Image caption: Alyssa Ferri collecting water samples at Lake Fayetteville. Photo courtesy of Brad Austin.

Understanding Microcystin Occurrence and Predictors at Lake Fayetteville

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Abstract: Harmful algal blooms (HABs) are widespread and can produce toxins. We wanted to understand if and why algae produce toxins at Lake Fayetteville, a highly productive recreational reservoir in Northwest Arkansas. We collected water samples weekly to monthly from March 2019–December 2021. We measured microcystin (MC), one of the most common algal toxins, and a range of physical, chemical, and biological water quality characteristics. Water quality follows seasonal patterns at Lake Fayetteville that affect MC production. Microcystin levels were sometimes potentially unsafe for human contact (i.e. >8 µg/L). Unsafe conditions were most likely in summer, but also possible in spring and fall. Thresholds in the data can predict when MC is likely to be higher or lower. We analyzed these thresholds across years and for each year individually. We also explored potential secondary thresholds in water quality characteristics after grouping data based on a primary threshold. The top recurring MC predictors were indicators of algal biomass, bioavailable supply nitrogen, and water temperature. Microcystin was greatest at greater algal biomass, especially greater cyanobacterial numbers. We found more MC when supply nitrogen was limited. Microcystin largely occurs when surface water temperature is ≥27°C. Water temperature is a master variable because it strongly affects both algal biomass and nitrogen availability. These themes are a starting point for solving the HABs puzzle at Lake Fayetteville. The observed MC predictors differed between years, however, presenting a challenge for HABs management. We must continue monitoring to understand this interannual variability and recognize patterns across years.

Key Points:

- Many water quality and environmental factors predicted microcystin production by harmful algal blooms at Lake Fayetteville.
- Physical, chemical, and biological characteristics of water quality vary seasonally, and these seasonal differences affect microcystin production.
- The magnitude and timing of microcystin production differed between years (2019 – 2021).
- The predictors for greater microcystin production also differed between years.
- We must continue monitoring to understand this interannual variability in microcystin controls.

Introduction

An algal bloom is considered harmful (HAB) when it produces toxins or other negative effects for human health and ecosystem function. These blooms are frequent and widespread in inland freshwaters and estuaries (Brooks et al., 2016). Their formation is associated with nutrient enrichment in a waterbody and its watershed (Anderson et al., 2002; Paerl et al., 2016; Wurtsbaugh et al., 2019).

Microcystin (MC) is one of the most common toxins and is produced by many types of cyanobacteria, the type of algae that often dominate HABs (Paerl et al., 2001). When MC is found in lakes and reservoirs, other toxins are also often present (Graham et al., 2010). The Environmental Protection Agency (EPA, 2019) has released guidance for states and tribes on what levels of cyanobacterial toxins pose a human health risk. The target for MC is 8 µg L^{-1}.

It is not well understood what causes HABs to produce toxins (Graham et al., 2004). Factors linked to MC production can differ depending on the scale of analysis, global location, and system-specific characteristics. Globally, total nitrogen (TN) is a predictor of MC, while total phosphorus (TP) is not (Buley et al., 2021). But, TP availability affects relative abundance of cyanobacterial biomass (Shan et al., 2020). In general, greater algal biomass coincides with greater MC in lakes (Yuan et al., 2014; Buley et al., 2021; Chaffin et al., 2021). The ratio, or relative availability, of N and P is also a predictor for MC globally (Harris et al., 2014), but may be secondary to large TN concentrations (Scott et al., 2013).

Lake Fayetteville, a recreational lake in Northwest Arkansas, has significant, recurrent algal blooms each year that are dominated by cyanobacteria (Meyer, 1971). We wanted to know if these blooms produce toxins and what the predictors are for toxin production. Our study objectives were: 1) collect a detailed long-term database of MC concentrations and water quality characteristics, and 2) explore relationships between MC and water quality characteristics to discover which factors or combinations of factors are most common when MC occurs at greater or lesser concentrations.

