

National Water Quality Program

Cyanobacteria, Cyanotoxin Synthetase Gene, and Cyanotoxin Occurrence Among Selected Large River Sites of the Conterminous United States, 2017–18



Scientific Investigations Report 2021–5121

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Cover photos. 1. A U.S. Geological Survey (USGS) gage house on the bank of the Connecticut River at Thompsonville, Connecticut. Photograph by Brittney Izbicki, USGS. 2. USGS hydrologists using a crane and DH-95 sampler from a bridge, Delaware River at Trenton, New Jersey. Photograph by Emily Wengrowksi, USGS. 3. An algal bloom on the Willamette River at Portland, Oregon. Photograph by Sean Payne, USGS. 4. Measuring streamflow with an acoustic doppler current profiler, Mississippi River at Hastings, Minnesota. Photograph by the USGS. 5. Using a DH-95 sampler on the Willamette River in downtown Portland, Oregon. Photograph by Michael Sarantou, USGS. 6. Hydrologic Technicians Brittney Izbicki and Theresa Armijo collecting water quality samples during high flow on the Connecticut River at Thompsonville, Connecticut. Photograph by Kaitlin Laabs, USGS.

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By Robert E. Zuellig, Jennifer L. Graham, Erin A. Stelzer, Keith A. Loftin, and Barry H. Rosen

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Conversion Factors

International System of Units to U.S. customary units

Multiply	By	To obtain
Length		
meter (m)	3.281	foot (ft)
kilometer (km)	0.6214	mile (mi)
Area		
square kilometer (km ²)	247.1	acre
square kilometer (km ²)	0.3861	square mile (mi ²)
Flow rate		
cubic meter per second (m ³ /s)	35.31	cubic foot per second (ft ³ /s)
Mass		
milligram (mg)	0.00003527	ounce, avoirdupois (oz)
microgram (μg)	0.0000003527	ounce, avoirdupois (oz)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:
 $^{\circ}\text{F} = (1.8 \times ^{\circ}\text{C}) + 32$.

Supplemental Information

Concentrations of chemical constituents in water are given in either milligrams per liter (mg/L) or micrograms per liter (μg/L).

Abbreviations

AVLD	absolute value logarithmic difference
chl _a	chlorophyll <i>a</i>
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
mRNA	messenger ribonucleic acid
NAWQA	National Water Quality Assessment (Project)
nQ	streamflow normalized to the 10-year mean daily streamflow for the day of sampling
NWIS	National Water Information System
qPCR	quantitative polymerase chain reaction
RPD	relative percentage difference
SSC	suspended sediment concentration
T	temperature
TN	total nitrogen
TP	total phosphorus
USGS	U.S. Geological Survey

Cyanobacteria, Cyanotoxin Synthetase Gene, and Cyanotoxin Occurrence Among Selected Large River Sites of the Conterminous United States, 2017–18

By Robert E. Zuellig,¹ Jennifer L. Graham,¹ Erin A. Stelzer,¹ Keith A. Loftin,¹ and Barry H. Rosen^{1,2}

Abstract

Cyanobacteria and associated cyanotoxins often affect recreational and drinking-water use but are understudied in rivers relative to lakes and reservoirs. The U.S. Geological Survey measured cyanobacteria, cyanotoxin synthetase genes, and cyanotoxins at 11 river sites throughout the conterminous United States in a multiyear pilot study during 2017–19 through the National Water Quality Assessment Project to better understand the occurrence of cyanobacteria and cyanotoxins in large inland and coastal rivers. Samples were collected during summer and early fall. Selected sites represented a range of environmental conditions and were sampled in accordance with National Water Quality Assessment Project schedules and protocols. This report focuses on the first 2 years of data collection (2017 and 2018) and describes occurrence of anatoxin-, cylindrospermopsin-, microcystin-, and saxitoxin-producing cyanobacteria, cyanotoxin synthetase genes (*anaC*, *cyrA*, taxa specific *mcyE*, and *sxtA*), and cyanotoxins (anatoxins, cylindrospermopsins, microcystins, and saxitoxins). In addition, this study evaluated (1) concordance on the basis of presence or absence between cyanobacteria, cyanotoxin synthetase genes, and cyanotoxin occurrence, and (2) correlations between these three measures and selected environmental variables.

Because the current (2021) study was a pilot-scale effort that leveraged an existing water-quality monitoring study designed to meet unrelated objectives, rigorous spatiotemporal, seasonal, and statistical analyses of factors associated with occurrence and dynamics of these three variables were not tenable. Nonetheless, results from this pilot effort can be used to help inform future studies designed to address cyanobacterial community dynamics in large rivers.

Potential cyanotoxin-producing cyanobacteria occurred more frequently in samples than cyanotoxin synthetase genes (34–72 percent compared to 8–58 percent of samples), and cyanotoxin synthetase genes occurred more often than

cyanotoxins (6–8 percent of samples), depending on cyanotoxin type and year. Concordance between the occurrence of potential cyanotoxin-producing cyanobacteria, cyanotoxin synthetase genes, and cyanotoxins varied by cyanotoxin type and which set of comparisons were being considered. Potential cyanotoxin-producing cyanobacteria were present without measurable genetic potential for cyanotoxin production (between 20 and 91 percent of the time), and cyanotoxin synthetase genes were present without cyanotoxins (between 88 and 100 percent of the time). Relations between potential cyanotoxin-producing cyanobacteria and water-quality and hydrologic variables suggest upstream source areas and downstream transport may affect cyanobacterial communities in these rivers.

Study findings demonstrate that cyanobacteria, cyanotoxin synthetase genes, and cyanotoxins are present in large U.S. rivers under ambient conditions and show that downstream transport and flushing likely affect relative abundance of potential cyanotoxin-producing cyanobacteria. Additionally, the results agree with existing literature that support the importance of water temperature, light, and nutrients—as moderated by hydrologic conditions—in shaping the structure of riverine cyanobacterial communities.

Introduction

Most cyanobacteria research in the United States has been conducted in lakes and reservoirs (Graham and others, 2004; Graham and others, 2010; Beaver and others, 2014; Loftin and others, 2016a), where excess cyanobacterial biomass and cyanotoxins often affect recreational and drinking-water use because of human health concerns. Ecosystem-level effects are also of concern where cyanobacteria and associated cyanotoxins can result in food-web disruption and animal intoxication (Brooks and others, 2016). National and regional assessments in the United States have demonstrated that cyanobacteria and cyanotoxins are common not only in lakes and reservoirs, but also in small streams (Fetscher and others, 2015; Loftin and others, 2016b), wetlands (EPA, 2016), and estuaries (Preece and others, 2017). Comparable assessments

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in large (nonwadeable) U.S. rivers have not been conducted, though several high-profile cyanobacteria-related events have occurred. For example, in 2011, cyanotoxins were detected throughout the entirety of the Kansas River, Kansas following releases from upstream reservoirs (Graham and others, 2012), and in 2015 and 2019, cyanotoxins affected hundreds of kilometers of the Ohio River in West Virginia (2015 only), Ohio, Indiana, and Kentucky (ORSANCO, 2020); these events caused recreational advisories and drinking-water treatment concerns. Occurrences such as these underscore the need for research on cyanobacterial community dynamics in large river systems.

