Assessing spatial distributions of cyanobacteria and microcystins in NH lakes with implications for lake monitoring

Amanda Lee Murby

University of New Hampshire, Durham

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Assessing spatial distributions of cyanobacteria and microcystins in NH lakes with implications for lake monitoring

Abstract
Cyanobacteria pose a threat to the health of humans and wildlife when concentrations are high and the species present are toxic. However, routine monitoring of toxic cyanobacteria in lakes is not typical in the United States and, currently, there are no standards on the allowable limit of cyanotoxins in N.H. water bodies. Phycocyanin (PC) fluorescence was evaluated as an indicator for cyanobacteria densities, with implications for monitoring cyanobacteria and microcystins in lakes. PC fluorescence predicted cultured cyanobacteria, ranging from 0--900,000 cells ml-1 (Adj R 2 0.99, p<0.001). PC fluorescence was also useful for estimating microcystin (MC) concentrations among lakes of varying trophic status (p<0.001) and was more significant than net cyanobacteria counts were for predicting microcystin concentrations (p=0.049). Synoptic, horizontal sampling revealed the significant variability in the spatial distributions of cyanobacteria and microcystins in N.H. lakes.

Keywords
Biology, Microbiology, Biology, Limnology, Water Resource Management

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ASSESSING SPATIAL DISTRIBUTIONS OF CYANOBACTERIA AND MICROCYSTINS IN N.H. LAKES WITH IMPLICATIONS FOR LAKE MONITORING

BY

AMANDA LEE MURBY
B.S. University of New Hampshire, 2006

THESIS

Submitted to the University of New Hampshire in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Zoology

December, 2009
This thesis has been examined and approved.

Thesis Director, James F. Haney
Professor of Zoology

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Associate Professor of Plant Biology

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Professor Emeritus of Zoology

Jeff Schloss,
Extension Professor of Water Quality

Date
10 December 2009
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I dedicate this thesis to my dog and friend, Ava.
# TABLE OF CONTENTS

**ACKNOWLEDGMENTS**

**LIST OF TABLES**

**LIST OF FIGURES**

**ABSTRACT**

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. MATERIALS AND METHODS</td>
<td>6</td>
</tr>
<tr>
<td>Preliminary Testing of the Phycocyanin Fluorescence Sensor</td>
<td>6</td>
</tr>
<tr>
<td>Sampling Sites</td>
<td>6</td>
</tr>
<tr>
<td>Deployment of Drogues</td>
<td>9</td>
</tr>
<tr>
<td>Field Sampling</td>
<td>9</td>
</tr>
<tr>
<td>MC Analysis via Enzyme-Linked Immunosorbent Assay (ELISA)</td>
<td>13</td>
</tr>
<tr>
<td>Statistical Analysis</td>
<td>14</td>
</tr>
<tr>
<td>III. RESULTS</td>
<td>15</td>
</tr>
<tr>
<td>Quantifying Cyanobacteria Abundance from Phycocyanin Fluorescence</td>
<td>15</td>
</tr>
<tr>
<td>Trophic Conditions of Study Lakes</td>
<td>18</td>
</tr>
<tr>
<td>Lake Cyanobacteria Concentrations</td>
<td>22</td>
</tr>
<tr>
<td>Lake Microcystin Concentrations</td>
<td>27</td>
</tr>
</tbody>
</table>
Spatial Distributions of Cyanobacteria & Microcystin in Lakes of Varying Trophic Status

Spatial Comparisons

Down-current vs. Up-current

Deep Site vs. Shoreline

Assessing Trends Between Trophic Status Parameters and Shoreline Cyanobacteria Dominance

Comprehensive Arcmap-Derived Spatial Distributions

Summer Spatial Distributions of Cyanobacteria & Microcystins in Barbadoes Pond

Comparison of Cyanobacteria Distributions with wind and water currents in Barbadoes Pond

IV. DISCUSSION

Quantifying Cyanobacteria and Microcystin from Phycocyanin Fluorometry

Spatial Comparisons of the Distribution of Cyanobacteria and Microcystins

Spatial Comparisons of Cyanobacteria and Microcystins in Barbadoes Pond

Trophic Relationships

Application of in situ Phycocyanin Fluorescence Monitoring: A Case Study in Willand Pond

LIST OF REFERENCES
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Physical features of the five New Hampshire lakes examined in the study</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>Mean total nitrogen, total phosphorus, chlorophyll a (extracted &amp; fluorescence), phycocyanin fluorescence, and Secchi disk depths</td>
<td>19</td>
</tr>
<tr>
<td>3</td>
<td>Genera of cyanobacteria (colonies ml(^{-1})) and free microcystins (ng L(^{-1})) measured from different regions of each of the study lakes</td>
<td>28</td>
</tr>
<tr>
<td>4a</td>
<td>Total net cyanobacteria (colonies ml(^{-1})) sampled from Barbadoes Pond</td>
<td>29</td>
</tr>
<tr>
<td>4b</td>
<td>Microcystins (ng L(^{-1})) measured from whole lake water in Barbadoes Pond</td>
<td>29</td>
</tr>
<tr>
<td>5</td>
<td>Microcystins (ng L(^{-1})) measured from integrated water collected along various transects</td>
<td>29</td>
</tr>
<tr>
<td>6</td>
<td>Microcystins ((\mu g) MC g(^{-1}) dwt) extracted from Daphnia</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>Microcystins (ng L(^{-1})) measured from sediment</td>
<td>31</td>
</tr>
<tr>
<td>FIGURE</td>
<td>PAGE</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>Figure 1. Transects marked at each of the study lakes</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Figure 2. Log-log regression of phycocyanin fluorescence (equivalent <em>Microcystis</em> cells ml$^{-1}$) with serial dilutions of natural colonies of <em>Microcystis aeruginosa</em> (cells ml$^{-1}$) collected from Barbados Pond, Madbury, NH</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Figure 3. Regression of phycocyanin fluorescence (equivalent <em>Microcystis</em> cells ml$^{-1}$) with serial dilutions of filaments of cultured <em>Anabaena flos-aquae</em> (cells ml$^{-1}$)</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Figure 4. Microcystins (bars) and total phosphorus (TP) concentrations (scatter &amp; line plot) from each of the study lakes, in order of trophic status characterized by TP</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Figure 5. Regression of total phosphorus (TP) with microcystins measured from whole lake water</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Figure 6. Regression of total nitrogen (TN) with microcystins measured from whole lake water</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Figure 7. Regression of Secchi disk depth (SDD) with microcystins measured from whole lake water</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Figure 8. Net phytoplankton abundances from separate regions of Barbados Pond on September 13, 2007. Bottom stacks (black) signify cyanobacteria abundance within net phytoplankton populations (Full stacks)</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Figure 9. Net phytoplankton abundances from three separate regions of Willand Pond on September 27, 2007. Bottom stacks (black) signify cyanobacteria abundance within net phytoplankton populations (Full stacks)</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Figure 10. Net phytoplankton abundances from three separate regions of Silver Lake on September 20, 2007. Bottom stacks (black) signify cyanobacteria abundance within net phytoplankton populations (Full stacks)</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Figure 11. Net phytoplankton abundances from three separate regions of Mirror Lake on October 11, 2007. Bottom stacks (black) signify cyanobacteria abundance within net phytoplankton populations (Full stacks)</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>
Figure 12. Net phytoplankton abundances from three separate regions of Sawyer Pond on October 4, 2007. Bottom stacks (black) signify cyanobacteria abundance within net phytoplankton populations (Full stacks).

Figure 13. Regression of phycocyanin fluorescence with net cyanobacteria measured from lakes of varying trophic status.

Figure 14. Regression of phycocyanin with net Microcystis (estimated cells ml⁻¹) measured from lakes of varying trophic status.

Figure 15. Regression of phycocyanin fluorescence with WLW microcystins measured from lakes of varying trophic status.

Figure 16. Regression of net cyanobacteria with whole lake water microcystins measured from lakes of varying trophic status.

Figure 17. Cyanobacteria concentrations measured (via PC fluorometry) from five different regions of Barbadoes Pond on September 13, 2007.

Figure 18. Cyanobacteria concentrations measured from five different regions of Willand Pond on September 27, 2007.

Figure 19. Cyanobacteria concentrations measured (via PC fluorometry) from five different regions of Silver Lake on September 20, 2007.

Figure 20. Cyanobacteria concentrations measured (via PC fluorometry) from five different regions of Mirror Lake on October 11, 2007.

Figure 21. Cyanobacteria concentrations measured (via PC fluorometry) from five different regions of Lower Sawyer Pond on October 4, 2007.

Figure 22. Cyanobacteria concentrations measured (via PC fluorometry) from the west and east embayment of Willand Pond on September 27, 2007.

Figure 23. Cyanobacteria concentrations (via PC fluorometry) compared between the down-current up-current areas of Barbadoes Pond on September 13, 2007.

Figure 24. Cyanobacteria concentrations (via PC fluorometry) compared between the down-current and up-current areas of Willand Pond on September 27, 2007.

Figure 25. Cyanobacteria concentrations (via PC fluorometry) compared between the down-current and up-current areas of Silver Lake on September 20, 2007.
Figure 26. Cyanobacteria concentrations (via PC fluorometry) compared between the down-current and up-current areas of Mirror Lake on October 11, 2007.

Figure 27. Cyanobacteria concentrations (via PC fluorometry) compared between the down-current and up-current areas of Sawyer Pond on October 4, 2007.

Figure 28. Cyanobacteria concentrations (via PC fluorometry) compared between the deep site and the shoreline of Barbadoes Pond on September 13, 2007.

Figure 29. Cyanobacteria concentrations (via PC fluorometry) compared between the deep site and the shoreline of Willand Pond on September 27, 2007.

Figure 30. Cyanobacteria concentrations (via PC fluorometry) compared between the deep site and the shoreline of Silver Lake on September 20, 2007.

Figure 31. Cyanobacteria concentrations (via PC fluorometry) compared between the deep site and the shoreline of Mirror Lake on October 11, 2007.

Figure 32. Cyanobacteria concentrations (via PC fluorometry) compared between the deep site and the shoreline of Lower Sawyer Pond on October 4, 2007.

Figure 33. Regression of shoreline PC: deep site PC with chlorophyll a (µg L\(^{-1}\)).

Figure 34. Regression of the ratio of shoreline PC: deep site PC with TP (µg L\(^{-1}\)).

Figure 35. Regression of the ratio of shoreline PC: deep site PC with TN (µg L\(^{-1}\)).

Figure 36. Regression of the ratio of shoreline PC: deep site PC with SDD (m).

Figure 37. Regression of the ratio of shoreline PC: deep site PC with MC (ng L\(^{-1}\)).

Figure 38. Image-maps indicating spatial changes in cyanobacteria and chlorophyll within Barbadoes Pond on September 13, 2007.

Figure 39. Arcmap image-maps indicating spatial changes in cyanobacteria and chlorophyll within Willand Pond on September 27, 2007.

Figure 40. Arcmap image-maps indicating spatial changes in cyanobacteria and chlorophyll within Silver Lake on September 20, 2007.

