant slippery (pers. obs.). The secretion from the cells may have an allelopathic effect on phytoplankton, however no studies about this seem to exist, and our hypothesis remains untested. Toxic compounds from other species, e.g., *E. crassipes* (Gross, 2003; Sharma et al., 1996), may also affect phytoplankton in Inlay Lake.

Despite the gaps in the knowledge of the Inlay Lake ecosystem, our study indicates that the high abundance of submerged macrophytes play an important role for maintaining a clear water state in Inlay Lake. In addition, the high number of submerged species with different seasonal strategies, functional traits, and phenology allows high submerged abundance in different areas and throughout the year, and may contribute to maintaining the resilience and stability of the ecosystem, as suggested earlier (Moss, 1998; Scheffer, 1998; Sayer et al., 2010; Liu et al., 2020). Fish, invertebrates, zooplankton and periphyton algae certainly play a role in the lake ecosystem stabilization, however, no comprehensive studies on these groups in Inlay Lake exist, and their importance in Inlay Lake remains unknown. The shallowness of the lake, which enables the submerged vegetation to cover a large bottom area also during periods with more turbid water, is a prerequisite for a macrophyte-dominated clear water state.

No comprehensive management plan for decreasing nutrient loads to the lake has been established. Considering the high catchment:lake surface area and the deforestation and hotel plans in the catchment area, which most probably will increase sediment and nutrient loads (Cooke et al., 2005), Inlay Lake may be close to or within the nutrient level range where alternative states can exist (Phillips et al., 2016). The richness and abundance of submerged macrophytes has been and is still an important characteristic of Inlay Lake. However, continued or increased nutrient load can result in decreased submerged species abundance and richness which makes the lake more vulnerable to cyanobacteria blooms (Cooke et al., 2005; Downing et al., 2001).

In temperate lakes, species richness of submerged and floating-leaved species seems to decrease when winter nitrate concentrations –

a proxy for nitrate loading – rise above 1–2 mg $NO_3$-N $L^{-1}$ (Barker et al., 2008; James et al., 2005). We do not know if these values also apply to tropical lakes, but one should be aware that nutrient concentrations in Inlay Lake, at least in some areas of the lake, may at times be close to such thresholds.

The use of lake macrophytes as mulch and fertilizers in the floating gardens is increasingly replacing the use of chemical fertilizers. This means that large amounts of lake macrophytes are harvested (Michalon, 2014). During our study period, we did not see any reduction in macrophyte biomass due to the harvest of submerged macrophytes, however no study on this aspect were conducted. The use of macrophytes may be minor compared to their abundance and regrowth. However, increasing this practice may endanger the natural balance between aquatic macrophytes and phytoplankton in Inlay Lake which can trigger a shift from the current macrophyte dominance towards a phytoplankton dominated lake (Scheffer et al., 2001).

We suggest that a year-long growing period and no dieback in autumn favour submerged macrophytes, which can thus maintain a heavy presence throughout the year. Since macrophytes do not have to compete with phytoplankton every spring as in temperate lakes, we suppose that their presence may be more stable than in temperate systems at similar nutrient level. Conversely, once a turbid phytoplankton-dominated state is established, it may be more stable in tropical areas compared to temperate areas. Stability in Inlay Lake may also be influenced by its extreme shallowness. The Osgood Index (OI) for Inlay Lake is 0.2–0.3 which is way below the OI≈6 threshold for polymixis (Mataraza and Cooke, 1997), strongly suggesting that Inlay Lake is particularly prone to storm-related mixing that could resuspend nutrients making them available for phytoplankton growth. Conversely, the heavy presence of submerged plants may reduce sediment resuspension despite the frequent polymixis (e.g., James and Barko, 1994; Vermaat et al., 2000).

To assess the consequences of increasing pressures, mainly nutrient load (from growing human population, agricultural areas, tourism and hotel establishments) and sedimentation load (due to deforestation) it is important to increase the knowledge about the Inlay Lake ecosystem and its catchment area. In addition, continuation of management activities in the lake (MOECAF, 2014, 2015) followed by further monitoring are needed. Aquatic macrophytes and phytoplankton respond to nutrients which enter the lake only periodically. Therefore, monitoring of these groups is desirable to assist in the management of Inlay Lake, as well as for other Myanmar lakes. The high alkalinity water in Inlay Lake is a premise for the high diversity of submerged species. We expect that increased eutrophication in low alkalinity lakes, e.g., more typical lakes in the lowland dry area in Myanmar (pers. obs.), may favour free-floating macrophyte species, which seem to have a weaker structuring role than submerged vegetation (Meerhoff et al., 2003).