Nutrients, water residence time, water temperature, and light are key drivers of phytoplankton biomass and community composition in aquatic systems (Søballe and Kimmel, 1987; Reynolds and Descy, 1996; Smith, 2003; Giblin and Gerrish, 2020). In general, conditions of abundant nutrients (Van Nieuwenhuysse and Jones, 1996; Heiskary and Markus, 2001), long residence times (Cha and others, 2017; Matson and others, 2020), warm water temperatures (Cha and others, 2017; Knowlton and Jones, 2000), and high light exposure (Giblin and Gerrish, 2020) are positively associated with phytoplankton biomass and favor dominance by cyanobacteria. The relative importance of environmental variables on phytoplankton biomass and community composition depends on site-specific factors such as meteorologic, hydrogeologic, geomorphic, and biotic interactions (Reynolds and Descy, 1996; Van Nieuwenhuysse and Jones, 1996; Heiskary and Markus, 2001; Smith, 2003; Chételat and others, 2006; Cha and others, 2017; Giblin and Gerrish, 2020). Hydrologic connectivity further complicates these relations in large rivers as phytoplankton may be exported from upstream source areas such as lakes, reservoirs, or backwaters and transported downstream (Knowlton and Jones, 2000; Graham and others, 2012; Otten and others, 2015; Preece and others, 2017; Giblin and Gerrish, 2020). Downstream transport of cyanobacteria and associated cyanotoxins from upstream lakes and reservoirs has been documented during cyanobacterial blooms (Graham and others, 2012; Otten and others, 2015; Preece and others, 2017), but physical processes, including transport and the influence of backwater areas, are understudied relative to other environmental influences in large rivers (Giblin and Gerrish, 2020; Matson and others, 2020; Xia and others, 2020).

Cyanobacterial production of cyanotoxins is strain-specific and the drivers of cyanotoxin production are not well understood. Microscopic identification of cyanobacteria indicates the presence of taxa with known cyanotoxin-producing strains, but does not distinguish between nontoxic and toxin-producing strains (Al-Tebrineh and others, 2012a; Pacheco and others, 2016; Bouma-Gregson and others, 2019). Measuring cyanotoxin synthetase genes in addition to cyanobacterial identification provides more detail about the potential for cyanotoxin production within a cyanobacterial community than cyanobacterial identification alone. In addition, the methods used to measure genes are sensitive enough that they may detect cyanobacteria that are too rare to be

detected using microscopy (Otten and others, 2015; Pacheco and others, 2016; Graham and others, 2020). However, direct measurement of cyanotoxins is the only definitive indicator of cyanotoxin presence in the environment. Combined, these three measures (cyanobacteria taxa with known cyanotoxin-producing strains, cyanotoxin synthetase genes, and cyanotoxins) provide a more detailed understanding of the relative abundance, spatiotemporal variability, and environmental conditions associated with the occurrence of cyanobacteria and nontoxic- and toxin-producing strains. Understanding the relations between these measures is complicated by the potential for cyanotoxin synthetase genes and cyanotoxins to persist in the environment for some length of time outside the source cyanobacteria cells, leading to spatial and temporal overlap of nonrelated viable cyanobacteria, cyanotoxin synthetase genes, and cyanotoxins (Graham and others, 2012; Otten and others, 2015; Pacheco and others, 2016; Preece and others 2017; Graham and others, 2020).

To better understand cyanobacterial occurrence in rivers and help inform future studies that will lead to better information and tools for early indicator, management, and mitigation strategies, the U.S. Geological Survey (USGS) collected samples at 11 large river sites located throughout the conterminous United States (fig. 1) through the National Water Quality Assessment (NAWQA) Project as part of a multiyear pilot study during 2017–19. Graham and others (2020) described the occurrence of cyanobacteria with known cyanotoxin-producing strains, cyanotoxin synthetase genes, and cyanotoxins during the first year (2017) of the study. Results from June through September 2017 indicated that, depending on cyanotoxin type, cyanobacteria with known cyanotoxin-producing strains frequently occurred (100 percent of sites out of 11 sites; 48–80 percent of samples out of 50 samples), cyanotoxin synthetase genes occurred less often (91 percent of rivers; 4–44 percent of samples), and cyanotoxins infrequently occurred (18 percent of rivers; 6–17 percent of samples). Occurrence and concentration of cyanotoxin synthetase genes and cyanotoxins were highest in the eutrophic midcontinent rivers when compared to other regions of the United States (Graham and others, 2020). This report expands on the Graham and others (2020) analysis by including data from the first 2 years (2017 and 2018) of the study to describe cyanobacteria, cyanotoxin synthetase gene, and cyanotoxin occurrence. In addition, concordance between cyanobacteria, cyanotoxin synthetase gene, and cyanotoxin occurrence and general relations between these three measures and environmental variables were evaluated.

Purpose and Scope

The NAWQA large river pilot study is the first effort to include cyanobacteria, cyanotoxin synthetase genes, and cyanotoxins in large rivers distributed throughout the United States (Graham and others, 2020). As such, the study allows the unique opportunity to compare the occurrence of

cyanotoxin synthetase genes relative to cyanobacteria with known cyanotoxin-producing strains and cyanotoxins in large rivers across a range of water-quality and hydrologic conditions. The purpose of this report is to describe (1) cyanobacteria, cyanotoxin synthetase gene, and cyanotoxin occurrence, (2) concordance between cyanobacteria, cyanotoxin synthetase gene, and cyanotoxin occurrence, and (3) relations between cyanobacteria, cyanotoxin synthetase genes, cyanotoxins, and selected environmental variables.

The scope of this report includes water-quality samples collected at 11 USGS streamflow-gaging stations in the conterminous United States, chosen from a set of routinely sampled locations as part of the USGS NAWQA Project (Lee and Henderson, 2020). Samples were collected between early summer and fall over 2 consecutive years (2017 and 2018). Sites were selected to represent large inland or coastal rivers and a broad range of environmental, water-quality, and streamflow conditions throughout the conterminous United States (fig. 1; table 1). All site watersheds were domestic except the Missouri River watershed, which partially lies in Canada. All rivers are used for water supply, though intakes were not necessarily proximal to site locations (Price and Maupin, 2014). All sites were nonwadeable, draining basins between 6,294 and 1,353,269 square kilometers, and influenced by upstream dams (table 1). Four sites had mainstem dams located within 5 kilometers (km) upstream (table 1); whereas one site (Trinity River) was approximately 4.8 km downstream from a wastewater treatment facility.

Methods

All sites were sampled during June through September 2017 and June through October 2018 based on preexisting NAWQA sampling schedules (Lee and Henderson, 2020). As a result, individual sites were sampled between two and nine times each year (table 1). Based on observed cyanobacteria occurrence in 2017, the sampling season was extended through October in 2018. Water temperature (T), total nitrogen (TN), total phosphorus (TP), and suspended sediment concentration (SSC) are all routine NAWQA analytes. For this study, chlorophyll *a* (chl_a) samples also were collected along with samples to assess phytoplankton community composition including cyanobacteria, genes present in cyanotoxin synthetase gene clusters, and cyanotoxins (Graham and others, 2020).

Sample Collection and Laboratory Analysis

Streamflow, T, TN, TP, and SSC data were downloaded from the USGS National Water Information System (NWIS) database (U.S. Geological Survey, 2021). Samples for all other analyses except cyanotoxin synthetase genes were collected in accordance with NAWQA sampling protocols that follow isokinetic equal width or depth integrated techniques to

ensure samples are representative of stream conditions (Lee and Henderson, 2020). Traditional cleaning protocols for field sampling equipment do not sufficiently remove all residual deoxyribonucleic acid (DNA; Harris and others, 2006).

Therefore, samples for cyanotoxin synthetase gene analysis were collected as near-surface grabs at the centroid of flow using a weighted basket sampler and bleached polypropylene bottles (Stelzer and others, 2013). Chl_a, phytoplankton, cyanotoxin synthetase gene, and cyanotoxin samples were processed and analyzed as described in Graham and others (2020, 2021a, b) and King and others (2020a, b). Chl_a, cyanotoxin synthetase gene, and cyanotoxin data are available in NWIS (U.S. Geological Survey, 2021), Graham and others (2021a), and King and others (2020a). Phytoplankton data are available in Graham and others (2021b) and King and others (2020b).

Chl_a (minimum reporting level: 0.10 microgram per liter [µg/L]) was analyzed fluorometrically at the USGS National Water Quality Laboratory using the U.S. Environmental Protection Agency method 445.0 (Arar and Collins, 1997). Phytoplankton samples were preserved with acidified Lugol's iodine and analyzed at the USGS Caribbean-Florida Water Science Center. Phytoplankton were concentrated by settling and then enumerated by natural unit (cell, colony, or filament) to the lowest possible taxonomic level as described in Rosen and others (2018).

