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*Case Study Article*

# Chlorophyll and Phycocyanin Raw Fluorescence May Inform Recreational Lake Managers on Cyanobacterial HABs and Toxins: Lake Fayetteville Case Study

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**Abstract:** Cyanobacterial harmful algal blooms (cyanoHABs) have been observed across the USA and worldwide, and even locally in Lake Fayetteville (Arkansas, USA) once we started monitoring for total microcystin. The goal of this research note was to present a framework that might help guide cyanoHAB and toxin public health advisories at Lake Fayetteville. We evaluated nonparametric change points (i.e., thresholds) and hierarchical structure (using classification and regression trees) between total microcystin concentrations, chlorophyll, and phycocyanin; chlorophyll-a is a pigment in all algae, while phycocyanin is specific to cyanobacteria. Pigment concentrations and raw fluorescence units (RFUs) all showed significant thresholds with total microcystin concentrations, basically showing that as concentration or RFUs increased above the thresholds that total microcystin was greater at Lake Fayetteville. The regression tree with total microcystin concentrations showed a first split with phycocyanin RFUs at 4524, and then when phycocyanin RFUs were greater there was an optimal range for the phycocyanin to chlorophyll RFU ratio (0.64-1.5). At this recreational lake, total microcystin concentrations were greatest when water samples met these criteria, providing a possible framework for when lake managers might suggest an increased risk for elevated cyanobacterial toxins.

**Keywords:** *cyanobacterial blooms, total microcystin, pigment raw fluorescence, lake management*

Cyanobacterial harmful algal blooms (cyanoHABs) are being observed across the USA and globally, and these present a threat to inland freshwaters and challenges for lake users and managers (Brooks et al. 2016). These cyanoHABs are often dominated by species capable of producing toxins under the right environmental conditions, including *Aphanizomenon*, *Cylindrospermopsis*, *Dolichospermum*, *Microcystis*, and *Oscillatoria* (*Planktothrix*) among others (see Carmichael 2001). The toxicity of these cyanoHABs and dominance of individual cyanobacterial species are driven by water temperatures, nutrient availability and ratios, and likely global climate change (Paerl and Otten 2016).

Cyanobacteria in freshwaters can produce several different types of toxins, which can result in everything from mild skin irritation to basic gastrointestinal issues and even acute pneumonia during recreational exposures (Falconer 1999). However, many studies focus on microcystin and its various forms in lakes and reservoirs because microcystins generally occur in lake waters when other classes of cyanobacterial toxins are present (Graham et al. 2010). Microcystin and its physicochemical and environmental drivers in lakes and reservoirs have been a research focus for the last couple decades, showing the importance of water temperature (Walls et al. 2018), nitrogen (N) supply (Wagner et al. 2021), and cyanobacterial

**Research Implications**

- Total microcystin concentrations were predicted using easily measured raw fluorescence of chlorophyll and phycocyanin in water samples from a recreational lake.
- Total microcystin concentrations were greatest when raw fluorescence units (RFUs) of phycocyanin exceeded 4254 and the ratio of chlorophyll and phycocyanin RFUs was between 0.64 and 1.5; these values could be rounded for management purposes.
- Public health advisories for cyanobacterial harmful algal blooms (cyanoHABs) and total microcystin concentrations could be issued based on RFUs of chlorophyll and phycocyanin, which is less expensive than toxin analysis.

biomass (Haggard et al. 2023). Recently, the Environmental Protection Agency (EPA) (2019) has released guidance on cyanobacterial toxins in recreational lakes and reservoirs for states and tribes, providing 8 µg $L^{-1}$ as the target guideline for total microcystin concentrations.

When recreational lakes and reservoirs exceed guidelines for cyanobacterial toxins (i.e., 8 µg $L^{-1}$ total microcystin) in Arkansas, the entity responsible for the lake deals with public health advisories and notices. The Arkansas HAB Response Program provides guidance on this topic (Arkansas HAB Workgroup 2019), but the episodic nature of toxins exceeding these guidelines makes it challenging to issue and retract public health advisories and notices. Plus, the general public is not well informed on the differences between nuisance algal blooms in freshwaters and those that might be toxic (i.e., cyanoHABs). Given this issue, the Arkansas Water Resources Center (AWRC), in collaboration with the Arkansas HAB Workgroup, published an educational fact sheet to help inform the general public on nuisance algal blooms and HABs in streams, ponds, and lakes (Austin et al. 2018).