Myanmar currently lacks systems for evaluating the ecological status of its surface waters, though there have been attempts to adopt Integrated Water Resources Management (IWRM) for this purpose through a number of recent government initiatives, including the Myanmar National Water Policy (NWP) and the Myanmar National Water Framework Directive (NWRC, 2014), inspired by the EU Water Framework Directive (EC, 2000; WFD, 2000). However, the success of the Myanmar National Water Framework depends on the knowledge about the different freshwater ecosystems in the country, with different biodiversity and stability drivers. The examination and maintenance of biodiversity and water quality in other lakes and reservoirs in Myanmar should be given more attention. Knowledge about aquatic macrophyte growth and understanding the mechanisms behind community shifts is of considerable importance for the management and the establishment of good ecological status for Myanmar's lakes and reservoirs.

## CRediT authorship contribution statement

**Thida Swe:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Paola Lombardo:** Formal analysis, Visualization, Writing - review & editing. **Andreas Ballot:** Conceptualization, Methodology, Visualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Supervision. **Jan-Erik Thrane:** Formal analysis, Visualization. **James Sample:** Formal analysis, Visualization. **Tor Erik Eriksen:** Writing - review & editing. **Marit Mjelde:** Conceptualization, Methodology, Visualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Supervision, Project administration.

## Declaration of Competing Interest

The authors report no conflict of interest

## Acknowledgements

This research was supported by the Project "Integrated Water Resources Management (IWRM) – Institutional Building and Training in Myanmar", a collaboration between Department of Forestry (FD), Ministry of Natural Resources and Environmental Conservation (MONREC) of Myanmar and the Norwegian Institute for Water Research (NIVA), funded by the Norwegian Ministry of Foreign Affairs and the Royal Norwegian Embassy in Myanmar. We also want to thank Mr. Zaw Myo Aung and Mr. Moe Myint Thu from Forest Department, the Ministry of Natural Resources and Environmental Conservation, who did the depth measurements in Inlay Lake. We are also grateful for the useful comments provided by the editor and two anonymous referees.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.limno.2021.125910.

## References

Akaishi, F., Satake, M., Otaki, M., Tominaga, N., 2006. Surface water quality and information about the environment surrounding Inle Lake in Myanmar. Limnology 7, 57–62. https://doi.org/10.1007/s10201-006-0165-1.

Allen, D.J., Smith, K.G., Darwall, W.R.T., 2012. The Status and Distribution of Freshwater Biodiversity in Indo-Burma. IUCN, Cambridge, UK and Gland, Switzerland x+158pp+4pp cover.

Aung, L., Zin, E., Theingi, P., Elvera, N., Aung, P., Han, T., Oo, Y., Skaland, R., 2017. Myanmar climate report, Norwegian Meteorological Institute MET report 9/2017. ISSN 2387-4201. Climate 105, p. 105.

Ballot, A., Mjelde, M., Swe, T., 2018. Integrated Water resources management in Myanmar. Assessing Ecological Status in Inlay Lake. NIVA-report 7301-2018.

Ballot, A., Swe, T., Mjelde, M., Cerasino, L., Hostyeva, V., Miles, C.O., 2020. Cylindrospermopsin- and deoxycylindrospermopsin-producing *Raphidiopsis raciborskii* and microcystin-producing *Microcystis* spp. in Meiktila Lake, Myanmar. Toxins 12, 232. https://doi.org/10.3390/toxins12040232.

Barker, T., Hatton, K., O'Connor, M., Connor, L., Moss, B., 2008. Effects of nitrate load on submerged plant biomass and species richness: results of a mesocosm experiment. Fundam. Appl. Limnol. 173, 89–100. https://doi.org/10.1127/1863-9135/2008/0173-0089.

Büdel, B., Gärtner, G., Krienitz, L., Schagerl, M., 2015. Süsswasserflora von Mitteleuropa, Freshwater Flora of Central Europe, Volume 1-19. Spektrum Akademischer Verlag Heidelberg.