Cyanotoxin synthetase genes were analyzed by quantitative polymerase chain reaction (qPCR) at the USGS Ohio Water Microbiology Laboratory in accordance with Stelzer and others (2013). Analyses targeted cyanotoxin synthetase genes present in the anatoxin (the *anaC* gene; Sabart and others, 2015), cylindrospermopsin (the *cyrA* gene; Al-Tebrineh and others, 2012b), microcystin (the *mcyE* gene; Rantala and others, 2006; Sipari and others, 2010), and saxitoxin (the *sxtA* gene; Al-Tebrineh and others, 2012b) gene clusters. These DNA-based qPCR assays determine presence and concentration of the cyanotoxin synthetase genes present, which means they only measure the genetic potential for cyanotoxin production. A cyanobacteria cell must transcribe its DNA-encoded toxin gene into messenger ribonucleic acid (mRNA) to initiate the biosynthetic process responsible for cyanotoxin production (Stelzer and others, 2013).

The *anaC*, *cyrA*, and *sxtA* gene assays were general and taxa independent. The *mcyE* gene assays targeted the potential microcystin-producing taxa associated with *Dolichospermum* (formerly *Anabaena*), *Microcystis*, and *Planktothrix* genera. Targeted assays for the *mcyE* gene were chosen because of laboratory quality assurance and control concerns with the general *mcyE* gene assay at the time of this study. TaqMan Universal PCR Master Mix (Life Technologies) was used for all assays except *anaC* and *Planktothrix mcyE*, which used SYBR Green PCR Master Mix (Life Technologies). Plasmid standards for each assay were used to establish standard curves for quantification. For *Dolichospermum mcyE*, *Microcystis mcyE*, and *Planktothrix mcyE* genes, plasmid standards were constructed by insertion of a polymerase chain reaction-amplified target sequence into a pCR4-TOPO cloning

Table 1. Drainage area, number of major dams in the drainage area, number of samples collected, mean streamflow and water temperature, and mean nutrient, suspended sediment, and chlorophyll *a* concentrations at U.S. Geological Survey streamflow-gaging stations sampled during June through September 2017 and June through October 2018.

[km², square kilometer; m³/s, meter cubed per second; °C, degrees Celsius; mg/L, milligram per liter; µg/L, microgram per liter; USGS, U.S. Geological Survey]

USGS streamflow-gaging station site name	USGS station identifier	Drainage area (km ²)	Major dams ¹	Number of samples		Streamflow ² (m ³ /s)		Water temperature ³ (°C)		Total nitrogen ^{3,4} (mg/L)		Total phosphorus ³ (mg/L)		Suspended sediment ³ (mg/L)		Chlorophyll <i>a</i> (µg/L)	
				2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Connecticut River at Thompsonville, Conn. ⁵	01184000	25,019	77	7	8	349	344	22.1	22.9	0.588	0.546	0.035	0.035	6.7	3.8	1.6	3.2
Delaware River at Trenton, N.J.	01463500	17,560	33	3	4	221	481	24.5	21.2	1.158	0.960	0.071	0.053	6.0	10.3	2.1	2.0
Susquehanna River at Conowingo, Md. ⁵	01578310	70,189	125	2	3	744	1,885	23.9	22.1	1.258	2.006	0.044	0.126	8.5	65.0	8.9	4.9
Chattahoochee River near Whitesburg, Ga.	02338000	6,294	46	5	7	77	117	24.8	23.0	3.148	2.328	0.156	0.144	93.8	152.3	2.3	4.4
Ohio River at Cannelton Dam at Cannelton, Ind. ⁵	03303280	251,229	709	2	3	2,233	3,403	25.6	23.4	1.699	1.313	0.159	0.080	63.0	26.7	3.4	4.1
Mississippi River at Hastings, Minn. ⁵	05331580	96,089	93	4	6	545	1,015	24.1	20.3	3.886	4.661	0.130	0.185	44.5	60.0	37.4	19.1
Kansas River at De Soto, Kans.	06892350	154,767	89	5	5	216	151	24.6	24.5	2.268	2.028	0.538	0.596	411.8	441.0	34.6	50.8
Missouri River at Hermann, Mo.	06934500	1,353,269	992	5	6	2,712	3,315	27.2	26.1	2.161	3.341	0.348	0.582	291.4	585.2	20.4	10.3
Trinity River below Dallas, Tex.	08057410	16,260	97	8	9	59	138	28.9	28.4	7.108	7.637	0.783	0.793	96.9	85.8	23.9	14.5
Sacramento River at Freeport, Calif.	11447650	61,445	135	4	5	583	419	21.2	21.5	0.177	0.185	0.037	0.046	21.0	11.3	4.1	2.1
Willamette River at Portland, Oreg.	14211720	29,008	37	5	6	365	245	21.6	20.1	0.549	0.565	0.058	0.057	7.0	6.2	4.6	5.6

¹Number of major dams in the drainage area obtained from Wicczorek and others (2018).

²All study sites had USGS streamflow-gaging stations. Streamflow data were downloaded from the USGS National Water Information System database (U.S. Geological Survey, 2021) using the eight-digit USGS station number presented in the table. Fifteen-minute interval data were used to calculate mean streamflow for the periods June through September 2017 and June through October 2018.

³Nutrient and suspended sediment data were downloaded from the USGS National Water Information System database (U.S. Geological Survey, 2021) using the eight-digit USGS station number presented in the table and were used to calculate means for the periods June through September 2017 and June through October 2018. The USGS National Water Information System database is the definitive dataset for the analyses presented in this report.

⁴Total nitrogen was calculated as the sum of particulate nitrogen and dissolved nitrogen.

⁵Site was within 5 kilometers downstream from a mainstem dam.

vector (Life Technologies). Plasmids for all other cyanotoxin synthetase gene targets were synthesized in cloning vectors by Integrated DNA Technologies. The copy number of each target was calculated using the DNA concentration measured by the Qubit dsDNA High Sensitivity Assay (Life Technologies) and the molecular weight of the plasmid. Standard curve information and assay detection limits are presented in King and others (2020a) and Graham and others (2021a).

Total anatoxins, cylindrospermopsins, microcystins (-adda specific assay), and saxitoxins were analyzed at the USGS Organic Geochemistry Research Laboratory using enzyme-linked immunosorbent assays (ELISA). Prior to analysis, whole-water samples were lysed by three sequential freeze-thaw cycles and filtered using 0.7-micrometer glass fiber filters (Loftin and others, 2008). Eurofins Abraxis ELISA kits were used for all cyanotoxin analyses. Minimum reporting levels were assay-dependent (anatoxins: 0.15 µg/L; cylindrospermopsins: 0.05 µg/L; microcystins: 0.15 µg/L; saxitoxins: 0.02 µg/L). The ELISA kit used for microcystins analysis is also cross-reactive to nodularin-R, and reported microcystin occurrence concentration may also include this cyanotoxin. However, though nodularin-R detections have been reported in U.S. freshwaters (for example, Graham and others, 2010; Foss and others, 2017), occurrence is rare.

Quality assurance and quality control samples were collected to evaluate variability in sample collection, processing, and laboratory techniques. About 15 percent of all samples collected were concurrent field replicates. Relative percentage difference (RPD) was used to evaluate differences in chl *a* and cyanotoxin concentrations in replicate water samples. The RPD was calculated by dividing the difference of concentration between the replicate pair by the mean of the concentration of the replicate pair and multiplying that value by 100, thereby creating a value that represents the percent difference of concentration between replicate samples (Zar, 1999).