Figure 41. Arcmap image-maps indicating spatial changes in cyanobacteria and chlorophyll within Mirror Lake on October 11, 2007.
Figure 42. Arcmap image-maps indicating spatial changes in cyanobacteria and chlorophyll within Lower Sawyer Pond on October 4, 2007

Figure 43. Regression of phycocyanin fluorescence with microcystin measured from whole lake water in Barbados Pond over the summer of 2007

Figure 44. Regression of net cyanobacteria with microcystin measured from whole lake water in Barbados Pond over the summer of 2007

Figure 45. Phycocyanin fluorescence measured from five different regions of Barbados Pond on June 21, 2007

Figure 46. Cyanobacteria concentrations measured from five different regions of Barbados Pond on July 10, 2007

Figure 47. Cyanobacteria concentrations (via PC fluorometry) measured from five different regions of Barbados Pond on July 21, 2007

Figure 48. Cyanobacteria concentrations measured from five different regions of Barbados Pond on August 24, 2007

Figure 49. Change in abundance and spatial distributions of cyanobacteria (via PC fluorometry) within Barbados Pond over the summer of 2007

Figure 50. Cyanobacteria concentrations (via PC fluorometry) compared between the deep site and the shoreline of Barbados Pond on June 21

Figure 51. Cyanobacteria concentrations (via PC fluorometry) compared between the deep site and the shoreline of Barbados Pond on July 10

Figure 52. Cyanobacteria concentrations (via PC fluorometry) compared between the deep site and the shoreline of Barbados Pond on July 21
Figure 53. Cyanobacteria concentrations compared between the deep site and the shoreline of Barbadoes Pond on August 24.

Figure 54. Cyanobacteria concentrations (via PC fluorometry) compared between the downwind and upwind areas of Barbadoes Pond on June 21.

Figure 55. Cyanobacteria concentrations (via PC fluorometry) compared between the downwind and upwind areas of Barbadoes Pond on July 10.

Figure 56. Cyanobacteria concentrations (via PC fluorometry) compared between the downwind and upwind areas of Barbadoes Pond on July 21.

Figure 57. Cyanobacteria concentrations (via PC fluorometry) compared between the downwind and upwind areas of Barbadoes Pond on August 24.

Figure 58. Cyanobacteria concentrations (via PC fluorometry) compared between the downwind and upwind areas of Barbadoes Pond on September 13.

Figure 59. Cyanobacteria concentrations (via PC fluorometry) compared between the down-current and up-current areas of Barbadoes Pond on July 21.

Figure 60. Cyanobacteria concentrations (via PC fluorometry) compared between the down-current and up-current areas of Barbadoes Pond on August 24.

Figure 61. *Microcystis aeruginosa* cells within mucilaginous colonies.

Figure 62. Surface bloom of Cyanobacteria (*Microcystis* and *Anabaena* spp.) collected from Willand Pond on June 12th, 2009.

Figure 63. Map displaying spatial distributions of cyanobacteria within Willand Pond, with aerial view of surrounding landscape.

Figure 64. Spatial distributions of phycocyanin fluorescence in ArcMap 9.2.

Figure 65. Spatial distributions of predicted microcystins ArcMap 9.2.
ABSTRACT

ASSESSING SPATIAL DISTRIBUTIONS OF CYANOBACTERIA AND MICROCYSTINS IN N.H. LAKES WITH IMPLICATIONS FOR LAKE MONITORING

BY

Amanda Lee Murby
University of New Hampshire, December, 2009

Cyanobacteria pose a threat to the health of humans and wildlife when concentrations are high and the species present are toxic. However, routine monitoring of toxic cyanobacteria in lakes is not typical in the United States and, currently, there are no standards on the allowable limit of cyanotoxins in N.H. water bodies. Phycocyanin (PC) fluorescence was evaluated as an indicator for cyanobacteria densities, with implications for monitoring cyanobacteria and microcystins in lakes. PC fluorescence predicted cultured cyanobacteria, ranging from 0-900,000 cells ml$^{-1}$ (Adj $R^2$ 0.99, $p<0.001$). PC fluorescence was also useful for estimating microcystin (MC) concentrations among lakes of varying trophic status ($p<0.001$) and was more significant than net cyanobacteria counts were for predicting microcystin concentrations ($p=0.049$). Synoptic, horizontal sampling revealed the significant variability in the spatial distributions of cyanobacteria and microcystins in N.H. lakes.

December, 2009
CHAPTER I

INTRODUCTION

Water quality and the preservation of natural freshwater ecosystems is a major concern in the world today. With climate changes and other anthropogenic affects on the environment, freshwater systems have increased in nutrients and productivity allowing algal blooms to occur more often (Gilbert & Burkholder, 2006; Chorus & Bartram, 1999). Blooms containing toxic cyanobacteria in freshwater systems are a risk to the public health of humans and animals (Cox et al., 2003; Carmichael et al., 2001; Falconer, 1989). Surface winds and water currents may concentrate cyanobacteria along shorelines, where dangerous levels of cyanotoxicity can reach more than 1000-fold above those found in the open water (Chorus, 2001).

Within a lake, localized blooms may develop as cyanobacteria become concentrated in particular regions. Localized surface blooms are common in mesotrophic lakes, whereas in highly eutrophic systems the algal community seems distributed homogenously as green, turbid water throughout the entire lake. Visible scums are usually a sign of intense cyanobacteria production and are often the basis for bloom reporting. However, visibility of surface scums may decrease or disappear completely once a bloom disperses. In addition, surface blooms can be blown around the lake or driven by natural lake currents, possibly creating patches of cyanobacteria to occur across the lake (Marce et al., 2007). Wind can also mix blooms into the epilimnion,
where these particles may eventually sink or be carried by currents. Since visible blooms
do not (typically) remain very long after being reported in N.H. lakes, it is difficult for
monitors to determine when cyanobacteria levels are safe for public use (Hudnell, 2008;

Given that many taxa of cyanobacteria produce toxins, high density blooms of
cyanobacteria are a public health concern when toxic species are present (Watanabe et
al., 1996; Rapala & Sivonen, 1997). General symptoms, caused by a wide variety of
cyanotoxins, include nausea, vomiting, giddiness, stomachache, diarrhea, cramps, body
pains and weakness (Carmichael & Gorham, 1977; Carmichael 1992). *Microcystis* is a
common cyanobacterium often found in New Hampshire lakes and known for producing
hepatotoxins, such as microcystins (Haney & Ikawa, 2000; Foxall, 1980; Foxall & Sasner,
1981; Botes *et al.*, 1982). Acute poisoning by ingesting hepatotoxins may lead to rapid
death due to liver hemorrhaging, as well as chronic poisoning resulting in liver cancer
and/or tumors (Carmichael *et al.*, 2001; Jochimsen *et al.*, 1998). While human deaths
have been reported due to cyanotoxins (Azevedo *et al.*, 2002, Dominos *et al*, 1999),
there is no guideline on the maximum acceptable concentration (MAC) of microcystins
(or other cyanotoxins) allowed in drinking water, nor are there consistent federal
regulations on public access to lakes with potentially harmful levels of microcystin in the
United States (Falconer 1999). Though some water quality groups use the standards set
by the World Health Organization (WHO) of 1 µg L⁻¹ microcystins in drinking water as a
general guideline (WHO, 2006). The Harmful Algal Research and Response National
Environmental Science Strategy (HARRNESS) is the newest national U.S. plan for
addressing harmful algal research and response, but they note that “there is no comprehensive source of information on the occurrence of harmful algal blooms in the U.S.” (HARRNESS, 2005).

Relationships between the distribution, size and toxicity of cyanobacteria are important to consider in the approach to monitoring cyanobacteria in lakes. Visible surface blooms are largely made up of colonial cyanobacteria, such as *Anabaena* and *Microcystis*. However, the abundance and spatial distributions of pico-cyanobacteria (0.2-2 µm) within lakes is not well-understood because most sampling for toxic cyanobacteria is done with net sizes of 20-50 µm mesh diameters. Pico-cyanobacteria, such as *Gleocapsa* sp. (as single-cells) and *Synechoccus* sp., have been reported as toxin-producers (Carmichael & Li 2006) although, are not typically evaluated in net samples from surface blooms.

Fluorometry is a valuable tool for determining algal abundance (Ahn, 2007; Gregor & Marsalek, 2003; Millie et al., 2002). In addition to chlorophyll *a* extractions, the use of chlorophyll *a* fluorescence has been widely accepted as a functional method for measuring lake productivity and algal biomass (Maxwell & Johnson, 2000). Because phycocyanin (PC) is an accessory pigment found almost exclusively in cyanobacteria, densities of cyanobacteria may be estimated via PC fluorescence. However, phycocyanin fluorometry is a relatively new technique for estimating cyanobacteria abundance and its application is currently under investigation. The stability of phycocyanin fluorescence has been questioned as the fluorescence response may change due to the adaptation of the cyanobacterial photosynthetic apparatus to different environmental conditions such
as light and nutrients (Brient et al., 2008; Beutler 2003). Yet, recent studies have also indicated the usefulness of PC fluorescence as an early detection method of cyanobacteria in drinking water supplies (Gregor et al. 2007; Izydorczyk et al. 2005; Lee et al. 1994). Others have validated that the use of a PC fluorescence sensor could help routinely monitor lakes for cyanobacteria. Furthermore, its application could determine detection limits of cyanobacteria (cells ml$^{-1}$) within lakes, defined at integral levels of cyanobacteria warnings (Ahn, 2007).

Tracking the spatial distributions of phycocyanin fluorescence has the potential to reveal the spatial dynamics of cyanobacteria and microcystins within lakes of varying trophic status. This information could be used to better understand the spatial patterns of toxic algal blooms, which have important implications for monitoring multiple sampling sites of a lake. Also, measurements with in situ PC fluorescence sensors could provide information on all sizes of cyanobacteria, including cells of pico-cyanobacteria, which are small enough to be grazed upon by some of the zooplankton (Chan et al., 2004; Ghadouani et al., 2004; Epp, 1996; Burns, 1987).

In the present study, microcystins (ng L$^{-1}$) were measured for the spatial assessment of toxic cyanobacteria, as determined by the ELISA method, due to its widespread occurrence throughout New Hampshire lakes (Haney &. Ikawa, 2000) and throughout the world (Chorus, 2001). Objectives of the study were to examine the patterns in the spatial and temporal distributions of cyanobacteria and microcystins in N.H. lakes with contrasting morphologies, trophic conditions and uses. Results of the study support the need for a synoptic sampling approach in routine monitoring of
cyanobacteria and cyanotoxins, which may enhance efforts in cyanobacteria monitoring within lake management plans in New Hampshire.

The hypotheses of the study were to test whether

1. Phycocyanin fluorescence would be a useful tool for measuring cyanobacteria concentrations across lakes of varying trophic status, with implications for lake monitoring.

2. Subsurface, horizontal distributions of cyanobacteria and microcystins would be significantly different within lakes, especially in mesotrophic-oligotrophic lakes.

3. Cyanobacteria would be more abundant in regions of the lake that were down-current and/or downwind and whether densities would be higher along the shoreline.
CHAPTER II

MATERIAL AND METHODS

Preliminary Testing of the Phycocyanin Fluorescence Sensor

A newly designed phycocyanin fluorescence sensor (Yellow Springs Instruments, Yellow Springs, OH, model 6131 Phycocyanin, blue-green algae sensor) was tested to determine the concentration of cyanobacteria (cells ml\(^{-1}\)). The sensor was calibrated with cultured, single-celled *Microcystis aeruginosa* (Utex #2385) and distilled water. Lab cultures of *Anabaena flos-aquae* (Utex #2557) were sub-sampled and enumerated via the hemacytometer method (Fisher Scientific Co.). A stock solution of the *Anabaena* cells was serial diluted with filtered well water to obtain solutions ranging from 0-900,000 cells ml\(^{-1}\). The probe was lowered into each dilution to assess the accuracy of the fluorescence readings (converted to equivalent *Microcystis* cells ml\(^{-1}\)). This was also done with natural colonies of *Microcystis aeruginosa*, collected from the shoreline of Barbadoes Pond with a 50 µm Nitex mesh net. Cells from natural colonies were enumerated and serial dilutions were made for testing of the phycocyanin fluorescence sensor to differentiate cells within natural colonies of cyanobacteria.

