Project Title: **Cyanotoxin presence and year-round dynamics in Falls Lake, NC**

EXECUTIVE SUMMARY

Key Questions

- Are cyanotoxins present in Falls Lake? When during the year are they present and where?
- Can spatiotemporal patterns in cyanotoxins be linked to environmental conditions?
- Which potential toxin-producing cyanobacterial taxa are present in Falls Lake? Does their spatiotemporal distribution link to toxin patterns and/or environmental factors?

Research Methods Monthly surface sampling from July 2019 through December 2021, in collaboration with the NC Department of Environmental Quality (NCDEQ), encompassed 11 stations across Falls Lake. Surface samples were collected to determine chlorophyll-a, dissolved toxins, and particulate toxin concentrations (microcystin, anatoxin, cylindrospermopsin, saxitoxin and β-Methylamino-L-alanine) and to collect DNA for high-throughput sequencing. Passive *in situ* toxin sampling devices were deployed at a subset of 4 stations across the lake to measure accumulated toxins. Environmental (e.g., temperature, and nutrients), meteorological and hydrological data are analyzed for relationships with cyanobacterial and toxin dynamics.

Findings

Maximal toxin concentrations from monthly collections did not exceed regulatory thresholds established by the World Health Organization(WHO) [1]. However, accumulated dissolved toxin levels, detected employing passive *in situ* samplers, indicated that monthly sampling is likely insufficient to document the full range in toxin dynamics.

Monthly, relatively low, microcystin (MC) levels did not align with the moderate risk of health effects from MC exposure suggested by the WHO based on chlorophyll-a (chl-a) levels. Thus, algal biomass alone is not a reliable indicator of cyanotoxin exposure risk in Falls Lake.

Co-occurrence of more than one cyanotoxin was observed in 14% of dissolved, 36% of particulate, and 43% of accumulated dissolved toxin samples alerting to the potential for chronic exposure to multiple toxins, especially during fall and summer seasons and in lower and tributary regions.

Note: Cyanobacterial composition analyses via high-throughput sequencing are currently underway, and the potential identification of key targets for monitoring approaches, as well as the final analyses of environmental linkages to community shifts are pending.

Management Implication and Recommendations

1) Continued monitoring of common toxins (of microcystin variants) on a weekly to biweekly basis at selected stations (upper, middle/tributary, lower lake) is highly recommended to resolve toxin dynamics beyond monthly snapshots. Lake research suggest that the eutrophic status of Falls Lake, make it prone to experience intensification of cyanobacterial harmful algal blooms (CyanoHABs) in response to climate change[2].

2) Conduct seasonal surveys (i.e., summer and fall) to determine food web accumulation of cyanotoxins in commonly caught/consumed fish. Toxin accumulation by passive samplers indicate the potential active accumulation of cyanotoxins through the food web.

Researchers:

Astrid Schnetzer and Emily Pierce, Department of Marine Earth and Atmospheric Sciences, NC State University; Steve Mohn, NC's Department of Environmental Quality, Division of Water Resources

PROJECT NARRATIVE

OVERVIEW

Cyanobacterial Harmful Algal Blooms (CyanoHABs) in North Carolina freshwater systems can adversely impact drinking water, fisheries, tourism and food web resilience. The main goals of this study are to examine the spatiotemporal dynamics of CyanoHABs in relation to cyanotoxins in Falls Lake. We determined algal growth together with cyanotoxin presence at multiple sampling sites throughout the lake and aimed to identify environmental conditions that favor algal growth and/or toxin production ("hot spots"). Furthermore, we focused on identifying the cyanobacterial taxa that dominate throughout the lake system and are associated with toxin presence and/or certain environmental conditions. All aspects of this study have been completed, except the analyses of sequencing data. Samples have been extracted and sent out for high-throughout sequencing and we expect to receive results within the next month.

RESEARCH METHODS

Sampling.