Burks, R.L., Lodge, D.M., Jeppesen, E., Lauridsen, T.L., 2002. Diel horizontal migration of zooplankton: costs and benefits of inhabiting the littoral. Freshw. Biol. 47, 343–365. https://doi.org/10.1046/j.1365-2427.2002.00824.x.

Canfield Jr., D.E., Phlips, E., Duarte, C.M., 1989. Factors influencing the abundance of blue-green algae in Florida lakes. Can. J. Fish. Aquat. Sci. 46, 1232–1237.

Carpenter, S.R., Lodge, D.M., 1986. Effects of submerged macrophytes on ecosystem processes. Aquat. Bot. 26, 341–370. https://doi.org/10.1016/0304-3770(86)90031-8.

Chambers, P.A., Lacoul, P., Murphy, K.J., Thomaz, S.M., 2008. Global diversity of aquatic macrophytes in freshwater. Hydrobiologia 595, 9–26. https://doi.org/10.1007/s10750-007-9154-6.

Colwell, R.K., 2013. EstimateS: Statistical Estimation of Species Richness and Shared Species from Samples. User's Guide and application published at: http://purl.oclc.org/estimates; last accessed 4 Dec 2020.

Cook, C.D., 1996. Aquatic and Wetland Plants of India: a Reference Book and Identification Manual for the Vascular Plants Found in Permanent or Seasonal Fresh Water in the Subcontinent of India South of the Himalayas. Oxford University Press.