Absolute value logarithmic difference (AVLD) was used to evaluate differences in cyanobacterial and cyanotoxin synthetase gene concentration between replicate pairs (Francy and others, 2015). The AVLD was calculated as follows:

$$\text{AVLD} = |\log_{10} R1 - \log_{10} R2|, \quad (1)$$

where *R1* is the concentration in replicate 1, and *R2* is the concentration in replicate 2. AVLD was used to evaluate differences for cyanobacterial data because RPD calculations are sensitive to rare taxa present in one of the replicate samples but not the other; and used to evaluate differences for cyanotoxin synthetase gene data because of the variability that may be introduced during processing of samples with high cyanobacterial concentrations (Stelzer and others, 2013; Francy and others, 2015). Replicate pairs with an AVLD value less than 1.0 were considered acceptable for this study.

Data Processing, Analysis, and Interpretation

Phytoplankton taxonomy was harmonized between years and used at the level of genus to ensure that observed patterns reflected community composition and not taxonomic inconsistencies in identification. Genera with known cyanotoxin-producing strains were grouped by associated cyanotoxin type (table 2). All associated genera were included in potential anatoxin-, cylindrospermopsin-, and saxitoxin-producing groups, but only *Dolichospermum*, *Microcystis*, and *Planktothrix* were included in the microcystin group because of the specificity of the cyanotoxin synthetase gene assays. Cyanotoxin data were reported as presence or absence, taxonomic data were reported as presence or absence or relative abundance, and gene data were reported as presence or absence or concentration depending on the analysis.

Concordance between cyanobacteria, gene, and cyanotoxin occurrence was determined for each cyanotoxin type by calculating the proportion of samples when genes and cyanotoxins were present relative to observations where cyanobacteria were present; when cyanobacteria and cyanotoxins were present relative to observations where genes were present; and when cyanobacteria and genes were present relative to observations where cyanotoxins were present. Each proportion was expressed as a percentage of observations where both constituents were detected. One hundred percent agreement indicated complete concordance between any two constituents, whereas values less than 100 percent indicated that the compared constituent was not detected in all samples where the principal constituent was detected.

Nonparametric Spearman rank-correlation analysis (Helsel and others, 2020) was used to evaluate the strength and form of the association between biological response and selected environmental variables. Biological response variables consisted of chl *a*, relative abundance of cyanobacteria associated with each toxin (anatoxin, cylindrospermopsin, microcystin, and saxitoxin), and gene concentration (*cyrA*, *sxtA*, and *mcyE*). Relative abundance, in percent, was calculated for each sample as cyanobacteria concentration associated with each toxin divided by total phytoplankton concentration. Environmental variables consisted of T, TN, TP, SSC, and streamflow normalized to the 10-year mean daily streamflow for the day of sampling (nQ). The resulting nQ value represented how streamflow on the day of sampling compared to the past 10-year mean daily streamflow. An nQ value of 1 indicated streamflow on the day of sampling was equal to the 10-year average; whereas, an nQ value above or below 1 indicated that the sampled streamflow was above or below the 10-year average. Mean daily streamflow data collected between January 1, 2007, and December 31, 2018, were downloaded from NWIS (U.S. Geological Survey, 2021). The *anaC* gene and cyanotoxins data were not included in the correlation analysis because of the small number of detections. Spearman rank-correlation coefficients were considered significant when p-values were less than or equal to 0.05.

Table 2. Cyanobacterial genera present in the 11 rivers sampled during June through September 2017 and June through October 2018 and cyanotoxins known to be produced by some strains.

[Genera are ordered from most to least common taxa, based on number of sites with the taxa present. ATX, anatoxins; CYL, cylindrospermopsins; MC, microcystins; SAX, saxitoxins; X, genus produces indicated cyanotoxin; —, no strains within the genera are known to produce the indicated cyanotoxin]

Genera	ATX ¹	CYL ¹	MC ¹	SAX ¹
<i>Pseudanabaena</i>	X	—	X	—
<i>Planktothrix</i>	X	—	X	X
<i>Dolichospermum</i>	X	X	X	X
<i>Aphanocapsa</i>	—	—	X	—
<i>Cuspidothrix</i>	X	—	—	X
<i>Merismopedia</i>	—	—	X	—
<i>Planktolyngbya</i>	—	—	—	—
<i>Aphanizomenon</i>	X	X	X	X
<i>Cylindrospermopsis</i>	—	X	—	X
<i>Limnothrix</i>	—	—	—	X
<i>Anabaenopsis</i>	—	—	X	—
<i>Eucapsis</i> ²	—	—	—	—
<i>Chroococcus</i>	—	—	—	—
<i>Dactylococopsis</i>	—	—	—	—
<i>Schizothrix</i>	—	—	—	—
<i>Phormidium</i> ²	X	—	X	X
<i>Snowella</i> ²	—	—	—	—
<i>Coelosphaerium</i>	—	—	—	—
<i>Microcystis</i> ³	X	—	X	—
<i>Raphidiopsis</i> ³	—	X	—	X
<i>Calothrix</i> ²	—	—	X	—
<i>Nostoc</i> ²	—	—	X	—
<i>Anabaena</i>	X	X	X	X
<i>Romeria</i>	—	—	—	—
<i>Oscillatoria</i> ³	X	X	X	X
<i>Cylindrospermum</i> ²	X	—	—	X
<i>Komvophoron</i> ²	—	—	—	—
<i>Synechococcus</i> ²	—	—	X	—
<i>Geitlerinema</i> ³	—	—	X	X

¹Potential for cyanotoxin production based on Graham and others (2008) and Bernard and others (2017).

²Not present in 2018 samples.

³Not present in 2017 samples.

Results of Quality Assurance and Quality Control Analysis

The median of individual replicate RPDs for *chl a* was 6 percent (range of 0–52 percent, 17 samples). Larger RPDs for *chl a* were the result of low *chl a* values, and the absolute difference between replicate pairs with RPDs greater than 20 percent never exceeded 0.7 µg/L. The medians of individual replicate RPDs for all cyanotoxins were 0 percent (range of 0–11 percent, 76 samples). Most replicate cyanotoxin concentrations were below minimum reporting levels and there were no instances where cyanotoxins were detected in one replicate but not the other. Larger RPDs for cyanotoxins were the result of low concentrations, and the absolute difference between replicate pairs with RPDs greater than 10 percent never exceeded 0.04 µg/L. About 20 percent of all cyanotoxin analyses included laboratory replicates. The medians of individual laboratory replicate RPDs for all cyanotoxins were 0 percent (range of 0–2 percent, 104 samples); the absolute difference between replicate pairs never exceeded 0.01 µg/L. The low variability of laboratory replicates compared to replicates collected in the field shows most of the observed variability in cyanotoxin concentrations was likely caused by sample collection and processing technique rather than analytical measurement error.

The medians of individual replicate RPDs for cyanobacteria (18 samples) and cyanotoxin synthetase gene (114 samples) abundances were both 0.3; replicate RPDs ranged from 0 to 1.1. About 89 percent of cyanobacteria comparisons and 97 percent of cyanotoxin synthetase gene comparisons had AVLs less than 0.7. All *chl a*, cyanotoxin, cyanobacteria, and cyanotoxin synthetase gene data were of acceptable quality for the purpose of this report.

Potential Cyanotoxin-Producing Cyanobacteria, Cyanotoxin Synthetase Gene, and Cyanotoxin Occurrence

During summer and fall 2017 and 2018, cyanobacteria, genes, and cyanotoxins were analyzed in samples collected during routinely scheduled NAWQA Project site visits. In total, 112 samples from 11 river sites were collected. Frequency of occurrence of cyanobacteria, genes, and cyanotoxins in rivers (geographic occurrence) and samples (overall occurrence) are summarized for each year and both years combined.

Potential Cyanotoxin-Producing Cyanobacteria

Cyanobacteria occurrence among rivers varied by year and associated cyanotoxin, but no distinct patterns were observed when data from 2017 and 2018 were combined (fig. 2A). For example, percent occurrence of taxa among rivers ranged between 64 percent (potential cylindrospermopsin-producing taxa) and 100 percent (potential anatoxin-producing taxa) in 2017 (fig. 2A, dark gray bars), and between 64 percent (potential microcystin-producing taxa) and 82 percent (potential anatoxin-, cylindrospermopsin-, and saxitoxin-producing taxa) in 2018 (fig. 2A, light gray bars). Cyanobacterial taxa associated with each cyanotoxin occurred at all sites when data from both years were combined (fig. 2A, black bars).