Sampling Sites

New Hampshire lakes are typically considered mesotrophic to oligotrophic when assessed with trophic scales used nationally (Forsberg & Ryding 1987). However, New Hampshire lakes with total phosphorous (TP) concentrations greater than 10 mg L\(^{-1}\)
could be considered eutrophic due to the association with periodic cyanobacteria blooms. In this study, five lakes representing a variety of lake types in N.H. (Table 1) were chosen to examine differences in the distributions of subsurface populations of cyanobacteria and microcystins. The lakes included Barbadoes Pond (BP) (eutrophic), Willand Pond (WP) (eutrophic), Silver Lake (SL) (meso-eutrophic), Mirror Lake (ML) (meso-oligotrophic), and Lower Sawyer Pond (LSP) (oligotrophic). In addition, Barbadoes Pond was sampled four times in the summer to assess temporal variations on the distributions of cyanobacteria and microcystins within a eutrophic N.H. lake (Haney & Ikawa, 2000).

<table>
<thead>
<tr>
<th>Lake</th>
<th>Town</th>
<th>Elev</th>
<th>Length (m)</th>
<th>Area (Ha)</th>
<th>Volume (Ha-m)</th>
<th>Mean Depth(m)</th>
<th>Max Depth(m)</th>
<th>Wtrshd Area (Ha)</th>
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<tbody>
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<td>132</td>
<td>1000</td>
<td>5.8</td>
<td>34.9</td>
<td>6.1</td>
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<td>Willand Pond</td>
<td>Somersworth</td>
<td>182</td>
<td>2700</td>
<td>34.8</td>
<td>162.7</td>
<td>4.7</td>
<td>11.2</td>
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<td>Silver Lake</td>
<td>Hollis</td>
<td>274</td>
<td>1800</td>
<td>13.6</td>
<td>36.7</td>
<td>2.7</td>
<td>7.5</td>
<td>124.1</td>
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<td>Mirror Lake</td>
<td>Tuftonoboro</td>
<td>510</td>
<td>5600</td>
<td>152.9</td>
<td>611.6</td>
<td>4.0</td>
<td>13.4</td>
<td>736.3</td>
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<td>Sawyer Pond</td>
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<td>1937</td>
<td>1900</td>
<td>19</td>
<td>231.4</td>
<td>12.2</td>
<td>29.6</td>
<td>310.8</td>
</tr>
</tbody>
</table>
Figure 1. Transects marked at each of the study lakes. a) Barbadoes Pond b) Willand Pond, c) Silver Lake d) Mirror Lake, e) Sawyer Pond. Lakes represented the seacoast (a&b), southern N.H. (c), lakes region (d), and white mountains region (e), of New Hampshire.
Deployment of Drogues

Current drogues (Jeff Schloss personal communication for design) were set from the deep site to determine direction of currents within each lake. Three drogues were deployed at depths of 0.5 m, 1.5 m and 3.0 m. Each drogue was equipped with a Garmin Geko 201, differential GPS unit to track the direction of epilimnetic water flow for a 1 h period. The prevailing direction of the subsurface, 0.5 m drogues were compared with the distributions of cyanobacteria across each lakes surface.

Field Sampling

A multi-parameter sonde (Yellow Springs Instruments Inc. (YSI) 6600 M V2, Yellow Springs, OH), coupled with a data logger (YSI 650), rapidly recorded the lake parameters in 3-second intervals to determine the chemical and physical properties of each lake. The sonde was equipped with sensors to measure depth (by pressure), temperature, dissolved oxygen (concentration and percent saturation), pH, specific conductance (corrected to 25 C), oxidation reduction potential, turbidity, and the fluorescence of chlorophyll a and phycocyanin. Multiple horizontal transects were tracked across the lake to cover a broad range of the surface area (Figure 1). The YSI 650 was set up to log coordinates along each transect (Garmin, GPSmap 76C). In addition, transects and sampling sites were marked with waypoints using the Garmin Geko, 201 GPS units. GPS data were uploaded in ArcView GIS 3.3 (Arview; ESRI Redlands, CA).
Integrated water was sampled along transects via a peristaltic pump. Tygon tubing was affixed at the depth and locations on the sensors on the multi-parameter sonde. The system was attached to a down-rigger and set at a depth just below the water surface (0.25-0.5 m). After each transect was completed, water samples were thoroughly mixed and transferred to 500 ml Nalgene bottles. Integrated water samples were put on ice, in a dark cooler and returned to the laboratory where they were frozen until MC ELISA analyses. Multi-parameter data were uploaded into Ecowatch and analyzed using Excel, SigmaPlot 11.0 and ArcMap 9.2.

In addition, three in-lake locations or “regions” were selected for collection of net plankton and water from the epilimnion (arbitrarily defined as 0-3m). Sediment from the benthos was also sampled in triplicate. The three sampling regions were chosen based on spatial orientation to parallel transects and to the deep site (which was often near the middle of the lake). Designated regions were intended to represent different areas of the lake for evaluating the spatial variation of cyanobacteria and microcystins. Regions were described as north (N), middle (M), and south (S) for Barbadoes Pond, Silver Lake and Mirror Lake and as east (E), middle (M), and west (W) for Willand Pond and Lower Sawyer Pond. Additionally, Secchi disk depths (m) were determined from the deep site (middle region) of the lake, using a black and white, 20 cm diameter disk and view scope.
**Plankton**

Vertical hauls of net plankton were collected from the epilimnion (0-3 m), using a 50 µm Nitex mesh net (25 cm diameter). The net was washed by dipping the net back into the water (to just below the opening to prevent additional volume collection) three times to ensure that all plankton (> 50 µm) were rinsed into the cod end. Live samples of plankton were put on ice until laboratory processing where *Daphnia* were isolated for MC ELISA. Samples were preserved with a 4% formalin:sucrose solution (Haney & Hall, 1973) for identification and enumeration of dominant net phytoplankton, including cyanobacteria.

Net phytoplankton were identified using Smith (1950) and confirmations from Alan Baker, Professor of Marine and Freshwater Biology at UNH. Subsamples were transferred to Palmer counting cells and enumerated under a compound microscope at 100 x magnification. Volume counted varied (100-300 ml) depending on phytoplankton densities in the sample. Net phytoplankton composition was determined after at least 200 organisms were counted from subsamples.

Live samples were viewed under a dissecting microscope and individual *Daphnia* were transferred to 1.5 ml centrifuge-tube vials (10-30 individuals/vial). *Daphnia* (0.02-0.045 g sample⁻¹) underwent three freeze/thaw cycles before the addition of 0.25 ml of 80% methanol as initial steps in the microcystin extraction procedure. Each centrifuge tube was mixed thoroughly via vortex and stored for 18-24 h at room temperature for
the extraction of microcystins. After extraction was completed, 0.75 ml of a phosphate buffer solution (PBS) was added, bringing the volume to 1 ml. Exactly 0.3 ml of the solution was syringe-filtered through a Whatman filter (13 mm diameter, 0.2 μm) and transferred to a centrifuge tube containing 0.45 ml of the 80% methanol, bringing the final methanol concentration to <10%. Samples were frozen until ELISA microcystin analyses.

**Water**

Epilimnetic water was sampled in triplicate from each region using a weighted tygon tube (2 cm diameter). Water was tested for total phosphorus (TP) and total nitrogen (TN) following the standard operating procedure and guidance of the New Hampshire Lakes Lay Monitoring Program (U.N.H., Durham New Hampshire) (Schloss & Craycraft, 2006). Whole lake water samples were frozen until MC ELISA was performed. Water samples (volume varied by lake) were also filtered through a 0.45 μm (47 mm diameter, Millipore HAWP) filter for extracted chlorophyll analyses. Filters were dried in desiccators and stored in darkness until extracted for chlorophyll. Chlorophyll a analysis methods included grinding filters with glass-tissue grinders and extracting with 90% acetone with MgCO₃ for 18-24 h (Lind, 1985; Vollenweider, 1969).

Water samples were freeze/thawed in triplicate. In order to increase the sensitivity of the ELISA analyses for microcystin detection, water samples were lyophilized (8-12 h) in a Labconco Freezone 4.5 (-50 C, 30 x 10⁻³ mbar) to concentrate a 10 ml sub-sample of the water into a powder. The powder was then re-hydrated with 1 ml Milli-Q distilled water, finally concentrating the water sample 10-fold. The 1 ml
filtrate was then syringe-filtered through a Whatman filter (13 mm diameter, 0.2 μm) into a 1.5 ml centrifuge tube. The water samples were frozen until ELISA microcystin analyses.

**Sediment**

Sediment was sampled in triplicate from each site to assess spatial differences in microcystins accumulating in the sediment. The Sediment Skimmer (beta version from Aquatic Research Instruments Inc., Hope ID, 2007) was lowered to the bottom of the lake. The messenger was released, allowing the trigger to open the stopper. Suction, created by released pressure, sampled the top layer of flocculent or loose sediment. Once at the surface, the sediment slurry sample was transferred to a 500 ml Nalgene bottle. Sediment samples were frozen until laboratory processing.

Sediment samples were thawed in the laboratory. As each sample was brought to room temperature, sediment settled so that the excess water from the sample could be poured off. Sediment samples were subjected to three freeze/thaw cycles. Sediment was sub-sampled (1 ml) and filtered through a Whatman filter (13 mm diameter, 0.2 μm). Samples were also frozen until ELISA microcystin analyses.

**MC Analysis via Enzyme-Linked Immunosorbent Assay (ELISA)**

Microcystin (MC) analyses were performed using an ELISA 96-well microcystin plate kit with MC standards of 160, 600, and 2500 ng L⁻¹ (Envirologix Quantiplate Kit, Envirologix Inc., Portland, ME). In order to extend the standard curve and improve the sensitivity of the test, provided standards were diluted with Milli-Q water to make up additional standards at concentrations of 25 and 250 ng L⁻¹ (all standards were run in
duplicate). Optical densities (OD) of each well were read on a Bio-tek (Winooski, VT) EL800 Plate Reader (sensitivity of +/- 0.010 Abs) at a wavelength of 450 nm.

**Statistical Analysis**

Data were filed in Excel and were graphed and analyzed using SigmaPlot 11.0. Analyses (t-test, ANOVA, Tukey's Post Hoc test) were performed using statistical tools in SigmaPlot 11.0 (SYSTAT Software Inc. Chicago IL). Log transformations were performed on the regressions and the highest $R^2$ values were chosen to be presented. Statistical spatial analysis tools were used in Arcmap 9.2 (Arview; ESRI Redlands, CA) to map and interpolate geo-referenced data obtained from the multi-parameter sonde coupled with GPS. Microcystin data were presented in KC Junior, a companion program to the Bio-tek microplate reader and a standard curve was derived based on optical densities read. Microcystin concentrations were calculated based on a standard curves generated in Sigma Plot 11.0 to determine the best fit curve, where all standard curves had an $R^2 > 98%$. 
Chapter III

RESULTS

Quantifying Cyanobacteria Abundance from Phycocyanin Fluorescence

Phycocyanin (PC) fluorescence (calibrated as equivalent *Microcystis aeruginosa* cells ml$^{-1}$) was linearly correlated with natural colonies of *Microcystis aeruginosa* cells ml$^{-1}$ (Fig. 1; Adj. $R^2 = 0.992; p <0.001; [\text{Phycocyanin fluorescence (equivalent } Microcystis cells ml}^{-1}]) = 3.362 + (0.566 * \log Microcystis cells ml}^{-1}])$. PC fluorescence overestimated the concentration of cells estimated from hemacytometer counts. The greatest error occurred at lower concentrations, where PC fluorescence predicted nearly four times the concentration of *Microcystis* cells ml$^{-1}$ (Fig. 2).

