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Research Note

Total Microcystin Concentration Variability in Water Samples and Recommended Minimum Volume (20 mL) for Freeze Thaw Cycles

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Abstract: Cyanobacterial harmful algal blooms (cyanoHABs) continue to be a monitoring and research focus, particularly on the occurrence of toxins like total microcystins. The objectives of this study were to evaluate sampling and analytical variability in measured total microcystin concentrations and then to evaluate the volume of raw water needed in the freeze thaw cycle to reduce sampling variability. Water samples were collected from a recreational lake with annual cyanoHABs, and then 2 mL was used in freeze thaw cycles before total microcystin analysis. Then, sample volumes used in the freeze thaw cycles varied from 2 to 300 mL for total microcystin analysis. With three separate experiments, we observed a great deal of sampling variability (when using 2 mL in the freeze thaw cycles) while analytical variability was much less. In fact, sampling variability could potentially account for temporal variability observed in the routine monitoring. However, when sample volume used in the freeze thaw cycles increased, total microcystin variability decreased. We recommend at least 20 mL to be used in the freeze thaw cycles when analyzing total microcystins in environmental samples.

Keywords: *total microcystins, ELISA analysis, freeze thaw volume, sampling variability*

Cyanobacterial harmful algal blooms (cyanoHABs) have been and continue to be a research focus across the USA and globe, where scientists are trying to understand the drivers in cyanobacterial toxin production. These blooms have been observed in freshwaters, mainly lakes, across the USA (Loftin et al. 2016b), but toxins can be measurable in streams (Loftin et al. 2016a; Graham et al. 2020; Austin and Haggard 2022). While toxins from cyanoHABs in lotic systems tend to be low, occurrences of elevated toxins, particularly total microcystins, have been reported in large rivers, e.g., Obryzyca River, Poland (Czyzewska et al. 2020) and Poteau River, Arkansas, USA (Haggard, B.E., unpublished data).

Microcystin in all its various forms (i.e., total microcystins) is one of the most studied cyanobacterial toxins in freshwaters, including lakes and lotic systems. Microcystins are usually present and often in high concentrations when other toxins like anatoxin, cylindrospermopsin, and saxitoxin are measured in water samples from lakes (Graham et al. 2010) and rivers (Loftin et al. 2016a; Czyzewska et al. 2020). Also, total microcystins (and nodularins) are easily measured by water labs using the enzyme linked immunoassay techniques (ELISA, Method 546; EPA 2016), but the ELISA Method is used by labs with skilled analysts. For these reasons, total microcystins are the focus of many studies on occurrence and drivers of cyanobacterial toxin production in freshwaters.

The ELISA technique for total microcystins has been shown to be quantitative, reliable, and quick (Nagata et al. 1997), although this technique is an indirect competitive assay or measure of this toxin. Generally, this technique can produce repeatable analytical results (Massey et al. 2020), and its method detection limit (MDL) is much less

Research Implications

- Variability in total microcystin concentrations was observed with repeated sampling and subsampling within an individual bottle.
- Some variability in total microcystin concentrations observed in lake studies might be due to sample volume used in freeze thaw cycles.
- Total microcystin variability decreased as sample volume used in three freeze thaw cycles increased.
- We suggest at least 20 mL for freeze thaw cycles, especially if water is not mechanically homogenized.

than recreational guidelines (e.g., 8 µg L^{-1} total microcystin; EPA 2019) and even slightly below drinking water limits (e.g., 0.3 µg L^{-1} for infants; EPA 2015). The method's freeze thaw cycles have been shown to produce strong recovery of intracellular total microcystins (Greenstein et al. 2021), so the use of raw water with this method provides a solid measure of extracellular (free in water) and intracellular total microcystin concentrations.

The volume from water samples from lakes and rivers used in the freeze thaw cycles varies (Table 1), but in general, most standard operating procedures, labs, and literature studies use 30 mL or less. Method 546 (EPA 2016) suggests 5 to 10 mL of well-mixed water be used in the freeze thaw cycles before ELISA analysis. We have a recreational lake (Lake Fayetteville, Northwest Arkansas) which experiences cyanoHABs each growing season, and on May 7, 2019 we measured 1.8 µg L^{-1} total microcystins at 13:00 and then over 11 µg L^{-1} at 16:00 (Figure 1). This variability between sampling events on the same day led to two questions: 1) could there be temporal variability in total microcystins during the cyanoHABs at this lake?, and 2) does the volume of raw water used in the freeze thaw cycles influence variability in measured concentrations of total microcystins? The purpose of our study was to answer these questions, but our efforts focused more on the second question to help guide future total microcystin analysis at the Arkansas Water Resources Center (AWRC) water quality lab.