Monthly surface sampling from July 2019 through December 2021, in collaboration with the NC Department of Environmental Quality (NCDEQ), encompassed 11 stations across Falls Lake (Fig. 1). Sampling did not occur in January or February of 2021 due to COVID-19. Surface samples (0 – 0.25 m) were collected in 1L PETG bottles and then transported to the lab within ~ 6 hours and processed to determine chlorophyll-a (chl-a), dissolved toxin, and particulate toxin concentrations and for DNA extraction and sequencing. As part of the NC Division of Water Resources (NCDWR) Ambient Monitoring Program, samples were also collected (depth-integrated from surface to 2x Secchi) to determine concentrations for ammonia (NH_3), nitrite plus nitrate (NO_2+NO_3), total Kjeldahl nitrogen (TKN), total phosphorus (TP), temperature, turbidity, pH, dissolved oxygen (DO), and conductivity (NCDWR standard operating manual). Solid Phase Adsorption Toxin Tracking (SPATT) devices were deployed at a subset of 4 stations across the lake to measure accumulated toxins (triangles in Fig. 1). Additional meteorological and hydrological data were obtained from the State Climate Office of North Carolina, NCDWR's online Drought Monitor History database, and the US Geological Survey (USGS) (in progress).

Chlorophyll a

For chl-a analysis ($\mu g\ L^{-1}$), approximately 50 mL of each sample were filtered onto µm Whatman GF/F filters and stored at -20°C until analysis. Filters were thawed and suspended in 7 mL of 100% acetone, sonicated for 5 seconds at 50% intensity (Fisher Scientific, Hampton, NH, USA, Model 120 Sonic Dismembrator) and extracted in the dark at -20°C for 24 hours[3]. Samples were run fluorometrically (Turner Designs Trilogy Laboratory Fluorometer) using the non-acidification method[4].

Cyanotoxin Analysis

Approximately 50 mL of each sample were filtered onto µm Whatman GF/F filters for the analysis of particulate toxins and 1.5 mL of filtrate collected in 2 mL glass autosampler vials for the analysis of dissolved toxins. Filters and filtrate were stored at -20°C until analysis. Filters were then extracted through one freeze/thaw cycle in 3 mL of Milli-Q water followed by a 30 second sonication at 50% intensity (Fisher Scientific, Hampton, NH, USA, Model 120 Sonic Dismembrator) [5]. SPATT units from sites NEU013B, LI01, NEU019E, and, NEU019P that were deployed monthly, were stored at -80°C until extraction following previously published protocols except elutes were combined into one sample prior to analysis[6,7]. Filter extracts and filtrate were analyzed for microcystin (MC), cylindrospermopsin (CYN), anatoxin (ANA), β-Methylamino-L-alanine (BMAA), and saxitoxin (SXT) (each in µg L^{-1}) and SPATT extracts were analyzed for MC, ANA, and CYN (ng toxin (g $resin^{-1}$) day^{-1}) using commercially available enzyme-linked immunosorbent (ELISA) kits (Gold Standard Diagnostics, Westminster, PA, USA): MC-ADDA (Product #520011; sensitive to MCY-LR, -YR, -LF, -RR, LW, and nodularin; LDL = 0.10 µg L^{-1}), CYN (Product #522011; sensitive to CYN, deoxy-CYN, 7-Epi-CYN; LDL = 0.04 µg L^{-1}), ANA (Product #520060; sensitive to anatoxin-a and homoanatoxin-a; LDL = 0.1 µg L^{-1}), STX (Product #52255B; sensitive to STX and other paralytic shellfish poison [PSP] toxins; LDL = 0.015 µg L^{-1}), and BMAA (Product #520040; sensitive to BMAA; limit of quantitation = 4 µg L^{-1}). ELISA microplates were read at 450 nm using a BioTek ELx800 Absorbance Microplate Reader (BioTek, Winooski, VT, USA).