The goal of this study is to present a management framework for HAB risk in recreational waters, based on preliminary analysis of relatively easy to measure water quality data or physicochemical properties measured in the recreational Lake Fayetteville, and how these relate to total microcystin concentrations. The specific objectives are to 1) describe total microcystin concentrations over time during the cyanoHAB events in 2020, 2) evaluate threshold responses and hierarchical structure between total microcystin and cyanobacterial or algal variables related to fluorescence, and 3) present a framework to help lake managers determine when the chances of cyanobacterial HABs with elevated toxins are greatest. This analysis focuses on sustained and prevalent HABs at Lake Fayetteville, where total microcystin concentrations varied more than an order of magnitude (Haggard et al. 2023).

## Methods

Lake Fayetteville is a small lake used for secondary contact recreational purposes (boating, fishing, and kayaking) in Fayetteville, Arkansas city limits, and the lake and surrounding park are managed by the municipality; for more information, see: https://www.fayetteville-ar.gov/3130/Lake-Fayetteville. The lake is relatively small with a surface of ~0.6 $km^2$ and a watershed area of 24 $km^2$. In 2019, the first lake advisory for cyanobacterial toxins (i.e., microcystin) was issued after the AWRC observed total microcystin concentrations exceeding recreational guidelines (8 µg $L^{-1}$; EPA 2019). The lake has been a research focus for watershed and lake nutrient budgets (Grantz et al. 2014), sediment phosphorus (P) sources (Haggard et al. 2023), and cyanobacterial HABs (Wagner et al. 2021; Haggard et al. 2023). However, cyanobacteria have dominated the phytoplankton community since 1968 or since initial impoundment (Meyer 1971).

Water samples have been collected since 2019; however, this study focused on water samples collected during calendar year 2020. Water samples were collected at three sites accessible by foot along the north shore, where the public has relatively easy access to the lake (see Haggard et al. 2023). Upon return to the AWRC water quality lab, water samples were analyzed for raw fluorescence of chlorophyll and phycocyanin using a CyanoFlour (hand-held fluorometer, Turner Designs, San Jose, CA, USA). At the lab, water was filtered and analyzed for chlorophyll-a pigment analysis (Method APHA 10200 H3, Turner Designs Fluorometer). A subsample of lake water (20-30 mL) was stored in an amber glass vial and put through three freeze thaw cycles before being analyzed for total microcystin concentration, using the enzyme linked immunoassay technique (Method EPA 546). The subsample volume for total microcystin was determined based on a study by Austin and Haggard (2023), which suggests that the minimum volume needed to reduce sampling variability is at least 20 mL.

We used these data to evaluate correlations between parameters (particularly chlorophyll-a pigment and chlorophyll/phycocyanin raw fluorescence units (RFUs)), thresholds between microcystin and algal parameters (nCPA; King and Richardson 2003; Qian et al. 2003), and any hierarchical structure in the microcystin relationships (CART; De'Ath and Fabricius 2000). The nCPA and CART analyses were performed in R 4.1.2 using the rpart package for CART analysis (Therneau and Atkinson 2019), requiring a minimum of five observations per terminal node and that each split increase the complexity parameter by at least 0.05. We used the deviance explained by each split relative to the total deviance of the model to approximate an $R^2$ for each split within the CART models (R. King, personal communication, 5 April 2022).

## Results

Total microcystin concentrations revealed an interesting pattern at Lake Fayetteville (Figure 1), showing little measurable microcystin (<0.200 µg $L^{-1}$) from March through early June 2020; the one exception was 0.277 µg $L^{-1}$ at the mid-lake site on May 19. After this period, total microcystin concentrations increased to an average of 4.692 µg $L^{-1}$ across all three sites on June 29, 2020. Total microcystin concentrations remained elevated (~1 µg $L^{-1}$ or greater) through late summer, August 18, 2020. After this time, total microcystin concentrations decreased to less than 0.200 µg $L^{-1}$ on September 14, 2020, and then a secondary peak in microcystin occurred in late fall, where mean total microcystin concentration reached 1.619 µg $L^{-1}$ on October 19, 2020. Microcystin remained elevated in November and then decreased to a mean less than 0.200 µg $L^{-1}$ in December 2020.

Chlorophyll-a pigment concentrations varied in 2020 from 2.6 to 96.4 µg $L^{-1}$, averaging 33.3 µg $L^{-1}$ across all sampling sites and dates in 2020; chlorophyll and phycocyanin RFUs were variable over time too (Figure 1). Chlorophyll-a concentration showed a bimodal peak in 2020, where chlorophyll-a pigment concentrations were greatest in late April through May and then increased again in mid-October through early November following lake turnover. Phycocyanin RFUs ($R^2$=0.54) were more strongly related to chlorophyll-a pigment concentrations than chlorophyll RFUs ($R^2$=0.10), and neither chlorophyll-a pigment concentration nor the RFUs of either phycocyanin or chlorophyll showed a linear relation to total microcystin concentrations.