Methods

Lake Fayetteville is a small recreational lake with a surface area of ~0.6 km^2 and catchment area of 24 km^2, which is managed by the City of Fayetteville, Arkansas. The first recorded study on this lake was in 1968 (Meyer 1971), which showed that the phytoplankton community was dominated by cyanobacteria at that time, and the annual pattern in dissolved nutrient supply was the same as today (Haggard et al. 2023a). Total microcystins were first measured on November 19, 2018 by a First-Year Engineering Honors Research Team under our mentorship and we were surprised to see total microcystins (0.442 µg L^{-1}) greater than MDL in late fall with colder water temperatures. We began routine cyanoHAB and total microcystins monitoring in March 2019, resulting in published studies on cyanoHABs, microcystin, and environmental drivers including Wagner et al. (2021) and Haggard et al. (2023a; 2023b). We have been collecting water samples from three access points along the north shore of the lake since 2019, and we sampled off the marina and kayak platforms near the dam in 2019 and 2020 to answer the question posed in this study (Figure 1). All water samples were collected approximately weekly, and each had total microcystins measured using the ELISA technique (Method 546; EPA 2016).

Our researchers collected additional water samples for total microcystins on select sampling dates beyond those collected for our routine monitoring. On June 11 and July 1, 2019, multiple water samples were collected 0.2 m below the water surface at the end of the kayak dock near the dam to evaluate potential sampling and analytical variability. On April 27, 2020, multiple water samples of the surface scum were collected to evaluate sampling and analytical variability; the surface scum was targeted intentionally, as it was likely to have greater toxin concentrations. Approximately 15 1-L samples were collected each date, and processed upon return to the lab. Each bottle had ~2 mL saved in 4 mL amber glass vials for the freeze thaw cycles, and one random bottle was subsampled ten times (~2 mL or less each time in 4 mL amber glass vials). Following three freeze thaw cycles, water with lysed contents

Table 1. Select references and labs providing volume used for freeze thaw cycles in analysis of total microcystins using enzyme linked immunosorbent assays techniques (based on Google Scholar search and eight pages viewed, as well as select personal communications).

ELISA Citation	Freeze Thaw Volume	Qualifier, If Any
Method 546; EPA 2016	5-10 mL	Well-mixed water
Thorpe and Brunet 2021	4 mL or less	Based on half of vial volume (8 mL)
Abdullahi et al. 2022	5 mL	Subsequent methanol extraction
Cullen 2009; Greenstien et al. 2021; Wilson 2022	~10 mL	Not applicable; Only small volume (µL) needed for Method 546 analysis
Wood et al. 2006	10 mL	100 mL water frozen initially; 10 mL two additional freeze thaw cycles
Ohio EPA 2018	20 mL	Additional preprocessing if chlorinated water sample
Klamath Blue Green Algae Working Group 2009	30 mL	Based on 25% of vial volume (120 mL or 4 oz vial in methods)
Nagata et al. 1997	30 mL	40 mL original volume
Olsen 2022	2-250 mL	If quick freeze thaw needed, the lesser volume in the range is used
Loftin et al. 2016a; 2016b	25-50 mL	Lysed sample filtered and stored; Frozen until analysis
Trout-Haney et al. 2016	750 mL or more	Entire volume three freeze thaw cycles and then centrifuged