Bacterial Composition Analysis (pending)

Bacterial community composition will be analyzed based on 16S rDNA gene sequencing. Surface water (50 mL) were filtered onto 0.7 µm Whatman GF/F filters and frozen at -20°C until further processing. DNA was extracted using the DNeasy PowerWater Kit (Qiagen, Germantown, MD, USA) and quantified using the Invitrogen Qubit 1X dsDNA high sensitivity assay kit and a Qubit 2 fluorometer (Thermo Fisher Scientific, Waltham, MA). Amplification and sequencing is currently underway through the University of Illinois Chicago Genomics Research Core using universal V4-V5 primers[8,9] with an Illumina Miseq machine (paired-end, 300x300bp). Sequences will be analyzed using the DADA2 pipeline[10] implemented in R v.4.3.0 using the Silva v138.1 taxonomic reference database[11].

Statistical Analysis

Data were compared over different spatiotemporal scales including season, year, station, and region. For this later approach, the 11 stations were classified into 4 general regions (Upper = NEU013, NEU013B; Middle = NEU0171B, NEU018E, NEU019E; Tributary = LC01, LLC01, LI01; Lower = NEU019L, NEU019P, NEU020D) [12]. Statistical analysis was completed using RStudio[13]. Data visualizations were produced using the ggplot2 package[14]. An ANOVA test within the AICcmodavg package[15] was used to assess differences in environmental parameters and toxin concentrations across regions and time. Tukey honest significant differences were computed to determine pairwise difference between significantly different groups using the R stats package.

The final component, cyanobacterial assemblage data from gene sequencing will be analyzed in relation to the toxin data and the environmental information. Results are expected to provide

important insight into which species are best targeted to monitor bloom activity within the lake using highly-sensitive molecular assays. Understanding the environmental drivers of genotype composition is expected to proof as a powerful predictive metric, especially in combination with toxin information, in systems that are prone to toxic HABs [2].

FINDINGS

Environmental Parameters

Temperature in Falls Lake fluctuated seasonally within a range of 7.6 °C to 33.4 °C with an average temperature of 21°C. pH ranged between 6.2 to 9.1 with an average value of 7.6. Conductance ranged between 1 µmhos cm^{-1} to 261 µmhos cm^{-1} with an average conductance of 97 µmhos cm^{-1}. Turbidity ranged between 2.9 to 50 NTU with an average value of 10.7 NTU (Fig. 2). $NO_2 + NO_3$ (NOX) (mg L^{-1}) were measured at a range of 0.02 mg L^{-1} to 0.38 mg L^{-1} with an average concentration of 0.05 mg L^{-1}. NH_3 concentrations (mg L^{-1}) ranged between 0.02 mg L^{-1} to 0.35 mg L^{-1} with an average value of 0.03 mg L^{-1}. TKN ranged between 0.3 mg L^{-1} to 1.3 mg $L^{-1}$with an average conductance of 0.73 mg L^{-1}. TP ranged between 0.02 mg L^{-1} to 0.15 mg L^{-1} with an average value of 0.05 mg L^{-1} (Fig. 3). Temperature showed a seasonal trend across the system with the highest monthly average temperatures occurring during the late summer and early fall ($p < 2 \times 10^{-16}$, ANOVA). Conversely, $NO_2 + NO_3$ and NH_3 also showed seasonal trends with higher concentrations observed in late fall and early winter ($p < 2 \times 10^{-16}$, $p = 3.9 \times 10^{-8}$ ANOVA). There were regional differences in turbidity with higher average monthly values in the upper lake region ($p = 1.2 \times 10^{-4}$, ANOVA). There were also regional differences in all average monthly nutrient values with higher TP, TKN, and $NO_2 + NO_3$ concentrations in the upper region and higher NH_3 concentrations in the lower region ($p < 2 \times 10^{-16}$, $p = 5.8 \times 10^{-10}$, $p = 0.04$, $p = 2.9 \times 10^{-10}$, ANOVA).