The cyanobacterial or algal fluorescence properties showed significant thresholds with total microcystin concentrations at Lake Fayetteville (Figure 2), including:

- 23.4 chlorophyll-a pigment mg $L^{-1}$ ($R^2$=0.10, P=0.013), where mean total microcystin was 0.30 µg $L^{-1}$ to the left (less than) of the threshold and 1.07 µg $L^{-1}$ to the right (greater than);
- 4172 chlorophyll RFUs ($R^2$=0.14, P=0.005), where mean total microcystin was 0.25 µg $L^{-1}$ to the left of the threshold and 1.14 µg $L^{-1}$ to the right;
- 4524 phycocyanin RFUs ($R^2$=0.18, P=0.001), where mean total microcystin was 0.27 µg $L^{-1}$ to the left of the threshold and 1.25 µg $L^{-1}$ to the right;
- 0.37 phycocyanin to chlorophyll-a (PC:CHL) RFU ratio ($R^2$=0.14, P=0.002), where mean total microcystin was 0.13 µg $L^{-1}$ to the left of the threshold and 1.09 µg $L^{-1}$ to the right; and
- pheophytin did not show a significant nonparametric change point with microcystin (P=0.273).

More information about physicochemical thresholds with total microcystin concentrations at Lake Fayetteville using this cyanoHAB database from March through September 2020 is available in Haggard et al. (2023).

When we considered hierarchical structure in the relation between these cyanobacterial or algal fluorescence properties and total microcystin concentrations, an interesting pattern emerged (Figure 3). The first split in this relation was with phycocyanin RFU and total microcystin concentrations using the change point defined above, and then water samples with phycocyanin RFUs above the threshold split twice with the PC:CHL RFU ratio. The least mean total microcystin concentration was 0.27 µg $L^{-1}$ when phycocyanin was less than 4524 RFUs. The next grouping of the water samples was when phycocyanin exceeded 4524 RFUs but had a PC:CHL RFU ratio greater than or equal to 1.5; the mean total microcystin concentration was 0.43 µg $L^{-1}$. When the PC:CHL RFU ratio was less than 1.5, the data split again with this ratio. So, water samples with phycocyanin greater than 4524 RFUs but a PC:CHL RFU ratio less than 0.64 had a mean total microcystin concentration of 1.1 µg $L^{-1}$. The greatest mean total microcystin concentration (2.6 µg $L^{-1}$) was observed when phycocyanin RFUs exceeded the first threshold and the PC:CHL RFU ratio was between 0.64 and 1.5. These multiple, sequential thresholds or this hierarchical structure