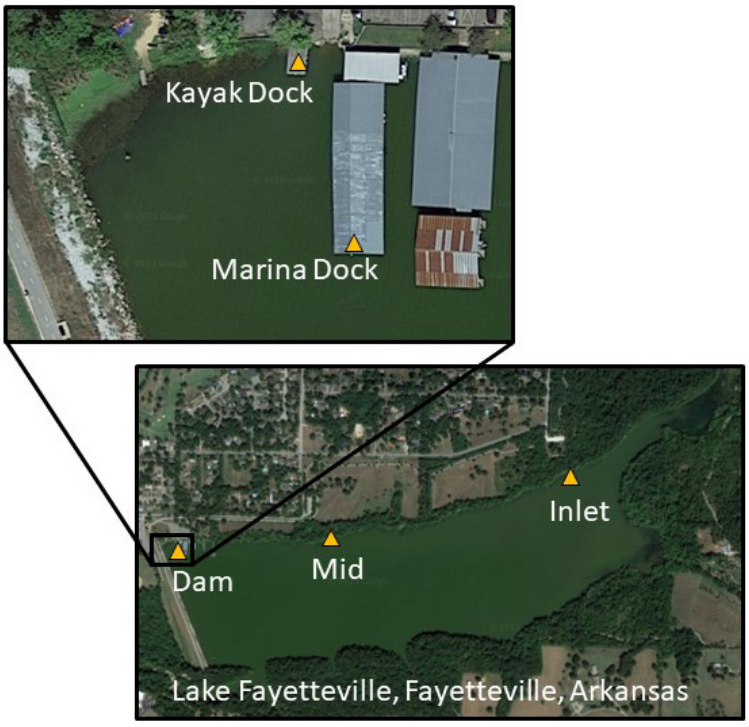

Figure 1. Map of sampling sites, marina dock, and kayak dock at Lake Fayetteville in Fayetteville, Arkansas.

was analyzed for total microcystins. One of the ten vials from subsampling was analyzed ten times; the vial was randomly selected. Variability across "between bottles," "within bottle," and "within vial" was compared by calculating the absolute residual for each individual concentration relative to the group mean. Mean absolute residuals were then compared across all bottles, within bottle, and within vial, using analysis of variance (ANOVA) with least significant difference (LSD) at an alpha of 0.05 ($p<0.05$). We used the software program, SigmaPlot Version 14.5 (Systat Software, Inc.) for all statistical comparisons.

On June 30 through July 1, 2019, we collected water samples to capture potential diurnal variability in total microcystin concentrations. The first water sample was collected at 17:00 from the end of the dock at the marina near the dam, and water samples were collected every hour until 23:00. Water samples were collected every 1.5 hr from 00:30 to 5:00 on July 1, and then sampling shifted back to hourly until 17:00 that day. A small volume (~2 mL) was put through freeze thaw cycles for total microcystin analysis from these samples.

On June 16th, 2020, we collected ~4 L of water from ~0.2 m below the surface at the end of the marina dock near the dam and near the shore, as well as sufficient volume of surface scum at both locations. The collected water was kept mixed at the lab by vigorously shaking each sample, while various subsample volumes were collected for the freeze thaw cycles. We used volumes of 2 mL (n=10), 5 mL (n=10), 10 mL (n=10), 20 mL (n=10), 60 mL (n=5), and 300 mL (n=3) for the below surface samples and the surface scum samples; a total of 48 subsamples for each, or 192 total for all four. All were put through three freeze thaw cycles and then analyzed for total microcystins. We assumed the true total microcystin concentration represented the mean of all subsample volumes, and then each individual concentration was converted to a Z-score to compare variability in sample volumes used in the processing and analysis. The Z-score allowed us to group each experiment, despite differences in measured total microcystin concentration before below surface and surface scum samples. Mean Z-scores of the various subsample volumes were compared using ANOVA, and means were separated using LSD ($p<0.05$).

Results and Discussion

Total microcystin concentrations were variable over time at Lake Fayetteville, showing a distinct bimodal pattern over 2019 and 2020 (Figure 2). For the most part, total microcystin concentrations are in relatively close agreement between the three sampling sites along the north shore of the lake across both study years. However, on occasion, total microcystin concentrations show increased variability between sites in 2019. As previously mentioned, lake samples from the same day (May 7, 2019) and site (dam), but just hours apart, had order of magnitude (1.78 versus 11.01 µg L^{-1}) differences in total microcystins. The second set of samples from that day were sent to the Wilson Lab at Auburn University (Table 1; https://www.wilsonlab.com/), where total microcystins at the dam site measured 4.23 µg L^{-1}. On May 27, 2019, total microcystin varied from 1.67 to 5.00 µg L^{-1} across the sites, and then the greatest measured total microcystin (15.38 µg L^{-1}) measured was observed on June 4, 2019, but other sites that day were less than 2 µg L^{-1}. This site variation persisted through late June, and then total microcystin differences between sites were less until October 29, 2019, when total microcystin varied from 2.00 µg L^{-1} at the dam to 0.31 µg L^{-1} or less at the other two sites. Considering the variability in total microcystin concentrations, we wanted to determine if these differences were real, or due to using subsample volumes on the low end of the range.