Chlorophyll a

Chl-a concentrations ranged between 1.1 µg L^{-1} to 65.7 µg L^{-1} with an average of 19.9 µg L^{-1} (Fig. 4). Levels rose above the 40 µg L^{-1} EPA threshold for impairment in only 5% of samples. Chl-a concentrations were not significantly different across seasons ($p = 0.17$, ANOVA). Chl-a concentrations were significantly different across regions with higher concentration observed in upper and tributary regions ($p = 2.5 \times 10^{-5}$, ANOVA). Chl-a was positively correlated with conductance, turbidity, TP and TKN and showed a negative relationship with temperature, pH and varying dissolved nitrogen sources (Table 2). Further analyses will include hydrological and meteorological data (pending).

Cyanotoxins

Maximal toxin concentrations from monthly discrete collections did not exceed regulatory thresholds established by the World Health Organization(WHO) [1]. Particulate MC concentrations ranged from below detection level (BDL)- 0.29 µg L^{-1} with an average of 0.03 µg L^{-1}. Particulate CYN and ANA levels ranged from BDL - 0.01 µg L^{-1} and BDL - 0.36 µg L^{-1} with

averages of 0.01 µg L^{-1} and 0.10 µg L^{-1}, respectively. Particulate BMAA concentrations ranged from below detection level (BDL)- 86.3 µg L^{-1} with an average of 4.1 µg L^{-1}. Particulate SXT was consistently BDL. Dissolved MC concentrations ranged from below detection level (BDL)- 0.39 µg L^{-1} with an average of 0.14 µg L^{-1}. Dissolved CYN and ANA levels ranged from BDL - 0.54 µg L^{-1} and BDL - 0.20 µg L^{-1} with averages of 0.12 µg L^{-1} and 0.16 µg L^{-1}, respectively. Dissolved BMAA concentrations ranged from below detection level (BDL)- 26 µg L^{-1} with an average of 10.3 µg L^{-1}. Dissolved SXT was consistently BDL. Overall, MC was most consistently detected across the varying sampling types (particulate, dissolved and accumulated dissolved (Table 1). Stepwise model selection to explain total discrete MC (part. + dis.) conc. using all environmental parameters produced a best model that included only temperature, NH_3, and turbidity which explained 10.8, 5.6, and 5.7 % of variance respectively.

Co-occurrence of more than one cyanotoxin was observed in 14% of dissolved, 36% of particulate, and 43% of accumulated dissolved toxin samples. Co-occurrence was more commonly observed during fall and summer seasons and in lower and tributary regions for particulate, dissolved, and accumulated dissolved forms (Fig. 5). Accumulated dissolved toxin levels, detected employing passive in situ samplers, indicated that monthly sampling may be insufficient to document the full range in toxin dynamics where episodic toxic events are not captured and/or the continuous presence of chronic toxin concentrations is underestimated. Of the three cyanotoxins assessed from SPATTs, MC and CYN were detected in 89% and 48% of samples respectively, whereas ANA was only detected in 2% of samples (Table 1). Because of the low positive SPATT sample size (n = 1), ANA was excluded from additional statistical analyses. Average accumulated MC concentration from SPATTs (38.5 ng (g resin)$^{-1}$ day^{-1}) was two orders of magnitude greater than average accumulated CYN concentrations from SPATTs (0.19 ng (g resin)$^{-1}$ day^{-1}) (Table 1 and Fig. 6). Average accumulated dissolved CYN concentrations from SPATTs did not vary across seasons or lake region, while accumulated dissolved MC differed across seasons and lake region with more accumulation in summer and fall in middle and lower lake regions (p =3.1e-5; p = 3.6e^{-3} resp., ANOVA, Fig. 6). Regional patterns in MC accumulation are inverse to regional patterns in chl-a which was higher in upper regions of the lake potentially indicating a spatial disconnect between algal biomass and toxin presence within the system.