PC fluorescence was a good predictor of laboratory cultured *Anabaena flos-aquae* (Fig 2; Adj. $R^2 = 0.998; p <0.001; [\text{Phycocyanin fluorescence (equivalent } Microcystis cells ml}^{-1}]) = 3837.451 + (1.033 * Anabaena cells ml}^{-1}])$. PC fluorescence accurately measured *Anabaena* cells in suspensions ranging from 0-900,000 cells ml$^{-1}$. There was very little difference between PC fluorescence (estimated as *Microcystis* (cells ml$^{-1}$)) and the concentration of *Anabaena* (cells ml$^{-1}$) (Fig. 3).
Figure 2. Log-log regression of phycocyanin fluorescence (equivalent *Microcystis* cells ml\(^{-1}\)) with serial dilutions of natural colonies of *Microcystis aeruginosa* (cells ml\(^{-1}\)) collected from Barbadoes Pond, Madbury, NH. (Adj. \(R^2 = 0.992, p < 0.001\)). [Phycocyanin fluorescence (equivalent *Microcystis* cells ml\(^{-1}\)) = 3.362 + (0.566 * log *Microcystis* cells ml\(^{-1}\))].
Figure 3. Regression of phycocyanin fluorescence (equivalent *Microcystis* cells ml$^{-1}$) with serial dilutions of filaments of cultured *Anabaena flos-aquae* (cells ml$^{-1}$). (Adj. $R^2 = 0.998$, $p <0.001$). [Phycocyanin fluorescence (equivalent *Microcystis* cells ml$^{-1}$) = 3837.451 + (1.033 * *Anabaena* cells ml$^{-1}$)].
Trophic Conditions of Study Lakes

Cyanobacteria were found in all five study lakes, in which total nitrogen (TN) concentrations ranged from 263-971 µg L\(^{-1}\) and total phosphorous (TP) ranged from 3.6-39.1 µg L\(^{-1}\). Chlorophyll \(a\) fluorescence and chlorophyll \(a\) concentrations (extracted chlorophyll) were in the range of 1.7–7.3 µg L\(^{-1}\) (converted equivalent) and 1.5–13.0 µg L\(^{-1}\), respectively. Phycocyanin fluorescence (equivalent Microcystis cells ml\(^{-1}\)) ranged between 2,330–74,166 cells ml\(^{-1}\). Secchi disk depth (SDD) measurements were from depths between 1.36-1.44 m (Table 2). Microcystins (MC) were measured from whole lake water (WLW) of the epilimnion where averages were 2.86-159.17 ng L\(^{-1}\) (equivalent MC-LR) (Figure 4). Whole lake water microcystins were linearly correlated with trophic level indicators such as TP, TN and SDD (Figs. 4-7).
Table 2. Mean total nitrogen, total phosphorus, chlorophyll a (extracted & fluorescence), phycocyanin fluorescence, and Secchi disk depth for the lakes sampled in the study.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Total Nitrogen (µg L(^{-1}))</th>
<th>Total Phosphorus (µg L(^{-1}))</th>
<th>Chlorophyll Extracted (µg L(^{-1}))</th>
<th>Chlorophyll Fluorescence (µg L(^{-1}))</th>
<th>Phycocyanin fluorescence (equivalent <em>Microcystis</em> cells ml(^{-1}))</th>
<th>Secchi Disk Depth (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbados Pond</td>
<td>971.3</td>
<td>39.1</td>
<td>6.9</td>
<td>7.3</td>
<td>74166</td>
<td>1.36</td>
</tr>
<tr>
<td>Willand Pond</td>
<td>782.1</td>
<td>23.4</td>
<td>13.0</td>
<td>4.1</td>
<td>9229</td>
<td>2.78</td>
</tr>
<tr>
<td>Mirror Lake</td>
<td>383.6</td>
<td>10.8</td>
<td>3.0</td>
<td>4.3</td>
<td>6308</td>
<td>4.48</td>
</tr>
<tr>
<td>Silver Lake</td>
<td>477.7</td>
<td>10.1</td>
<td>2.4</td>
<td>2.5</td>
<td>15004</td>
<td>7.00</td>
</tr>
<tr>
<td>Sawyer Pond</td>
<td>262.5</td>
<td>3.6</td>
<td>1.5</td>
<td>1.7</td>
<td>2330</td>
<td>11.44</td>
</tr>
</tbody>
</table>
Figure 4. Microcystins (bars) and total phosphorus (TP) concentrations (scatter & line plot) from each of the study lakes, in order of trophic status characterized by TP.

Figure 5. Regression of total phosphorus (TP) with microcystins measured from whole lake water. (p<0.001; Adj. $R^2 = 0.985$; [Microcystins (ng L$^{-1}$) = -11.179 + (4.564 * TP (µg L$^{-1}$))]. Data symbols indicate study lake, i.e. BP (Barbadoes Pond), WP (Willand Pond), SL (Silver Lake), ML (Mirror Lake), and LSP ((Lower) Sawyer Pond).
Figure 6. Regression of total nitrogen (TN) with microcystins measured from whole lake water. (p<0.001; Adj. $R^2 = 0.978$; [Microcystins (ng L$^{-1}$) = -57.756 + (0.219 * TN μg L$^{-1}$)]. Data symbols indicate study lakes as defined in Fig 5.

Figure 7. Regression of Secchi disk depth (SDD) with microcystins measured from whole lake water. (p<0.001; Adj. $R^2 = 0.626$, [Microcystins (ng L$^{-1}$) = 143.131 - (13.830 * SDD)]. Data symbols indicate study lakes as defined in Fig 5.
Lake Cyanobacteria Concentrations

*Microcystis aeruginosa* was abundant in each of the study lakes except for the ultra-oligotrophic lake, Lower Sawyer Pond (Table 3). The only genus of cyanobacteria observed in Lower Sawyer Pond was *Gleocapsa* sp., which was relatively sparse in quantity, but representing one of the dominant phytoplankton genera in the lake. Cyanobacteria were abundant and diverse in the other four study lakes and consistently sampled among a series of collection dates from Barbadoes Pond in the summer of 2007 (Table 3 & 4). Mirror Lake had a diverse cyanobacteria community in the epilimnion, including *Microcystis aeruginosa*, *Merismopedia* sp., *Lyngbya* sp. and *Oscillatoria agardhii*, the latter occurring in high densities between 8 and 10 meters within the water column. Silver Lake was replete with *M. aeruginosa*, *Aphanocapsa* sp., *Anabaena* sp. and *Lyngbya* sp. Willand Pond had high numbers of *M. aeruginosa* and *Aphanocapsa* sp., the two dominant cyanobacteria and principle phytoplankton in the lake as a whole. In the fall, Barbadoes Pond was abundant with *M. aeruginosa*, *Aphanocapsa* sp., *Aphanothece* sp., and *Aphanizomenon* sp. (Table 3). However, populations of cyanobacteria were not as diverse in the summer, when *M. aeruginosa* and *Aphanocapsa* sp. were the only net cyanobacteria consistently sampled, peaking in concentrations at the end of August (Table 4a).
Net phytoplankton and cyanobacteria densities were (overall) significantly different from each of the three regions sampled (ANOVA, p<0.05, Figs. 8-12). However, there was no significant difference in either net phytoplankton or net cyanobacteria between the north and south regions of Barbadoes (t-test, Net phytoplankton p=0.754, Cyanobacteria p=0.364, Fig. 8). In Willand Pond, cyanobacteria densities were significantly different from each region, however net phytoplankton was only significantly higher in the west (ANOVA, p<0.05, Fig. 9). Silver lake had significantly higher levels of cyanobacteria from the south sampling site. Net phytoplankton differed significantly between the north and south regions but both were statistically similar to densities from the middle region in Silver lake (ANOVA, p<0.05, Fig. 10). Cyanobacteria were significantly higher in the middle region sampled from Mirror Lake, though there were no differences between the north and south sites. Net phytoplankton sampled from the middle was significantly different from densities sampled from the south region, but was not different from counts of net phytoplankton in the north sampling site of Mirror Lake (ANOVA, p<0.05, Fig. 11). Finally, there was no significant difference between net phytoplankton densities in the three regions of Sawyer Pond (Fig. 12). Cyanobacteria densities were significantly higher from the west sampling site over densities counted from the east and middle sampling sites of the lake (ANOVA, p<0.05, Fig. 12).
Figure 8. Net phytoplankton abundances from separate regions of Barbadoes Pond on September 13, 2007. Bottom stacks (black) signify cyanobacteria abundance within net phytoplankton populations (Full stacks) (t-test, Net phytoplankton $p=0.754$, Cyanobacteria $p=0.364$).

Figure 9. Net phytoplankton abundances from three separate regions of Willand Pond on September 27, 2007. Bottom stacks (black) signify cyanobacteria abundance within net phytoplankton populations (Full stacks) (one-way ANOVA, Tukey’s Test, Net phytoplankton $p=0.002$, Cyanobacteria $p<0.001$). Letters indicate significance $p<0.05$. 

---

**Barbadoes Pond**

<table>
<thead>
<tr>
<th>Total net phytoplankton (colonies ml$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>South</td>
</tr>
<tr>
<td>North</td>
</tr>
<tr>
<td><strong>Net phytoplankton</strong> $P = 0.754$</td>
</tr>
<tr>
<td><strong>Cyanobacteria</strong> $P = 0.364$</td>
</tr>
</tbody>
</table>

**Willand Pond**

<table>
<thead>
<tr>
<th>Total net phytoplankton (colonies ml$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>East</td>
</tr>
<tr>
<td>Middle</td>
</tr>
<tr>
<td>West</td>
</tr>
<tr>
<td><strong>Net phytoplankton</strong> $P = 0.002$</td>
</tr>
<tr>
<td><strong>Cyanobacteria</strong> $P &lt; 0.001$</td>
</tr>
</tbody>
</table>

```python
24
```
Figure 10. Net phytoplankton abundances from three separate regions of Silver Lake on September 20, 2007. Bottom stacks (black) signify cyanobacteria abundance within net phytoplankton populations (Full stacks) (one-way ANOVA, Tukey’s Test, Net phytoplankton $p=0.006$, Cyanobacteria $p=0.003$). Letters indicate significance $p<0.05$.

Figure 11. Net phytoplankton abundances from three separate regions of Mirror Lake on October 11, 2007. Bottom stacks (black) signify cyanobacteria abundance within net phytoplankton populations (Full stacks) (one-way ANOVA, Tukey’s Test, Net phytoplankton $p=0.006$, Cyanobacteria $p=0.003$). Letters indicate significance $p<0.05$. 
Figure 12. Net phytoplankton abundances from three separate regions of Sawyer Pond on October 4, 2007. Bottom stacks (black) signify cyanobacteria abundance within net phytoplankton populations (Full stacks) (one-way ANOVA, Tukey's Test, Net phytoplankton p = 0.196 (NS), Cyanobacteria p = 0.001). Letters indicate significance p<0.05.
**Lake Microcystin Concentrations**

Microcystin (MC) levels were significantly variable within lakes. Average microcystins were in the range of 2.86-159.17 ng L\(^{-1}\) in epilimnetic water sampled from lakes of varying trophic status (Table 3). Considering the high densities of net cyanobacteria in summer samples from Barbadoes Pond (Table 4a), MC levels were relatively low, ranging from 5.22 –193.33 ng L\(^{-1}\) (Table 4b). Microcystins measured from water sampled along transects were comparable at levels between 1.20–281.20 ng L\(^{-1}\) (Table 5). The coefficient of variation (% CV) of the horizontal distributions of microcystins was consistently high among the lakes, varying from 62% of the mean in Barbadoes Pond to 79% of the mean in Lower Sawyer Pond (Table 5). The coefficient of variation of microcystins generally decreased with trophic status, with the lowest % CV in the most eutrophic-mesotrophic lakes (BP and WP) and the highest variability in the most oligotrophic study lake, Sawyer Pond.