Cyanobacteria occurrence among samples varied by associated cyanotoxin, but less so by year (fig. 3A). In 2017 and 2018, percent occurrence of potential anatoxin- and saxitoxin-producing taxa was greater than occurrence of potential cylindrospermopsin- and microcystin-producing taxa (fig. 3A). Additionally, percent occurrence was generally similar between years for any given associated cyanotoxin. For example, potential cylindrospermopsin producers occurred in 48 percent of samples in 2017 compared to 44 percent in 2018, whereas potential anatoxin producers occurred in 72 percent of samples in 2017 compared to 63 percent in 2018 (fig. 3A).

The percent occurrence of potential microcystin-producing cyanobacteria was underrepresented in these analyses. Numerous cyanobacterial taxa have known microcystin-producing strains (table 2). However, synthetase gene assays for microcystin only targeted strains associated with two genera, *Microcystis* and *Planktothrix*. In a more broadly-scoped analysis of the 2017 data collected from these rivers that included additional microcystin-producing genera, Graham and others (2020) found that cyanobacterial genera with known microcystin-producing strains occurred in 91 percent of sites and 78 percent of samples.

Cyanotoxin Synthetase Genes

The percent occurrence of synthetase genes among rivers varied by associated cyanotoxin; however, percent occurrences among rivers were similar in both years (fig. 2B). For example, the ranked order of percent occurrence of synthetase genes in rivers ($anaC < cyrA < mcyE < sxtA$) was the same in both years. Overall percent occurrence of synthetase genes was lower in 2017 (low, *anaC* in 9 percent of rivers; high, *sxtA* in 73 percent of rivers) than 2018 (low, *anaC* in 36 percent of rivers; high, *sxtA* in 100 percent of rivers) for all measured genes (fig. 2B, light gray bars compared to dark gray bars). Consequently, there was no change in pattern when gene data from both years were combined (fig. 2B, black bars). The *anaC* gene occurred about 2.5 times less often in 2017 and 2018 (36 percent of rivers) than other measured genes (90–100 percent of rivers).

The percent occurrence of synthetase genes among samples also varied by associated cyanotoxin, and percent occurrences were similar in both years (fig. 3B). Percent occurrence of synthetase genes among all samples increased in the same ranked order in both years ($anaC < cyrA < sxtA < mcyE$), where values were lower in 2017 (low, *anaC* in 4 percent of samples; high, *mcyE* in 44 percent of samples) than in 2018 (low, *anaC* in 11 percent of samples; high, *mcyE* in 70 percent of samples; fig. 3B, light gray bars compared to dark gray bars). There was no change in pattern when gene data from both years were combined (fig. 3B, black bars). Differences in percent occurrence of each gene were more pronounced among samples than among rivers. In both years combined, the *anaC* gene occurred (8 percent of samples) about 3 times less often than the *cyrA* gene (29 percent of samples) and about 7 times less often than the *sxtA* (51 percent of samples) and *mcyE* (58 percent of samples) gene.

Individual sites were sampled from two to nine times per year during this pilot study. The small number of samples at some sites and lack of parity across sites precludes a meaningful analysis of environmental factors associated with the observed differences in gene occurrence between years. Higher overall occurrence of genes in 2018 than 2017 may have been influenced by regional (climate and weather) and site-specific factors (water residence time, nutrients, upstream source areas; Reynolds and Descy, 1996; Smith, 2003; Chételat and others, 2006). However, there were no consistent or obvious patterns in select environmental variables among years (table 1).

Cyanotoxins

Microcystins were the only cyanotoxin detected by ELISA in rivers sampled during 2017 and 2018. Overall, 36 percent of rivers had detectable microcystin (fig. 2C). Percent occurrence of microcystins was lower in 2017 (18 percent of rivers) than in 2018 (27 percent of rivers). Although occurrence in rivers was higher in 2018 than in 2017, occurrence in samples was similar between years (6–8 percent of samples; fig. 3C).

Concordance Between Potential Cyanotoxin-Producing Cyanobacteria, Cyanotoxin Synthetase Gene, and Cyanotoxin Occurrence

Concordance between the occurrence of cyanobacteria, genes, and cyanotoxins varied by cyanotoxin type and which set of comparisons were being considered (table 3). When considering samples where cyanobacteria were present, concordance with the gene ranged from 9 to 80 percent (average

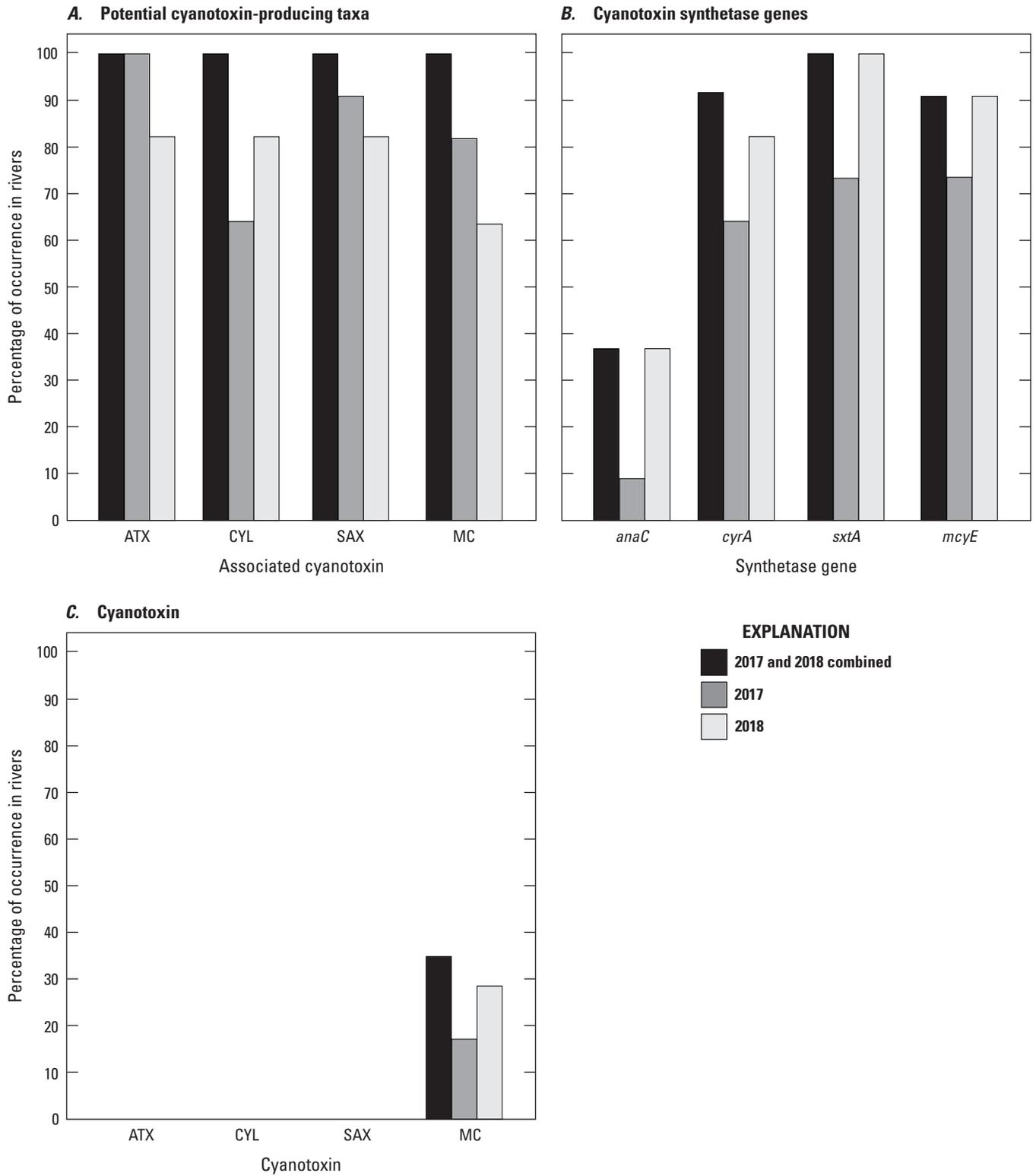


Figure 2. Graphs showing percentage of occurrence of constituents among rivers in 2017 and 2018 and combined. *A*, Potential cyanotoxin-producing taxa; *B*, Cyanotoxin synthetase genes; and *C*, Cyanotoxins. ATX, anatoxin; CYL, cylindrospermopsin; SAX, saxitoxin; MC, microcystin; *anaC*, anatoxin gene; *cyrA*, cylindrospermopsin gene; *sxtA*, saxitoxin gene; *mcyE*, microcystin gene. Synthetase gene assays for anatoxin, cylindrospermopsin, and saxitoxin were general in scope whereas the synthetase gene assays for microcystin were specific for the genera *Planktothrix* and *Microcystis*.