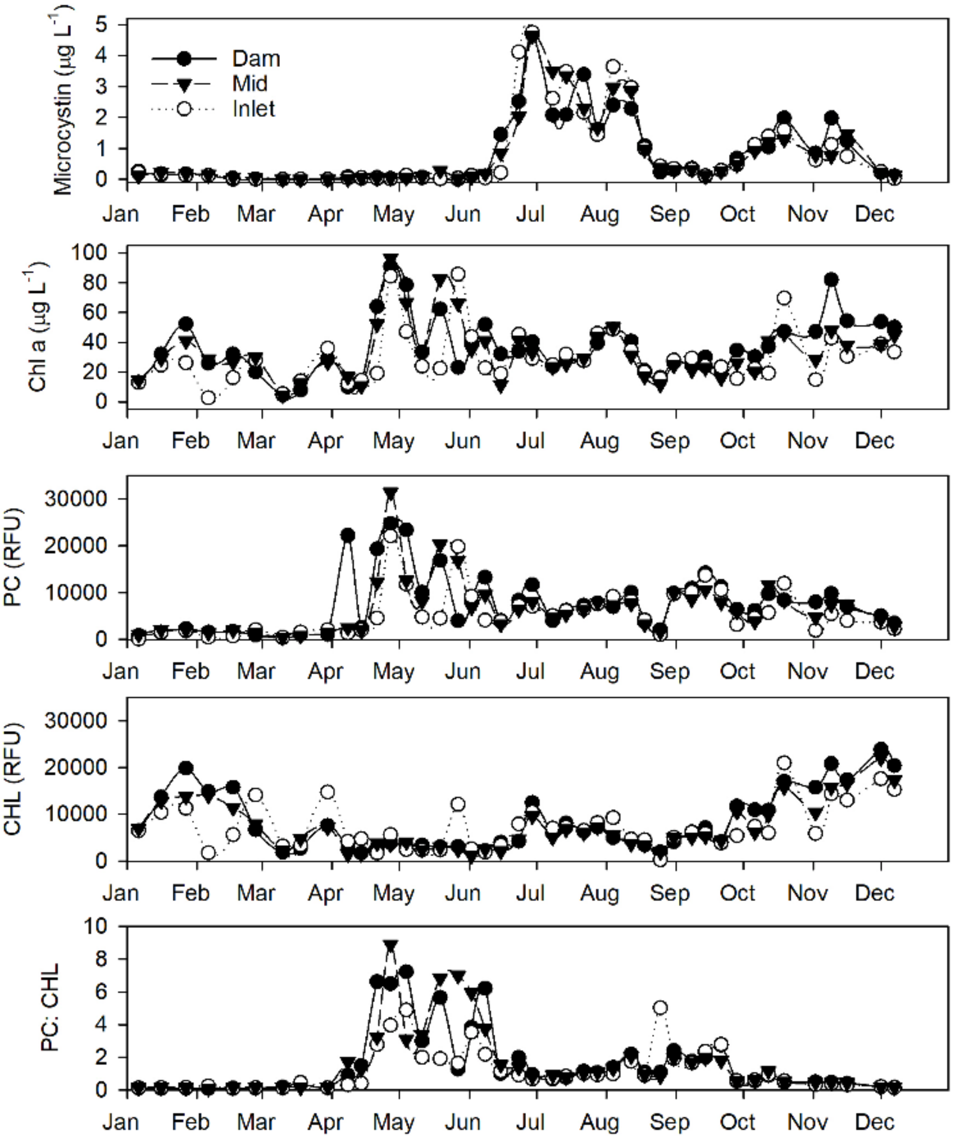

**Figure 1.** Time series graph of total microcystin, chlorophyll-a pigment and raw fluorescence of chlorophyll and phycocyanin at Lake Fayetteville, Arkansas, calendar year 2020.

explained more than half of the deviation in total microcystin concentrations at Lake Fayetteville during calendar year 2020.

## Discussion

When lakes have elevated microcystin concentrations and exceed recreational guidelines (8 µg $L^{-1}$; EPA 2019), the next step is public notice by the authority in charge of the water body. At Lake Fayetteville, the City of Fayetteville has issued public health and contact advisories, and the signage went up in 2019 when total microcystin concentrations hit 11 µg $L^{-1}$ in one lake sample on May 7, 2019 (mean of two samples that day shown in Wagner et al. 2021). However, the next week total microcystin concentrations dropped below EPA guidance levels at Lake Fayetteville, and then total microcystin concentrations (15 µg $L^{-1}$, June 4, 2019; Wagner et al. 2021) once again exceeded EPA guidance levels. Total microcystin concentrations were highly variable in the 2019 growing season, but that could be due to the variability in the cyanoHABs that year or due to the smaller volumes (~2 mL) used in the freeze thaw cycles for analysis (Austin and Haggard 2023).

The cyanoHABs in 2020 were sustained for a longer period during the growing season, but lake water below the water surface was always less than EPA guidance for total microcystin concentrations. The year 2020 was also the first year where we had a complete database of total microcystin data paired with RFUs for chlorophyll and phycocyanin, and total microcystin was measured using at least 20 mL following the three freeze thaw cycles (Austin and Haggard 2023). While microcystin below the water surface never exceeded EPA guidelines, we did observe surface scums of these cyanoHABs, especially at the marina on the northwest corner of Lake Fayetteville. These surface scums concentrate

