

# Deep Cyanobacteria Layers: An Overlooked Aspect of Managing Risks of Cyanobacteria

Kevin J. Erratt, Irena F. Creed,\* Erika C. Freeman, Charles G. Trick, Judy Westrick, Johnna A. Birbeck, L. Cynthia Watson, and Arthur Zastepa



Cite This: *Environ. Sci. Technol.* 2022, 56, 17902–17912



Read Online

ACCESS |

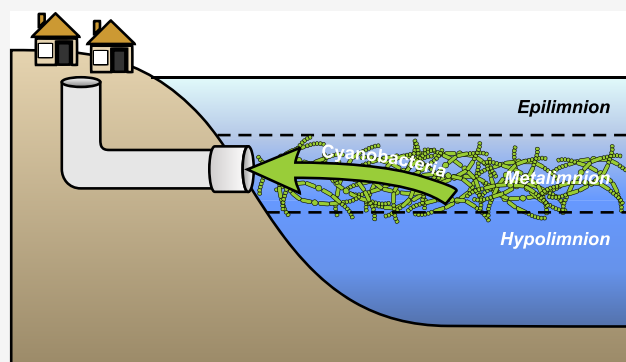
Metrics & More

Article Recommendations

Supporting Information

**ABSTRACT:** The risk of human exposure to cyanotoxins is partially influenced by the location of toxin-producing cyanobacteria in waterbodies. Cyanotoxin production can occur throughout the water column, with deep water production representing a potential public health concern, specifically for drinking water supplies. Deep cyanobacteria layers are often unreported, and it remains to be seen if lower incident rates reflect an uncommon phenomenon or a monitoring bias. Here, we examine Sunfish Lake, Ontario, Canada as a case study lake with a known deep cyanobacteria layer. Cyanotoxin and other bioactive metabolite screening revealed that the deep cyanobacteria layer was toxigenic [ $0.03 \mu\text{g L}^{-1}$  microcystins (max) and  $2.5 \mu\text{g L}^{-1}$  anabaenopeptins (max)]. The deep layer was predominantly composed of *Planktothrix isothrix* (exhibiting a lower cyanotoxin cell quota), with *Planktothrix rubescens* (exhibiting a higher cyanotoxin cell quota) found at background levels. The co-occurrence of multiple toxigenic *Planktothrix* species underscores the importance of routine surveillance for prompt identification leading to early intervention. For instance, microcystin concentrations in Sunfish Lake are currently below national drinking water thresholds, but shifting environmental conditions (e.g., in response to climate change or nutrient modification) could fashion an environment favoring *P. rubescens*, creating a scenario of greater cyanotoxin production. Future work should monitor the entire water column to help build predictive capacities for identifying waterbodies at elevated risk of developing deep cyanobacteria layers to safeguard drinking water supplies.

**KEYWORDS:** cyanobacteria, cyanotoxins, deep cyanobacteria layer, *Planktothrix*, water security



## 1. INTRODUCTION

Near-surface cyanobacteria proliferations have drawn considerable public attention due to their visibility.<sup>1,2</sup> However, not all cyanobacteria proliferations occur near the surface.<sup>3–5</sup> A prime example is the “deep cyanobacteria layer”. These are cyanobacteria communities located below the surface where sufficient light penetrates and the disturbance effects of wind mixing are meager. These deep-water proliferations of cyanobacteria are often unreported due to their inconspicuous nature.<sup>6,7</sup> Sometimes referred to as metalimnetic cyanobacteria layers,<sup>8,9</sup> metalimnetic blooms,<sup>10,11</sup> or deep chlorophyll maxima or layers (a general term used for all phylogenetic groups),<sup>12</sup> these deep-water abundances often occur within the relatively narrow metalimnetic region.<sup>13,14</sup> Unlike near-surface cyanobacteria proliferations, deep cyanobacteria layers seldom lead to public responses.<sup>15</sup> Yet deep cyanobacteria layers may consist of toxin-producing genera, posing risks to drinking water,<sup>13,16</sup> as drinking water withdrawal systems are often constructed at depths that may align with potential deep cyanobacteria layers.<sup>17,18</sup>

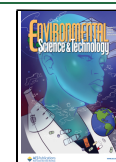
Deep cyanobacteria layers represent a ubiquitous concern in lakes worldwide.<sup>7,19</sup> However, research points to geographical biases in reporting, with only 18% of reported deep cyanobacteria layers in North America and 77% in Europe (Figure 1). Of the 136 articles identified in our literature survey, 43% arise from exploring two significant European waterbodies (e.g., Lake Zürich, Switzerland, and Lac du Bourget, France). The early 2000s signifies the onset of increasing scientific literature focused on deep cyanobacteria layers. Wood and colleagues’ literature survey on cyanobacteria occurrences between the years 2008 and 2018 shows approximately 150 publications on benthic cyanobacteria and 1100 publications on planktonic cyanobacteria.<sup>5</sup> Of those planktonic reports, we identified only 47 deep cyanobacteria

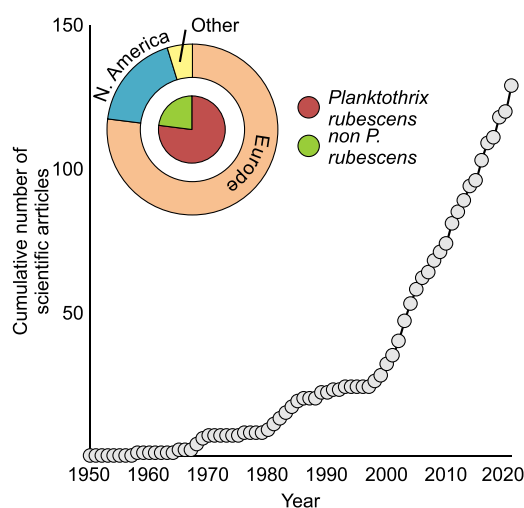
**Received:** September 22, 2022

**Revised:** November 8, 2022

**Accepted:** November 9, 2022

**Published:** November 22, 2022





**Figure 1.** Cumulative number of published scientific articles focused on deep cyanobacteria layers (gray circles), the proportion of literature from different continents, and the proportion of literature focused on *Planktothrix rubescens*. Data were extracted from the following scientific databases: Web of Science Core Collection, Scopus, Google Scholar, and ScienceDirect. The keywords used are presented in Table SI.1. Articles were reviewed to confirm evidence of a deep cyanobacteria layer. Articles that referenced a previously identified deep cyanobacteria layer were excluded from the tally.

layer publications over that decade (representing less than 5% of all planktonic cyanobacteria articles). Cyanobacteria research has become increasingly focused on opposing ends of lakes (i.e., the top and bottom). Notably absent is the “middle”, which leads to a biased assessment of water quality, which may in turn underestimate the risks of cyanobacteria to public health.

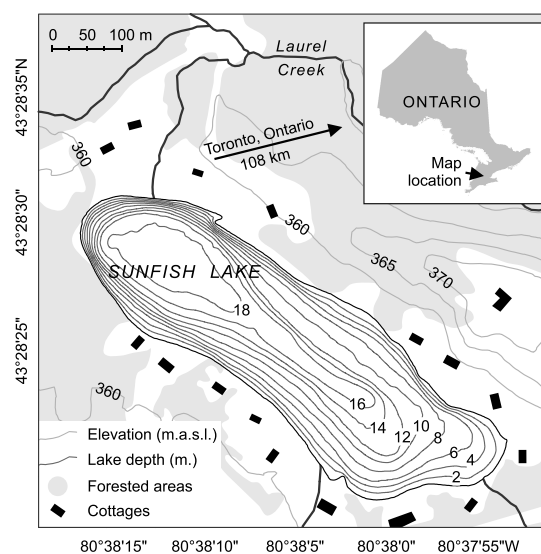
Deep cyanobacteria layers are a relatively understudied phenomenon. Knowledge of deep cyanobacteria layers is based mainly on studies from a few lakes (e.g., Lake Zürich and Lac du Bourget), a narrow geographic distribution (i.e., European emphasis), and a single species [i.e., *Planktothrix rubescens* (*P. rubescens*), with 77% of research focused on this cyanobacterium (Figure 1)]. Although this research has advanced our scientific understanding of this deep-water phenomenon,<sup>4,15</sup> establishing scientific consensus from a narrow research scope overlooks the inherent variability of deep cyanobacteria layers within and among lakes. As a result, there is limited knowledge regarding the global prevalence, taxonomic composition, and toxin-producing potential of these deep-water phenomena. These knowledge gaps underscore the need for increased research focused on assessing public health risks, especially in an era of unprecedented global change.

Deep cyanobacteria layers are designated as “known unknowns” in risk management. A known unknown is a concern that society (or the scientific community) has recognized. Yet the impacts of that risk remain unrecognized, often the result of inadequate research.<sup>20</sup> The known unknown distinction suggests a blind spot in cyanobacteria management, and research priorities need to broaden to incorporate the whole lake to avoid “missing the middle”.<sup>18</sup> Further research on deep cyanobacteria layers, particularly outside of Europe and including organisms aside from *P. rubescens*, will advance our understanding of these deep-water events and help build predictive capabilities for identifying waterbodies at proportionately higher risk.