We did the first sampling variability experiment (Figure 3) at Lake Fayetteville on June 11, 2019, when total microcystin concentrations in the routine sampling varied from 0.34 µg L^{-1} at the mid-lake site to 2.96 µg L^{-1} near the dam. The sampling variability results showed significant differences and high variability, including:

- total microcystins varied from 0.39 to 2.98 µg L^{-1} across the 16 bottles collected, averaging 1.21 µg L^{-1} (± 0.60 standard deviation, SD) across all bottles that day from the kayak dock;
- total microcystins varied from 0.04 to 1.65 µg L^{-1} across the 9 vials used to subsample one bottle, averaging 0.51 µg L^{-1} (± 0.48 SD) across all vials; and
- total microcystins varied from 0.15 to 0.32

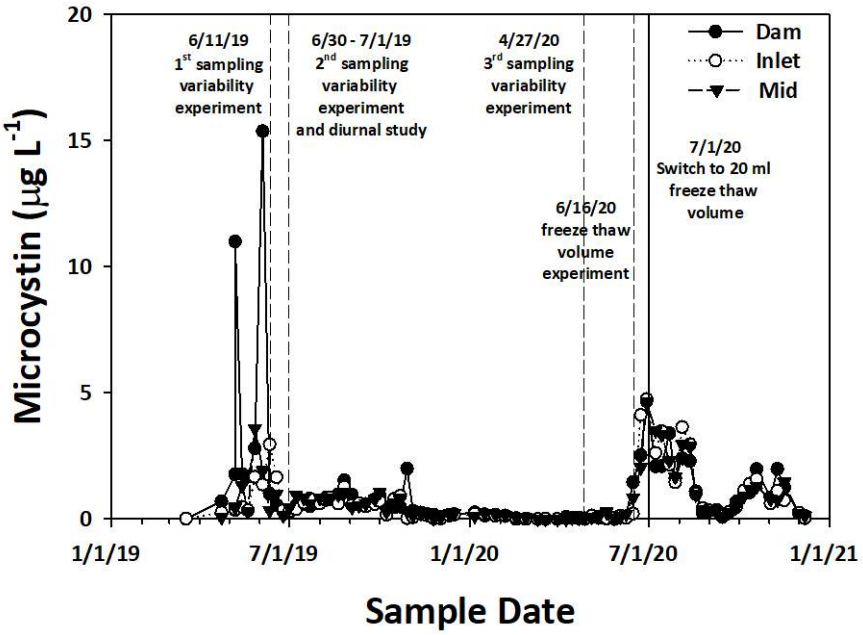

Figure 2. Microcystin concentrations measured during routine monitoring of three sites along the north shore of Lake Fayetteville from March 2019 through December 2020. Dashed vertical lines align with when each experiment was conducted over the two-year period. The solid vertical line shows when the lab switched freeze thaw sample volumes from 2 to 20 ml.

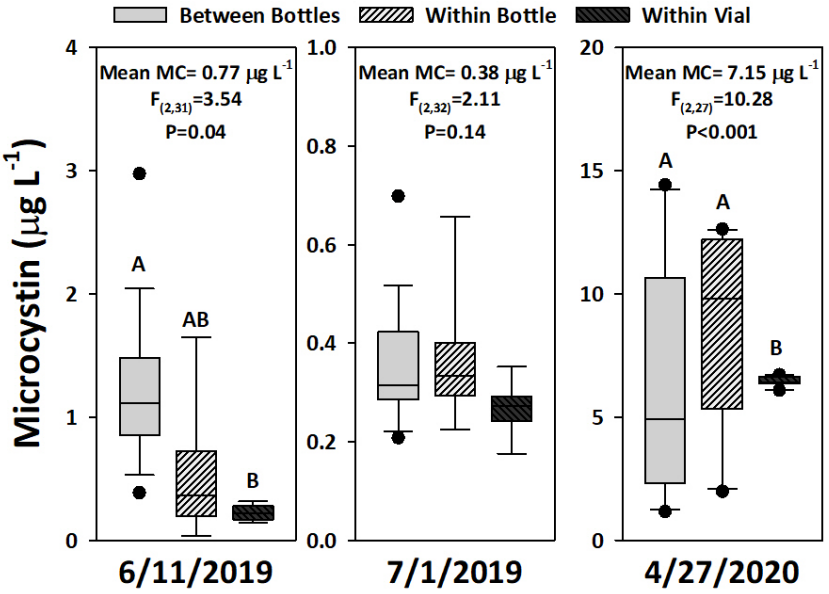

Figure 3. Box plots depicting variability in measured microcystin concentrations from sampling (Between Bottles), sample processing (Within Bottles), and analytical (Within Vial), when mean microcystin concentrations are moderate (6/11/2019), low (7/1/2019), and high (4/27/2020); letters above box plots show significant differences in the absolute value of the residuals (i.e., variability) for each experiment (ANOVA, LSD).