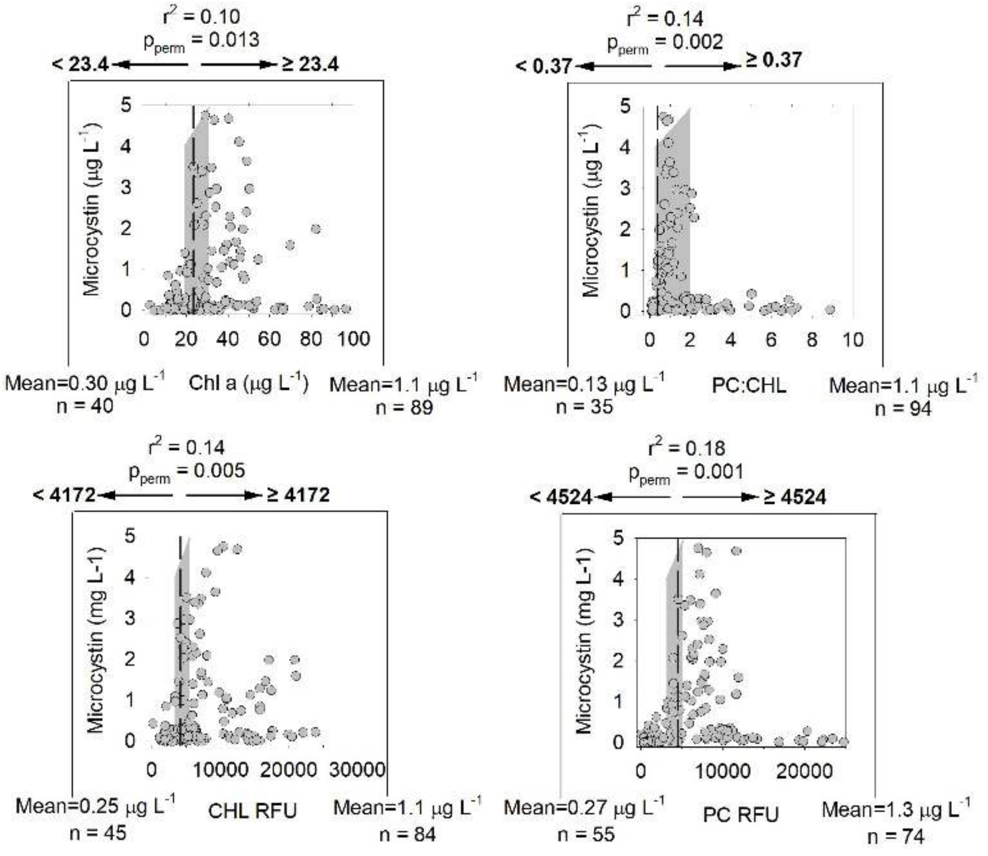

**Figure 2.** Plots showing significant thresholds (vertical dashed line) based on nonparametric change point analysis between total microcystin concentrations and cyanobacterial and algal fluorescence properties, including chlorophyll-a pigment concentrations, chlorophyll (CHL) raw fluorescence units (RFUs), phycocyanin (PC) RFUs, and the ratio of CHL to PC RFUs; the grey shaded area represents the 90% confidence interval about the threshold.