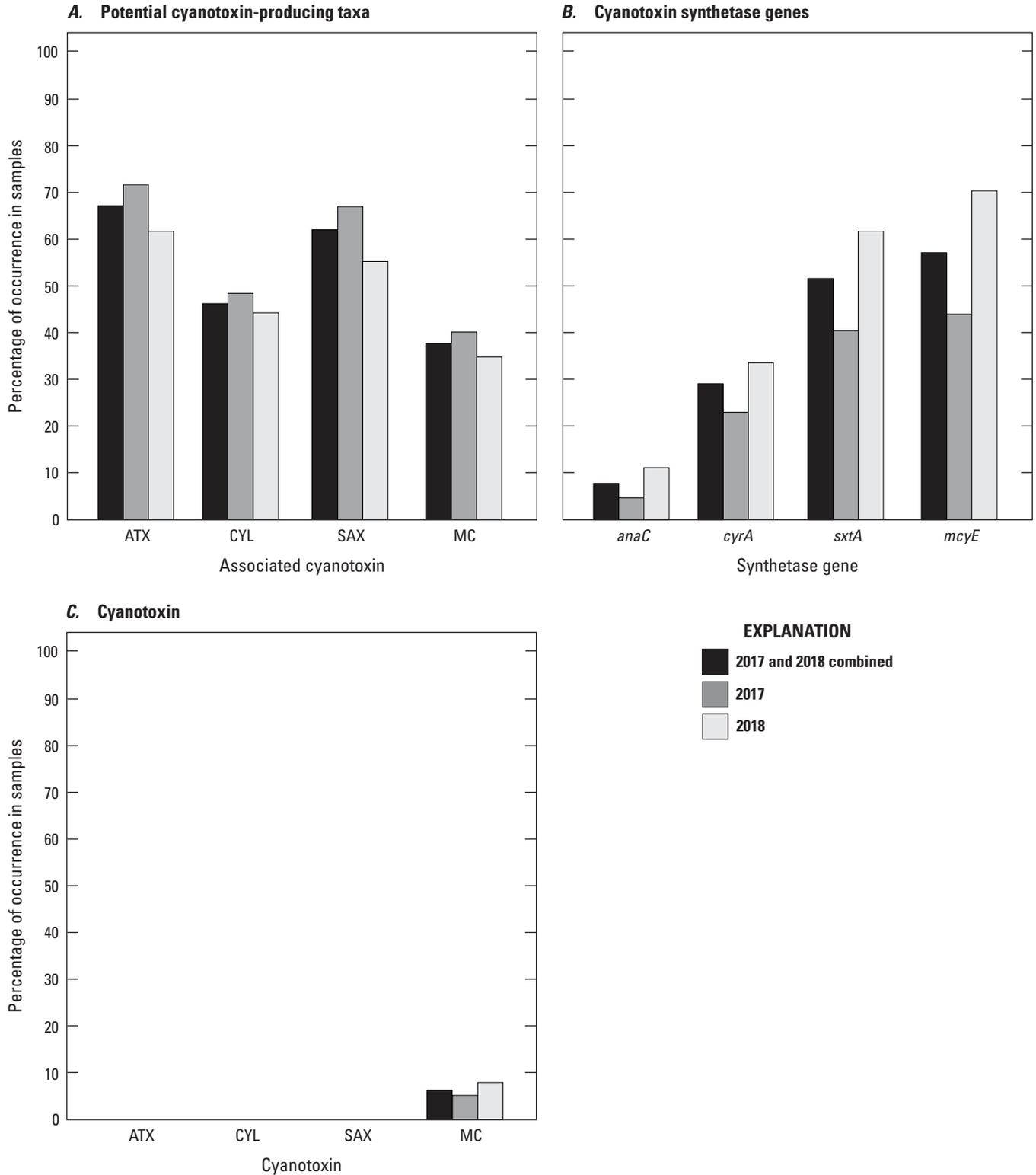


Figure 3. Graphs showing percentage of occurrence of constituents among samples in 2017 and 2018 and combined. *A*, Potential cyanotoxin-producing taxa; *B*, Cyanotoxin synthetase genes; and *C*, Cyanotoxins. Synthetase gene assays for anatoxin, cylindrospermopsin, and saxitoxin were general in scope whereas the synthetase gene assays for microcystin were specific for the genera *Planktothrix* and *Microcystis*. ATX, anatoxin; CYL, cylindrospermopsin; SAX, saxitoxin; MC, microcystin; *anaC*, anatoxin gene; *cyrA*, cylindrospermopsin gene; *sxtA*, saxitoxin gene; *mcyE*, microcystin gene.

Table 3. Percent concordance between the occurrence of potential cyanotoxin-producing taxa, cyanotoxin synthetase genes, and cyanotoxins in samples where taxa were present, cyanotoxin synthetase genes were present, or cyanotoxins were present.

[Cyanotoxin synthetase gene assays for anatoxin, cylindrospermopsin, and saxitoxin were general in scope, whereas the microcystin assays were specific for the genera *Planktothrix* and *Microcystis*. Total number of samples collected was 112. n, number of samples with variable detected; nd, not detected]

Cyanotoxin	Percent concordance when potential cyanotoxin-producing taxa were present			Percent concordance when synthetase genes were present			Percent concordance when cyanotoxins were present		
	n	Genes	Cyanotoxins	n	Taxa	Cyanotoxins	n	Taxa	Genes
Anatoxins	75	9	0	9	78	0	0	nd	nd
Cylindrospermopsins	51	18	0	32	28	0	0	nd	nd
Saxitoxins	68	65	0	57	77	0	0	nd	nd
Microcystins	41	80	12	65	51	12	8	62	100

43 percent). The lowest concordance was observed for anatoxins and the highest for microcystins. Microcystins may have had the highest concordance because the taxa-specificity of the qPCR assays used for analysis allowed a more targeted comparison than the more general assays for the other three cyanotoxin types. Overall, concordance between cyanobacteria and cyanotoxins was low: anatoxins, cylindrospermopsins, and saxitoxins were not detected, and microcystins were only detected in 12 percent of samples with *Microcystis* or *Planktothrix* present.