Methods

Study Site and Field Methods

Lake Fayetteville is a small (surface area <1 km^2), shallow (mean depth = 3 m, maximum depth = 10 m), and hypereutrophic reservoir located in Northwest Arkansas (Figure 1). Lake Fayetteville impounds 24 km^2 of the Clear Creek watershed (Hydrologic Unit Code 12 = 111101030201). The reservoir was constructed in 1948 for drinking water supply, but the lake and adjacent public lands now serve as a recre-

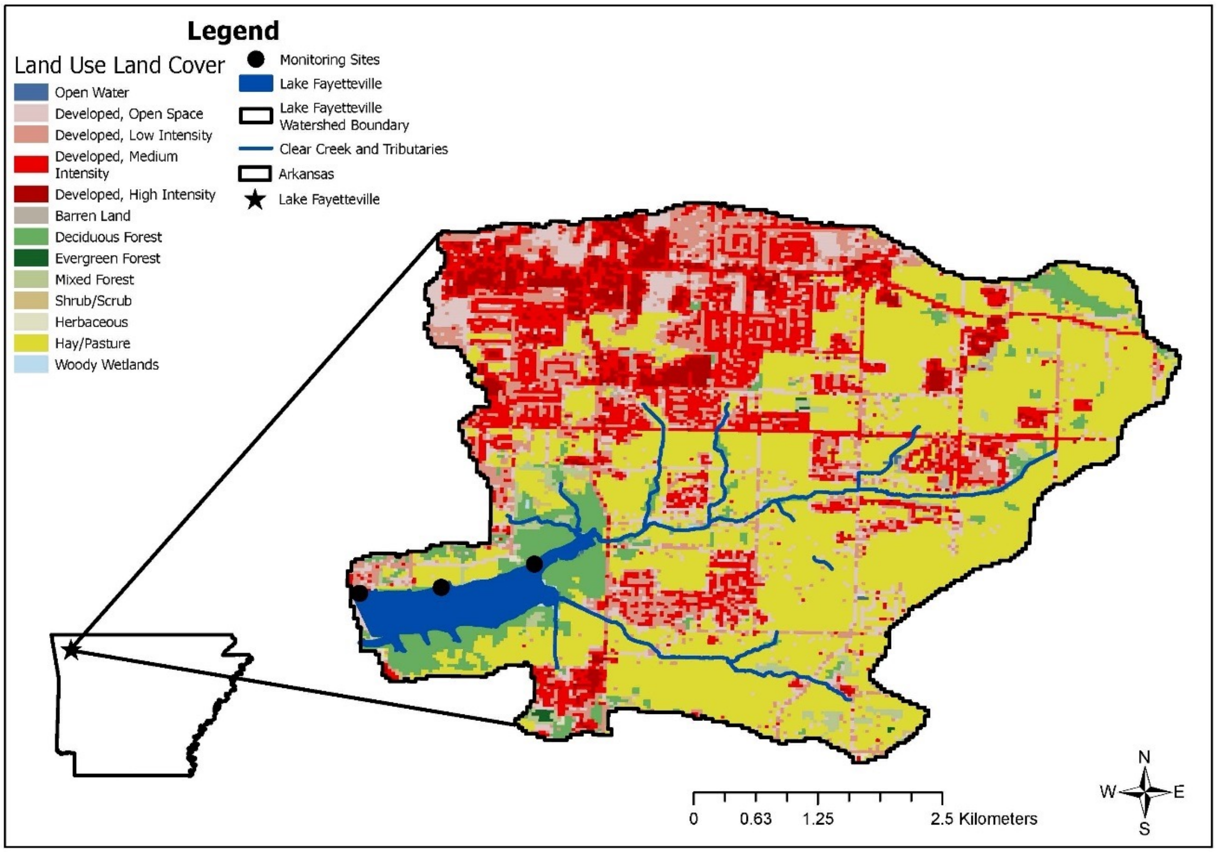

Figure 1: Sampling sites for routine lake monitoring at Lake Fayetteville, Arkansas (March 2019 – December 2021). Watershed land use was estimated using the 2019 National Land Cover Database in Model My Watershed (https://modelmywatershed.org/).

ational area. Primary contact recreation is not permitted, but fishing and boating are common.