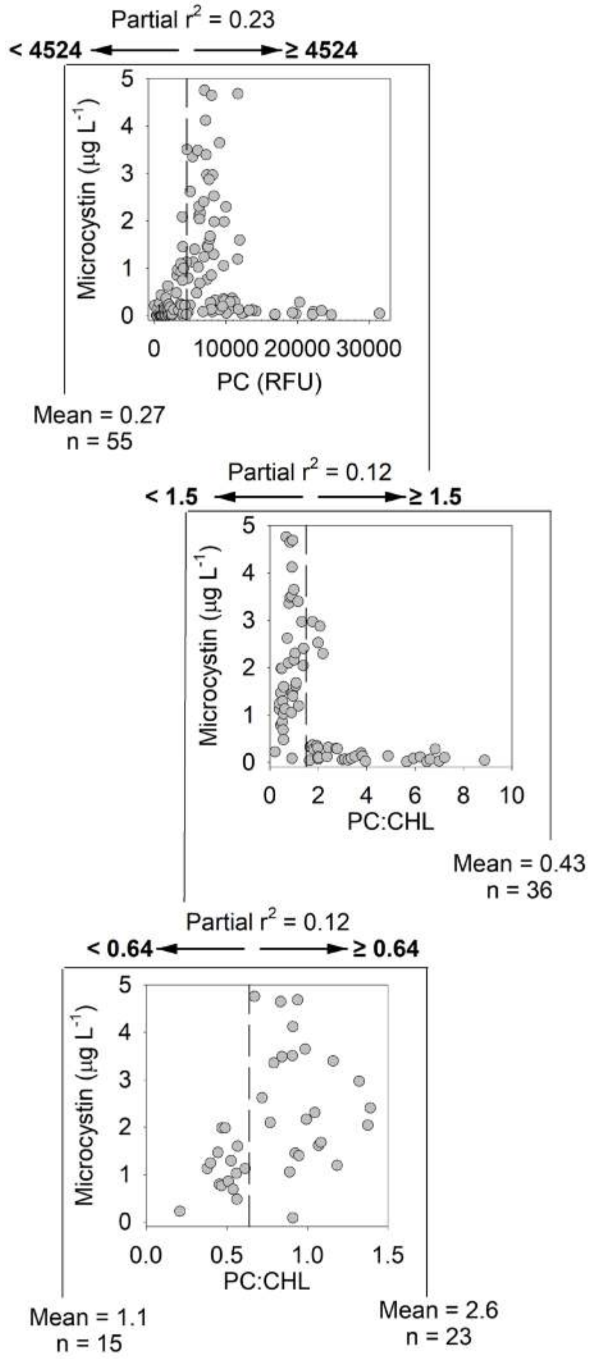

**Figure 3.** Thresholds and hierarchical structure in splits with total microcystin concentrations at Lake Fayetteville using only raw fluorescence of chlorophyll (CHL) and phycocyanin (PC) at all three sites during calendar year 2020; the top graph shows the first threshold between total microcystin and PC RFUs, the middle graph shows data exceeding PC 4524 RFUs and its threshold with PC:CHL, and the bottom graph shows data less than a PC:CHL of 1.5 and its final thresholds with PC:CHL of 0.64.

the cyanobacteria which can produce cyanotoxins such as microcystins (Plaas and Paerl 2021).

Ideally, lake managers would have access to total microcystin concentrations in the lake waters. While microcystin can be measured by laboratory services, it can be costly for municipalities, and the data are not necessarily quickly available to make decisions on public advisories. The turnaround time for many commercial water labs is up to eight days for total microcystin analysis (based on web search), and field test strips and or kits have their own challenges to accurately estimate total microcystin concentrations (Aranda-Rodriguez et al. 2015). Therefore, lake managers need access to a decision matrix based on more easily measurable and readily available data. These data could include phycocyanin and chlorophyll RFUs, which could be measured in grab samples like this study, or provided continuously by deployed data sondes (e.g., see Izydorczyk et al. 2005). However, these data sondes, when deployed near the surface in lakes, can have light-induced quenching of fluorescence, resulting in errors in measurement of phycocyanin and chlorophyll (Rousso et al. 2021).

The regression tree splits in microcystin, phycocyanin, and PC:CHL RFU ratios might help provide some guidance on when to expect the greatest microcystin concentrations in the lake water, especially at Lake Fayetteville. Microcystin in below-surface lake water at Fayetteville was consistently less than recreational guidelines in 2020, but it showed significant thresholds and hierarchical structure with cyanobacterial fluorescence properties. When phycocyanin RFUs were greater than 4524 and PC:CHL was between 0.64 and 1.5, microcystin was greatest at Lake Fayetteville. Alternatively, total microcystin is likely to be elevated or of potential concern when phycocyanin RFU exceeds 4524 and the PC:CHL RFU ratio is between 0.64 and 1.5. These parameters can be [relatively] easily and rapidly measured using the CyanoFlour (Turner Designs, San Jose, CA) or other handheld fluorometers, providing lake managers an opportunity to issue initial advisories or even rate the risk of elevated microcystin in recreational lakes dominated throughout the growing season by cyanobacteria.

The use of RFUs for chlorophyll and phycocyanin to forecast total microcystin concentrations and cyanoHABs might be advantageous for recreational lakes because phycocyanin RFU and cyanobacterial biovolumes show a strong positive correlation (Thomson-Laing et al. 2020). While other studies have suggested that chlorophyll and phycocyanin RFUs do not necessarily correlate with pigment analysis for chlorophyll-a and phycocyanin, respectively (e.g., see Chaffin et al. 2018), we observed a relatively strong correlation between phycocyanin RFU and chlorophyll-a pigment concentrations. This suggests that phycocyanin RFU is likely a good proxy for cyanobacterial biomass at Lake Fayetteville. The regression tree observed with these parameters at Lake Fayetteville may be a potential management or decision tool for HAB advisories. However, we need to expand this database to see if these thresholds vary across years at this lake and across lakes. These data could also be paired with other parameters that are relatively easy to measure, including conductivity, dissolved oxygen, pH, and water temperature.

Since Lake Fayetteville is used for recreational purposes including fishing, the potential bioaccumulation of microcystin would be a concern with fish consumption. Microcystin in its various forms has been detected in fish tissues in other lakes, where these tissues likely exceeded total daily intake guidelines (Gurbuz et al. 2016). Globally, microcystin concentrations in the water column and fish tissues show a positive relation (Flores et al. 2018), suggesting that elevated concentrations in the lake water would correlate with increased concentrations in the fish at Lake Fayetteville. It is also likely that microcystin accumulates in the bottom and shoreline sediments at Lake Fayetteville, and it often persists in sediments for many days following algal blooms and toxin production (Preece et al. 2021). Benthic cyanobacteria are also an emerging concern in cyanoHABs (Burford et al. 2020), potentially requiring consideration in recreational lakes. We do not know what the microcystin concentrations are in benthic cyanobacteria, sediments, and fish tissues from Lake Fayetteville, but the widespread occurrence of this toxin in the water and likely accumulation of this toxin in the sediments and fish should give rise to potential public health concerns.

