PROJECT TITLE

Title:	A Quantitative Assessment of the Spatiotemporal Dynamics of Algae, Cyanobacteria, Cyanotoxins, and Water Quality in Lower Bolton Lake
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Summary

Cyanobacterial blooms are a major ecological and human health problem in all 50 states, with considerable economic impacts, and are ostensibly caused by anthropogenic eutrophication of surface waters. To gain a greater understanding of the underlying processes related to cyanobacteria and cyanotoxins in Connecticut lakes, we monitored Lower Bolton Lake, a waterbody with historic cyanobacterial events, semi-monthly from May through October 2021 for key water quality/nutrient parameters, cyanotoxin (anatoxin-a, cylindrospermopsin, microsystins, and nodularin) concentrations, and cyanobacteria and algal densities. Cyanobacterial blooms occurred from May through October and resulted in detectible microcystin concentrations throughout the study, with a total microcystin concentration of 9.4 ppb observed in October, exceeding the USEPA recreational criteria of 8 ppb. No samples contained detectible concentrations of anatoxin-a, cylindrospermopsin, or nodularin. Cyanobacteria density and cyanotoxin concentrations were highest in the surface water samples and exhibited a significant positive correlation. A model to predict microcystin concentrations was developed using chemical and physical water quality parameters (nitrogen and phosphorus series, chlorophyll, conductivity, dissolved oxygen, pH, secci depth, and temperature). Using the measured nitrogen and phosphorus parameters, this model was able to determine only 51.2% of the variation in the total microcystin concentration in Lower Bolton Lake. When we optimized the model to include the density of the cyanobacteria *Microcystis spp*., and other key nitrogen/phosphorus and real-time measurements, the model was able to determine 76.2% of the variation in the total microcystin concentration, and over 91% of the variation in microcystin-LR and -LA. Although this model has not been tested or validated on other Connecticut lakes, it can serve as a prototype for the development of monitoring programs that can be implemented elsewhere in Connecticut.

Introduction

Blooms of cyanobacteria (CB) are a major environmental problem in all 50 states and typically are caused by anthropogenic eutrophication of surface waters (CTDPH 2017). During the summer of 2012, local residents raised concern over a bloom of algae and CB in Lower Bolton Lake. At that time, Connecticut Department of Energy and Environmental Protection (CTDEEP) did not have a formal monitoring program for CB in lakes and ponds, lacking regulatory guidance and baseline data to conduct a formal assessment of the biological, physical, or chemical factors that led to the bloom. The CB and cyanotoxins (CTx) that they produce encompass several broad groups and include hepatotoxins such as microcystin (MC), neurotoxins, and anatoxin-a (Carmichael 2001), and represent ecological and human health concerns, with considerable economic impacts related to drinking water, recreation, and tourism. Although studies have shown that different microcystin analogs are produced at different temperatures, pH levels, and light conditions (Song et al. 1998, Tonk et al. 2005, Sivonen 2009), the temporal and spatial variability of CB populations may appear to be idiosyncratic due to a lack of comprehensive long-term monitoring as well as considerable variability among lakes in many physical, chemical, and biological characteristics (Graham et al. 2008).

As a direct result of the 2012 CB bloom, the Connecticut Department of Public Health (CTDPH) and CTDEEP published guidance for local health departments (CTDPH 2017), establishing a monitoring and assessment framework for response to blooms that included visual assessments. The guidance suggested a concentration threshold of 15 µg/L for total MCs, with the Environmental Protection Agency (EPA) releasing guidance recommending a threshold for total MCs of 4µg/L (EPA 2016). In 2017, CTDEEP continued a targeted assessment for CB and CTx at state parks and recreation areas that resulted in CTDEEP closing Kettletown and Indian Wells State Parks to recreational use. As in previous years, the lakes were initially closed due to visual inspection and densities of algae and CB, but one factor differed significantly from previous years. In 2017, 12 samples were collected and analyzed (CTDEEP unpublished data), and of those, one third exceeded the EPA limit for MC of 4 µg/L, and 3 of these exceeded the 15 µg/L CT guidance. During the summers of 2018 and 2021, Lower Bolton Lake was once again closed to recreational activities due to exceedances of algal and CB density thresholds, demonstrating that this is a continuing problem.