Microcystins were also measured from zooplankton and sediment sampled from three regions of each lake. Microcystins (µg MC g\(^{-1}\) dwt) extracted from *Daphnia* ranged from undetectable in the east and west regions of Sawyer Pond to 38.2 µg MC g\(^{-1}\) dwt from *Daphnia* in the north region of Barbadoes Pond (Table 6). Microcystins from *Daphnia* of Barbadoes Pond were considerably higher than MC levels in the other study lakes. Microcystins were relatively high and variable in the sediment, with the lowest levels measured from Sawyer Pond and the highest from Willand Pond (Table 7).
Table 3. Genera of cyanobacteria (colonies ml\(^{-1}\)) and free microcystins (ng L\(^{-1}\)) measured from different regions of each of the study lakes.

<table>
<thead>
<tr>
<th>Lake:</th>
<th>Barbados Pond</th>
<th>Willand Pond</th>
<th>Silver Lake</th>
<th>Mirror Lake</th>
<th>Sawyer Pond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site:</td>
<td>North</td>
<td>South</td>
<td>East</td>
<td>Middle</td>
<td>West</td>
</tr>
<tr>
<td><strong>Cyanobacteria:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Microcystis</em></td>
<td>164.2</td>
<td>190.0</td>
<td>64.6</td>
<td>40.1</td>
<td>97.7</td>
</tr>
<tr>
<td><em>Aphanocapsa</em></td>
<td>2.0</td>
<td>2.7</td>
<td>8.2</td>
<td>4.0</td>
<td>14.5</td>
</tr>
<tr>
<td><em>Anabaena</em></td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>Oscillatoria</em></td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>Lyngbya</em></td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>Aphanathece</em></td>
<td>0.3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>Aphanizomenon</em></td>
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<td>0.3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td><em>Merismopedia</em></td>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><em>Gloeocapsa</em></td>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Total net cyanobacteria (colonies ml(^{-1})</strong></td>
<td>166.5</td>
<td>193.0</td>
<td>72.8</td>
<td>44.2</td>
<td>112.2</td>
</tr>
<tr>
<td><strong>Microcystins (ng L(^{-1})</strong></td>
<td>156.7</td>
<td>159.2</td>
<td>143.8</td>
<td>101.8</td>
<td>95.5</td>
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**Table 4a: Total net cyanobacteria (colonies m$^{-1}$) sampled from Barbados Pond**

<table>
<thead>
<tr>
<th></th>
<th>North</th>
<th>Middle</th>
<th>South</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-Jun</td>
<td>321.43</td>
<td>164.15</td>
<td>261.91</td>
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<tr>
<td>10-Jul</td>
<td>52.23</td>
<td>14.08</td>
<td>27.67</td>
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<tr>
<td>21-Jul</td>
<td>35.63</td>
<td>9.91</td>
<td>19.29</td>
</tr>
<tr>
<td>24-Aug</td>
<td>577.83</td>
<td>466.23</td>
<td>497.27</td>
</tr>
<tr>
<td>13-Sep</td>
<td>166.48</td>
<td>na</td>
<td>192.99</td>
</tr>
</tbody>
</table>

**Table 4b: Microcystins (ng L$^{-1}$) measured from whole lake water in Barbados Pond**

<table>
<thead>
<tr>
<th></th>
<th>North</th>
<th>Middle</th>
<th>South</th>
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<tr>
<td>21-Jun</td>
<td>20.43</td>
<td>15.50</td>
<td>12.92</td>
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<tr>
<td>10-Jul</td>
<td>27.41</td>
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<td>21-Jul</td>
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<tr>
<td>24-Aug</td>
<td>114.84</td>
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</tr>
<tr>
<td>13-Sep</td>
<td>156.69</td>
<td>na</td>
<td>159.17</td>
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</table>

**Table 5: Microcystins (ng L$^{-1}$) measured from integrated water collected along various transects (T).**

<table>
<thead>
<tr>
<th>Lake &amp; Transect</th>
<th>Barbados Pond</th>
<th>Silver Lake</th>
<th>Willand Pond</th>
<th>Sawyer Pond</th>
<th>Mirror Lake</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>178.30</td>
<td>57.85</td>
<td>41.01</td>
<td>4.67</td>
<td>5.63</td>
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<tr>
<td>T2</td>
<td>83.16</td>
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<td>6.87</td>
</tr>
<tr>
<td>T3</td>
<td>185.74</td>
<td>59.63</td>
<td>85.56</td>
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<td>63.57</td>
</tr>
<tr>
<td>T4</td>
<td>58.16</td>
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<td>Table 6: Microcystins (µg MC g(^{-1}) dwt) extracted from Daphnia.</td>
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Table 7: Microcystins (ng L$^{-1}$) measured from sediment.

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<th>SE</th>
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<td>28.62</td>
<td>15.30</td>
<td>23.77</td>
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</table>
 Spatial Distributions of Cyanobacteria & Microcystin 

in Lakes of Varying Trophic Status 

The phycocyanin fluorescence sensor was used for determining spatial distributions of cyanobacteria in five lakes of varying trophic status. Phycocyanin fluorescence was highly correlated with the concentration of net cyanobacteria among each of the study lakes (Adj $R^2 = 0.612$, $p < 0.001$) (Fig 13). Although Barbadoes Pond appears to have a considerable influence on the regression, Cook’s distance and leverage values were acceptable (Cook’s Distance = 0.00207 & 0.151, leverages were 0.514 & 0.441 for north and south sites, respectively). Phycocyanin fluorescence was also highly correlated with the concentrations of net Microcystis (estimated cells per colony; cells ml$^{-1}$) (Adj $R^2 = 0.812$, $p < 0.001$, $n=14$) and microcystins (ng L$^{-1}$) (Adj $R^2 = 0.643$, $p < 0.001$, $n=14$) (Fig 14 and 15, respectively). Although similarly related, the correlation was significant with Microcystis colonies as well (Adj $R^2 = 0.677$, $p < 0.001$, $n=14$). Additionally, the concentration of microcystins was also strongly correlated with the concentration of net cyanobacteria in the five lakes with an Adj $R^2$ of 0.628 and p-value $<0.001$ (Fig 16).
Figure 13. Regression of phycocyanin fluorescence with net cyanobacteria measured from lakes of varying trophic status (Adj R² = 0.612, p < 0.001) [net cyanobacteria (colonies ml⁻¹) = 16.583 + (0.00239 * Phycocyanin fluorescence (equivalent Microcystis cells ml⁻¹))].

Figure 14. Regression of phycocyanin with net Microcystis (estimated cells ml⁻¹) measured from lakes of varying trophic status (Adj R² = 0.812, p < 0.001) [Microcystis (cells ml⁻¹) = 260.4 + (0.59 * Phycocyanin fluorescence (equivalent Microcystis cells ml⁻¹))]. Overlapping points include Sawyer Pond (E,M,W) and Silver Lake (N,M,S) at the low end of the curve and Barbadoes Pond (N,S) at the high end.
Figure 15. Regression of phycocyanin fluorescence with WLW microcystins measured from lakes of varying trophic status (Adj $R^2 = 0.643$, $p < 0.001$). [Microcystins (ng L$^{-1}$) = -354.621 + (105.096 * Phycocyanin fluorescence (equivalent Microcystis cells ml$^{-1}$)).

Figure 16. Regression of net cyanobacteria with whole lake water microcystins measured from lakes of varying trophic status (Adj $R^2 = 0.628$, $p < 0.001$) [Microcystins (ng L$^{-1}$) = 23.2 + (0.74 * net cyanobacteria (colonies ml$^{-1}$))].
**Spatial Comparisons**

PC fluorescence values were averaged among regions obtained from transect measurements from each lake. Cyanobacteria concentrations differed significantly in all lakes (P<0.001). In Barbadoes Pond, the southwest side had significantly higher PC values than other regions (Fig 17). There were considerable variations of PC fluorescence in regions of Willand Pond, averaging highest at the north side of the lake and lowest at the west where a surface bloom was previously sighted in the summer (Fig 18). Due to the complex shape of Willand Pond, levels of cyanobacteria from two major embayments were also compared. The east embayment had significantly higher levels of cyanobacteria than the west (t-test, Fig 22).

Cyanobacteria concentrations within Silver Lake differed significantly between the five regions. Higher cyanobacteria levels were measured in the southern end of the lake, while the east side had the lowest cyanobacteria levels (ANOVA, p< 0.001, Fig 19). Mirror Lake had significant differences in the spatial distributions of cyanobacteria concentrations from each region as well (ANOVA, p< 0.001, Fig 20). Subsurface cyanobacteria ranged from 3,500-10,000 cells ml$^{-1}$. Although Mirror Lake had relatively low levels of cyanobacteria at the surface, it contained a deep bloom of *Oscillatoria*, where PC levels peaked to nearly 300,000 cells ml$^{-1}$ between 8 and 10 meters.
Cyanobacteria were not abundant in Sawyer Pond, yet there were significant differences in spatial distributions of cyanobacteria in this lake (Fig 21). The northeast end had the highest PC values and was significantly different from all other areas. The lowest PC values were obtained from the northwest side, where a lean-to and camping area is located.

![Barbadoes Pond](image)

Figure 17. Cyanobacteria concentrations measured (via PC fluorometry) from five different regions of Barbadoes Pond on September 13, 2007 (p < 0.001).
Figure 18. Cyanobacteria concentrations measured from five different regions of Willand Pond on September 27, 2007 (ANOVA, p < 0.001).

Figure 19. Cyanobacteria concentrations measured (via PC fluorometry) from five different regions of Silver Lake on September 20, 2007 (ANOVA, p < 0.001).
Figure 20. Cyanobacteria concentrations measured (via PC fluorometry) from five different regions of Mirror Lake on October 11, 2007 (ANOVA, p <0.001).

Figure 21. Cyanobacteria concentrations measured (via PC fluorometry) from five different regions of Lower Sawyer Pond on October 4, 2007 (ANOVA, p <0.001).
Figure 22. Cyanobacteria concentrations measured (via PC fluorometry) from the west and east embayment of Willand Pond on September 27, 2007 (t-test, p < 0.001).
**Down-current vs. Up-current**

Down-current directions were determined by the prevailing direction of current drogues deployed from the middle of the lake. There were significant differences in the spatial distribution of cyanobacteria when comparing down-current and up-current directions of Barbadoes Pond (t-test, $p=0.006$, Fig 23). Cyanobacteria levels were significantly higher in down-current areas of Willand Pond than in up-current areas of the lake (t-test, $p=0.001$, Fig 24). PC measurements compared from down-current areas did not differ from up-current area on Silver Lake (Fig 25). This was also true for Mirror Lake (Fig 26). There were, however, significantly higher levels of cyanobacteria among down-current regions of Sawyer Pond than in up-current areas ($p < 0.001$) (Fig 27).

![Barbadoes Pond](image)

**Figure 23.** Cyanobacteria concentrations (via PC fluorometry) compared between the down-current and up-current areas of Barbadoes Pond on September 13, 2007 (t-test, $p = 0.006$).
Figure 24. Cyanobacteria concentrations (via PC fluorometry) compared between the down-current and up-current areas of Willand Pond on September 27, 2007 (t-test, $p < 0.001$).

Figure 25. Cyanobacteria concentrations (via PC fluorometry) compared between the down-current and up-current areas of Silver Lake on September 20, 2007 (t-test, $p = 0.194$).
Figure 26. Cyanobacteria concentrations (via PC fluorometry) compared between the down-current and up-current areas of Mirror Lake on October 11, 2007 (t-test, \( p = 0.356 \)).