When considering only samples where genes were present, concordance with cyanobacteria taxa ranged from 28 to 78 percent (average 58 percent; table 3). The lowest concordance was observed for cylindrospermopsins and the highest observed for microcystins. Again, microcystins may have had the highest concordance because of the taxa-specificity of the qPCR assays used for analysis compared to the more general assays used for the other three cyanotoxin types. As observed for cyanobacteria, concordance between genes and cyanotoxins was low. Microcystins were only detected in 12 percent of samples with the *mcyE* gene present. This comparison indicates that genes were often detected without detection of an associated cyanobacteria with known potential for cyanotoxin production. In particular, the *cyrA* gene was detected without associated cyanobacteria 72 percent of the time compared to 22–49 percent of the time for other genes. Genes may be present in the environment without associated cyanobacteria because increased sensitivity of the qPCR assays may have allowed detection of organisms present in low concentrations in the environment that went undetected by microscopy (for example, picoplankton), presence of unrecognized potential producers, or downstream transport of gene fragments (qPCR measures target gene fragments rather than intact, complete genes; Otten and others, 2015; Pacheco and others, 2016; Preece and others, 2017; Graham and others, 2020). Differences between cyanobacteria and gene presence may also be a result of the different sampling approaches used for these analyses (integrated compared to near-surface grabs, respectively). Near-surface grab samples may not be

representative of overall stream conditions if water is not well mixed, or suspended materials, such as cyanobacteria, are not uniformly distributed. In a study on the Kansas River, Graham and others (2012) found that cyanobacterial concentration varied substantially depending on whether near-surface, single integrated vertical, or equal-width integrated samples were collected. The comparability of integrated and near-surface grab samples is therefore dependent on in-stream conditions.

Microcystins were the only cyanotoxin detected in this study and were only present in a small percentage of samples (fig. 3C). When microcystins were detected, 62 percent of samples had cyanobacteria present (*Microcystis* or *Planktothrix*) and 100 percent of samples had the *mcyE* gene present (table 3). High concordance may be because of gene specificity; however, in this study, microcystins were never detected in the environment without the presence of the associated gene.

In general, cyanobacteria occurred more often than genes, and genes occurred more often than cyanotoxins. Between 20 and 91 percent of samples had cyanobacteria present without the associated gene (the genetic potential for cyanotoxin production); and between 88 and 100 percent of samples had genes present without the associated cyanotoxin. These results support previous study findings that indicate cyanotoxins are the best indicator of immediate human health risks, and that cyanobacteria and genes may serve as indicators of the potential for a cyanobacterial community to produce cyanotoxins (Pacheco and others, 2016).

Complete concordance among cyanobacteria, gene, and cyanotoxin occurrence was not expected because cyanobacteria communities are comprised of toxic and nontoxic strains. Occurrence of genes depends on strain composition of a cyanobacterial community, which cannot be determined using microscopy. Similarly, cyanotoxins may not be produced even though genes are present. The presence of genes does, however, provide more detail about the potential for cyanotoxin production within a cyanobacterial community than phytoplankton taxonomy alone, and may be used as an early monitoring tool (Pacheco and others, 2016). For example, potential

anatoxin-producing cyanobacteria were observed in all rivers and were among the most commonly occurring cyanobacteria, but the *anaC* gene was only observed in 36 percent of rivers and occurred less often than other genes (figs. 2 and 3). The *anaC* gene data indicate that the potential for anatoxin occurrence in these rivers is substantially lower than indicated by taxonomic data alone. Graham and others (2020) did, however, detect a low level of anatoxin (0.1 µg/L) by liquid chromatography with tandem mass spectrometry in one sample collected during 2017; the *anaC* gene was also present in that sample. Routinely measuring genes and phytoplankton community composition will provide a more detailed understanding of the relative abundance, spatiotemporal variability, and environmental conditions associated with the occurrence of nontoxin- and toxin-producing strains.

Association Between Biological Response and Selected Environmental Variables

Overall, the strength of significant associations between biological response and environmental variables were relatively weak, never exceeding a Spearman correlation coefficient of 0.66 (fig. 4). Chla was positively associated with TP, TN, and T, and negatively associated with nQ, reflecting the broad relations between these key drivers and phytoplankton biomass observed in lotic ecosystems worldwide (Smith, 2003). Relations between the relative abundance of cyanobacteria and environmental variables were generally similar among potential cyanotoxin-producing groups (fig. 4). All cyanobacteria groups were significantly and positively associated with SSC. Cyanobacteria groups were also all positively associated with nQ and nutrient concentrations, although only some associations were significant (fig. 4). Except for *sxtA*, genes were not significantly correlated with environmental variables. The *sxtA* gene was significantly and positively associated with nQ and SSC (fig. 4). The positive associations of cyanobacteria and the *sxtA* gene with nQ and SSC counter the general expectation that cyanobacteria are less abundant when residence times are short and light availability (as indicated by SSC in this analysis) is low (Søballe and Kimmel, 1987; Reynolds and Descy, 1996; Giblin and Gerrish, 2020). Higher streamflows and elevated SSC concentrations are indicative of potential flushing events; therefore, these positive associations also may reflect the importance of downstream transport on cyanobacterial communities in this study (Knowlton and Jones, 2000).

Descriptive Association Between Cyanobacteria and Streamflow

Whereas the design of this pilot study precludes a rigorous analysis of the influence of downstream transport on cyanobacterial communities in these rivers, a descriptive analysis suggests potential linkages. Four sites were identified where cyanobacteria contributed more than 20 percent to the total phytoplankton concentration (relative cyanobacterial abundance values greater than 20 percent) to determine if there was any anecdotal evidence of corresponding increases in relative abundance with increases in streamflow. The association between cyanobacteria and streamflow was explored by examining plots of relative abundance by sample date over a continuous plot of streamflow values during the same sampling period. Anecdotal association between cyanobacteria and streamflow was noted when relative abundance values increased and aligned with a noticeable rise in streamflow.

Increases in cyanobacteria relative abundance were often observed when sampling dates coincided with an abrupt increase in streamflow (fig. 5A–D). For example, sample dates coincided with abrupt increases in streamflow at 5 of 12 sampling dates at the Chattahoochee River and 3 of 10 at the Kansas River, where relative abundance of cyanobacteria increased compared to samples collected from dates without increases in streamflow (fig. 5A, B). None of the 10 sample dates directly coincided with abrupt changes in streamflow at the Mississippi River site. However, six sampling events took place during a high streamflow period that occurred between June and August in 2018, which were associated with the highest cyanobacteria relative abundance values at that site (fig. 5C). At the Trinity River site, only one sample date coincided with an abrupt increase in streamflow, which was also when the highest cyanobacteria relative abundance value occurred at that site (fig. 5D). These observations suggest the importance of upstream source areas on the occurrence of potential cyanotoxin-producing cyanobacteria in these rivers. Reservoirs, backwaters, and benthic cyanobacteria may all act as upstream source areas of cyanobacteria during changing streamflow conditions. Elucidating upstream source areas and downstream transport of cyanobacteria requires studies specifically designed to address these processes.

Limitations

Study findings demonstrate that cyanobacteria, genes, and cyanotoxins are present in large U.S. rivers under ambient conditions (figs. 2, 3) and illustrate the likely importance downstream transport and flushing has on cyanobacterial communities (fig. 5). Additionally, the results and existing literature generally support the importance of water