The recent presence of elevated algal and CB densities, concentrations of CTx, and uncertainty about which physicochemical characteristics drive bloom formation and toxin production, demonstrate a critical need to expand and enhance existing monitoring. A more comprehensive synoptic assessment of CB and CTx, coupled with a well-defined water quality assessment and determination of densities of CB and algae, should lead to increased understanding of the spatiotemporal dynamics and drivers of blooms.

Objective(s)

The objectives of this project were to 1) Quantify the association between environmental characteristics, species densities of algae and CB, and concentrations of CTx, as well as their spatiotemporal variation. 2) Develop a predictive model to understand how spatiotemporal variation in concentrations of CTx is related to variation in densities of CB, densities of algae, or water quality characteristics.

Results/Discussion

Methods

Water samples were obtained from Lower Bolton Lake twice a month from mid-May until mid-October. Sampling events were performed as close as possible to the 7th and 21st of each month. For sampling locations, the lake was divided into three zones, with each zone including five equidistant sampling sites (*Figure 1*). Sampling sites were arranged within each zone to maximize distance between points, reduce spatial autocorrelation, and represent different depths of the lake.

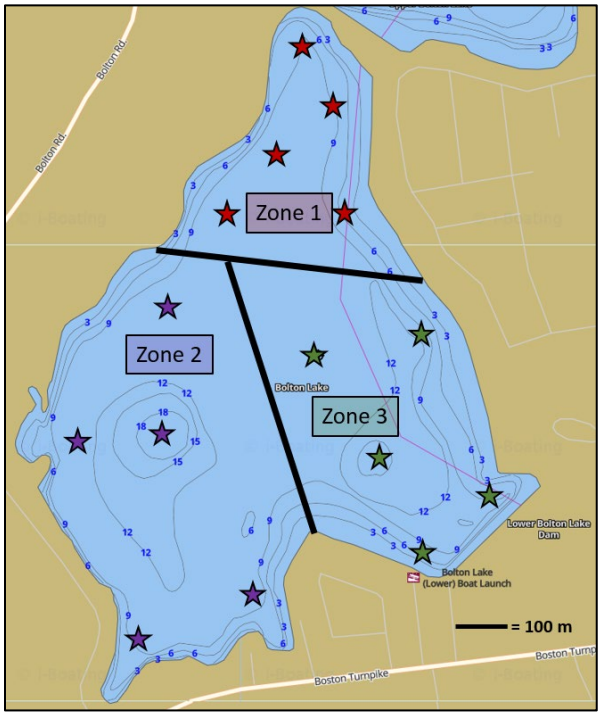

Figure 1. Map of Lower Bolton Lake and sampling design.

During each sampling event, we first traveled to the deepest sampling site of each zone (i.e. three sites) and performed a water quality profile using a YSI ProDSS multiprobe meter. We measured temperature (C), dissolved oxygen (% and mg/L), conductivity (µS/cm), pH, and nitrate [Chris: I feel like I'm missing something and don't have the field notebook to check) every half meter from the surface to the bottom of the lake. In addition, we measured water clarity (Secchi disk depth; m) at the three locations. Using the water quality profiles, we determined the current location of three stratified layers within the lake: epilimnion, metalimnion, and hypolimnion. We then took water samples at the epilimnion (0.5 m below the surface), metalimnion (in the approximate middle), and hypolimnion (0.5 m above the bottom) at all fifteen sampling sites (i.e. five sites in each of three zones) using a beta bottle (i.e. Kemmerer sampler) and pre-cleaned 125m HDPE bottle. For each site that was too shallow to contain all three stratified layers, we randomly determined an additional sampling site within that zone in areas that were deep enough to contain the needed layers. We took water samples at the additional points so that each zone had a total of five samples representing each layer (i.e.

epilimnion, metalimnion, and hypolimnion). The five samples characterizing each layer within each zone were composited together so there were three final composites for each zone (i.e. one representing each layer in each zone). In addition, the inlet to Lower Bolton Lake from Middle Bolton Lake was sampled and analyzed separately. Samples were stored at -20° C prior to chemical analyses of other water quality parameters within the laboratory.