## Conclusions

At Lake Fayetteville, total microcystin concentrations were variable across the calendar year, but sustained above 1 $\mu g\ L^{-1}$ in the early and late periods of the growing season in 2020. The algal or cyanobacterial fluorescence variables were not linearly related to total microcystin concentrations at this lake, but each variable did show a significant threshold or non-parametric change point. The hierarchical structure between total microcystin concentrations and phycocyanin RFU and the PC:CHL RFU ratio provides guidance on when elevated microcystin might be present at Lake Fayetteville. When lake water exceeds 4524 RFUs for phycocyanin and has a PC:CHL RFU ratio between 0.64 and 1.5, lake managers might want to consider issuing public health advisories for cyanoHABs and elevated toxins relative to recreational contact guidelines.

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## Author Bio and Contact Information

**Dr. Brian Haggard** (corresponding author) has been focused on water quality issues since his graduate studies, and he has a keen interest in cyanoHABs. He is currently the director of the Arkansas Water Resources Center and a professor in the Biological and Agricultural Engineering Department with the University of Arkansas System Division of Agriculture. Prior to his current positions, he worked for the US Geological Survey and USDA Agricultural Research service in hydrology and water quality. He conceptualized the data collection and this sampling and analytical variability research project at Lake Fayetteville, as well as drafted the manuscripts. He has a Ph.D. in Biosystems Engineering from Oklahoma State University, a M.S. in Agronomy from the University of Arkansas, and a B.S. in Life Sciences from the Missouri University of Science and Technology. He may be contacted at haggard@uark.edu or Engineering Hall 203, University of Arkansas, Fayetteville, AR 72701, USA.

**Mrs. Erin Grantz** is a program manager and data scientist with the Arkansas Water Resources Center, and she is currently working on her Ph.D. in Environmental Dynamics at the University of Arkansas. She helped manage the data analysis for this project at Lake Fayetteville, including the development of the classification and regression trees. She has a M.S. and B.S. in Crop, Soil and Environmental Sciences from the University of Arkansas, and her graduate research includes nutrient mass balances at Lake Fayetteville. She may be contacted at egrantz@uark.edu or 1371 West Altheimer Drive, Don Tyson Center for Agricultural Sciences, Fayetteville, AR 72704, USA.

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## References

Aranda-Rodriguez, R., Z. Jin, J. Harvie, and A. Cabecinha. 2015. Evaluation of three field test kits to detect microcystins from a public health perspective. *Harmful Algae* 42: 34-42.

Arkansas HAB Workgroup. 2019. Harmful Algal Bloom Management Plan. Arkansas State Waters, Arkansas Department of Energy and Environment – Division of Environmental Quality. Available at: https://www.adeq.state.ar.us/water/pdfs/HAB-ResponsePlan-Manual-bookmarks-2019-12-12-Final.pdf. Accessed February 1, 2023.

Austin, B.J., B. Olsen, T. Wentz, and B.E. Haggard. 2018. Algal Blooms in Arkansas Streams, Ponds, and Lakes. Arkansas Water Resources Center FS-2018-02. Available at: https://scholarworks.uark.edu/awrcfs/9?utm_source=scholarworks.uark.edu%2Fawrcfs%2F9&utm_medium=PDF&utm_campaign=PDFCoverPages. Accessed February 1, 2023.

Austin, B.J. and B.E. Haggard. 2023. Total microcystin concentration variability in water samples and recommended minimum volume for freeze thaw cycles. *Journal of Contemporary Water Research and Education* (this Issue).

Brooks, B.W., J.M. Lazorchak, M.D.A. Howard, M.V. Johnson, S.L. Morton, D.A.K. Perkins, E.D. Reavie, G.I. Scott, S.A. Smith, and J.A. Steevens. 2016. Are harmful algal blooms becoming the greatest inland water quality threat to public health and aquatic ecosystems? *Environmental Toxicology and Chemistry* 35: 6-13.

Burford, M.A., C.C. Carey, D.P. Hamilton, J. Huisman, H.W. Paerl, S.A. Wood, and A. Wulff. 2020. Perspective: Advancing the research agenda for improving understanding of cyanobacteria in a future of global change. *Harmful Algae* 91: 101601.