This study was carried out in Sunfish Lake, Ontario, Canada. Duthie and Carter<sup>21</sup> previously encountered a deep cyanobacteria layer composed predominately of *Planktothrix* sp. (previously *Oscillatoria* sp.) in the late 1960s at Sunfish Lake. Further, the paleorecord suggests a contemporary increase in cyanobacteria abundance, specifically *Planktothrix* sp.<sup>22</sup> The aims of this study are to (a) characterize the deep cyanobacteria layer in Sunfish Lake, (b) evaluate the presence of toxins associated with the deep cyanobacteria layer to establish potential health risks, and (c) isolate the dominant cyanobacteria species and characterize the toxin-producing potential of these species to establish toxin–producer relationships.

## 2. MATERIAL AND METHODS

**2.1. Site Description.** Sunfish Lake (43°28′25″N, 80°38′01″W) is situated in the township of Wilmot, adjacent to Waterloo, Ontario, Canada. A small surface area (about 19 ha) paired with an unusually steep shoreline creates a lake morphology that supports meromixis<sup>21,22</sup> (Figure 2). A 1,000



**Figure 2.** Bathymetry and surrounding landscape of Sunfish Lake (adapted from Ontario Ministry of Natural Resources and Forestry, Bathymetry, Line, 1968).

m buffer surrounding the lake is dominated by farmland (54.6% area) followed by mixed deciduous forests (36.4% area).<sup>22</sup> The shoreline is well developed, with high cottage development occurring around the 1970s.<sup>23</sup> Sunfish has one major outflow in the northeast corner (start of Laurel Creek) and two small outflows in the southwest portion.<sup>21</sup> The lake is mesotrophic (i.e., average total phosphorus during the open water season of about 10  $\mu\text{g L}^{-1}$ ), narrow, and has two deep recesses. The northwestern facing recess reaches a maximum depth of 19.8 m, and the southeastern recess reaches a maximum depth of 16.0 m.<sup>22</sup>

**2.2. Lake Sampling and Processing.** In 2017 and 2019, water samples were collected monthly throughout the open water season (May–October). In-situ measurements of temperature ( $^{\circ}\text{C}$ ), dissolved oxygen ( $\text{mg L}^{-1}$ ), chlorophyll, and phycocyanin (PC) fluorescence ( $\mu\text{g L}^{-1}$ ) were recorded at 1-m intervals using a YSI EXO2 Multiparameter Sonde (Yellow Springs, Ohio, USA). Water samples were collected using a Van Dorn water sampler using a discrete profile

sampling approach. In-situ PC measurements determined the depth of the deep cyanobacteria layer. Samples (1 L) were collected above the deep cyanobacteria layer (1, 3, and 5 m), at the deep cyanobacteria layer (7, 8, and 9 m), and below the deep cyanobacteria layer (11 m). Sub-samples (50 mL) were used to calculate *Planktothrix* filament counts using the Utermöhl method.<sup>24</sup> Secchi depth was used to estimate the compensation depth by multiplying Secchi depth by 2.5, which corresponds to 1% light irradiance relative to surface waters.<sup>25</sup> Extractions of phycobiliproteins were analyzed using a modified method described in Erratt et al.<sup>26</sup> Sub-samples (500 mL) were passed through a Whatman GF/F filter and kept at  $-20\text{ }^{\circ}\text{C}$  prior to analysis. Phycobiliproteins were extracted with phosphate buffer (0.1 M, pH of 6.8) solvent. Filters were submerged in 5 mL solvent and macerated using a bead beater ( $3 \times 10\text{ s}$  cycles) supplied with 0.1 and 0.5 mm zirconia/silica beads. The macerated product was stored at  $4\text{ }^{\circ}\text{C}$  for 24 h followed by purification through centrifugation (10,000  $g$  for 5 min). The supernatant was passed through a  $0.22\text{ }\mu\text{m}$  syringe filter and PC (620 nm) and phycoerythrin (PE) (545 nm) were measured using spectrophotometry. Phycobilin concentrations were estimated using equations described in Lawrence et al.<sup>27</sup> Relative thermal resistance (RTR) was calculated using Wetzel and Likens,<sup>25</sup> where RTR is the relative difference of energy involved in mixing two water layers and therefore is dimensionless (equation 1):  $\text{RTR} = [(d_2 - d_1) \times 10^6]/8$ , where water temperature values were converted to density values using standard conversion tables ([https://www.simetric.co.uk/si\\_water.htm](https://www.simetric.co.uk/si_water.htm)),  $d_1$  is the density of water in the upper 5 m portion of the water column, and  $d_2$  is the density of water below the deep cyanobacteria layer (i.e.,  $>8\text{ m}$  from surface).<sup>25</sup>

**2.3. Cell Isolation and Identification.** Two phenotypically distinct *Planktothrix* species—*P. isothrix* (PC-rich) and *P. rubescens* (PE-rich)—were isolated from the deep cyanobacteria layer. *Planktothrix* cultures were maintained as non-axenic, unialgal strains on Wright's Cryptophyte medium<sup>28</sup> at  $20 \pm 2\text{ }^{\circ}\text{C}$  under a continuous light flux of  $10 \pm 1\text{ }\mu\text{mol photons m}^{-2}\text{ s}^{-1}$  (daylight fluorescent light). Isolates were gently hand-stirred daily to aid in cell development. Cyanobacteria isolates were sent to Algal Taxonomy and Ecology Inc. (Winnipeg, MB, CA) for taxonomic identification.

**2.4. Toxin and Bioactive Metabolites.** Microcystin and cyanopeptide standards and samples were analyzed using a Thermo Scientific TSQ Altis triple quadrupole mass spectrometer (Waltham, MA, USA) with a TriPlus RSH EQuan 850 system. For online concentration analysis, standards and samples were loaded onto a concentration column (Thermo Scientific Hypersil GOLD Q 2.1  $\times$  20 mm,  $12\text{ }\mu\text{m}$ ) using a TriPlus RSH autosampler equipped with a 10 mL sampling syringe, at a flow rate of 1.5 mL/min using 0.1% formic acid in water. One milliliter of the standard or samples was loaded onto the concentrator column, and the column was washed with mobile phase for the first minute. Then, after 1 min, the switching valve enabled the concentrator column to be back flushed with the analytical column gradient onto the analytical column (Hypersil GOLD C18 column 2.1  $\times$  50 mm,  $1.9\text{ }\mu\text{m}$ ), where the analytes were separated in under 6 min. Gradient programs and mass spectrometer instrument settings are in Supporting Information. Retention times, precursor and quantification and qualification ions are listed in the Supporting Information (Figure S2.1). Standard curve

dynamic range, detection limits, minimum reporting limits, % relative standard deviation, and % recovery are in the Supporting Information (Figures S2.1–2.4).