µg L^{-1} when the same random vial was analyzed several times.

The variability in total microcystin concentrations between bottles and within one bottle was much greater than that observed within one vial. These observations suggest that analytical variability of the ELISA method was low, as seen in other studies (Massey et al. 2020), particularly in relation to sampling variability. This leads to the questions regarding sampling variability both from the lake and sample processing within labs, i.e., subsample volume used in freeze thaw cycles.

On June 30, 2019, we looked at diurnal variability in total microcystin concentrations below the water surface from the marina dock near the Lake Fayetteville dam (Figure 4); water samples were collected every 1 to 1.5 hr for 24 hr. The concentrations of total microcystin ranged from ~0.1 µg L^{-1} at 16:00 July 1, 2019, to ~0.8 µg L^{-1} 12:00 the night before (June 30), but the variability in concentrations did not fit any diurnal patterns. For example, total microcystin concentrations were not greater just below the water surface at night when buoyancy might be increased in some cyanobacteria (Ibelings et al. 1991) nor were the concentrations greater during day when increased photosynthetically active radiation (PAR) has been positively correlated with microcystin production and content in a cyanobacteria (Wiedner et al. 2003). Even though potential diurnal patterns might be opposite, we observed variability in total microcystin concentrations, although mean total microcystin was less than 0.3 µg L^{-1} across the 24 hr period. We need to qualify that these represent water samples where only 2 mL or less was used in the freeze thaw process.

During this diurnal study, we repeated the sampling variability experiment at 15:00 on July 1, 2019. Again, sampling variability results showed no differences between subsampling and analysis but total microcystins varied two-fold to an order of magnitude difference (Figure 3), including:

- total microcystins varied from 0.21 to 0.70 µg L^{-1} across the 17 bottles collected, averaging 0.35 µg L^{-1} (± 0.12 SD) across all bottles that day from the kayak dock;
- total microcystins varied from 0.22 to 2.02 µg L^{-1} across the 10 vials used to subsample one bottle, averaging 0.53 µg L^{-1} (± 0.54 SD) across all vials; and
- total microcystins varied from 0.18 to 0.35

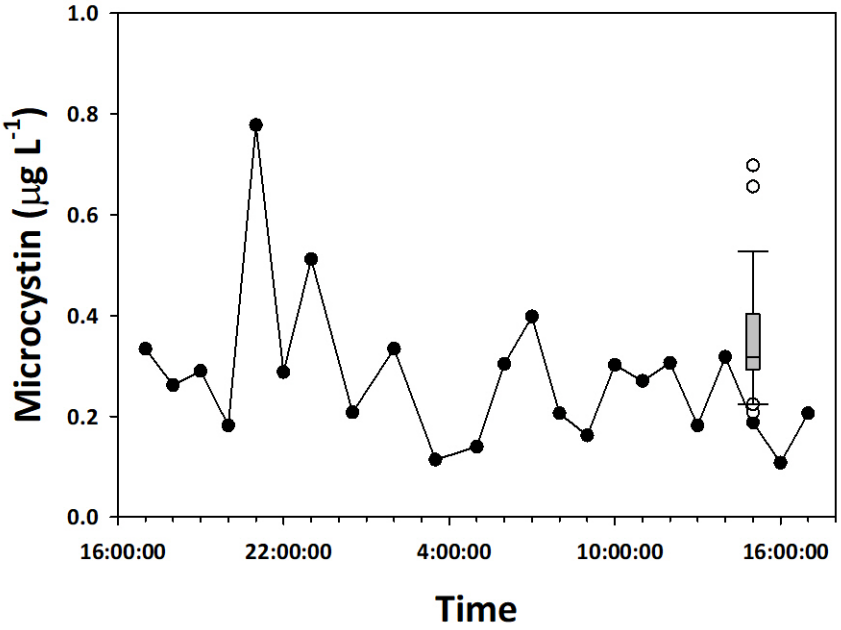

Figure 4. Diurnal variability in microcystin over a 24 hr period starting at 17:00 on 6/30/2019 to 17:00 on 7/1/2019; box plot at 15:00 on 7/1/2019 shows bottle and within bottle variability from the second sampling variability experiment, and open symbols represent the data outside the 90th percentile.