Carmichael, C.W. 2001. Health effects of toxin-producing cyanobacteria: "The CyanoHABs". *Human and Ecological Risk Assessment: An International Journal* 7(5): 1393-1407.

Chaffin, J.D., T.W. Davis, D.J. Smith, M.M. Baer, and G.J. Dick. 2018. Interactions between nitrogen form, loading rate, and light intensity on Microcystis and Planktothrix growth and microcystin production. *Harmful Algae* 73: 84-97.

De'Ath, G. and K.E. Fabricius. 2000. Classification and regression trees: A powerful yet simple technique for ecological data analysis. *Ecology* 81(11): 3178-3192.

Environmental Protection Agency (EPA). 2019. Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin. Office of Water, Health and Ecological Criteria Division, EPA 822-R-19-001, Washington, D.C. Available at: https://www.epa.gov/sites/default/files/2019-05/documents/hh-rec-criteria-habs-document-2019.pdf. Accessed February 1, 2023.

Falconer, I.R. 1999. An overview of problems caused by toxic blue-green algae (cyanobacteria) in drinking and recreational water. *Environmental Toxicology* 14(1): 5-12.

Flores, N.M., T.R. Miller, and J.D. Stockwell. 2018. A global analysis of the relationship between concentrations of microcystins in water and fish. *Frontiers in Marine Science* 5: 00030.

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(19): 7361-7368.

Grantz, E.M., B.E. Haggard, and J.T. Scott. 2014. Stoichiometric imbalance in rates of nitrogen and phosphorus retention, storage, and recycling can perpetuate nitrogen deficiency in highly-productive reservoirs. *Limnology and Oceanography* 59(6): 2203-2216.

Gurbuz, F., O.Y. Uzunmehmetoğlu, Ö. Diler, J.S. Metcalf, and G.A. Codd. 2016. Occurrence of microcystins in water, bloom, sediment and fish from a public water supply. *Science of the Total Environment* 562: 860-868.

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

Izydorczyk, K., M. Tarczynska, T. Jurczak, J. Mrowczynski, and M. Zalewski. 2005. Measurement of phycocyanin fluorescence as an online early warning system for cyanobacteria in reservoir intake water. *Environmental Toxicology* 20(4): 425-430.

King, R.S. and C.J. Richardson. 2003. Integrating bioassessment and ecological risk assessment: An approach to developing numerical water-quality criteria. *Environmental Management* 31: 795-809.

Meyer, R.L. 1971. *A Study of Phytoplankton Dynamics in Lake Fayetteville as a Means of Assessing Water Quality*. Arkansas Water Resources Center Technical Report Number PUB010A, Fayetteville, AR.

Paerl, H.W. and T.G. Otten. 2016. Duelling 'CaynoHABs': Unravelling the environmental drivers controlling dominance and succession among diazotrophic and non-N2-fixing harmful cyanobacteria. *Environmental Microbiology* 18(2): 316-324.

Plaas, H.E. and H.W. Paerl. 2021. Toxic cyanobacteria: A growing threat to water and air quality. *Environmental Science and Technology* 55(1): 44-64.

Preece, E.P., W. Hobbs, F.J. Hardy, L. O'Garro, E. Frame, and F. Sweeney. 2021. Prevalence and persistence of microcystin in shoreline lake sediments and porewater, and associated potential for human health risk. *Chemosphere* 272: 129581.

Qian, S.S., R.S. King, and C.J. Richardson. 2003. Two statistical methods for the detection of environmental thresholds. *Ecological Modelling* 166: 87-97.

Rousso, B.Z., E. Bertone, R.A. Stewart, K. Rinke, and D.P. Hamilton. 2021. Light-induced fluorescence quenching leads to errors in sensor measurement of phytoplankton chlorophyll and phycocyanin. *Water Research* 198: 117133.

Therneau, T. and B. Atkinson. 2019. rpart: Recursive Partitioning and Regression Trees. R Package version 4.1-19. Available at: https://rdrr.io/cran/rpart/. Accessed February 1, 2023.

Thomson-Laing, G., J. Puddick, and S.A. Wood. 2020. Predicting cyanobacterial biovolumes from phycocyanin fluorescence using a handheld fluorometer in the field. *Harmful Algae* 97: 101869.

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.

Walls, J.T., K.H. Wyatt, J.C. Doll, E.M. Rubenstein, and A.R. Rober. 2018. Hot and toxic: Temperature regulates microcystin release from cyanobacteria. *Science of The Total Environment* 610-611: 786-795.