Cook, C.D.K., Lüönd, R., 1982. A revision of the genus *Nechamandra* (*Hydrocharitaceae*). Aquat. Bot. 13, 505–513. https://doi.org/10.1016/0304-3770(82)90075-4.
Cooke, G.D., Welch, E.B., Peterson, S., Nichols, S.A., 2005. Restoration and Management of Lakes and Reservoirs, 3rd ed. CRC – Taylor & Francis, Boca Raton, FL, USA, p. 612. ISBN: 9781566706254.
Dalu, T., Nhiwatiwa, T., Clegg, B., 2012. Aquatic macrophytes in a tropical African reservoir: diversity, communities and the impact of reservoir-level fluctuations. Trans. R. Soc. S. Afr. 67, 117–125.
Dong, B.L., Qin, B.Q., Gao, G., Cai, X.L., 2014. Submerged macrophyte communities and the controlling factors in large, shallow Lake Taihu (China): sediment distribution and water depth. J. Great Lakes Res. 40, 646–655. https://doi.org/10.1016/j.jglr.2014.04.007.
Downing, J.A., Watson, S.B., McCauley, E., 2001. Predicting Cyanobacteria dominance in lakes. Can. J. Fish. Aquat. Sci. 58, 1905–1908. https://doi.org/10.1139/cjfas-58-10-1905.
EC, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Off. J. Eur. Commun. L327, 72.
Eriksen, T.E., Friberg, N., Brittain, J.E., Soli, G., Ballot, A., Arstein-Eriksen, E., Blakseth, T.A., Braaten, H.F.V., 2021. Ecological condition, biodiversity and major environmental challenges in a tropical river network in the Bago District in South-central Myanmar: first insights to the unknown. Limnologica 86. https://doi.org/10.1016/j.limno.2020.125835.
Everall, N.C., Johnson, M.F., Wood, P., Mattingley, L., 2018. Sensitivity of the early life stages of a mayfly to fine sediment and orthophosphate levels. Environ. Pollut. 237, 792–802. https://doi.org/10.1016/j.envpol.2017.10.131.
Frodge, J.D., Thomas, G.L., Pauley, G.B., 1990. Effects of canopy formation by floating and submergent aquatic macrophytes on the water quality of two shallow Pacific Northwest lakes. Aquat. Bot. 38, 231–248. https://doi.org/10.1016/0304-3770(90)90008-9.
Furuichi, T., 2008. Catchment Processes and Sedimentation in Lake Inle, Southern Shan State, Myanmar, Report to Forest Department, Myanmar. Centre for Resource and Environmental Studies, the Australian National University, Canberra, p. 69. Final Report, March 2008.
Gopal, B., 1990. Aquatic weed problems and management in Asia, 1990. In: Pieterse, A. H., Murphy, K.J. (Eds.), Aquatic Weeds. University Press, Oxford, p. 593.
Gross, E.M., 2003. Allelopathy of aquatic autotrophs. Crit. Rev. Plant Sci. 22, 313–339.
Gross, E.M., Erhard, D., Ivanyi, E., 2003. Allelopathic activity of *Ceratophyllum demersum* L. and *Najas marina* ssp *intermedia* (Wolfgang) Casper. Hydrobiologia 506, 583–589. https://doi.org/10.1023/b:Hydr.0000008539.32622.91.
Gross, E.M., Hilt, S., Lombardo, P., Mulderij, G., 2007. Searching for allelopathic effects of submerged macrophytes on phytoplankton - state of the art and open questions. Hydrobiologia 584, 77–88. https://doi.org/10.1007/s10750-007-0591-z.
Hellsten, S., Willby, N., Ecke, F., Mjelde, M., Phillips, G., Tierney, D., Poikane, S., 2014. In: Poikane, S. (Ed.), Northern Lake Macrophyte Ecological Assessment Methods. Water Framework Directive Intercalibration Technical Report. Joint Research Centre Technical Reports. JRC, p. 104. https://doi.org/10.2788/75735. Report EUR 26513 EN.
Hilt, S., Lombardo, P., 2010. Effects of macrophytes on phytoplankton: nutrient uptake versus allelopathy. Verh. Int. Ver. Theor. Angew. Limnol. 30, 1317–1320.
Hilt, S., Ghobrial, M.G.N., Gross, E.M., 2006. In situ allelopathic potential of *Myriophyllum verticillatum* (Haloragaceae) against selected phytoplankton species. J. Phycol. 42, 1189–1198. https://doi.org/10.1111/j.1529-8817.2006.00286.x.
Htwe, T.N., 2015. Changes of Traditional Farming Systems and Their Effects on Land Degradation and Socio-economic Conditions in the Inle Lake Region, Myanmar. Dissertation submitted to the Faculty of Organic Agricultural Sciences/ Department of Organic Plant Production and Agroecosystems Research in the Tropics and Subtropics. University of Kassel, Witzenhausen, Germany.