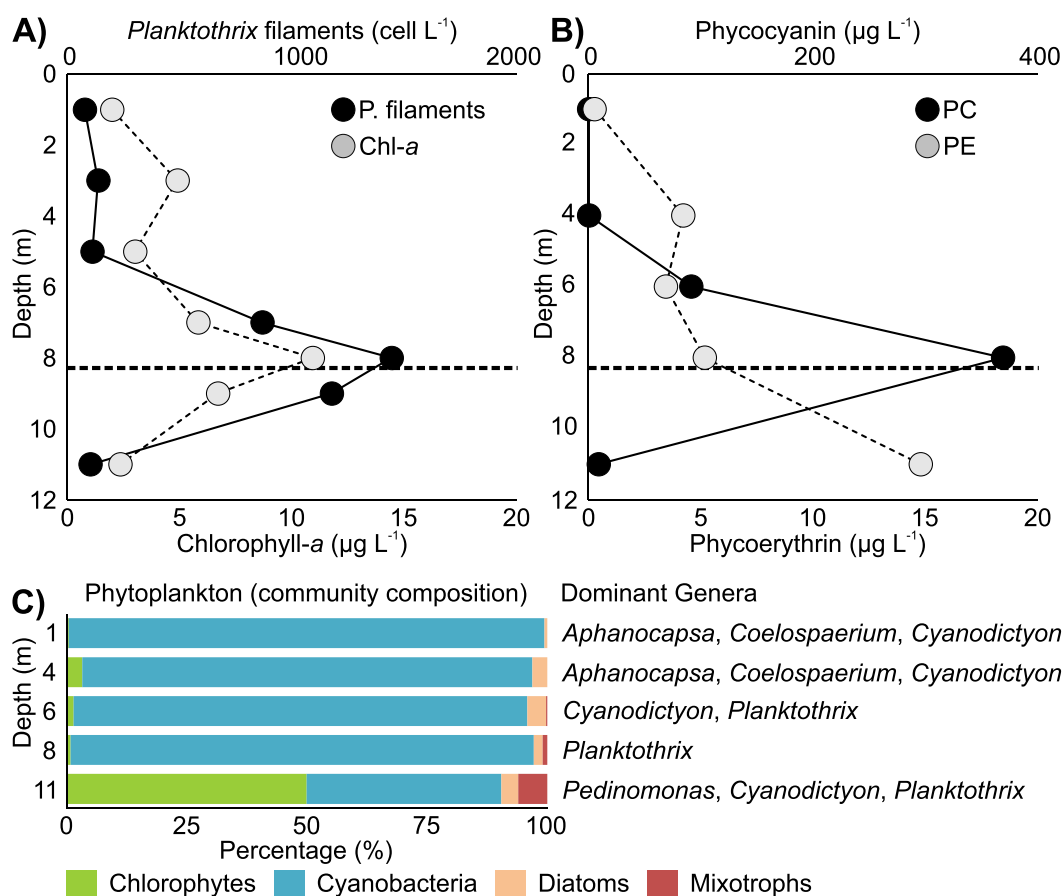
Anatoxins and cylindrospermopsin standards were analyzed using a Thermo Scientific TSQ Altis triple quadrupole mass spectrometer (Waltham, MA, USA) with a TriPlus RSH EQuan 850 system. A  $10\text{ }\mu\text{L}$  injection volume was used for analysis. Analytes were separated on a Hypersil GOLD aQ C18 2.1  $\times$  100 mm,  $1.9\text{ }\mu\text{m}$ . Mobile phases used are (i) 100 mM acetic acid in water and (ii) 100 mM acetic acid in methanol. Gradient program and instrument settings are in the Supporting Information. Retention times, precursor, and quantification and qualification ions are listed in the Supporting Information (Figure S2.5). Standard curve dynamic range, detection limits, minimum reporting limits, % relative standard deviation, and % recovery are in the Supporting Information (Figures S2.5–2.8).

Control samples and analytical standards were quantified after every 20 samples during each run. Control standards included a negative control (blank) and positive control as well as duplicate and fortified samples. For each standard, a quantitative and qualitative ion transition was chosen for use with selected reaction monitoring (SRM). Supporting Information (Figures S2.1 and 2.5) provides a list of the SRM transitions for each cyanotoxin and cyanopeptide. Quantification was accomplished using TraceFinder EFS 5.1 (Thermo Fisher Scientific, Waltham, MA, USA, 2021).

**2.5. DNA Extraction and PCR Amplification.** DNA was isolated using the Qiagen DNeasy Powerwater kit according to kit instructions. Quantitative PCR (qPCR) was used for the detection and quantification of gene copy numbers for three toxins, namely, microcystin (*mycE*), cylindrospermopsin (*cyrA*), and saxitoxin (*sxtA*). The PhytoXigene kit (Diagnostic Technology, Australia) was used for the qPCR assays and was carried out following the manufacturer's protocol. The lyophilized master mix containing all necessary PCR components was reconstituted in nuclease-free water and aliquoted into separate PCR tubes. Each qPCR reaction consisted of  $20\text{ }\mu\text{L}$  of the rehydrated master mix and  $5\text{ }\mu\text{L}$  of template DNA (10–50 ng) and was performed in a Bio-Rad CFX96 cyler (Bio-Rad, Hercules, CA). The PCR cycling conditions consisted of an initial denaturation at  $95\text{ }^{\circ}\text{C}$  for 2 min, then denaturation at  $95\text{ }^{\circ}\text{C}$  for 15 s and 40 cycles, and annealing-extension at  $60\text{ }^{\circ}\text{C}$  for 40 s. Gene copies per sample were determined using a standard curve (target gene copy number vs Ct) constructed for each target gene. Standard curves for all target genes were developed using five standards (100–1,000,000 copies) purchased from PhytoXigene.

Digital PCR (dPCR) was used to provide absolute quantification of anabaenopeptin gene copies. The forward and reverse primers used for detecting the anabaenopeptin gene were apnDTe-F (5'-GACACGCCTTCTATTTTCGAGA-3') and apnDTe-R (5'-ACGCGAAATAAAACAA-TAGGGG-3').<sup>29</sup> Each dPCR reaction consisted of  $7.5\text{ }\mu\text{L}$  of QuantStudio 3D dPCR master mix v2 (Thermo Fisher Scientific, Canada),  $0.75\text{ }\mu\text{L}$  of 250 nM probe,  $1.4\text{ }\mu\text{L}$  of each 900 nM forward and reverse primers,  $2\text{ }\mu\text{L}$  of template DNA (10–50 ng), and  $1.95\text{ }\mu\text{L}$  nuclease-free water. The reaction mixtures were loaded onto a QuantStudio 3D dPCR 20 K chip v2 (Thermo Fisher Scientific, Canada) with the aid of a QuantStudio 3D chip loader (Thermo Fisher Scientific, Canada). A ProFlex thermocycler (Thermo Fisher Scientific, Canada) was used for PCR amplification with the cycling





**Figure 3.** Depth profiles of select physiological variables on July 7, 2017: (A) chlorophyll-*a* (gray circles) and *Planktothrix* filaments (closed circles); (B) phycoerythrin (gray circles) and phycocyanin (closed circles); and (C) phytoplankton community composition with dominant genera identified. The dashed line represents compensation depth (1% irradiance).

conditions consisting of 96 °C for 10 min, 40 cycles of 60 °C for 2 min, 98 °C for 30 s, and a final step of 60 °C for 2 min. The chips with amplified products were read on a QuantStudio 3D dPCR instrument and the results were analyzed using the QuantStudio AnalysisSuite software (v3.2, 2019) (Thermo Fisher Scientific, Canada).

### 3. RESULTS AND DISCUSSION

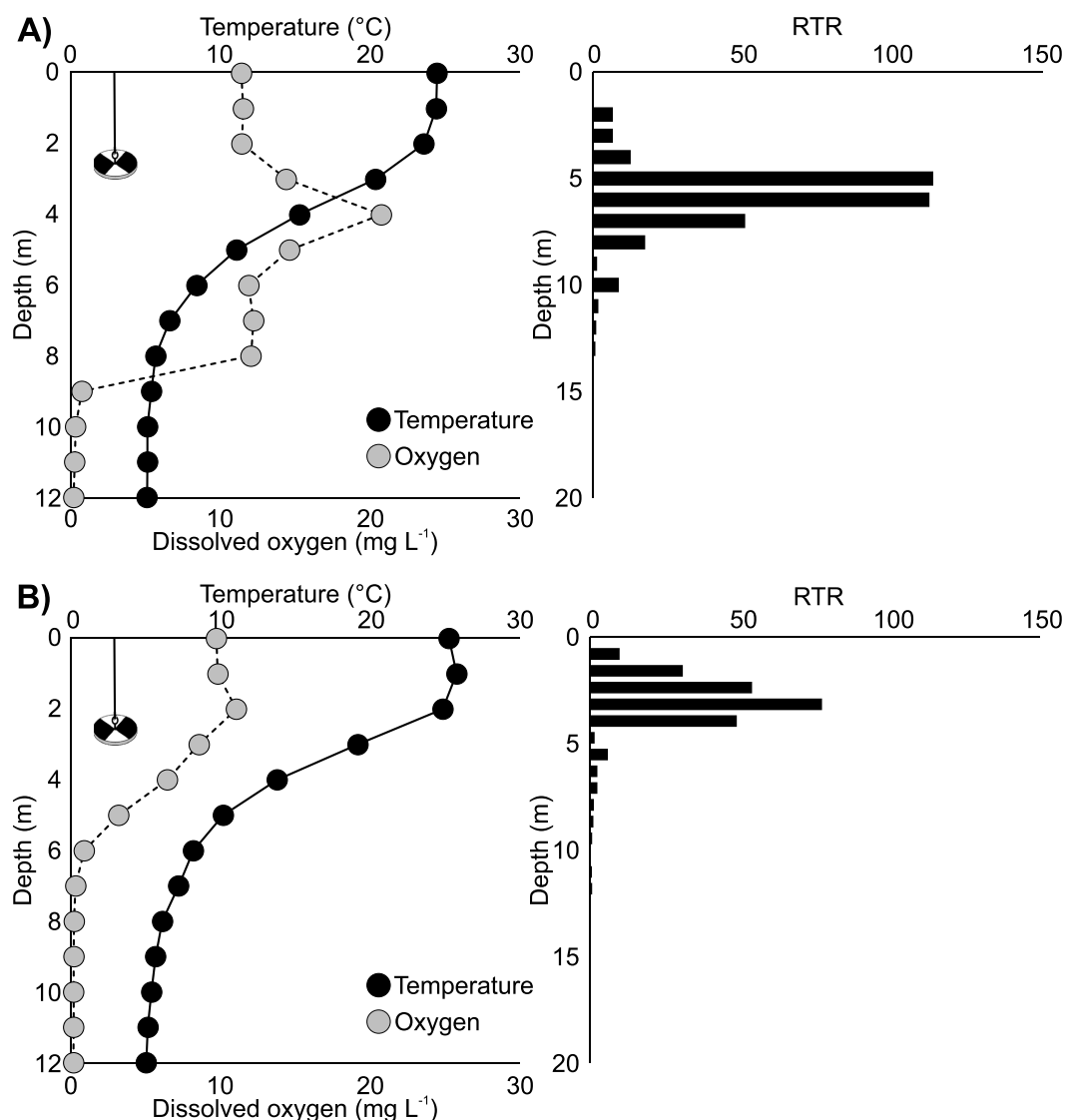
**3.1. Deep Cyanobacteria Layer and Its Physiological Composition.** A deep cyanobacteria layer was observed in Sunfish Lake at 8 m (Figure 3A) and *P. isothrix* was the dominant cyanobacterium. *P. isothrix* was largely confined to the metalimnion, between 6 to 9 m, with maximum abundance (~1,500 filaments mL<sup>-1</sup>) observed at 8 m (Figure 3A). A similar phenomenon was observed in Sunfish Lake roughly 50 years prior, with a deep cyanobacteria layer dominated by *P. isothrix* (~1,000 filaments mL<sup>-1</sup>) occurring at 8 m.<sup>21</sup> The physiological composition of Sunfish Lake was comparable to earlier reports,<sup>21</sup> with low biomass reported in the surface waters during summer, consisting mainly of cyanobacteria: Synechococcales (e.g., *Aphanocapsa*, *Cyanodictyon*, and *Coelosphaerium*). Other notable phytoplankton genera found at appreciable abundance were diatoms (e.g., *Fragilaria* and *Stephanodiscus*), cryptophytes (e.g., *Cryptomonas* and *Rhodomonas*), and chlorophytes (*Pedinomonas*), with the latter predominately residing at lower depths (>8 m) (Table S1). Sunfish Lake displayed cyanobacteria dominance (>50% phytoplankton assemblage) throughout the water column,