The composited sample within each zone provided an integrative indication of CB and algal species taxonomic composition and relative abundances. The technique used for quantitative determination of algal and CB density was based on an enumeration method using a compound microscope and Sedgwick-Rafter counting cell. Taxonomic keys, including Bellinger and Sigee, were used in the identification of algal species (2015). CESE utilized a novel, rapid analytical method (Bogialli et al. 2005, Kaloudis et al. 2013, Provatas et al. 2014) using liquid chromatography/ tandem mass spectroscopy (UPLC/MS/MS) to quantify concentrations of MC analogs, while maintaining high sensitivity for each of the compounds. The most recent microcystin method detection limits (MDLs) range from 0.02-0.05 ng/mL. A full suite of physicochemical characteristics was measured on the water samples, and included chlorophyll, nitrogen and phosphorus series (ammonia, nitrate, nitrite, ortho-phosphorus, total nitrogen, total phosphorus), and pH. CESE is certified by the CTDPH for the analysis of all these characteristics and each method corresponds to approved USEPA methods and quality assurance guidance.

Results

Cyanobacterial blooms occurred from May through October and resulted in detectible microcystin concentrations throughout the study, with a total microcystin concentration of 9.4 ppb observed in October, exceeding the USEPA recreational criteria of 8 ppb. No samples contained detectible concentrations of anatoxin-a, cylindrospermopsin, or nodularin.

On July 28, 2021, the swimming area in Lower Bolton Lake was closed for recreational use due to the presence of a large, significant algae/cyanobacterial bloom. The Eastern Highlands Health District closed the area due to algae and CB cell counts (*Figure 2*). This project collected samples that bracketed the date of the closure (July 22 and August 10) and although the area was closed for swimming, the total MC concentrations were below the EPA threshold of 4 ppb, with a maximum value of 0.5 ppb. It was not until later in the season, specifically, our last collection event on October 15, 2021 where concentrations of MCs exceeded the EPA threshold, with concentrations of 9.47 ppb (zone 2), 7.81 ppb (Zone 1), and 1.02 ppb (zone 1).

There were no significant differences in mean concentrations of measured parameters, including water quality, cyanobacterial counts, or MC concentrations, by lake zone, except for ortho-phosphorus ($p<0.001$), which had the highest concentrations at the outlet from Middle Bolton Lake when compared to the other three zones (0.099 ppm vs. 0.067 ppm).

Figure 2. Closure of Lower Bolton Lake in July 2021 due to an algae bloom. (Photograph courtesy J. Lech)

Mean concentrations of Microcystins, both total and individual, and cyanobacteria density did significantly differ by depth, with the highest concentrations measured in the surface (epilimnion) samples, and the mid (metalimnion) and bottom (hypolimnion) samples significantly lower in concentration ($p<0.05$).

Table 1. Mean concentrations of Microcystins (ppb) and Cyanobacteria (cells/mL) in Lower Bolton Lake.

Depth	Microcystin-RR	Microcystin-LR	Microcystin-LA	Total Microcystins	Cyanobacteria
Epilimnion	0.696	0.156	0.100	0.951	10387.6
Metalimnion	0.161	0.091	0.059	0.311	8527.4
Hypolimnion	0.115	0.076	0.047	0.238	6647.8

To develop the model to understand how spatiotemporal variation in concentrations of CTx is related to variation in densities of CB, densities of algae, or water quality characteristics we utilized a generalized linear mixed model (GLMM). Models were run separately for each group of explanatory variables and for each microcystin as well as for total microcystins. For all analyses, day and zone were used as random factors to account for spatiotemporal autocorrelation and repeated measures (Willig 1994). We first evaluated the effects of nitrogen and phosphorus species on microcystin concentration, other water quality parameters on microcystin concentration, or cyanobacteria density on microcystin concentrations (Table 2).

A total of 8 parameters (**in bold**) showed significant ($p<0.5$) correlation between the parameter and the concentration of individual MCs or total MCs. For each of the three overarching groups evaluated (nitrogen and phosphorus, water quality, cyanobacteria), the model was able to determine between 39.1 and 73.3% of the variation in total and individual CTx.