Huber-Pestalozzi, G., 1969. Das Phytoplankton des Süßwassers: Systematik und Biologie. In: Euglenophyceen, 4. Schweizerbart'sche Verlagsbuchhandlung (Nägele u. Obermiller), Berlin, Stuttgart.
Ito, Y., Barfod, A.S., 2014. An updated checklist of aquatic plants of Myanmar and Thailand. Biodivers. Data J. e1019. https://doi.org/10.3897/BDJ.2.e1019.
James, W.F., Barko, J.W., 1994. Macrophyte influences on sediment resuspension and export in a shallow impoundment. Lake Reserv. Manag. 19, 95–102. https://doi.org/10.1080/07438149409354180.
James, C., Fisher, J., Russell, V., Collings, S., Moss, B., 2005. Nitrate availability and hydrophyte species richness in shallow lakes. Freshw. Biol. 50, 1049–1063. https://doi.org/10.1111/j.1365-2427.2005.01375.x.
Jasser, I., 1995. The influence of macrophytes on a phytoplankton community in experimental conditions. Hydrobiologia 306, 21–32. https://doi.org/10.1007/bf00007855.
Jeppesen, E., Søndergaard, M., Søndergaard, M., Christoffersen, K., 1998. The Structuring Role of Submerged Macrophytes in Lakes. Springer Verlag, New York, USA.
Jeppesen, E., Søndergaard, M., Mazzeo, N., Meerhoff, M., Branco, C.C., Huszar, V., Scasso, F., Reddy, M.V., 2005. Lake restoration and biomanipulation in temperate Lakes: relevance for subtropical and tropical Lakes. In: Reddy, M.V. (Ed.), Restoration and Management of Tropical Eutrophic Lakes. Science Publishers, Enfield, N.H, pp. 341–359.
Kolada, A., Hellsten, S., Søndergaard, M., Mjelde, M., Dudley, B., van Geest, G., Goldsmith, B., Davidson, T., Bennion, H., Nõges, P., 2012. WISER Deliverable D3.2-3: Report on the Most Suitable Lake Macrophyte Based Assessment Methods for Impacts of Eutrophication and Water Level Fluctuations. WISER project (Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery), p. 114. Technical Report, February 2012.
Komárek, J., Anagnostidis, K., 1999. In: Ettl, H., Gärtner, G., Heynig, H., Mollenhauer, D. (Eds.), Cyanoprokaryota 1. Chroococcales. – 19/1. Süsswasserflora von Mitteleuropa Gustav Fischer, Jena-Stuttgart-Lübeck-Ulm, p. 548.
Komárek, J., Fott, B., 1983. Chlorophyceae (Grünalgen), O. Chlorococcales. In: Huber Pestalozzi, G. (Ed.), Die Binnengewässer. Das Phytoplankton des Süßwassers, Systematik und Biologie. Hälfte. E. Schweizerbart' sche Verlagsbuchhandlung (Nägele und Obermiller), Stuttgart, 7 Teil. 1.
Körner, S., Nicklisch, A., 2002. Allelopathic growth inhibition of selected phytoplankton species by submerged macrophytes. J. Phycol. 38, 862–871. https://doi.org/10.1046/j.1529-8817.2002.t01-1-02001.x.
Kotai, J., 1972. Instructions for Preparation of Modified Nutrient Solution Z8 for Algae. - Publication B-11/69. Norwegian Institute for Water Research, Blindern, Oslo, Norway.
Kullander, S.O., 2012. Box 3.2. Inlé lake, Allen DJ, Smith KG, Darwall WRT, compilers. The Status and Distribution of Freshwater Biodiversity in Indo-burma. IUCN, Cambridge, UK and Gland, Switzerland.
Lacoul, P., Freedman, B., 2006. Relationships between aquatic plants and environmental factors along a steep Himalayan altitudinal gradient. Aquat. Bot. 84, 3–16. https://doi.org/10.1016/j.aquabot.2005.06.011.
Lansdown, R.V., 2012. Box 7.4 Inlé Lake, Myanmar, Allen DJ, Smith KG, Darwall WRT, compilers. The Status and Distribution of Freshwater Biodiversity in Indo-burma. IUCN, Cambridge, UK and Gland, Switzerland.
La-Ongsri, W., 2008. Taxonomic Revision and Ethnobotany of the Families Nelumbonaceae and Nymphaeaceae in Thailand. Chiang Mai University, Thailand.
Lewis, W.M.J., 2000. Basis for the protection and management of tropical lakes. Lakes Reserv. Res. Manag. 5, 35–48.
Liu, H., Zhou, W., Li, X.W., Chu, Q.S., Tang, N., Shu, B.Z., Liu, G.H., Xing, W., 2020. How many submerged macrophyte species are needed to improve water clarity and quality in Yangtze floodplain lakes? Sci. Total Environ. 724, 10. https://doi.org/10.1016/j.scitotenv.2020.138267.