We collected water samples weekly to monthly from March 2019 to December 2021 at three public- access sites along the windward side of the reservoir (Figure 1). Samples were collected from just below the water surface (~ 0.15 m) in acid-washed bottles after triple rinsing in the field. Sampling occurred consistently during late morning to afternoon (~11 am – 2 pm). Water temperature, dissolved oxygen, pH, and conductivity were measured on site.

Laboratory Analysis

At the Arkansas Water Resources Center Water Quality Lab (WQL), sample aliquots were processed and stored according to standard methods. All WQL chemical analyses use approved EPA methods, when available (https://awrc.uada.edu/water-quality-lab/). Total N and P were analyzed by colorimetry following pH adjustment ($pH < 2$) with sulfuric acid (H_2SO_4) and digestion in persulfate. Nitrate+nitrite-nitrogen (NO_x-N), soluble reactive phosphorus (SRP), and ammonium-nitrogen (NH_4-N) were analyzed by colorimetry following filtration through a 0.45 µm membrane and pH adjustment ($pH < 2$) with H_2SO_4.

Algal biomass was collected on a Whatman GF-F 0.7 µm glass fiber filter and extracted in the freezer in 90% acetone for at least 24 hours. The concentration of the pigment chlorophyll-a (CHL-a) and its degradate pheophytin (PHEO) were then measured by fluorometry. Beginning June 25, 2019, the raw fluorescence units (RFU) of chlorophyll (CHL) and phycocyanin (PC), a pigment in cyanobacteria, was also measured after subtracting the background RFU of filtered (0.45 µm) lake water.

Total microcystin (MC) concentration in raw lake water was measured using enzyme-linked immunosorbent assays after three freeze-thaw cycles to lyse cells and release intracellular microcystin. Before June 2020, we processed 2 mL for MC analysis, but we used 20 mL afterward to reduce method sensitivity to sub-sampling variability (Austin and Haggard, 2022).

Statistical analysis

We calculated several additional variables for statistical analysis. Particulate nitrogen (PN) and phosphorus (PP) fractions are the total fractions of N and P less dissolved “supply” forms (i.e., NO_x-N + NH_4-N and SRP). Molar N:P ratios were calculated for the total, particulate, and supply fractions by dividing the atomic mass of N by P. The PC:CHL ratio equals PC RFU divided by CHL RFU.

Mean MC and predictor variable values were calculated across the three sites on each date as inputs for the statistical analysis. Each date was assigned to a meteorological season (Spring = March 1 – May 31, Summer = June 1 – August 31, Fall = September 1 – November 30, and Winter = December 1 – February 28), as well as to growing season (April 1 – October 31) or off season (November 1 – March 31). We calculated antecedent precipitation (24-hr, 72-hr, and 1-wk) for each date using daily precipitation data from the U.S. Geological Survey gage 071948095 (https://waterdata.usgs.gov/nwis).

We used nonparametric change point analysis (nCPA) to identify differences in mean MC response ($p < 0.05$) at threshold values of nutrients, algal community metrics, physicochemical variables, and antecedent precipitation (King and Richardson, 2003; Qian et al., 2003). We used classification and regression tree (CART) analysis to look for secondary thresholds for MC response (De’Ath and Fabricius, 2000). Months, seasons, and years were included as potential predictors in CART analysis, which is compatible with categorical variables. We pruned our models by requiring a split in the data to increase model complexity by at least 0.05 (out of 1.0) to reduce overfitting.

The nCPA and CART analyses were carried out using all project data across years and separately on data from each calendar year. A minimum of 7 datapoints were required on each side of all thresholds in both nCPA and CART. We used RStudio Desktop (version 2021.09.2+382, RStudio Team, 2021) with the library rpart (Therneau and Atkinson, 2019) for CART analysis.