apart from greater depths (>11 m) where chlorophyte abundance increased (Figure 3C).

The Secchi depth at Sunfish Lake was comparable between 2017 (3.4 m) and 2019 (3.2 m) (Figure 4A,B), suggesting a compensation depth of approximately 8 m.<sup>25</sup> The deep cyanobacteria layer was situated near the light compensation depth (Figure 3A). Deep-living cyanobacteria have evolved to flourish under low light and cooler temperatures (typical of the metalimnion). The presence of phycobiliproteins enhances photosynthetic efficiency under low light, allowing deep-living cyanobacteria to exist within this relatively uncompetitive niche.<sup>29,30</sup> Maximum phycobiliprotein content, PC 369 μg L<sup>-1</sup> and PE 5.2 μg L<sup>-1</sup>, coincided with the deep cyanobacteria layer, with PC levels approximately 60 times greater than PE (Figure 3B). This finding corroborates our microscopic enumeration, with *P. isothrix* being the dominant cyanobacterium and *P. rubescens* occurring at background levels (Section 3.3).

Cyanobacteria inhabiting the deep layers require the simultaneous occurrence of three abiotic factors to materialize: (a) sufficient nutrients; (b) sufficient light; and (c) thermal stability.<sup>4,6</sup> Mesotrophic conditions provide the “sweet spot” where there are sufficient nutrients for biomass development and sufficient light to drive photosynthesis.<sup>6,7,14</sup> With further eutrophy, the potential for forming a deep cyanobacteria layer is hindered, resulting from insufficient light.<sup>6</sup>

Both meromictic and holomictic lakes can support deep cyanobacteria layers.<sup>6,21</sup> Meromictic lakes are uncommon, but

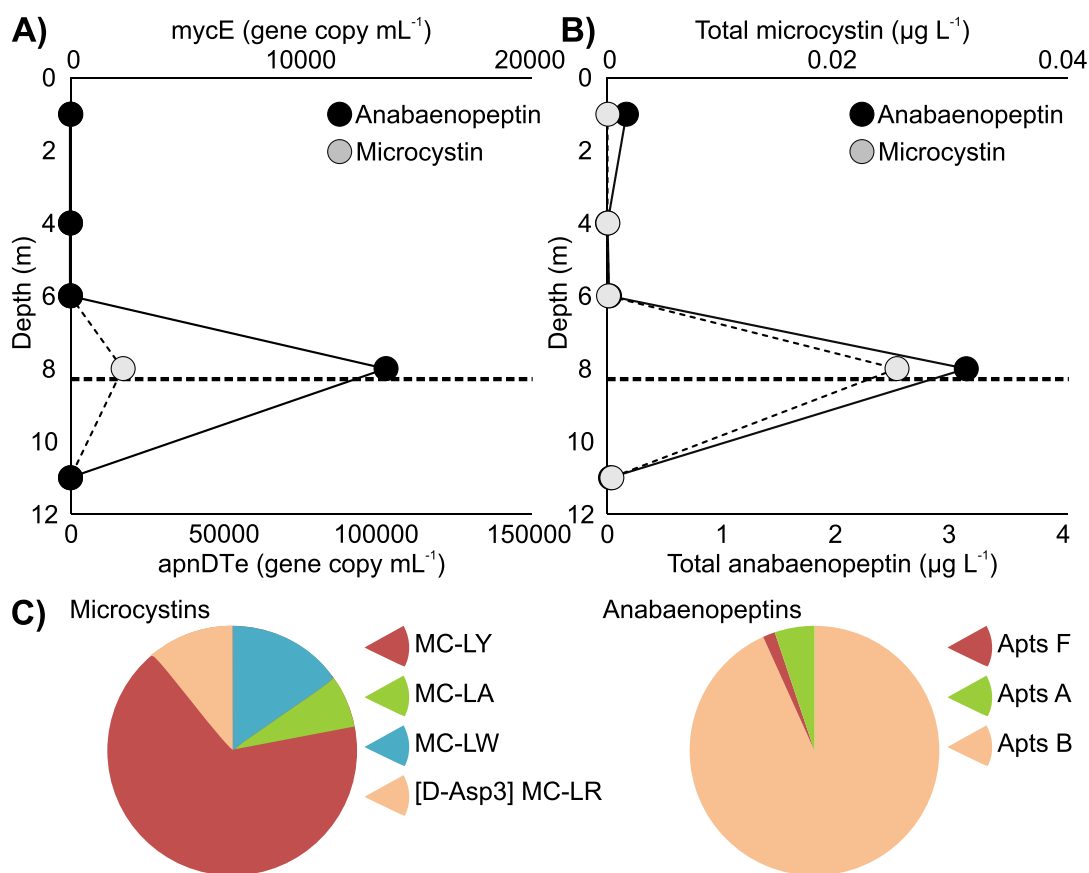


**Figure 4.** Depth profiles of select physical variables: (A) oxygen (gray circles) and temperature (closed circles) on July 7, 2017 (deep cyanobacteria layer present) with RTR; (B) oxygen (gray circles) and temperature (closed circles) on July 12, 2019 (deep cyanobacteria layer absent) with RTR.

if the three prerequisites for deep cyanobacteria are met (i.e., sufficient nutrients, light, and thermal stability), these lakes can support cyanobacteria accumulating at greater depths. In meromictic lakes on unmanaged landscapes (without human activity), the lack of intermixing of water layers inhibits the periodical recharge of nutrient-enriched deep water into the surface layer and may restrict deep cyanobacteria layer development.<sup>31</sup> However, meromictic lakes, such as Sunfish Lake, situated on managed landscapes (with human activity) may experience larger external nutrient loads that keep the lake in a moderately enriched state, supporting deep cyanobacteria layer development.<sup>22</sup> Lake mixing strongly influences nutrient concentrations and thermal stability, which dictates the duration and intensity of stratification.<sup>4,7</sup> The lack of deep water-column mixing in meromictic lakes may favor stable thermal characteristics, which would be beneficial for deep cyanobacteria aggregating at depth. However, the nutrient prerequisite would not be satisfied, hindering deep cyanobacteria layer development.

In Sunfish Lake, nutrient and light conditions were similar between a year where a deep cyanobacteria layer developed

(2017) and a year where there was no discernable deep cyanobacterial layer (2019). A notable difference between 2017 and 2019 was altered thermal characteristics. In 2017 (deep cyanobacteria layer present), the maximum RTR was lower in the water column (6 m) (Figure 4A), suggesting the depth of stratification was deeper. In 2019 (deep cyanobacteria layer absent), the maximum RTR was higher in the water column (4 m), suggesting the depth of stratification was shallower (Figure 4B). Multiple environmental variables influence stratification depth, such as temperature, wind, and chemical composition (e.g., salinity), and these factors remain to be further explored in Sunfish Lake to help understand the environmental factors altering thermal characteristics that may lead to the development of deep cyanobacteria layers. We propose that greater thermal stability occurring at lower depths observed in 2017 created an optimal environmental niche for deep cyanobacteria layer development (i.e., a low turbulent environment situated at optimal irradiance). Here, we suggest that the deep cyanobacterial layer may be ephemeral and not necessarily an integral component of a lake.