Lombardo, P., Mjelde, M., Kallqvist, T., Brettum, P., 2013. Seasonal and scale-dependent variability in nutrient- and allelopathy-mediated macrophyte-phytoplankton interactions. Knowl. Manag. Aquat. Ecosyst. 409, 10. https://doi.org/10.1051/kmae/2013055.
Lwin, Z., Sharma, M., 2012. Environmental management of the Inle lake in Myanmar. Hydro Nepal J. Water Energy Environ. 11, 57–60.
Lyche-Solheim, A., Feld, C.K., Birk, S., Phillips, G., Carvalho, L., Morabito, G., Mischke, U., Willby, N., Sondergaard, M., Hellsten, S., Kolada, A., Mjelde, M., Bohmer, J., Miler, O., Pusch, M.T., Argillier, C., Jeppesen, E., Lauridsen, T.L., Poikane, S., 2013. Ecological status assessment of European lakes: a comparison of metrics for phytoplankton, macrophytes, benthic invertebrates and fish. Hydrobiologia 704, 57–74. https://doi.org/10.1007/s10750-012-1436-y.
Madsen, T.V., Sandjensen, K., 1991. Photosynthetic carbon assimilation in aquatic macrophytes. Aquat. Bot. 41, 5–40. https://doi.org/10.1016/0304-3770(91)90037-6.
Mataraza, L.K., Cooke, G.D., 1997. A test of a morphometric index to predict vertical phosphorus transport in lakes. Lake Reserv. Manage. 13, 328–337.
Meerhoff, M., Mazzeo, N., Moss, B., Rodriguez-Gallego, L., 2003. The structuring role of free-floating versus submerged plants in a subtropical shallow lake. Aquat. Microb. Ecol. 37, 377–391. https://doi.org/10.1023/B:AECO.0000007041.57843.0b.
Melzer, A., 1999. Aquatic macrophytes as tools for lake management. Hydrobiologia 395, 181–190. https://doi.org/10.1023/a:1017001703033.
Michalon, M., 2014. The Gardener and the Fisherman in Globalization: the Inle Lake (Myanmar), a Region under Transition. Master Thesis. University Lyon 2 Lumière.
Michalon, M., Gunnell, Y., Lejot, J., Mialhe, F., Aung, T., 2019. Accelerated degradation of Lake Inle (Myanmar): a baseline study for environmentalists and developers. Land Degrad. Dev. 30, 928–941.
Mjelde, M., Ballot, A., 2016. Conservation of biodiversity and improved management of protected areas in Myanmar. Aquatic Macrophytes and Phytoplankton in Moeyingyi Reservoir. November 2015. NIVA-report SNO 6975-2016.
Mjelde, M., Faafeng, B.A., 1997. *Ceratophyllum demersum* hampers phytoplankton development in some small Norwegian lakes over a wide range of phosphorus concentrations and geographical latitude. Freshw. Biol. 37, 355–365. https://doi.org/10.1046/j.1365-2427.1997.00159.x.
Mjelde, M., Ballot, A., Swe, T., 2018. Conservation of biodiversity and improved management of protected areas in Myanmar. Aquatic Macrophytes and Phytoplankton in Indawgyi Lake, p. 41. NIVA-report 7253-2018.
Mjelde, M., Swe, T., Langangen, A., Ballot, A., 2021. A contribution to the knowledge of charophytes in Myanmar; morphological and genetic identification and ecology notes. Bot. Lett. 168, 102–109. https://doi.org/10.1080/23818107.2020.1847189.
MOECAF, 2014. 5 Years Action Plan for the Sustainability of Inle Lake and Environmental Conservation in Myanmar. Ministry of Environmental Conservation and Forestry, Forest Department.
MOECAF, 2015. Inle Lake Conservation 5-year Action Plan (2015-2016 to 2019-2020). Prepared by: Ministry of Environmental Conservation and Forestry. Union of Myanmar, Nay Pyi Taw. May 2015.
Moss, B., 1998. Ecology of Fresh Waters—Man and Medium, Past to Future, 3rd ed. Blackwell Science, Oxford, UK.
Nair, D.M.N., 1960. Botanical Survey of the Southern Shan States: With a Note on the Vegetation of the Inle Lake. Burma Research Society. University of Rangoon, Burma, p. 173.
Nesheim, I., Wathne, B.M., Ni, B., Tun, Z.L., 2016. Myanmar: pilot introducing the national water framework directive. Water Solut. 1, 18–28.
Newcombe, G., Chorus, I., Falconer, I.R., Lin, T.-F., 2012. Cyanobacteria: impacts of climate change on occurrence, toxicity and water quality management. Water Res. 46, 1347–1348.