Results and Discussion

Temporal patterns

Physical, chemical, and biological water quality characteristics followed predictable seasonal patterns at Lake Fayetteville each year (Figure 2). The seasonal cycle of water temperatures shapes the timing of blooms, with the greatest algal biomass, as CHL-a (Kasprzak et al, 2008), in the late spring to early summer. During these blooms, PC:CHL was usually >1, suggesting cyanobacteria dominance (Thomson-Laing et al., 2020). Smaller blooms in the off season tended to have lower PC:CHL.

Water temperature and algal biomass, in turn, shape a seasonal nutrient cycle at Lake Fayetteville (Grantz et al. 2014). Each year, NH_4-N and SRP were consistently near or below reporting limits in surface waters, except immediately after lake mixing in the fall. These compounds accumulate all summer in lower lake layers, then mix with the surface layer in the fall. While also mostly absent during the growing season, NO_x-N concentrations were substantial during the off season each year. Less algal growth allows NO_x-N to build up from NH_4-N transformation and inflows from Clear Creek. Total nutrients also showed weak seasonal cycles. Seasonality in TN was greater than for TP and is driven by NO_x-N.

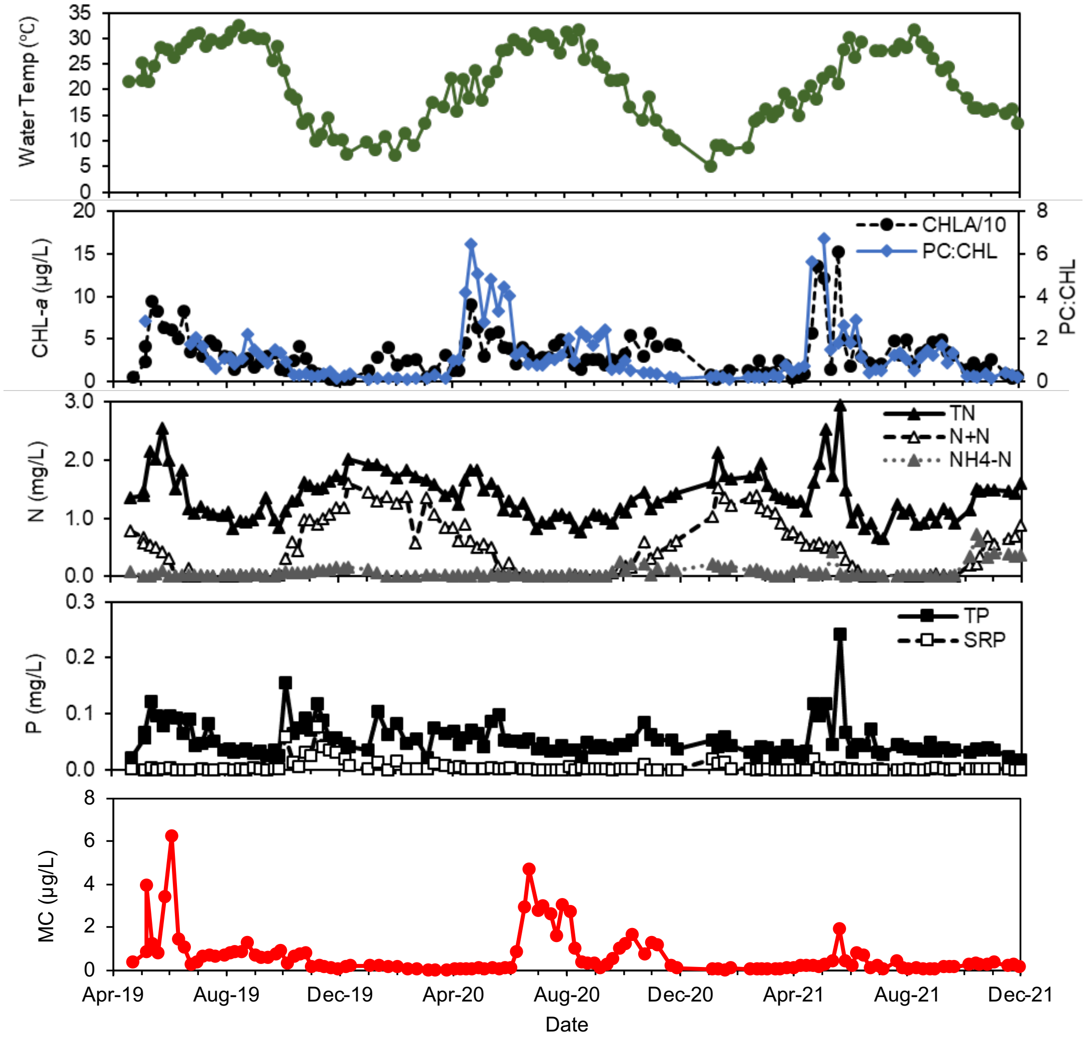

Figure 2: The mean of select physical, chemical, and biological water quality characteristics at Lake Fayetteville shown through time (April 2019 to December 2021).

We can see connections between the timing and magnitude of MC production and water temperature, algal metrics, and nutrients in the timeseries in Figure 2. Peaks in MC occurred throughout the growing season, when water temperatures and algal biomass are greatest and supply nutrients were least. Concentrations in individual samples sometimes exceeded the recreational guideline of 8 µg/L, suggesting water was potentially unsafe for human contact at those sites (data not shown). Maximum MC concentrations occurred earlier in spring in 2019, while 2020 had a more significant fall peak. We observed an interplay between PC:CHL and MC, with peak MC concentrations following peak PC:CHL by approximately a month.