µg L^{-1} when the same random vial was analyzed several times.

These observations showed that the diurnal variability in total microcystins was within the sampling variability (0.21 to 0.70 µg L^{-1}), as well as the analytical variability (0.18 to 0.35 µg L^{-1}) when total microcystins were relatively low at this lake. Therefore, we really cannot make any conclusions about diurnal variability, when this experiment showed sampling variability may account for observed changes.

Next, we wanted to evaluate sampling variability like the previous two experiments, when total microcystin concentrations were extremely high and potentially exceeded recreational guidelines (i.e., 8 µg L^{-1}; EPA 2019). On April 27, 2021, we repeated the experiment showing:

- total microcystins varied from 1.17 to 14.43 µg L^{-1} across the 10 bottles collected, averaging 6.22 µg L^{-1} (± 4.68 SD) across all bottles that day from the kayak dock;
- total microcystins varied from 1.98 to 12.63 µg L^{-1} across the 10 vials used to subsample one bottle, averaging 8.77 µg L^{-1} (± 3.88 SD) across all vials; and
- total microcystins varied from 6.09 to 6.72 µg L^{-1} when the same random vial was analyzed several times (Figure 2).

These results showed that when total microcystin concentrations approached the recreational guidelines, measured concentrations were highly variable across the bottles and within one bottle. In fact, 10 out of 20 measured total microcystin concentrations in the bottles and within the one bottle exceeded 8 µg L^{-1}. However, analytical variability using this method was low, especially relative to sampling variability.

Now, we knew that sampling variability within the source water or within an individual bottle was much greater than analytical variability across a range from low to high concentration, when freeze thawing only used 2 mL. Our next question was – what is the minimum volume needed in the freeze thaw process to reduce sampling variability? The final experiment gave us the answer, or at least guidance, where total microcystins measured from 2 mL vials after three freeze thaw cycles had the greatest variance (i.e., mean Z-scores) from the mean across all analyzed vials (Figure 5). The Z-scores (relative to total microcystin analyses) significantly decreased as we increased sample volume used in the freeze thaw cycles, showing

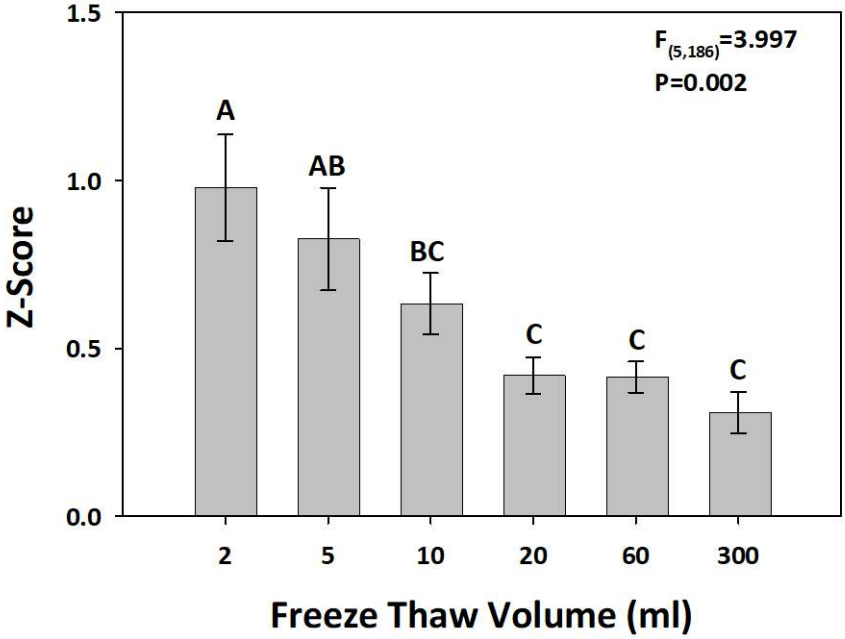

Figure 5. Mean Z-score (±1 standard error) based on microcystin freeze thaw volume (ml); Z-score's representing the amount of variability in measured microcystin concentrations relative to the overall mean for each freeze thaw volume; letters show differences in mean Z-scores across the volumes used in freeze thaw.

that at least 20 mL was needed for total microcystin analysis.