Novarino, G., 2011. Phylum cryptophyta (cryptopmonads). In: John, D.M., Whitton, B. A., Brook, A.J. (Eds.), Freshwater Algal Flora of the British Isles. Cambridge University Press.
NWRC, 2014. Myanmar National Water Policy. Mr. Htun Lwin Oo, Secretary of the National Water Resources Committee (NWRC), Ntional Water Resources Committee, Ministry of Transport, Building (5), Nay Pyi, Taw, Myanmar.
Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2020. Vegan: Community Ecology Package Version 2.5-7 accessed on 15 Dec. 2020. https://rdrr.io/cran/vegan/.(last.
Ondiba, R., Omondi, R., Musa, S., Nyakeya, K., Oyoo-Okoth, E., Abwao, J., 2018. Environmental constraints on macrophyte distribution and diversity in a tropical endorheic freshwater lake (Lake Baringo, Kenya). Int. J. Fish. Aquat. Stud. 6, 251–259.
OpenStreetMap, 2018. Data file retrieved from www.openstreetmap.org. Accessed on 12 May 2018.
Osgood, R.A., 1988. Lake mixis and internal phosphorous dynamics. Arch. Hydrobiol. 113, 629–638.
Panda, S., Sahoo, H., Subudhi, H., Sahu, A., Mishra, P., 2016. Ecofloristic diversity of Ansupa Lake, Odisha (India) with special reference to aquatic macrophytes. J. Biodiv. Photon. 116, 537–552.
Pelton, D.K., Levine, S.N., Braner, M., 1998. Measurements of phosphorus uptake by macrophytes and epiphytes from the LaPlatte river (VT) using $^{32}$P in stream microcosms. Freshw. Biol. 39, 285–299. https://doi.org/10.1046/j.1365-2427.1998.00281.x.
Phillips, G.L., Eminson, D., Moss, B., 1978. A mechanism to account for macrophyte decline in progressively eutrophicated freshwaters. Aquat. Bot. 4, 103–126. https://doi.org/10.1016/0304-3770(78)90012-8.
Phillips, G., Willby, N., Moss, B., 2016. Submerged macrophyte decline in shallow lakes: what have we learnt in the last forty years? Aquat. Bot. 135, 37–45. https://doi.org/10.1016/j.aquabot.2016.04.004.
Pieterse, A.H., Murphy, K.J., 1990. Aquatic Weeds. University Press, Oxford.
Quinn, G.P., Keough, M.J., 2002. Experimental Design and Data Analysis for Biologists. Cambridge university press, Cambridge, UK, p. 537.
RCoreTeam, 2020. R: a Language and Environment for Statistical Computing. URL. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Rørslett, B., 1991. Principal determinants of aquatic macrophyte richness in northern European lakes. Aquat. Bot. 39, 173–193. https://doi.org/10.1016/0304-3770(91)90031-y.
Rosén, G., 1981. Phytoplankton indicators and their relations to certain chemical and physical factors. Limnologica 13, 263–296.
Saluja, R., Garg, J.K., 2017. Macrophyte species composition and structure along littoral region in relation to limnological variables of a tropical wetland ecosystem. Chem. Ecol. 33, 499–515. https://doi.org/10.1080/02757540.2017.1328502.
Sand-Jensen, K., Madsen, T.V., 1991. Minimum light requirements of submerged freshwater macrophytes in laboratory growth experiments. J. Ecol. 79, 749–764. https://doi.org/10.2307/2260665.
Sayer, C.D., Davidson, T.A., Jones, J.I., 2010. Seasonal dynamics of macrophytes and phytoplankton in shallow lakes: a eutrophication-driven pathway from plants to plankton? Freshwat. Biol. 55, 500–513. https://doi.org/10.1111/j.1365-2427.2009.02365.x.
Scheffer, M., 1998. Ecology of Shallow Lakes. Kluwer Academic Publishers, Dordrecht, NL. Reprinted with corrections 2004.
Scheffer, M., Hosper, S.H., Meijer, M.L., Moss, B., Jeppesen, E., 1993. Alternative equilibria in shallow lakes. Trends Ecol. Evol. 8, 275–279. https://doi.org/10.1016/0169-5347(93)90254-m.
Scheffer, M., Carpenter, S., Foley, J.A., Folke, C., Walker, B., 2001. Catastrophic shifts in ecosystems. Nature 413, 591–596. https://doi.org/10.1038/35098000.
Schneider, S.C., Cara, M., Eriksen, T.E., Goreska, B.B., Imeri, A., Kupe, L., Lokoska, T., Patceva, S., Trajanovska, S., Trajanovski, S., Talevska, M., Sarafiloska, E.V., 2014. Eutrophication impacts littoral biota in Lake Ohrid while water phosphorus concentrations are low. Limnologica 44, 90–97. https://doi.org/10.1016/j.limno.2013.09.002.
Schneider, S.C., Sample, J.E., Moe, J.S., Petrin, Z., Meissner, T., Hering, D., 2018. Unravelling the effect of flow regime on macroinvertebrates and benthic algae in regulated versus unregulated streams. Ecohydrology 11, 15. https://doi.org/10.1002/eco.1996.