Figure 27. Cyanobacteria concentrations (via PC fluorometry) compared between the down-current and up-current areas of Sawyer Pond on October 4, 2007 (t-test, \( p = 0.001 \)).
**Deep Site vs. Shoreline**

There were no differences between the spatial distribution of cyanobacteria in the deep site and shoreline areas of Barbadoes Pond (Fig 28). However, cyanobacteria from deep site and shoreline areas differed significantly in the other lakes. The shoreline of Willand Pond had higher levels of cyanobacteria than the deep site ($p = 0.01$) (Fig 29). However, the deep site had higher cyanobacteria concentrations than were measured from the shoreline area of Silver Lake, Mirror Lake and Sawyer Pond (Figs 30, 31 & 32, respectively). Statistical comparisons between deep site and shoreline cyanobacteria were performed with t-test ($p<0.05$) in SigmaPlot 11.0.

![Figure 28. Cyanobacteria concentrations (via PC fluorometry) compared between the deep site and the shoreline of Barbadoes Pond on September 13, 2007 (t-test, $p = 0.546$).](image-url)
Figure 29. Cyanobacteria concentrations (via PC fluorometry) compared between the deep site and the shoreline of Willand Pond on September 27, 2007 (t-test, \( p = 0.010 \)).

Figure 30. Cyanobacteria concentrations (via PC fluorometry) compared between the deep site and the shoreline of Silver Lake on September 20, 2007 (t-test, \( p < 0.001 \)).
Figure 31. Cyanobacteria concentrations (via PC fluorometry) compared between the deep site and the shoreline of Mirror Lake on October 11, 2007 (t-test, $p = 0.008$).

Figure 32. Cyanobacteria concentrations (via PC fluorometry) compared between the deep site and the shoreline of Lower Sawyer Pond on October 4, 2007 (t-test, $p = 0.010$).
Assessing Trends Between Trophic Status Parameters and Shoreline Cyanobacteria Dominance

There were positive trends between cyanobacteria dominance along the shore (ratio of shoreline PC:Deep PC) and lake trophic status indicators, including TP, TN, CHL and SDD, however, only Secchi disk transparency was significant (Figs 33-36, respectively). The more eutrophic lakes tended to aggregate cyanobacteria along the shoreline, whereas more oligotrophic lakes generally had relatively higher concentrations of cyanobacteria in the deep regions. Additionally, microcystins were positively, but not significantly, correlated with the shoreline PC: Deep PC ratio. Mirror Lake was often an outlier (Figs 33, 34, 35 & 37).

Figure 33. Regression of the ratio of shoreline PC: deep site PC with chlorophyll a (μg L\(^{-1}\)).

$\text{ShorelinePC:DeepPC} = 0.741 + (0.0565 \times \text{CHL}), \ p=0.078, \ \text{Adj R}^2 = 0.598.$
Figure 34. Regression of the ratio of shoreline PC: deep site PC with TP (µg L⁻¹).

\[ \text{ShorelinePC/DeepPC} = 0.774 + (0.00985 \times \text{TP}), \quad p=0.094, \quad \text{Adj } R^2 = 0.548. \]

Figure 35. Regression of the ratio of shoreline PC: deep site PC with TN (µg L⁻¹).

\[ \text{ShorelinePC/DeepPC} = 0.690 + (0.000444 \times \text{TN}), \quad p=0.134, \quad \text{Adj } R^2 = 0.441. \]
Figure 36. Regression of the ratio of shoreline PC: deep site PC with SDD (m).
[ShorelinePC:DeepPC = 1.170 - (0.0414 * SDD), p = 0.009, Adj R² = 0.902.]

Figure 37. Regression of the ratio of shoreline PC: deep site PC with MC (ng L⁻¹).
[ShorelinePC:DeepPC = 0.806 + (0.00204 * Microcystin) p=0.125, Adj R² = 0.465.]
Comprehensive ArcMap-Derived Spatial Distributions

To provide a more complete display of surface variability of cyanobacteria and chlorophyll concentrations, phycocyanin (PC) and chlorophyll a fluorescence transect data were 2-D spatially interpolated (Kriging) then mapped in Arcmap 9.2 (Figs 38-42). The Arcmap-images indicate a broad range of both PC and chlorophyll fluorescence within lakes. Cyanobacteria ranged from 50,000-115,000 cells ml\(^{-1}\) in Barbadoes Pond, where higher PC fluorescence (cells ml\(^{-1}\)) were measured at the south end of the lake, but overall were patchy in distribution (Fig 38).

Figure 38. Image-maps indicating spatial changes in cyanobacteria and chlorophyll within Barbadoes Pond on September 13, 2007. Data were compiled and spatially interpolated in ArcMap 9.2.
Spatial distribution maps display significant surface variability of cyanobacteria concentrations across Willand Pond (Fig 39). The northern region and the lower east embayment of the lake had noticeably higher cyanobacteria concentrations than in other areas of the lake. PC fluorescence values ranged from 6,000-35,000 cells ml\(^{-1}\) in Willand Pond.

![Cyanobacteria](image1.png) ![Chlorophyll](image2.png)

Figure 39. Image-maps indicating spatial changes in cyanobacteria and chlorophyll within Willand Pond on September 27, 2007. Data were compiled and spatially interpolated in ArcMap 9.2.
Spatial distributions maps of PC fluorescence also revealed surface variability of cyanobacteria in Silver Lake, indicating that relatively small bodies of water may also be vulnerable to the occurrence of localized blooms of cyanobacteria (Fig 40). Additionally, spatial patches of cyanobacteria were noted in Mirror Lake and Sawyer Pond, though the PC fluorescence was relatively low at the surface. Cyanobacteria (as measured by PC fluorescence) ranged from 3,500-10,000 cells ml$^{-1}$ in Mirror Lake and from 900-5,000 cells ml$^{-1}$ in Sawyer Pond (Figs. 41 & 42, respectively).

![Image Maps](image.png)

Cyanobacteria

Chlorophyll

Figure 40. Image-maps indicating spatial changes in cyanobacteria and chlorophyll within Silver Lake on September 20, 2007. Data were compiled and spatially interpolated in ArcMap 9.2.
Figure 41. Image-maps indicating spatial changes in cyanobacteria and chlorophyll within Mirror Lake on October 11, 2007. Data were compiled and spatially interpolated in ArcMap 9.2.

Figure 42. Image-maps indicating spatial changes in cyanobacteria and chlorophyll within Lower Sawyer Pond on October 4, 2007. Data were compiled and spatially interpolated in ArcMap 9.2.
Summer Spatial Distributions of Cyanobacteria & Microcystins in Barbadoes Pond

PC fluorescence was a good predictor of cyanobacteria in the laboratory experiments and for net cyanobacteria in lakes of varying trophic status; however, phycocyanin fluorescence was not significantly correlated with the density of net cyanobacteria in Barbadoes Pond (p=0.480). Similarly, PC fluorescence and concentrations of net *Microcystis* were not significantly correlated (p=0.248), which would be expected since *Microcystis* was the dominant colonial cyanobacteria found in Barbadoes Pond. Phycocyanin fluorescence was, however, correlated with the concentration of microcystins (Fig 43, p=0.049, Adj $R^2= 0.23$). Similarly, net cyanobacteria concentrations were also correlated with the concentration of microcystins (Fig 44, p=0.051, Adj $R^2= 0.22$).
Figure 43. Regression of phycocyanin fluorescence with microcystin measured from whole lake water in Barbadoes Pond over the summer of 2007 (Adj. $R^2 = 0.227$, p=0.049).

Figure 44. Regression of net cyanobacteria with microcystin measured from whole lake water in Barbadoes Pond over the summer of 2007 (Adj. $R^2 = 0.222$, p=0.051).
Since PC fluorescence and net cyanobacteria from Barbadoes Pond were not significantly correlated, it should be noted that variations in PC fluorescence in Barbadoes Pond probably represent a significant fraction of small cyanobacteria present throughout the summer. The spatial distributions of PC fluorescence differed significantly ($p < 0.001$) on all sampling dates at Barbadoes Pond (Figs 45-48). There was a consistent pattern on ranking phycocyanin fluorescence levels from each designated region of the lake, where the northwest end, southeast end and southwest side were typically higher in PC and the northeast side usually had the lowest PC fluorescence of all regions. The center (deep site) of Barbadoes Pond never had the highest PC concentrations compared to other regions. Also, the central region had significantly lower PC values than those found from regions with the highest PC concentrations (ANOVA, Tukey’s Test (letters indicate significance $p<0.05$)) (Figs 45-48). These distributions are also displayed in Arcmap-images, creating an adequate visualization on the spatial distributions of sub-surface PC fluorescence (cyanobacteria) in Barbadoes Pond over the summer of 2007 (Fig 49).
Figure 45. Phycocyanin fluorescence measured from five different regions of Barbadoes Pond on June 21, 2007 (ANOVA, Tukey’s Test (same results for Ranks Dunn’s Method), p <0.001).

Figure 46. Cyanobacteria concentrations measured from five different regions of Barbadoes Pond on July 10, 2007 (ANOVA, Tukey’s Test p <0.001).
Figure 47. Cyanobacteria concentrations (via PC fluorometry) measured from five different regions of Barbadoes Pond on July 21, 2007 (ANOVA, Tukey’s Test, p <0.001).

Figure 48. Cyanobacteria concentrations measured from five different regions of Barbadoes Pond on August 24, 2007 (ANOVA, Tukey’s Test, p <0.001).
Figure 49. Change in abundance and spatial distributions of cyanobacteria (via PC fluorometry) within Barbadoes Pond over the summer of 2007. Note the variation in scale range for each map, also listed within the content of the thesis.
Cell concentrations were highly variable within the Barbadoes Pond. On June 21, cyanobacteria ranged from 16,500-60,000 cells ml\(^{-1}\); July 10 from 4,000-73,000 cells ml\(^{-1}\); July 21 from 14,000-465,000 cells ml\(^{-1}\); and on August 24 from 12,000-133,000 cells ml\(^{-1}\). September PC fluorescence values were also mapped for comparison of the spatial distributions over time, ranging from 50,000-115,000 cells ml\(^{-1}\). Though cyanobacteria concentrations varied, there were typically higher levels at either ends of Barbadoes Pond (NW & SE ends, Fig 49).

Cyanobacteria abundances were also compared between the deep site and the shoreline since these are typical sites monitored on lakes. Cyanobacteria densities were consistently higher along the shoreline than at the deep site of Barbadoes Pond for all sampling dates (p<0.001; Figs 50-53).

**Figure 50.** Cyanobacteria concentrations (via PC fluorometry) compared between the deep site and the shoreline of Barbadoes Pond on June 21, 2007 (t-test, p <0.001).
Figure 51. Cyanobacteria concentrations (via PC fluorometry) compared between the deep site and the shoreline of Barbadoes Pond on July 10, 2007 (t-test, p < 0.001).

Figure 52. Cyanobacteria concentrations (via PC fluorometry) compared between the deep site and the shoreline of Barbadoes Pond on July 21, 2007 (t-test, p < 0.001).
Comparison of Cyanobacteria Distributions

with Wind and Water Currents in Barbadoes Pond

The effects from wind, currents and natural lake morphometry were considered when assessing spatial distributions of cyanobacteria in lakes. Wind data from the UNH weather station was used to determine prevailing wind direction on each sampling date for Barbadoes Pond. Downwind areas of the lake did not consistently have higher cyanobacteria than upwind areas. Up- and downwind ends of the lake differed significantly in cyanobacteria concentrations, as determined by phycocyanin fluorescence, on four out of five sampling dates at Barbadoes Pond. The downwind
section of the lake had higher concentrations of cyanobacteria on June 21 and had significantly higher concentrations downwind on July 10, (Figs 54 & 55, respectively). Later in the lake sampling season, on July 21, August 24 and September 13 sampling dates, upwind ends had higher cyanobacteria concentrations (PC fluorescence) than downwind areas (Figs 56-58, t-test, p<0.05).