Thresholds in microcystin predictors

We observed MC response thresholds to multiple physical, chemical, and biological water quality characteristics both across and within years at Lake Fayetteville (Table 1). The water quality characteristics with the strongest nCPA thresholds differed between the temporal data groupings, however. The thresholds in Table 1 are ranked from high to low based on R^2, which approximately shows the amount of variability in MC explained by the threshold from 0 – 1.00 (i.e. 0 – 100%).

But, water quality characteristics also recurred as predictors for MC. Water temperature is a master variable that affects cyanobacterial growth (Yang et al., 2020) and nutrient dynamics (Grantz et al., 2014). In 2020 and across years, MC was greater when water temperature was ≥ 27°C. Others have shown increasing MC with increasing water temperature (Brutemark et al., 2015). It is unclear if the relationship is based on greater MC production, or simply greater cyanobacterial biomass (Peng et al., 2018).

Cyanobacterial biomass, as PC RFU, was a top predictor for MC in all years and across years. Thresholds ranged from 1989 – 6909 RFU, showing that more cyanobacteria mean more MC. The ratio of PC to CHL was also a top predictor across years and in 2019 and 2021, though not in 2020.

Table 1: The top five thresholds for MC response to physical, chemical, and biological characteristics of water quality at Lake Fayetteville based on nonparametric change point analysis both across and within years. Thresholds are ranked from high to low based on the R^2, which approximately shows the amount of variability explained by the threshold on a scale of 0 – 1.00. Both DIN and NO_x-N were analyzed as potential MC predictors, but only the threshold with the highest R^2 is shown for each timeframe. Results for these two variables were nearly identical, reflecting that NO_x-N is almost always the dominant component of DIN.