**Figure 5.** Depth profiles of chemical variables on July 7, 2017: (A) gene copies of microcystin-mycE (gray circles) and anabaenopeptin-apnDTe (closed circles); (B) concentrations of microcystin (gray circles) and anabaenopeptin (closed circles); and (C) pie charts showing microcystin and anabaenopeptin congener composition at 8 m. Dashed line represents compensation depth (1% irradiance). Note: The following toxins were not found or were found below the detection limit: microcystin(MC)-YR, MC-HtyR, MC-LR, MC-HilR, MC-WR, MC-LF, oscillamide Y, micropeptin 1106, cyanopeptolin 1007, cyanopeptolin 1040 MB, nodularin, aeruginosamide B, aeruginosamide C, oscillagin A, anatoxin-a, dihydroanatoxin-a, homoanatoxin, L-phenylalanine-D5, cylindrospermopsin, 7-epi-cylindrospermopsin, and 7-deoxy-cylindrospermopsin.

### 3.2. Toxin and Bioactive Metabolite Profiles in the Deep Cyanobacteria Layer.

The deep cyanobacteria layer coincides with maximum toxin concentrations, with total microcystin ( $0.03 \mu\text{g L}^{-1}$ ) and anabaenopeptin ( $2.5 \mu\text{g L}^{-1}$ ) peaking at 8 m (Figure 5B). Toxin concentrations mirrored gene copy numbers, with maximum microcystin ( $13,841 \text{ copy mL}^{-1}$ ) and anabaenopeptin ( $103,556 \text{ copy mL}^{-1}$ ) occurring at 8 m (Figure 5A). Saxitoxin was detected at 6 m in low copy numbers ( $5 \text{ gene copy mL}^{-1}$ ), but cylindrospermopsin was not detected with qPCR. While understanding total toxin concentrations (i.e., toxin quantity) is important, understanding the congener composition (i.e., toxin quality) is also important, given the differential toxicities of congeners. Microcystin congeners exhibit a wide spectrum of toxicity. For instance, microcystin LA is up to 12 times more toxic than other frequently encountered variants, such as RR.<sup>32</sup> Emerging research points to other microcystin congeners of concern (e.g., LW and LF) that exhibit greater toxicity at the cellular level and require further testing.<sup>33,34</sup> While the suite of microcystin congeners present in Sunfish Lake are at low concentrations, they consist of relatively potent congeners (Figure 5C).<sup>33</sup>

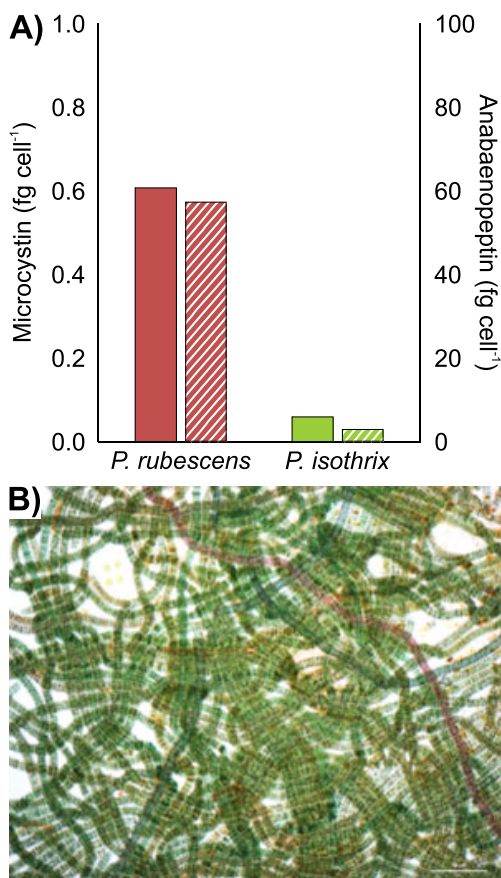
Direct water withdrawal paired with a deep cyanobacteria layer containing toxins creates a scenario of concern due to the greater potential for human exposure. Roughly half of the residents of Sunfish Lake draw water directly from the lake

(i.e., no central water treatment facility), with most water withdrawal occurring at depths greater than 3 m. While some residents still use Sunfish Lake as a drinking water source in combination with household water purification units, it has become less common, with more residents using lake water for non-potable sources (e.g., showers, dishwashing) (K. Thomason, pers. comm., June 13, 2022). An earlier investigation observed a deep cyanobacteria layer at 8 m during July, 1967,<sup>21</sup> aligning with our observations. However, over an 18-month observation period, the depth of the deep cyanobacteria layer exhibited a high degree of variability, migrating between 3 and 10 m.<sup>21</sup> This variability in the vertical positioning of deep cyanobacteria layers has been observed in other systems, such as Lake Zürich and Lac du Bourget, fluctuating between 8 and 25 m.<sup>35,36</sup> Vertical variability observed among deep-living cyanobacteria reveals that health concerns can migrate throughout the water column. This observation underscores the importance of routine monitoring to track cyanobacteria movement and modify water withdrawal accordingly to minimize the likelihood of human exposure.

### 3.3. Managing Risks Associated with Toxins and Bioactive Metabolites in Deep Cyanobacteria Layers.

Different cyanobacteria exhibit different potentials to produce toxins.<sup>37</sup> Within a cyanobacteria bloom, monospecific populations are uncommon.<sup>38</sup> Although one type of cyanobacterium may be dominant, cryptic species—endemic taxa

detected at low concentrations—are also important. While cryptic species occur at low levels, they may thrive with changing environmental conditions caused by climate change or in response to lake management activities.<sup>39</sup> In Sunfish Lake, *P. rubescens* occurred at low levels in the *P. isothrix*-dominated deep cyanobacteria layer (Figure 6B). However,



**Figure 6.** (A) Anabaenopeptin (striped) and microcystin (solid) concentrations of *P. isothrix* and *P. rubescens* isolates. (B) Light micrograph of the Sunfish Lake's deep cyanobacteria layer collected at 8 m on July 7, 2017. Note the low abundance of the more toxic *P. rubescens* (red filaments) in a community dominated by less toxic *P. isothrix* (green filaments). Scale bar 50 μm.

toxin profiles of the two *Planktothrix* isolates revealed that *P. rubescens* exhibited 10 times higher microcystin (0.64 fg cell<sup>-1</sup>) and 25 times higher anabaenopeptin (53.8 fg cell<sup>-1</sup>) than *P. isothrix* (microcystin: 0.06 fg cell<sup>-1</sup>; anabaenopeptin: 2.1 fg cell<sup>-1</sup>) (Figure 6A). Microcystin gene copy numbers were more than two times higher for *P. rubescens* (92,336 gene copy mL<sup>-1</sup>) compared to *P. isothrix* (42,360 gene copy mL<sup>-1</sup>), while anabaenopeptin gene copy numbers were close to four times higher for *P. rubescens* (2,006,666 gene copy mL<sup>-1</sup>) than for *P. isothrix* (520,727 gene copy mL<sup>-1</sup>).

In European lakes, *Planktothrix* populations dominated by red phenotypes exhibited a higher proportion of toxic genotypes (specifically microcystin potential), whereas populations dominated by green phenotypes or co-occurring (i.e., red and green phenotypes) exhibited a higher proportion of non-toxic genotypes.<sup>40</sup> *P. rubescens* is often associated with microcystin production<sup>41,42</sup> and has been observed to contain the highest microcystin content per cell relative to other commonly observed toxigenic cyanobacteria,<sup>43</sup> whereas *P.*

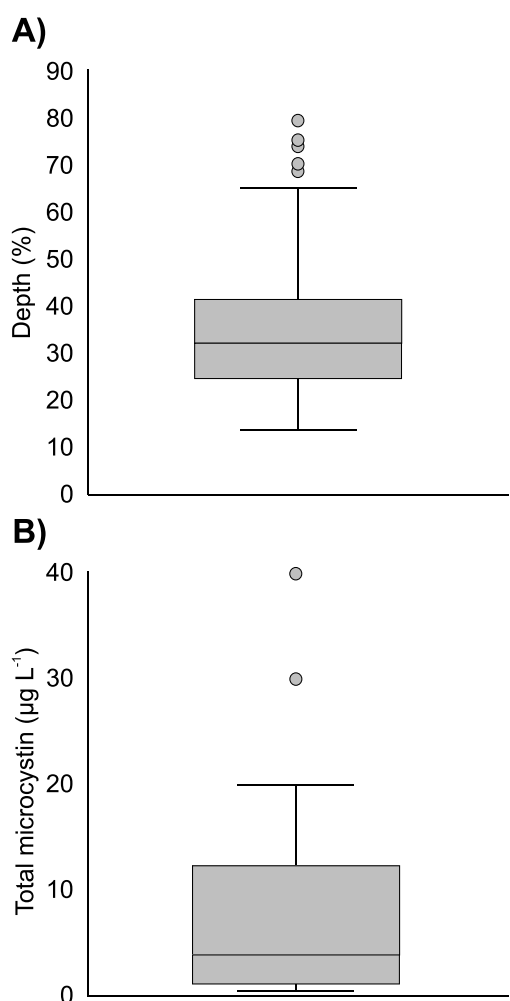
*isothrix* is often associated with low microcystin production [i.e., concentrations well below Health Canada's drinking water guideline (1.5 μg L<sup>-1</sup>)].<sup>9,44</sup> Here, we showcase that examining cyanobacterial assemblages is a central feature of monitoring for current risks and anticipating future risks. For instance, microcystin levels were below drinking water guidelines on July 7, 2017. However, changes in environmental conditions could favor the predominance of *P. rubescens*, creating a scenario where microcystin levels could rise.