Sharma, A., Gupta, M.K., Singhal, P.K., 1996. Toxic effects of leachate of water hyacinth decay on the growth of *Scenedesmus obliquus* (chlorophyta). Water Res. 30, 2281–2286. https://doi.org/10.1016/0043-1354(96)00113-3.
Skuja, H., 1949. Zur Süsswasseralgenflora Burmas, 14. Nova Acta Regia Societatis Scientarium Upsaliensis Ser. IV, pp. 65–187.
Su, M., Jassby, A.D., 2000. Inle: a large Myanmar lake in transition. Lakes Reserv. Res. Manag. 5, 49–54.
Swe, T., Miles, C.O., Cerasino, L., Mjelde, M., Kleiven, S., Ballot, A., 2021. *Microcystis, Raphidiopsis raciborskii and Dolichospermum smithii*, toxin producing and non-toxigenic cyanobacteria in Yezin Dam, Myanmar. Limnologica 90, 125901. https://doi.org/10.1016/j.limno.2021.125901 (2021).
Timms, R.M., Moss, B., 1984. Prevention of growth of potentially dense phytoplankton populations by zooplankton grazing, in the presence of zooplanktivorous fish, in a freshwater wetland ecosystem. Limnol. Oceanogr. 29, 472–486. https://doi.org/10.4319/lo.1984.29.3.0472.
Tóth, V.R., Herodek, S., 2011. Seasonal shift of dominance in a submerged rooted macrophyte community of Lake Balaton. Ann. Limnol.-Int. J. Lim. 47, 141–150. https://doi.org/10.1051/limn/2011002.
Triest, L., 1988. A revision of the genus *Najas* L.(Najadaceae) in the Old World (Najadaceae). Koninklijke Academie voor Overzeese Wetenschappen. Klass voor Natuur-en Geneeskundige Wetenschappen. Verhandelingen in-8. Nieuwe Reeks, Boek 22, afl. I, Brussel, p. 172 + plates.
Utermöhl, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt. Int. Ver. Theor. Angew. Limnol. 9, 1–38.
Van Donk, E., Gulati, R.D., Iedema, A., Meulemans, J.T., 1993. Macrophyte-related shifts in the nitrogen and phosphorus contents of the different trophic levels in a biomanipulated shallow lake. Hydrobiologia 251, 19–26. https://doi.org/10.1007/bf00007160.
Vermaat, J.E., Santamaría, L., Roos, P.J., 2000. Water flow across and sediment trapping in submerged macrophyte beds of contrasting growth form. Arch. Hydrobiol. 148, 549–562. https://doi.org/10.1127/archiv-hydrobiol/148/2000/549.
Vestergaard, O., Sand-Jensen, K., 2000. Alkalinity and trophic state regulate aquatic plant distribution in Danish lakes. Aquat. Bot. 67, 85–107. https://doi.org/10.1016/s0304-3770(00)00086-3.
Viana, D.S., Santamaria, L., Schwenk, K., Manca, M., Hobaek, A., Mjelde, M., Preston, C. D., Gornall, R.J., Croft, J.M., King, R.A., Green, A.J., Figuerola, J., 2014. Environment and biogeography drive aquatic plant and cladoceran species richness across Europe. Freshw. Biol. 59, 2096–2106. https://doi.org/10.1111/fwb.12410.
WFD, 2000. The EU Water Framework Directive - Integrated River Basin Management for Europe. - Directive 2000/60/EC.
Wiegleb, G., 1990. A redescription of *Potamogeton distinctus* (Potamogetonaceae) including remarks on the taxonomic structure of the *P. nodus* group. Plant Syst. Evol. 169, 245–259. https://doi.org/10.1007/bf00937678.
Wiegleb, G., Kaplan, Z., 1998. An account of the species of *Potamogeton* L. (Potamogetonaceae). Folia Geobot. 33, 241–316. https://doi.org/10.1007/bf03216205.
Wolowski, K., 2011. Phylum Euglenophyta. In: John, D.M., Whitton, B.A., Brook, A.J. (Eds.), Freshwater Algal Flora of the British Isles. Cambridge University Press.
Wong, D.W., 2017. Interpolation: inverse-distance weighting. In: Richardson, D., Castree, N., Goodchild, M., Kobayashi, A., Liu, W., Marston, R. (Eds.), International Encyclopedia of Geography: People, the Earth, Environment and Technology. Wiley Online Library, pp. 1–7. https://doi.org/10.1002/9781118786352.wbieg0066.
Wood, R.D., Imahori, K., 1965. A Revision of the Characeae: Monograph of the Characeae. Part I & II. Verlag J. Cramer, Weinhein, Germany.
Zar, H., 2009. Biostatistical Analysis, 5th ed. Pearson/Prentice Hall, Upper Saddle River, NJ, USA.

Doctoral dissertation no. 153
**2023**

—

**The ecology of phytoplankton with focus on cyanobacteria and of aquatic macrophytes in selected lakes and reservoirs in Myanmar**

—

Thida Swe

—

ISBN 978-82-7206-741-9 (print)
ISBN 978-82-7206-742-6 (online)

usn.no