Year	Variable	Units	Threshold	Median	p	R^2	Mean MC < Threshold (µg/L)	Mean MC ≥ Threshold (µg/L)
All Years	PC*	RFU	4530	4530	0.001	0.177	0.234	0.916
	Temp	°C	27.0	27.0	0.001	0.172	0.315	1.062
	NO_3-N	mg/L	0.50	0.50	0.001	0.157	0.844	0.162
	CHL-a	µg/L	23.4	23.4	0.001	0.112	0.252	0.821
	PC:CHL*		0.37	0.37	0.002	0.106	0.154	0.704
2019	PC*	RFU	1989	2348	0.001	0.413	0.176	0.684
	CHL*	RFU	2272	2278	0.001	0.337	0.155	0.649
	PC:CHL*		0.39	0.39	0.001	0.235	0.264	0.647
	PHEO	µg/L	5.18	5.18	0.001	0.208	0.559	1.425
	NO_3-N	mg/L	0.69	0.69	0.004	0.191	0.892	0.187
2020	Temp	°C	27.0	27.0	0.001	0.395	0.354	1.930
	NO_3-N	mg/L	0.32	0.27	0.001	0.287	1.415	0.179
	TN	mg/L	1.37	1.31	0.001	0.259	1.312	0.118
	Supply N:P		84.1	84.1	0.001	0.213	1.406	0.229
	PC	RFU	4524	4524	0.001	0.179	0.265	1.251
2021	72-hr Precip	cm	1.91	1.91	0.001	0.271	0.140	0.452
	PC:CHL		2.39	2.15	0.001	0.237	0.155	0.533
	CHL-a	µg/L	59.1	59.1	0.002	0.214	0.155	0.498
	NO_3-N	mg/L	0.71	0.71	0.003	0.173	0.242	0.056
	PC	RFU	6909	6719	0.003	0.169	0.161	0.496

*Raw fluorescence was added in June 25, 2019, so 2019 and all years had fewer observations for these variables compared to others.

In 2019 and across years, MC was greater on average above PC:CHL ~0.40. This ratio is <1, suggesting even minor cyanobacterial presence may be enough for MC to reach detectable levels. In contrast, the 2021 threshold in PC:CHL >2 associates greater MC with cyanobacterial dominance.

Algal biomass, as CHL-a, was a top threshold across years and in 2021. The threshold across years (CHL-a = 23.4 µg/L) is in range with the transition from productive to highly productive systems (Nuremberg et al., 1996), while the 2021 threshold (CHL-a = 59.1 ug/L) is well over limits suggesting high productivity. Other studies have shown that the probability of exceeding 1 µg L^{-1} MC increases with increasing nutrients and CHL-a (Yuan et al., 2014).

Finally, bioavailable supply nitrogen, as NO_x-N, appeared in the top thresholds across years and in all years. Below thresholds ranging from 0.32 – 0.71 mg/L, MC was greater in all analyses. Prior lab experiments with Lake Fayetteville waters also showed that the magnitude of the supply N affects toxin production (Wagner et al., 2021).

The CART analyses identified secondary thresholds in water quality characteristics. Here, we focus on the most data-rich all-years model. Individual year models are available in Ferri, 2021. The primary threshold in the all-years model was in water temperature, mirroring the nCPA threshold (Figure 3). The greatest MC tended to occur at ≥ 27°C. Samples collected at ≥ 27°C then split by year, where MC in 2020 was greater on average than in 2019 and 2021. For the 2019 and 2021 data, TN was a tertiary predictor, with greater MC when TN ≥ 1.7 mg/L (MC = 2.22 vs. 0.50 µg/L). For 2020, CHL RFU was the tertiary predictor, with greater MC above a threshold of 3570 RFU. Samples with ≥ 3570 RFU further split by TN:TP, where a ratio <59 was associated with in the greater MC (MC = 3.48 vs. 1.9 µg/L).

Conclusions

The driving factors behind HABs formation and toxin production are well known to be highly complex (Graham et al., 2004). No one environmental or water quality factor explained when and why algal blooms produced MC at Lake Fayetteville. Threshold-type analyses identified many physical, chemical, and biological characteristics of water