**3.4. Managing Risks Associated with Toxins and Bioactive Metabolites.** Despite a correlation between maximum toxin levels and the depth of the cyanobacteria layer, uncertainty surrounding the toxin-producing cyanobacteria remains. Phytoplankton communities are diverse, and cryptic species may be responsible for toxin production (Section 3.3). Isolating cyanobacteria from deep cyanobacteria layers helps to strengthen the link between toxin presence and the cyanobacteria producer. In Sunfish Lake, we demonstrated the toxin-producer link by complementing field data with screening of corresponding cyanobacteria isolates for cyanotoxins and other bioactive metabolites. Metabolite profiles (including microcystins) between lake samples and laboratory cultured cyanobacterial isolates from the deep cyanobacteria layer mirrored one another, providing evidence that suggests *Planktothrix* isolates were responsible for the microcystins and anabaenopeptins observed. Using this approach, scientists can build an inventory of known toxigenic deep-water cyanobacterium (beyond *P. rubescens*), which would be a useful tool for lake managers when evaluating potential risks.

Microcystin has been repeatedly used as a universal indicator of cyanobacterial risk. Monitoring efforts commonly measure microcystin when assessing deep cyanobacteria layers,<sup>17,42</sup> with metalimnion microcystin concentrations showing variability (0–40 μg L<sup>-1</sup>) (Figure 7B). The overreliance on microcystin as a sole metric for cyanobacterial risk has resulted in other cyanotoxins such as anatoxins, beta-*N*-methylamino-*L*-alanine, and other bioactive metabolites (e.g., anabaenopeptin and aeruginosins) to be overlooked in routine monitoring, which may underestimate public health risks.<sup>37,45</sup> Isolation studies of deep cyanobacteria layer species have revealed novel microcystin congeners,<sup>46</sup> anatoxin-a,<sup>47</sup> and bioactive metabolites, such as oscillapeptin J,<sup>48</sup> planktocylin<sup>49</sup> aeruginosins, and anabaenopeptins.<sup>50</sup> Despite lacking complementary field data, these isolation studies illustrate that specific cyanobacterium within deep cyanobacteria layers have the potential to produce a variety of cyanotoxins and other bioactive metabolites. A diverse suite of harmful metabolites produced in deeper waters suggests scientist and lake managers should incorporate “vertical tuning” in toxin analyses. Like regional tuning (i.e., modifying toxin screening to reflect frequently encountered compounds in geographic regions and using scientific advancements to update protocols),<sup>3</sup> “vertical tuning” would incorporate toxins and bioactive metabolites commonly associated with deep cyanobacteria layers. However, as deep cyanobacteria layers are a relatively understudied topic, further research is required to build this toxin inventory.

There is a growing need to evaluate the prevalence and inherent risk of compounds other than microcystin.<sup>49</sup> For example, anabaenopeptins were reported at concentrations (2.5 μg L<sup>-1</sup>) comparable to other North American and European waterbodies.<sup>44,51</sup> Zastepa et al.<sup>44</sup>'s investigation of *P. isothrix*-dominated deep cyanobacteria layers in Georgian Bay, Canada presents a similar narrative to Sunfish Lake, with deep





**Figure 7.** (A) Box–whisker plot of the vertical distribution of deep cyanobacteria layers reported in published studies ( $n = 52$ ). (B) Box–whisker plot of microcystin levels reported in the deep cyanobacteria layers in the same published studies ( $n = 12$ ). Data compiled from the 134 research articles collected for Figure 1. Data collected by examining the following scientific databases: Web of Science Core Collection, Scopus, Google Scholar, and ScienceDirect. Keywords used are presented in Table S1.1. Percent depth is calculated by dividing the maximum depth of the sampling location by the depth of the deep cyanobacteria layer. This value indicates where the deep cyanobacteria layer was situated in the water column (i.e., lower values indicate higher in the water column, while higher values (e.g., 80%) indicate lower in the water column) relative to the sampling depth.

cyanobacteria layers forming around 7–9 m with a larger proportion of anabaenopeptins (max.  $6.6 \mu\text{g L}^{-1}$ ) relative to microcystin (max.  $0.4 \mu\text{g L}^{-1}$ ). Despite not having any documented adverse toxicological effect on humans or larger vertebrates, anabaenopeptins and many other bioactive metabolites produced by cyanobacteria are known to interact with more proximal members of aquatic ecosystems.<sup>44,45</sup> For example, anabaenopeptins have been hypothesized to function as an antiparasitic mechanism, with protease inhibition impeding the digestive processes of cyanobacterial parasites.<sup>52</sup>

When managing cyanobacteria-associated risks, one critical aspect is developing a predictive understanding of where and when cyanobacteria dominance will occur. Deep cyanobacteria layers are challenging to monitor as they show considerable

vertical variation. For example, our literature survey indicates deep cyanobacteria layers occurred between 13.3 and 79.6% depth relative to the sampling depth (Figure 7A). The seasonal and year-to-year incidence and duration of deep cyanobacteria layers are largely unknown, aside from a few study lakes. Even when we are cognizant of these deep-water events, monitoring programs can miss capturing deep cyanobacteria layers. For example, in Europe, the European Union Water Framework Directive protocol is to sample the euphotic zone, defined as  $2.5 \times$  Secchi depth, as an integrated sample, which has been demonstrated to overlook deep cyanobacteria layers.<sup>53</sup>

**3.5. Filling the Gap: The Importance of Comprehensive Monitoring.** Deep cyanobacteria layers remain a clandestine component of aquatic environments. Without surface scum or visible water discoloration, it becomes a case of “out of sight, out of mind”. For example, despite earlier documentation of a deep cyanobacteria layer in Sunfish Lake,<sup>21</sup> lake monitoring at lower depths remained absent for over 50 years. Local knowledge regarding these deep-water phenomena was largely forgotten. Community awareness was revived when a comprehensive lake survey conducted by the authors rediscovered the deep cyanobacteria layer in 2017, which sparked concern as residents withdraw water directly from Sunfish Lake. Our findings raise awareness about improper monitoring in lakes with drinking water supplies, which can overlook deep cyanobacteria layers, creating a hidden public health concern. Further, we highlight the importance of cataloguing cyanobacteria community composition, as different cyanobacteria exhibit different toxigenic potential and understanding these differences can assist in determining risks.

Historically, scientific interest has been in near-surface cyanobacterial proliferations and benthic cyanobacteria.<sup>5,54</sup> Concentrating research and management efforts on the top and bottom of lakes contributes to “missing the middle” in risk assessments. Avoidance depends on awareness; without adequate monitoring, important phylogenetic features may go unnoticed. Technological advancements can now facilitate the rapid evaluation (presence or absence) of deep cyanobacteria layers. The ever-increasing use and availability of high frequency sensors capable of monitoring the entire water column (e.g., vertical profilers and autonomous underwater vehicle) will become an indispensable tool to reveal and characterize deep cyanobacteria layers.

Without a predictive understanding of when and where deep cyanobacteria layers will manifest, comprehensive lake monitoring assessments [examining the whole depth of the system (i.e., top to bottom)] are essential for better representing the risks associated with cyanobacteria throughout the water column, especially for waterbodies functioning as drinking water sources. This holistic monitoring approach is multifaceted by (a) safeguarding human health by uncovering deep cyanobacteria layers and prompting risk avoidance measures; (b) supplementing existing knowledge, which will in turn aid in building predictive capacities, uncovering more potentially toxigenic cyanobacterium; and (c) revealing how human and climate-mediated change influence deep cyanobacteria layers. New insight into deep cyanobacteria layers is crucial for developing a comprehensive understanding of the risks of cyanobacteria to public health, and the basis of suitable cyanobacteria mitigation or adaptation strategies.