Figure 54. Cyanobacteria concentrations (via PC fluorometry) compared between the downwind and upwind areas of Barbadoes Pond on June 21, 2007 (t-test, p = 0.671).
Figure 55. Cyanobacteria concentrations (via PC fluorometry) compared between the downwind and upwind areas of Barbadoes Pond on July 10, 2007 (t-test, p = 0.030).

Figure 56. Cyanobacteria concentrations (via PC fluorometry) compared between the downwind and upwind areas of Barbadoes Pond on July 21, 2007 (t-test, p <0.001).
Figure 57. Cyanobacteria concentrations (via PC fluorometry) compared between the downwind and upwind areas of Barbadoes Pond on August 24, 2007 (t-test, p < 0.001).

Figure 58. Cyanobacteria concentrations (via PC fluorometry) compared between the downwind and upwind areas of Barbadoes Pond on September 13, 2007 (t-test, p < 0.001).
Comparisons on the Distribution of Cyanobacteria

With Water Currents

Cyanobacteria concentrations (via PC fluorometry) were also compared between down-current and up-current areas in Barbadoes Pond. Down-current directions were determined by the prevailing direction traveled by the 0.5 m-depth current drogue. Consequently, up-current was defined as the opposite direction traveled by the drogue. Down-current areas of the lake did not have significantly higher cyanobacteria levels than up-current regions of Barbadoes Pond overall. However on July 21, areas of Barbadoes Pond that were down-current were significantly higher in PC fluorescence than up-current areas (Fig 59, t-test, p<0.001). Conversely, up-current areas had significantly higher PC fluorescence values on August 24 (Fig 60, t-test, P<0.001). Ultimately, there were no consistent results that support down-current ends of the lake as areas more vulnerable to accumulations of cyanobacteria as measured by phycocyanin fluorescence.
Figure 59. Cyanobacteria concentrations (via PC fluorometry) compared between the down-current and up-current areas of Barbadoes Pond on July 21, 2007 (t-test, p < 0.001).

August 24, 2007

Figure 60. Cyanobacteria concentrations (via PC fluorometry) compared between the down-current and up-current areas of Barbadoes Pond on August 24, 2007 (t-test, p < 0.001).
CHAPTER IV

DISCUSSION

Quantifying Cyanobacteria and Microcystin from Phycocyanin Fluorometry

The recent development of (in situ) probes, designed to measure the fluorescence emitted by phycocyanin, has allowed for a rapid assessment on the density of cyanobacteria (Ahn et al., 2007; Gregor et al., 2005; Izydorczyk et al., 2005; Leboulanger, 2002). In the current study, phycocyanin (PC) fluorescence was significantly correlated with cyanobacteria concentrations under laboratory conditions. Densities of both the field-collected (Microcystis) and the lab-cultured (Anabaena) cyanobacteria were highly correlated with PC fluorescence, supporting the sensor as a tool for measuring cyanobacteria (Figs. 2 & 3).

The phycocyanin fluorescence sensor provided an accurate estimate of the abundance of laboratory cultured Anabaena flos-aquae. Though phycocyanin fluorescence overestimated the concentration of field-collected Microcystis aeruginosa, there was a high adj. $R^2$ of 0.99 in the log-log relationship (Fig. 2). There are a few possible causes for the high estimates. First, Microcystis cells are difficult to differentiate within dense colonies, perhaps causing an underestimation in counts (Figure 61). Also, because the colonies were collected from the field, it is possible that pico-cyanobacteria were present and contributing to phycocyanin fluorescence. Finally, fluorescence tends to be more closely correlated with cell volume than number of cells (Brient et al., 2008). Agusti & Phillips (1992) found that cyanobacteria, including Microcystis colonies, and
filament of both *Anabaena* and *Oscillatoria*, tended to reduce their internal pigment as colony size increased. In the same study, the efficiency of light absorption increased among single-cells, including unicellular *Microcystis* (Agusti & Phillips, 1992). Consequently, cell size and the amount of fluorescence per cell may differ depending on the variety of *Microcystis* colonies sampled from the field.

Despite the possibility that not all cells fluoresce equally, phycocyanin fluorescence data were highly correlated with net cyanobacteria densities obtained by microscopic counts (Fig. 13; Adj $R^2 = 0.612$; $p < 0.001$). Phycocyanin (PC) fluorescence was also an accurate predictor of both *Microcystis* and microcystins, which may be expected since microcystins are produced by *Microcystis* (Fig. 15). Net cyanobacteria were also significantly correlated with microcystins and had nearly equivalent statistical significance as PC fluorescence did with microcystins (Fig. 16). However, results from summer sampling in Barbadoes Pond support that there is a higher correlation between PC fluorescence and microcystins than between net cyanobacteria counts and microcystins (Figs 43 & 44, respectively). In lakes where pico-cyanobacteria produce microcystins, PC fluorescence could potentially be a much better predictor of the cyanobacteria and microcystins in whole lake water than net sampling.

![Figure 61. *Microcystis aeruginosa* cells within mucilaginous colonies.](image)
Spatial Comparisons of the Distribution of Cyanobacteria and Microcystins

There were significant differences in the horizontal distribution of cyanobacteria based on both, net sampling and in situ PC fluorometry (p<0.001). Net cyanobacteria were abundant in each of the study lakes, though no visible surface scum was seen during sampling. Significant spatial variability in net cyanobacteria concentrations indicates that a single sampling site may not represent the conditions of the lake as a whole. Cyanobacteria monitoring strategies, based on net counts, should consider the likely variations in cell concentrations between sampling sites.

Since cyanobacteria populations vary between sampling sites, it is expected that microcystins would vary as well. *Microcystis aeruginosa*, a ubiquitous toxic cyanobacteria species, was present in all of the lakes with the exception of Lower Sawyer Pond. The only net cyanobacteria sampled from this lake were *Gleocapsa* sp., also capable of producing microcystins (Carmichael & Li, 2006). However, toxin production can vary between species and individual colonies (Falconer, 1999; Harada & Tsuji, 1998). Cyanobacteria varied considerably in the proportion of microcystin-producing genotypes in a Berlin lake (Lake Wannsee), where 73% of *M. aeruginosa* contained the *mcyB* gene and no colonies of *M. wesenbergii* produced the toxin genotype (Kurmayer *et al.*, 2002). Microcystin production can also vary between red and green pigmented *Planktothrix agardhii* (equivalent to *Oscillatoria agardhii* in this study) (Henrikson, 1996). The high degree of patchiness in cyanobacteria and microcystin
concentrations indicates the inadequacy of extrapolating the conditions of the entire lake from a single sampling site.

There was spatial variability in microcystins in all study lakes as indicated by the high percentages of the coefficient of variation within lakes (Table 5). The degree of variability was consistently high in all lakes, from 62% of the mean in Barbadoes Pond to 79% of the mean in Lower Sawyer Pond (Table 5). Though there were considerable variations in microcystins measured from integrated water from transects, the mean range among all lakes was comparable to microcystins measured in whole lake water from the three sampling locations (Table 3). This may indicate that three sampling regions are adequate for measuring mean microcystins in small-scale lakes. However multiple sampling is beneficial for determining the spatial variability (i.e. % CV) as it reveals the degree of variability of microcystins within lakes, even in relatively small water bodies. Interestingly, percent variability was negatively correlated with lake trophic status, as variability in horizontal distributions of microcystins decreased with eutrophication. This trend supports the assumption that the horizontal that horizontal distributions of microcystins will differ significantly within a lake, especially in mesotrophic-oligotrophic lakes where net cyanobacteria are lower in abundance.

The significant spatial variations of microcystins in lakes were evident in other lake components as well. Results indicate that microcystins were variable in the *Daphnia* and sediment measured from each sampling location (Tables 6 & 7, respectively). The standard errors among microcystins measured from sediment were high, probably due
to the seemingly random distribution of cyano-mats in lakes. Also, microcystin levels were higher in the sediment than in water and *Daphnia*. Cyanobacteria, such as *Microcystis*, tend to accumulate in the benthos and have been documented to dominate the microbial community (Bostrom *et al.*, 1989). Detecting microcystins in *Daphnia* is significant because it suggests that *Daphnia* are grazing on edible sizes of cyanobacteria, which may primarily be the pico-cyanobacteria (Phillips, 2002; Johnson, 1999; Capron, 1995, Haney, 1987). This supports the postulation that pico-cyanobacteria were probably present in the study lakes, an interesting topic that should be further investigated.

Most of the information on toxic cyanobacteria is based on large, colonial net forms such as *Anabaena flos aquae* and *Microcystis aeruginosa*. However, net phytoplankton do not represent the entire cyanobacteria community as they do not include small cyanobacteria size classes, such as nano- (2-50 µm) and pico-plankton (0.2-2 µm), that very likely contribute to the PC fluorescence and cyanotoxins within the lake (Lavalle & Pick, 2002; Pick, 2000). Cyanobacteria usually dominate the abundance of autotrophic picoplankton in lakes (Pick, 1991). Additionally, there are many factors that influence the abundance of pico-cyanobacteria such as light, temperature, total nitrogen, total phosphorus and grazing (Callieri & Stockner, 2002; Stockner, 1988; Fogg 1986). Unfortunately, there is little known about the ecological role of pico-cyanobacteria and their contribution to the load of cyanotoxins in a lake. Routine assessment of phytoplankton abundance rarely includes all size components, including
pico-phytoplankton missed by net sampling. A phycocyanin fluorescence sensor, capable of measuring all sizes of cyanobacteria in whole lake water, has an advantage over the net collection method for studies concerned with cyanobacteria. Continued studies are needed to examine the PC fluorescence and toxicity of the different size fractions of cyanobacteria to assess the relative importance of pico-cyanobacteria in lakes of different trophic classifications.

Synoptic measurements of cyanobacteria concentrations, via PC fluorometry, disclose significant variations in the spatial distributions of subsurface communities. To assess these differences, statistical comparisons between regions were examined, including comparisons of downwind vs. upwind, down-current vs. up-current, and deep site vs. shoreline (t-test, p<0.05). Early studies suggest that wind has a dual function, first as a primary force influencing horizontal distribution changes of algal blooms and, secondly causing a more homogenous distribution vertically within the epilimnion (Small, 1963; Pomeroy et al., 1956; Johnson 1949). It has been suggested that monitoring of primary production in lakes would be improved by considering wind effects on the distribution of phytoplankton (Small 1963). Baker and Baker (1976) determined that wind altered the phytoplankton composition, as cyanobacteria tended to drift downwind and other phytoplankton groups remained homogenous. Interestingly, cyanobacteria tended to aggregate in blooms more often than other phytoplankton, which was attributed to short-term, low-wind conditions (Baker & Baker 1976). However, in the present study, the data did not support the model that
downwind areas of a lake consistently have higher cyanobacteria and was therefore not a reliable predictor for the distribution of cyanobacteria. Although there are situations where accumulations of surface blooms are wind-blown along or onto the shore, in some cases the complex hydrodynamics of water movement is not simple and requires elaborate mathematical modeling (Schimiz & Imberger, 2008; George & Edwards, 1976; Baines & Knapp, 1965). Additionally, the lag time or response for phytoplankton patches to change direction may take weeks in large lakes (Verhagen, 1994). It is possible that a lag-time from wind affects could have influenced this spatial comparison on the distributions of cyanobacteria and phytoplankton, since the time scale for patch formation in the small lakes of this study was not determined.