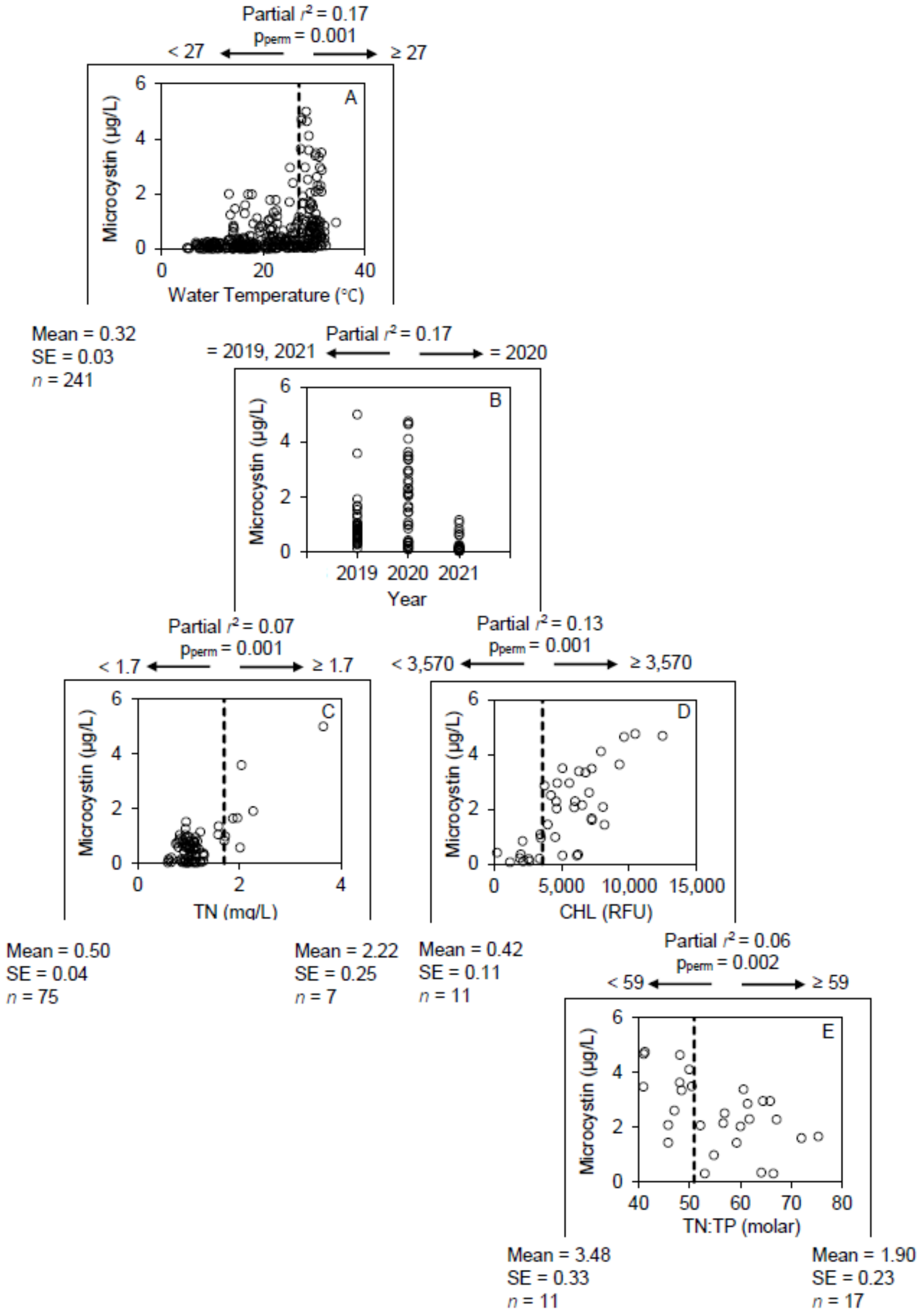

Figure 3: Classification and regression tree model for all years at Lake Fayetteville showing change points (dashed vertical lines) for microcystin response.

quality that predicted greater or less MC. The magnitude and timing of MC production and primary MC predictors differed between years 2019 – 2021. Nevertheless, a suite of interrelated factors is emerging from the multiple timescales of analysis, namely water temperature, algal/cyanobacterial biomass and relative abundance, and supply N availability. These water quality characteristics influence each other over seasonal cycles and appear to influence MC. Continued monitoring is needed to understand this interannual variability in MC levels and predictors in order to solve the HABs puzzle at Lake Fayetteville.

Acknowledgements

This material is based upon work supported by the United States Geological Survey under grant agreement No. G16AP00040 and No. G21AP10581-01, and administered by the Arkansas Water Resources Center. The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the opinions or policies of the U.S. Geological Survey.

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