Our recommended minimum volume (i.e., 20 mL) needed in freeze thaw cycles fit with our review of research, state, and federal labs analyzing for total microcystins using the ELISA kits; these volumes ranged from 2 to 750 mL or more (Table 1). Method 546 (EPA 2016) suggests 5 to 10 mL of well-mixed water – we have mixed manually, inverting sample bottles several times. If mechanical mixing (e.g., frother) was used, we may have observed that smaller volumes for the freeze thaw cycles could work. The challenge with larger volumes is typically freeze space limitations; one possible option would be to freeze thaw larger volumes and then store back smaller volumes of (potentially filtered) water for total microcystin analysis.

In 2020, we switched from 2 mL for freeze thaw to 20 mL, and we did notice possible reduced sampling variability throughout that growing season (Figure 2). Total microcystin concentrations did vary between the three sites along the north shore at Lake Fayetteville, where maximum range was 2.037 to 4.115 µg L^{-1} on June 23, 2020, near the beginning of the cyanoHABs and 0.995 to 2.869 µg L^{-1} on August 18, 2020 near the end of the toxic bloom. If the minimum volume of 20 mL for freeze thaw does reduce sampling variability, then this might explain why such strong hierarchical structure existed between total microcystin concentrations and physiochemical properties at Lake Fayetteville in 2020 (Haggard et al. 2023a; 2023b).

Conclusions and Recommendations

Total microcystin concentrations in lake water samples can be influenced greatly by sampling variability, which might obscure data patterns or relationships (e.g., temporal variability). We showed that sampling variability may be high where natural variability in cyanobacterial blooms exists either below the water surface or in the surface scum. Therefore, we recommend at least 20 mL of sample volume be used in the three freeze thaw cycles for total microcystin analysis using ELISA kits; if the water is well-mixed mechanically, and not just by inversion, then smaller volumes might work. When we switched to 20 mL sample volume for freeze thaw, we saw reduced differences in total microcystin concentrations between sites (Figure 2) and strong relations between this toxin and physiochemical properties measured in the water.

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References

Abdulla, H., Y. Tanimu, S.A. Akinyemi, M.C. Bittencourt-Oliveira, and M.A. Chia. 2022. Assessment of microcystins in surface water and irrigated vegetables in Kwaru stream, Hayin Danmani, Kaduna-Nigeria. *Environmental Science and Pollution Research* 29: 78303-78313.

Austin, B.J. and B.E. Haggard. 2022. Measurable microcystin in Ozark streams was rare during summer 2018 baseflow conditions. *Agriculture and Environmental Letters* 7(1): e20069. Available at: https://acsess.onlinelibrary.wiley.com/doi/full/10.1002/ael2.20069. Accessed February 8, 2023.

Cullen, B. 2009. Sampling and Analysis Plan for the Regional Examination of Harmful Algal Blooms (REHAB), King County Water and Land Division. Department of Natural Resources and Parks, Seattle, WA, USA.

Czyzewska, W., M. Piontek, and K. Luszczynska. 2020. The occurrence of potential harmful cyanobacteria and cyanotoxins in the Obrrzyca River (Poland), a source of drinking water. *Toxins* 12(5): 284. Available at: https://doi.org/10.3390/toxins12050284. Accessed February 8, 2023.

Environmental Protection Agency (EPA). 2015. Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins. EPA 820R15100.

Environmental Protection Agency (EPA). 2016. Method 546: Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-Linked Immunosorbent Assay. EPA 815-B-16-011.

Environmental Protection Agency (EPA). 2019. Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin. EPA 822-R-19-001.

Graham, J.L., K.A. Loftin, M.T. Meyer, and A.C. Ziegler. 2010. Cyanotoxin mixtures and taste-and-odor compounds in cyanobacterial blooms from the Midwestern United States. *Environmental Science and Technology* 44: 7361-7368.

Graham, J.L., N.M. Dubrovsky, G.M. Foster, L.R. King, K.A. Loftin, B.H. Rosen, and E.A. Stelzer. 2020. Cyanotoxin occurrence in large rivers of the United States. *Inland Waters* 10(1): 109-117.

Greenstein, K.E., A. Zamyadi, and E.C. Wert. 2021. Comparative assessment of physical and chemical cell lysis methods for total microcystin-LR analysis. *Toxins* 13(9): 596.