Water current movements were monitored in 1-hour deployments on each of the lakes, indicating the complexity of subsurface currents. Down-current directions were determined by the direction traveled by the 0.5 m drogue because it was more closely related to depths of probe data collection. However, since drogue deployments were short term, they probably did not accurately indicate where surface waters would ultimately carry and accumulate particles such as cyanobacteria. Although they are causally related, the direction of wind and currents are not always directly correlated, thereby making it difficult to predict the movement of water based on wind velocity and direction. Wind and currents must be evaluated as part of many factors including speed, direction, duration and the size of particles in the water (Webster, 1990; George and Edwards, 1976). Additionally, the movement of water is not unidirectional. Langmuir
spirals are common on lakes where wind moves water at the surface causing water to move in both clock-wise and counterclockwise directions simultaneously (Langmuir, 1938). Furthermore, since there were no visible scums, it is possible that the wind mixed particles, including cyanobacteria, into the epilimnion rather than aggregating along the shoreline. Even in large lakes, this process is relatively rapid and may occur within a day (Verhagen, 1994). The rapid disappearance of surface scums could suggest the process of epilimnetic mixing, perhaps on a scale of minutes to hours in small lakes.

Routine lake monitoring is often based on water collected at the middle/deep site of a lake. Thus, in this study, cyanobacteria concentrations from the deep site (within the middle region) were compared with other areas of the lake. Cyanobacteria concentrations differed between the middle and other locations of the lake. These values, however, were not consistently higher or lower in any location (Figs 17-21, p<0.001). The middle of the lake never had the highest PC values compared with four other surrounding regions. With the exception of Silver Lake, the middle regions of each lake had significantly lower PC fluorescence-estimated cyanobacteria than levels sampled from other regions (p<0.05). This finding supports the need for comprehensive sampling of cyanobacteria in lakes where distributions may be patchy.

**Spatial Comparisons of Cyanobacteria and Microcystins in Barbadoes Pond**

Because variations in subsurface PC fluorescence were unique to individual lakes, evaluating spatial distributions in Barbadoes Pond over time was useful for tracking trends in the distribution for a single lake. Distributions of cyanobacteria were
notably different throughout the summer in Barbadoes Pond (Fig 49). The lake is a particularly good model-lake for studying cyanobacteria due to the persistence of high concentrations of cyanobacteria, typically *Microcystis*, which typically re-occurs each year. Barbadoes Pond also has a simple shape and basin, simplifying interpretations of the distribution of cyanobacteria and microcystins. From previous visits to Barbadoes Pond, surface scums were often conspicuous at the south end of the lake, which was typically down-wind (personal observation). Although this may, in part, be related to the fact that the public access is at the south end of Barbadoes Pond and thus is more frequently visited or observed than the more isolated north end. Nonetheless, results from PC fluorometry indicated that the north end of the lake had consistently higher cyanobacteria levels (Figs 45-48). It may be important to note that the north region of Barbadoes Pond also includes shoreline data obtained by the multi-parameter sonde. Though, overall, distributions of cyanobacteria were heterogeneously represented across the sub-surface of this lake, cyanobacteria may be more abundant along the shoreline of the northern region in Barbadoes Pond (Fig 49).

**Trophic Relationships**

Levels of cyanobacteria (PC fluorescence) were higher along the shore in Barbadoes Pond and Willand Pond, but only significantly higher in Willand Pond (Figs 28 & 29, respectively). Phycocyanin fluorescence revealed significantly higher cyanobacteria concentrations among the deep site over the shoreline in Silver Lake, Mirror Lake, and Sawyer Pond (Figs. 30, 31, 32, respectively). There appeared to be
trends between the shoreline cyanobacteria dominance (ratio of shoreline to deep site cyanobacteria concentrations (PC fluorescence) and lake trophic indicators (Figs 33-37). Secchi disk depth, however, was the only trophic parameter that significantly correlated with shoreline cyanobacteria dominance ratio (Fig 36, p=0.009). This may be expected since both SDD and cyanobacteria measurements are related to the amount of particles in the water. The intriguing relationship between shoreline cyanobacteria dominance and eutrophication should be tested on a larger data set to further evaluate the significance of this finding. It would be interesting to test whether this is due to the composition of the cyanobacteria or the natural shape and characteristics of the lake. This would make sense, if a) the size of cyanobacteria may shift according to trophic classification with pico-cyanobacteria more abundant in meso-oligo lakes and b) if pico-cyanobacteria are dominant in the middle of the lake. Carrick and Schelske (1997) found that concentrations of pico-cyanobacteria varied from <100 ml⁻¹, in a eutrophic lake dominated by blooms of cyanobacteria, to over 100,000 ml⁻¹ in oligo-mesotrophic lakes. However in eutrophic lakes, net cyanobacteria which form surface blooms may be more susceptible to wind action and therefore tend to aggregate along the shore (Reynolds et al., 1981). From an applied standpoint, if eutrophic lakes always have higher cyanobacteria along the shore than the middle of the lake, there is a greater need to sample cyanobacteria from the shoreline in these lakes.

Variable concentrations of cyanobacteria were spatially represented by mapping PC fluorescence across each of the lakes in ArcMap 9.2. Chlorophyll a fluorescence
levels were also patchy in distribution across each lakes surface and were created as a visual reference, since methods in measuring chlorophyll a are typical in monitoring (Figs. 37-41). These maps display data interpolated through statistical spatial analyses and provide a comprehensive view of the degree of variability across the lakes surface. Mapping synoptic parameters provide a way to visualize sub-surface blooms, even when “blooms” are not visible by eye.

Microcystin and cyanobacteria concentrations were both highly correlated with TP, TN, and SDD (Figs 5, 6 & 7 respectively). Relationships between TP and/or TN and microcystin concentrations have been correlated in other studies as well (Rolland et al., 2005; Downing et al., 2001; Oh et al., 2001; Kotak et al., 2000; Rapala & Sivonen, 1997). Curiously, some lakes may have consistently high levels of cyanobacteria and cyanotoxins despite deep SDD and/or low TP & chlorophyll levels. Mirror Lake, for example, is considered to be meso-oligotrophic based on a SDD of 4.48 m, chlorophyll of 4.3 µg L\(^{-1}\) and total phosphorous values at 10.8 µg L\(^{-1}\) (Forsberg & Ryding, 1980; Table 2), despite the occurrence of a deep bloom of *Oscillatoria agardhii* that persisted in this lake prior to the study. Also, Silver Lake had relatively low phosphorus levels and oligomesotrophic characteristics (Table 2), but cyanobacteria, typically *Microcystis aeruginosa*, have been abundant and frequently formed surface blooms in this lake (Phillips, 2002; Capron, 1995). From the standpoint of cyanobacteria monitoring and public health, it may be useful to develop a trophic state index that uses cyanobacteria and cyanotoxins as additional criteria to consider in assessing lake water quality.
Application of in situ Phycocyanin Fluorescence Monitoring: A Case Study in Willand Pond

On June 12, 2009 Willand Pond was closed after a visible surface scum, containing potentially toxic cyanobacteria (i.e. *Anabaena flos-aquae*), was collected along the shore, near the public boat launch (Figure 62). Cyanobacteria warning signs were posted by NH DES and a public advisory was issued by the city of Dover due to the high percentage of cyanobacteria (> 50%) in the bloom. By June 16th, NH DES personnel from the Beach Program concluded from net samples that cyanobacteria were less than 50% and that the bloom had dissipated. The lake was then re-opened to the public.

The rate at which cyanobacteria can decompose from the water depends on a number of factors, such as temperature, light, the type of cyanobacteria and its concentration within the lake (Paerl and Huisman., 2008.; Tsuji et al., 1994). Generally, it takes days to weeks for a bloom to dissipate and decompose (Watanabe et al., 1996). It is also possible that the water may be more toxic following a bloom since the cells may release toxins as they decompose, thereby making the toxin more available for exposure (Sasner et al., 1984). The rate at which microcystins degrade has been from 2 days to 3 weeks, varying due to differences in environmental conditions (Hitzfeld et al., 2000; Jones and Orr, 1994).

Horizontal transects with the in situ phycocyanin sensor were conducted in Willand Pond on June 16th, 2009 following the summer bloom event. PC fluorescence (equivalent *Microcystis* cells ml$^{-1}$) varied from 1,000–835,500 cells ml$^{-1}$ in multiple
subsurface transects (Figs. 64 & 65). Though the bloom was no longer visible at the
public boat launch, synoptic sampling determined high concentrations of cyanobacteria
at the opposite end of the lake, with highest concentrations distributed in the southeast
region (Figure 64). To provide direct information on the cyanotoxicity in Willand Pond,
microcystins were also mapped using the predictive equation,

\[ MC (\text{ng} \ L^{-1}) = -354.6 + (105.1 \times \log PC (\text{equivalent } \text{Microcystis cells} \ \text{ml}^{-1}) \]

revealing spatial heterogeneity of predicted microcystins within the water following a
bloom (Figure 65). Highest predicted microcystins were also in the southeast region
where most of the lakeshore homes are located.

This example illustrates that unsafe levels of cyanobacteria and potentially
dangerous levels of microcystins were present in Willand Pond (compared to levels set
by the Massachusetts Department of Health at a standard of 70,000 cyanobacteria cells
ml\(^{-1}\) for recreational use. Integrated maps depicting cyanobacteria concentrations, as
well as predicted levels of cyanotoxins, could provide a valuable resource for regulating
lake-use during periods of cyanobacteria blooms. Additionally, water should be
synoptically sampled for cyanotoxin measurements to "ground truth" with actual
toxicity measurements. Measured microcystins could have been only a fraction of the
full toxin load in lakes where there were multiple species of cyanobacteria present. For
example, *Aphanizomenon* sp., sampled from the south location of Barbadoes Pond, is
capable of producing neurotoxins, different in structure and detection method of
microcystins (Sasner *et al.*, 1984). A larger data set of lakes with a broad range in trophic
status and cyanobacteria abundance should be included to further test the predictive MC model.

Although synoptic sampling, via PC fluorometry has considerable potential for cyanobacteria monitoring, it may not be practical to assume that most lake associations and volunteer monitors have the time, expertise and money to purchase and employ such equipment. Routine assessments of cyanobacteria could be made by state officials or through citizen-based monitoring in lakes with re-occurring cyanobacteria problems and/or lakes with declining water quality due to eutrophication.

The use of an in situ submersible phycocyanin fluorescence probe, following a reported cyanobacteria bloom, may be an effective method for evaluating when conditions are safe for public use. Mapping the distribution of cyanobacteria in the entire lake provides a useful tool for visualizing the spatial distributions of subsurface lake cyanobacteria populations. An integrated map of predicted cyanotoxin levels also allows for a more comprehensive evaluation of “areas of risk” within the entire lake and would thus permit notification of lake users in the impacted area.

To accurately assess cyanobacteria concentrations in lakes, a monitoring plan that includes both synoptic measurements of PC fluorescence and cyanobacteria counts may be more accurate than one method or one sampling site alone. Additionally, it would be useful to test whether other cyanotoxins are correlated with phycocyanin fluorescence. In proposals for routine monitoring of cyanobacteria, tracking the dynamics and spatial distributions of cyanobacteria populations should be considered in order to obtain a full range of measurements for potentially toxic cyanobacteria in N.H. 

80
lakes. The PC fluorescence method for determining cyanobacteria may be useful for predicting all sizes of potentially toxic species. PC fluorescence could serve as a quick, rapid assessment of lake cyanobacteria, microcystins and potentially other cyanotoxins 

*in situ.*

Figure 62. Surface bloom of Cyanobacteria (*Microcystis* and *Anabaena* spp.) collected from Willand Pond on June 12th, 2009.

Figure 63. Map displaying spatial distributions of cyanobacteria within Willand Pond, with aerial view of surrounding landscape.
Figure 64. Spatial distributions of phycocyanin fluorescence, compiled in ArcMap9.2.

Figure 65. Spatial distributions of predicted microcystins, compiled in ArcMap9.2.
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NH Department of Environmental Services (NHDES):
http://www.des.state.nh.us/GW/gw0602.htm
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88