## ■ ASSOCIATED CONTENT

### SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.est.2c06928>.

(1) Descriptors used for identifying scientific articles to build our literature survey (Table SI.1); (2) phyecological breakdown of major phytoplankton identified during the deep cyanobacteria layer year (July 7, 2017) (Table SI.2); and (3) methodology for toxin analysis including the gradient program and instrument settings (Tables SI.3–10) (PDF)

## ■ AUTHOR INFORMATION

### Corresponding Author

**Irena F. Creed** – School of Environment & Sustainability, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5E2, Canada; Department of Physical & Environmental Sciences, University of Toronto, Toronto, Ontario M1C 1A4, Canada; [orcid.org/0000-0001-8199-1472](https://orcid.org/0000-0001-8199-1472); Email: [irena.creed@utoronto.ca](mailto:irena.creed@utoronto.ca)

### Authors

**Kevin J. Erratt** – School of Environment & Sustainability, University of Saskatchewan, Saskatoon, Saskatchewan S7N 5E2, Canada

**Erika C. Freeman** – Ecosystems and Global Change Group, Department of Plant Sciences, University of Cambridge, Cambridge CB2 1TN, U.K.

**Charles G. Trick** – Department of Health & Society, University of Toronto, Toronto, Ontario M1C 1A4, Canada

**Judy Westrick** – Lumigen Instrument Center, Wayne State University, Detroit, Michigan 48202, United States

**Johnna A. Birbeck** – Lumigen Instrument Center, Wayne State University, Detroit, Michigan 48202, United States

**L. Cynthia Watson** – Environment and Climate Change Canada, Canada Centre for Inland Waters, Burlington, Ontario L7S1A1, Canada

**Arthur Zastepa** – Environment and Climate Change Canada, Canada Centre for Inland Waters, Burlington, Ontario L7S1A1, Canada

Complete contact information is available at <https://pubs.acs.org/doi/10.1021/acs.est.2c06928>

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

We thank the Sunfish Lake Association (in particular Kevin Thomason and Nancy Carlisle-Weaver) for sharing their public health concerns regarding the lake and for their assistance with field work. We thank Hedy Kling at Algal Taxonomy and Ecology Inc. for providing insight on cyanobacteria taxonomy, Jennifer Stockdill and Satya Rama Krishna Boddu at Wayne State University for synthesizing the cyanopeptide, Anabaenopeptin F, and Celine Siong at Environment and Climate Change Canada for assistance with the PCR analysis. This work was funded by NSERC Discovery Grants 06579-2014 to I.F.C. and 4458-2016 to C.G.T. and an NSERC CREATE Algal Bloom Abatement through Technology and Education (ABATE) (448172-2014) awarded to I.F.C. and C.G.T. Additional funding sources include NIEHS P30 ES020957

and an NSERC CGS-D and GWF PhD Excellence Scholarship awarded to K.J.E.

## ■ REFERENCES

- (1) Paerl, H. W.; Ustach, J. F. Blue-green algal scums: An explanation for their occurrence during freshwater blooms. *Limnol. Oceanogr.* **1982**, *27*, 212–217.
- (2) Winter, J. G.; DeSellas, A. M.; Fletcher, R.; Heintsch, L.; Morley, A.; Nakamoto, L.; Utsumi, K. Algal blooms in Ontario, Canada: Increases in reports since 1994. *Lake Reserv. Manage.* **2011**, *27*, 107–114.
- (3) Erratt, K. J.; Creed, I. F.; Trick, C. G. Harmonizing science and management options to reduce risks of cyanobacteria. *Harmful Algae* **2022**, *116*, No. 102264.
- (4) Dokulil, M. T.; Teubner, K. Deep living *Planktothrix rubescens* modulated by environmental constraints and climate forcing. In *Phytoplankton responses to human impacts at different scales*; Springer: Dordrecht, 2012; 29–46.
- (5) Wood, S. A.; Kelly, L.; Bouma-Gregson, K.; Humbert, J. F.; Laughinghouse, H. D.; Lazorchak, J.; McCallister, T.; McQueen, A.; Pokrzywinski, K.; Puddick, J.; Quiblier, C. Toxic benthic freshwater cyanobacterial proliferations: challenges and solutions for enhancing knowledge and improving monitoring and mitigation. *Freshw. Biol.* **2020**, *65*, 1824–1842.
- (6) Jacquet, S.; Briand, J. F.; Leboulanger, C.; Avois-Jacquet, C.; Oberhaus, L.; Tassin, B.; Vinçon-Leite, B.; Paolini, G.; Druart, J. C.; Anneville, O.; Humbert, J. F. The proliferation of the toxic cyanobacterium *Planktothrix rubescens* following restoration of the largest natural French lake (Lac du Bourget). *Harmful Algae* **2005**, *4*, 651–672.
- (7) Knapp, D.; Fernández Castro, B.; Marty, D.; Loher, E.; Köster, O.; Wüest, A.; Posch, T. The red harmful plague in times of climate change: blooms of the cyanobacterium *Planktothrix rubescens* triggered by stratification dynamics and irradiance. *Front. Microbiol.* **2021**, *12*, No. 705914.
- (8) Konopka, A. Metalimnetic cyanobacteria in hard-water lakes: buoyancy regulation and physiological state. *Limnol. Oceanogr.* **1989**, *34*, 1174–1184.
- (9) Perkins, S. *Going beneath the surface: The ecology of metalimnetic cyanobacteria layers in three New Hampshire Lakes*; MSc Thesis. University of New Hampshire: New Hampshire, USA, 2019.
- (10) Lenard, T. Metalimnetic bloom of *Planktothrix rubescens* in relation to environmental conditions. *Oceanol. Hydrobiol. Stud.* **2009**, *38*, 45–53.
- (11) Trbojević, I.; Blagojević, A.; Kostić, D.; Marjanović, P.; Krizmanić, J.; Popović, S.; Simić, G. S. Periphyton development during summer stratification in the presence of a metalimnetic bloom of *Planktothrix rubescens*. *Limnologica* **2019**, *78*, No. 125709.
- (12) Selmečzy, G. B.; Tapolczai, K.; Casper, P.; Krienitz, L.; Padišák, J. Spatial and niche segregation of DCM-forming cyanobacteria in Lake Stechlin (Germany). *Hydrobiologia* **2016**, *764*, 229–240.
- (13) Briand, J. F.; Jacquet, S.; Flinois, C.; Avois-Jacquet, C.; Maisonnète, C.; Leberre, B.; Humbert, J. F. Variations in the microcystin production of *Planktothrix rubescens* (Cyanobacteria) assessed from a four-year survey of Lac du Bourget (France) and from laboratory experiments. *Microb. Ecol.* **2005**, *50*, 418–428.
- (14) Walsby, A. E.; Schanz, F.; Schmid, M. The Burgundy-blood phenomenon: a model of buoyancy change explains autumnal water blooms by *Planktothrix rubescens* in Lake Zürich. *New Phytol.* **2006**, *169*, 109–122.
- (15) Chorus, I.; Fastner, J.; Welker, M. Cyanobacteria and cyanotoxins in a changing environment: concepts, controversies, challenges. *Water* **2021**, *13*, 2463.
- (16) Salmaso, N.; Copetti, D.; Cerasino, L.; Shams, S.; Capelli, C.; Boscaini, A.; Valsecchi, L.; Pozzoni, F.; Guzzella, L. Variability of microcystin cell quota in metapopulations of *Planktothrix rubescens*: causes and implications for water management. *Toxicol.* **2014**, *90*, 82–96.