Table 2. Evaluation of the effects of water quality and cyanobacterial density on microcystin concentrations in Lower Bolton Lake.

	Microcystins			
	RR	LR	LA	Total
Nitrogen and Phosphorus				
Ammonia	**0.008**	**< 0.001**	**< 0.001**	**0.007**
NOX	0.255	0.179	0.744	0.199
Total Nitrogen	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Ortho Phosphorus	**0.019**	0.278	0.109	**0.040**
Total Phosphorus	0.095	**< 0.001**	**< 0.001**	0.055
Model R^2	0.500	0.391	0.496	0.512
Water Quality Paramters				
Chlorophyll	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Dissolved Oxygen	0.871	0.858	0.732	0.906
Conductivity	0.751	**0.002**	**0.008**	0.514
pH	0.101	0.554	0.800	0.132
Temperature	0.862	0.729	0.961	0.976
Secci Depth	0.856	0.269	0.368	0.721
Model R^2	0.582	0.693	0.674	0.650
Cyanobacteria spp.				
Dolichospermum	0.871	0.213	0.228	0.703
Chrysosporum	0.084	0.096	0.072	0.100
Microcystis	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Chroococcus	0.129	**< 0.001**	**< 0.001**	**0.033**
Planktolyngbya	0.754	0.227	0.301	0.778
Planktothrix	0.335	0.810	0.318	0.357
Model R^2	0.571	0.733	0.690	0.603

We next optimized the model by isolating the eight variables that were found significant in the prior analysis (ammonia, total nitrogen, orthophosphorus, total phosphorus, chlorophyll, conductivity, and cell density of *Microcystis spp*. and C*hroococcus spp*.) and again used GLMM to develop a predictive model using just these parameters (Table 3). This analysis determined that for MC-LR and MC-LA, these 8 variables could explain over 90% of the variability associated with the concentration of these microcystins. For total microcystins, the model determined

that these 8 variables could explain over 76.2% of the variability associated with the concentration of these microcystins in Lower Bolton Lake.

Table 3. Evaluation of the effects of the significant water quality and cyanobacterial density on microcystin concentrations in Lower Bolton Lake.

	Microcystins			
	RR	LR	LA	Total
Ammonia	0.052	**0.018**	**0.004**	**0.019**
Total Nitrogen	**0.049**	**< 0.001**	**< 0.001**	**0.013**
Ortho Phosphorus	0.714	0.064	0.591	0.527
Total Phosphorus	0.516	**< 0.001**	**0.001**	0.353
Chlorophyll	**0.015**	0.257	**0.002**	**0.007**
Conductivity	0.216	0.230	0.480	0.303
Microcystis spp.	**0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Chroococcus spp	0.826	0.571	0.410	0.677
Model R^2	0.663	0.917	0.910	0.762

Conclusions

Cyanobacterial blooms occurred at Lower Bolton Lake from May through October, for which the Eastern Highlands Health District closed the lake to recreational swimming. The investigators were very fortunate to have a cyanobacterial bloom during the course of this study, since this was a one year project. There were in detectible microcystin concentrations throughout the course of this study, with a total microcystin concentration of 9.4 ppb observed in October, exceeding the USEPA recreational criteria of 8 ppb. No samples contained detectible concentrations of anatoxin-a, cylindrospermopsin, or nodularin. As would be expected, cyanotoxin concentrations were highest in the surface water samples and cyanotoxins exhibited a significant positive correlation with CB cell density. We were able to develop a lake-specific model to predict microcystin concentrations using chemical and physical water quality parameters as well as algae and CB density. When we optimized the model to include the density of the cyanobacteria, *Microcystis spp*., and other key nitrogen/phosphorus and real-time measurements, the model was able to determine 76.2 of the variation in the total microcystin concentration, and over 91% of the variation in microcystin-LR and -LA. It is important to point out that this model has not been tested or validated on other Connecticut lakes, and may not be applicable to these other systems. This study can, however, serve as a prototype for the development of monitoring programs that can be implemented elsewhere in Connecticut.

Acknowledgements

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