MANAGEMENT IMPLICATIONS

This study is the first to show that cyanobacterial communities in Falls Lake are linked with the recurrence of multiple cyanotoxins throughout the year and across several lake regions. These findings fall in line with an increasing number of studies that have confirmed the ubiquitous nature of cyanotoxins, their simultaneous presence in varying environments and the need for further research to characterize the conditions that favor toxin production[16-19]. The continued development and employment of highly sensitive toxin-tracking approaches (e.g., SPATTs), together with an expanding tool-kit for genomic testing, will be essential for further examination of cause–effect

relationships and providing the knowledge needed to predict the likelihood for current and future toxin exposure via varying exposure pathways in Falls Lake. Our study clearly demonstrates that further monitoring and expanded research (e.g., food web contamination study and determination of MC congener composition) in Falls Lake is warranted to protect the lake's dedicated uses. Falls Lake provides drinking water for over 500,000 people and serves as a significant recreational site for swimming, boating, and fishing[20].

Guidelines by the WHO to assess risks (low, moderate and high) from MC exposure are based on either direct measurements of MC concentration, the determination of chl-a or cyanobacterial density[1]. However, applying these three metrics, a water body can be at risk based on one, but not all, of these criteria. For instance, for over 1100 lakes in the US, agreement for risk assessment based on all three parameters was only observed for 27% of the systems[21]. Our findings from Falls Lake agree with these reports and indicate that MC exposure risk based on chl-a measurements (moderate risk) do not agree with those based on actual microcystin measurements (low risk). Thus, the inclusion of toxin measurements when chl-a values indicate active bloom conditions is a critical tool for continued monitoring across Falls Lake.

Another major knowledge gap that has not been addressed for Falls Lake but warrants further study, is the congener composition of MC as the most prevalent toxin detected. Cyanobacteria can potentially produce 100s of MC congeners of varying toxicity but bloom events are typically associated with a subset of dominant congeners[22-24]. Environmental testing, rodent, and human toxicity studies, however, have primarily focused on a single MC congener (MC-LR) [1]. A recent study by our research group within the Albemarle Sound region indicated that MC-LR might be present together with less toxic variants including MC-RR or MC-YR. This information is key since all WHO guidelines for drinking water, recreation and consumption are based on MC-LR and none of the co-occurring MC congeners. Gaining an understanding of variability in MC concentrations and congener composition in Falls Lake as well as in tested fish is needed to accurately assess exposure risks from lake uses. Additional key findings in this study alert to other important issues related to CyanoHABs in Falls Lake that, if unaddressed, may have more severe implications for public health.

1) While monthly discrete toxin concentrations did not rise above regulatory thresholds for MC, the detection of multiple types of cyanotoxins currently prevent an accurate assessment of chronic exposure to multiple toxins (including MC congener mixtures).

2) The results on accumulated dissolved toxins based on in situ tracking devices indicated that our monthly sampling approach may well have missed peak toxin concentrations or the occurrence of multiple events between discrete sampling efforts.

3) Toxin accumulation based on passive toxin trackers are a good indicator of the potential for active toxin accumulation through the food web. There is currently no information on whether commonly caught fish from Falls Lake are positive for cyanotoxins.

A comprehensive review of CyanoHAB research suggests that the eutrophic status of Falls Lake, make it prone to the intensification of blooms in response to climate change and, thus, increasing risk of toxin exposure[2]. Given our results so far, we highly recommend continued, higher-frequency monitoring of cyanotoxins in Falls Lake at least within the middle/tributary and lower

portions of the lake. In addition, monitoring should expand to determine MC congener composition in both water (intracellular and dissolved fractions) as well as in aquatic organisms commonly caught for human consumption. Since this is the first comprehensive study that focuses on cyanotoxin dynamics in Falls Lake we believe the information to be of value for residents, monitoring agencies and recreational users. We hope our findings will inform future investigations, adapted monitoring approaches and an expansion of targeted testing to accurately assess both current and future risks to lake uses and public health for Falls Lake.

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