- (17) Bogialli, S.; Nigro di Gregorio, F.; Lucentini, L.; Ferretti, E.; Ottaviani, M.; Ungaro, N.; Cannarozzi de Grazia, M. Management of a toxic cyanobacterium bloom (*Planktothrix rubescens*) affecting an Italian drinking water basin: a case study. *Environ. Sci. Technol.* **2013**, *47*, 574–583.
- (18) Le Vu, B.; Vinçon-Leite, B.; Lemaire, B. J.; Bensoussan, N.; Calzas, M.; Drezen, C.; Deroubaix, J. F.; Escoffier, N.; Degres, Y.; Freissinet, C.; Groleau, A. High-frequency monitoring of phytoplankton dynamics within the European water framework directive: application to metalimnetic cyanobacteria. *Biogeochemistry* **2011**, *106*, 229–242.
- (19) Nürnberg, G. K.; LaZerte, B. D.; Olding, D. D. An artificially induced *Planktothrix rubescens* surface bloom in a small kettle lake in Southern Ontario compared to blooms world-wide. *Lake Reserv. Manag.* **2003**, *19*, 307–322.
- (20) Flage, R.; Aven, T. Emerging risk—conceptual definition and a relation to black swan type of events. *Reliab. Eng. Syst. Saf.* **2015**, *144*, 61–67.
- (21) Duthie, H. C.; Carter, J. C. H. The meromixis of Sunfish Lake, Southern Ontario. *J. Fish. Res. Board Can.* **1970**, *27*, 847–856.
- (22) Erratt, K.; Creed, I. F.; Favot, E. J.; Todoran, I.; Tai, V.; Smol, J. P.; Trick, C. G. Paleolimnological evidence reveals climate-related pre-eminence of cyanobacteria in a temperate meromictic lake. *Can. J. Fish. Aquat. Sci.* **2022**, *79*, 558–565.
- (23) Brown, S. R.; McIntosh, H. J. The fossil history of sulphur phototrophs in a meromictic lake ecosystem. *Acta Acad. Abo.* **1987**, *47*, 83–95.
- (24) Paxinos, R.; Mitchell, J. G. A rapid Utermöhl method for estimating algal numbers. *J. Plankton Res.* **2000**, *22*, 2255–2262.
- (25) Wetzel, R. G.; Likens, G. *Limnological analyses*; Springer Science & Business Media, 2000.
- (26) Erratt, K. J.; Creed, I. F.; Trick, C. G. Comparative effects of ammonium, nitrate and urea on growth and photosynthetic efficiency of three bloom-forming cyanobacteria. *Freshw. Biol.* **2018**, *63*, 626–638.
- (27) Lawrenz, E.; Fedewa, E. J.; Richardson, T. L. Extraction protocols for the quantification of phycobilins in aqueous phytoplankton extracts. *J. Appl. Phycol.* **2011**, *23*, 865–871.
- (28) Andersen, R.A. *Algal culturing techniques*; Elsevier: New York, 2005; 578.
- (29) Scheffer, M.; Rinaldi, S.; Gragnani, A.; Mur, L. R.; van Nes, E. H. On the dominance of filamentous cyanobacteria in shallow, turbid lakes. *Ecology* **1997**, *78*, 272–282.
- (30) Stomp, M.; Huisman, J.; Vörös, L.; Pick, F. R.; Laamanen, M.; Haverkamp, T.; Stal, L. J. Colourful coexistence of red and green picocyanobacteria in lakes and seas. *Ecol. Lett.* **2007**, *10*, 290–298.
- (31) Lau, M. P.; Valerio, G.; Pilotti, M.; Hupfer, M. Intermittent meromixis controls the trophic state of warming deep lakes. *Sci. Rep.* **2020**, *10*, 12928.
- (32) Van de Waal, D. B.; Verspagen, J. M.; Lüring, M.; Van Donk, E.; Visser, P. M.; Huisman, J. The ecological stoichiometry of toxins produced by harmful cyanobacteria: an experimental test of the carbon-nutrient balance hypothesis. *Ecol. Lett.* **2009**, *12*, 1326–1335.
- (33) Liu, J.; Van Oosterhout, E.; Faassen, E. J.; Lüring, M.; Helmsing, N. R.; Van de Waal, D. B. Elevated pCO<sub>2</sub> causes a shift towards more toxic microcystin variants in nitrogen-limited *Microcystis aeruginosa*. *FEMS Microbiol. Ecol.* **2016**, *92*, No. fiv159.
- (34) Chernoff, N.; Hill, D.; Lang, J.; Schmid, J.; Le, T.; Farthing, A.; Huang, H. The comparative toxicity of 10 microcystin congeners administered orally to mice: clinical effects and organ toxicity. *Toxins (Basel)* **2020**, *12*, 403.
- (35) Walsby, A. E.; Schanz, F. Light-dependent growth rate determines changes in the population of *Planktothrix rubescens* over the annual cycle in Lake Zürich, Switzerland. *New Phytol.* **2002**, *154*, 671–687.
- (36) Yankova, Y.; Villiger, J.; Pernthaler, J.; Schanz, F.; Posch, T. Prolongation, deepening and warming of the metalimnion change habitat conditions of the harmful filamentous cyanobacterium *Planktothrix rubescens* in a prealpine lake. *Hydrobiologia* **2016**, *776*, 125–138.
- (37) Merel, S.; Walker, D.; Chicana, R.; Snyder, S.; Baurès, E.; Thomas, O. State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. *Environ. Int.* **2013**, *59*, 303–327.
- (38) Hutchinson, G. E. The paradox of the plankton. *Am. Nat.* **1961**, *95*, 137–145.
- (39) Trainer, V. L.; Moore, S. K.; Hallegraef, G.; Kudela, R. M.; Clement, A.; Mardones, J. I.; Cochlan, W. P. Pelagic harmful algal blooms and climate change: Lessons from nature's experiments with extremes. *Harmful Algae* **2020**, *91*, 101591.
- (40) Ostermaier, V.; Schanz, F.; Köster, O.; Kurmayer, R. Stability of toxin gene proportion in red-pigmented populations of the cyanobacterium *Planktothrix* during 29 years of re-oligotrophication of Lake Zürich. *BMC Biol.* **2012**, *10*, 100.
- (41) Akcaalan, R.; Young, F. M.; Metcalf, J. S.; Morrison, L. F.; Albay, M.; Codd, G. A. Microcystin analysis in single filaments of *Planktothrix* spp. in laboratory cultures and environmental blooms. *Water Res.* **2006**, *40*, 1583–1590.
- (42) Cerasino, L.; Shams, S.; Boscaini, A.; Salmaso, N. Multiannual trend of microcystin production in the toxic cyanobacterium *Planktothrix rubescens* in Lake Garda (Italy). *Chem. Ecol.* **2016**, *32*, 492–506.
- (43) Fastner, J.; Erhard, M.; Carmichael, W. W.; Sun, F.; Rinehart, K. L.; Röncke, H.; Chorus, I. Characterization and diversity of microcystins in natural blooms and strains of the genera *Microcystis* and *Planktothrix* from German freshwaters. *Arch. Hydrobiol.* **1999**, *145*, 147–164.
- (44) Zastepa, A.; Miller, T. R.; Watson, L. C.; Kling, H.; Watson, S. B. Toxins and other bioactive metabolites in deep chlorophyll layers containing the cyanobacteria *Planktothrix cf. isothrix* in two Georgian Bay embayments, Lake Huron. *Toxins* **2021**, *13*, 445.
- (45) Janssen, E. M. L. Cyanobacterial peptides beyond microcystins—A review on co-occurrence, toxicity, and challenges for risk assessment. *Water Res.* **2019**, *151*, 488–499.
- (46) Grach-Pogrebinsky, O.; Sedmak, B.; Carmeli, S. Seco [d-Asp3] microcystin-RR and [d-Asp3, d-Glu (Ome) 6] microcystin-RR, two new microcystins from a toxic water bloom of the cyanobacterium *Planktothrix rubescens*. *J. Nat. Prod.* **2004**, *67*, 337–342.
- (47) Viaggiu, E.; Melchiorre, S.; Volpi, F.; Di Corcia, A.; Mancini, R.; Garibaldi, L.; Bruno, M. Anatoxin-a toxin in the cyanobacterium *Planktothrix rubescens* from a fishing pond in northern Italy. *Environ. Toxicol.* **2004**, *19*, 191–197.
- (48) Blom, J. F.; Bister, B.; Bischoff, D.; Nicholson, G.; Jung, G.; Süßmuth, R. D.; Jüttner, F.; Oscillapeptin, J. A new grazer toxin of the freshwater cyanobacterium *Planktothrix rubescens*. *J. Nat. Prod.* **2003**, *66*, 431–434.
- (49) Baumann, H. I.; Keller, S.; Wolter, F. E.; Nicholson, G. J.; Jung, G.; Süßmuth, R. D.; Jüttner, F. Planktoicyclin, a cyclooctapeptide protease inhibitor produced by the freshwater cyanobacterium *Planktothrix rubescens*. *J. Nat. Prod.* **2007**, *70*, 1611–1615.
- (50) Schwarzenberger, A.; Kurmayer, R.; Martin-Creuzburg, D. Toward disentangling the multiple nutritional constraints imposed by *Planktothrix*: The significance of harmful secondary metabolites and sterol limitation. *Front. Microbiol.* **2020**, *11*, No. 586120.
- (51) Beversdorf, L. J.; Rude, K.; Weirich, C. A.; Bartlett, S. L.; Seaman, M.; Kozik, C.; Miller, T. R. Analysis of cyanobacterial metabolites in surface and raw drinking waters reveals more than microcystin. *Water Res.* **2018**, *140*, 280–290.
- (52) Rohrlack, T.; Christiansen, G.; Kurmayer, R. Putative antiparasite defensive system involving ribosomal and nonribosomal oligopeptides in cyanobacteria of the genus *Planktothrix*. *Appl. Environ. Microbiol.* **2013**, *79*, 2642–2647.
- (53) Moustaka-Gouni, M.; Sommer, U.; Katsiapi, M.; Vardaka, E. Monitoring of cyanobacteria for water quality: doing the necessary right or wrong? *Mar. Freshw. Res.* **2020**, *71*, 717–724.
- (54) Quiblier, C.; Wood, S. A.; Echenique-Subiabre, I.; Heath, M.; Villeneuve, A.; Humbert, J.-F. A review of current knowledge on toxic

benthic freshwater cyanobacteria—ecology, toxin production and risk management. *Water Res.* **2013**, *47*, 5464–5479.