Haggard, B.E., E. Grantz, B.J. Austin, A.L. Lasater, L. Haddock, A. Ferri, N.D. Wagner, and J.T. Scott. 2023a. Microcystin shows thresholds and hierarchical structure with physiochemical properties at Lake Fayetteville, Arkansas, May through September 2020. *Journal ASABE* 66(2): 307-317.

Haggard, B.E., E. Grantz, B.J. Austin, N.D. Wagner, and J.T. Scott. 2023b. Chlorophyll and phycocyanin raw fluorescence may inform recreational lake managers on cyanobacterial HABs and toxins: Lake Fayetteville case study. *Journal of Contemporary Water Research and Education* 177:63-71.

Ibelings, B.W., L.R. Murm and A.E. Walsby. 1991. Diurnal changes in buoyancy and vertical distribution in populations of Microcystis in two shallow lakes. *Journal of Plankton Research* 13: 419-436.

Klamath Blue Green Algae Working Group. 2009. Standard Operating Procedures, Environmental Sampling for Cyanobacteria for Cell Enumeration, Identification and Toxin Analysis. Developed for the 2009 AIP Measure 12 Water Quality Monitoring Activities.

Loftin, K.A., J.M. Clark, C.A. Journey, D.W. Kolpin, P.C. Van Metre, D. Carlisle, and P.M. Bradley. 2016a. Spatial and temporal variation in microcystin occurrence in wadeable streams in the southeastern United States. *Environmental Toxicology and Chemistry* 35: 2281-2287.

Loftin, K.A., J.L. Graham, E.D. Hilborn, S.C. Lehmann, M.T. Meyer, J.E. Dietze, and C.B. Griffith. 2016b. Cyanotoxins in inland lakes of the United States: Occurrence and potential recreational health risks in the EPA National Lakes Assessment 2007. *Harmful Algae* 56: 77-90.

Massey, I.Y., P. Wu, J. Wei, J. Luo, P. Ding, H. Wei, and F. Yang. 2020. A mini-review on detection methods of microsystins. *Toxins* 12(10): 641.

Meyer, R. 1971. *A study of phytoplankton dynamics in Lake Fayetteville as a means of assessing water quality*. Arkansas Water Resources Center Publication No. 10-A, 42 pp.

Nagata, S., T. Tsutsumi, A. Hasegawa, F. Yoshida, and Y. Ueno. 1997. Enzyme immunoassay for direct determination of microcystins in environmental water. *Journal of AOAC International* 80: 408-417.

Ohio EPA. 2018. Ohio EPA Total (Extracellular and Intracellular) Microcystins – ADDA by ELISA Analytical Methodology. Ohio EPA DES 701.0, Version 2.3.

Olsen, B. 2022. Division of Environmental Quality, Arkansas Department of Energy and Environment. Personal Communication July 8, 2022.

Thorpe, A. and C. Brunet. 2021. Standard Operating Procedure for: Microcystin (ELISA), University of Missouri Limnology Lab.

Trout-Haney, J.V., Z.T. Wood, and K.L. Cottingham. 2016. Presence of the cyanotoxin microcystin in Arctic lakes of Southwestern Greenland. *Toxins* 8(9): 256.

Wagner, N.D., E. Quach, S. Buscho, A. Ricciardelli, A. Kannan, S.W. Naung, G. Phillip, B. Sheppard, L. Ferguson, A. Allen, C. Sharon, J.R. Duke, R.B. Taylor, B.J. Austin, J.K. Stovall, B.E. Haggard, C.K. Chambliss, B.W. Brooks, and J.T. Scott. 2021. Nitrogen form, concentration, and micronutrient availability affect microcystin production in cyanobacterial blooms. *Harmful Algae* 103: 102002.

Wiedner, C., P.M., Visser, J. Fastner, J.S. Metcalf, G.A. Codd, and L.R. Mur. 2003. Effect of light on the microcystin content of Microcystis strain PCC 7806. *Applied and Environmental Microbiology* 69: 1475-1481.

Wilson, A. 2022. WilsonLab.com, Auburn University. Personal Communication July 6, 2022.

Wood, S.A., P.T. Holland, D.J. Stirling, L.R. Briggs, J. Sprosen, J.G. Ruck, and R.G. Wear. 2006. Survey of cyanotoxins in New Zealand water bodies between 2001 and 2004. *New Zealand Journal of Marine and Freshwater Research* 40: 585-597.