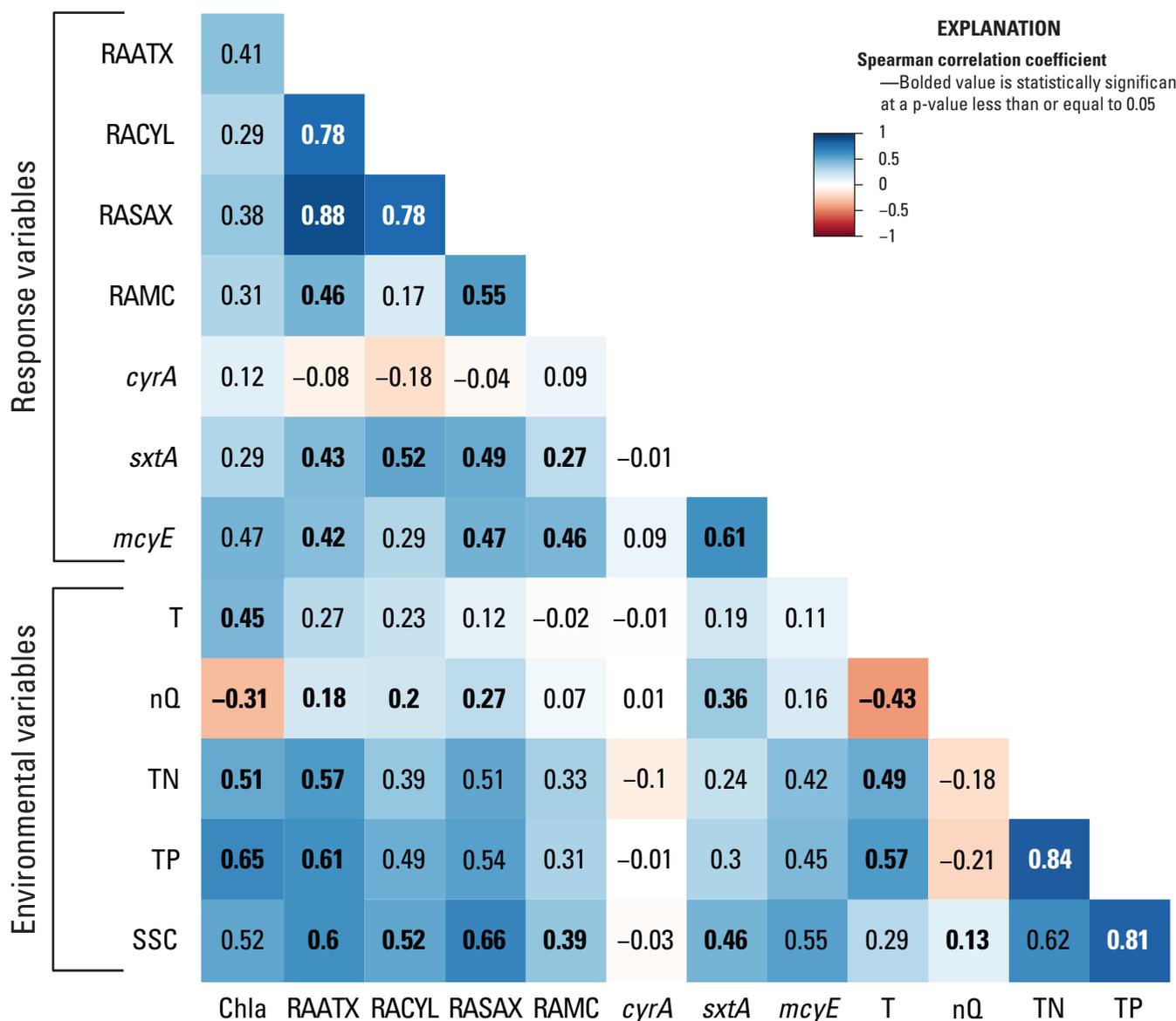


Figure 4. Correlation matrix showing Spearman correlation coefficients between biological response and environmental variables measured at 11 U.S. river sites during June and October of 2017 and 2018. Chla, chlorophyll-*a*; RAATX, relative abundance of potential anatoxin producers; RACYL, relative abundance of potential cylindrospermopsin producers; RASAX, relative abundance of potential saxitoxin producers; RAMC, relative abundance of the potential microcystin producers *Microcystis* and *Planktothrix*; *cyrA*, *cyrA* gene present in the cylindrospermopsin synthetase gene cluster; *sxtA*, *sxtA* gene present in the saxitoxin synthetase gene cluster; *mcyE*, *mcyE* gene present in the microcystin synthetase gene cluster (sum of *Microcystis*- and *Planktothrix*-specific *mcyE* genes); T, water temperature; nQ, streamflow normalized to the 10 year mean daily streamflow for the day of sampling; TN, total nitrogen; TP, total phosphorus; SSC, suspended sediment concentration.

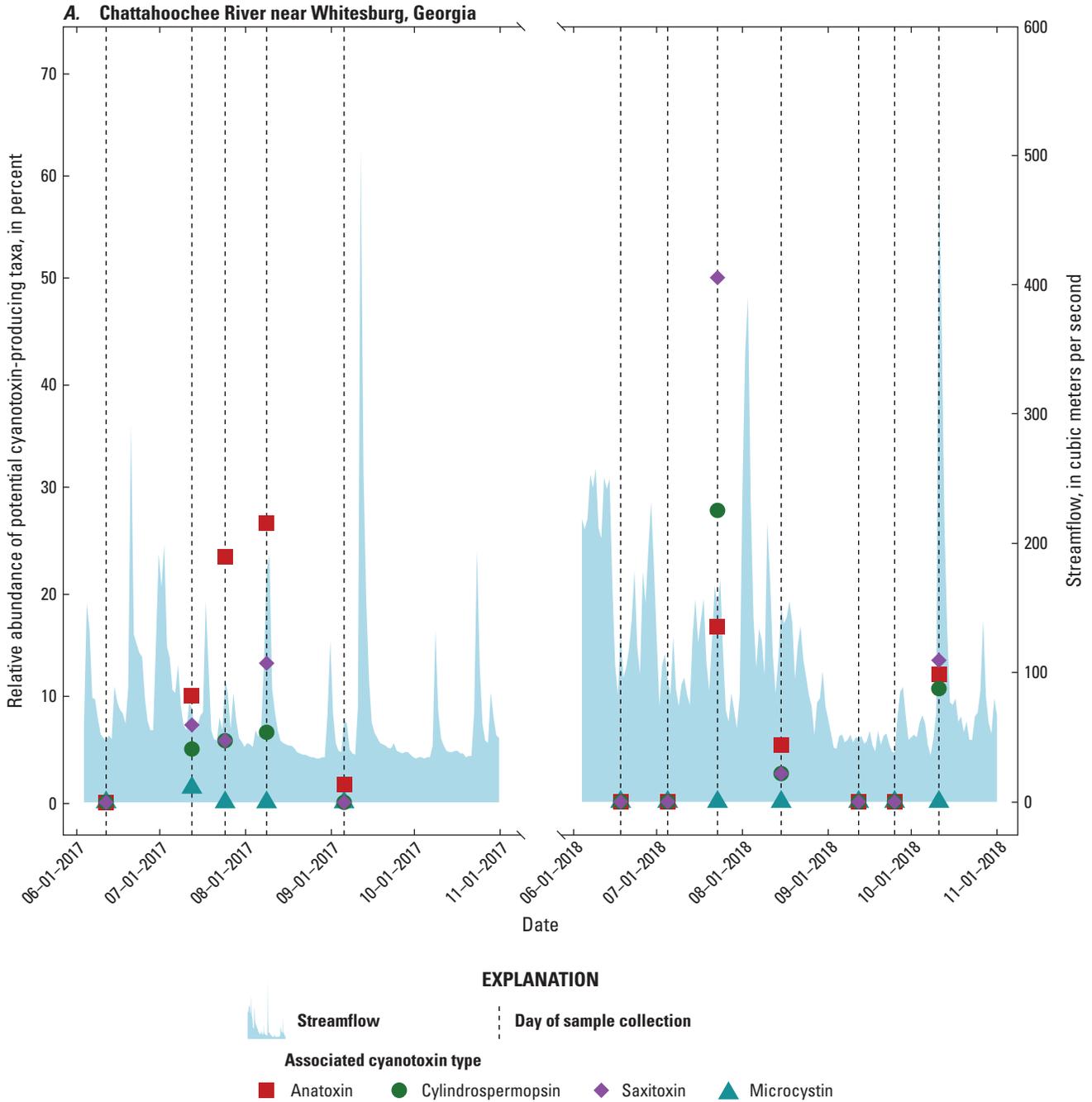


Figure 5. Graphs showing relative abundance of potential cyanotoxin-producing taxa grouped by associated cyanotoxin type and streamflow collected June through September 2017 and June through October 2018. *A*, Chattahoochee River near Whitesburg, Georgia (U.S. Geological Survey station 02338000); *B*, Kansas River at De Soto, Kansas (U.S. Geological Survey station 06892350); *C*, Mississippi River below lock and dam #2 at Hastings, Minnesota (U.S. Geological Survey station 05331580); and *D*, Trinity River below Dallas, Texas (U.S. Geological Survey station 08057410).

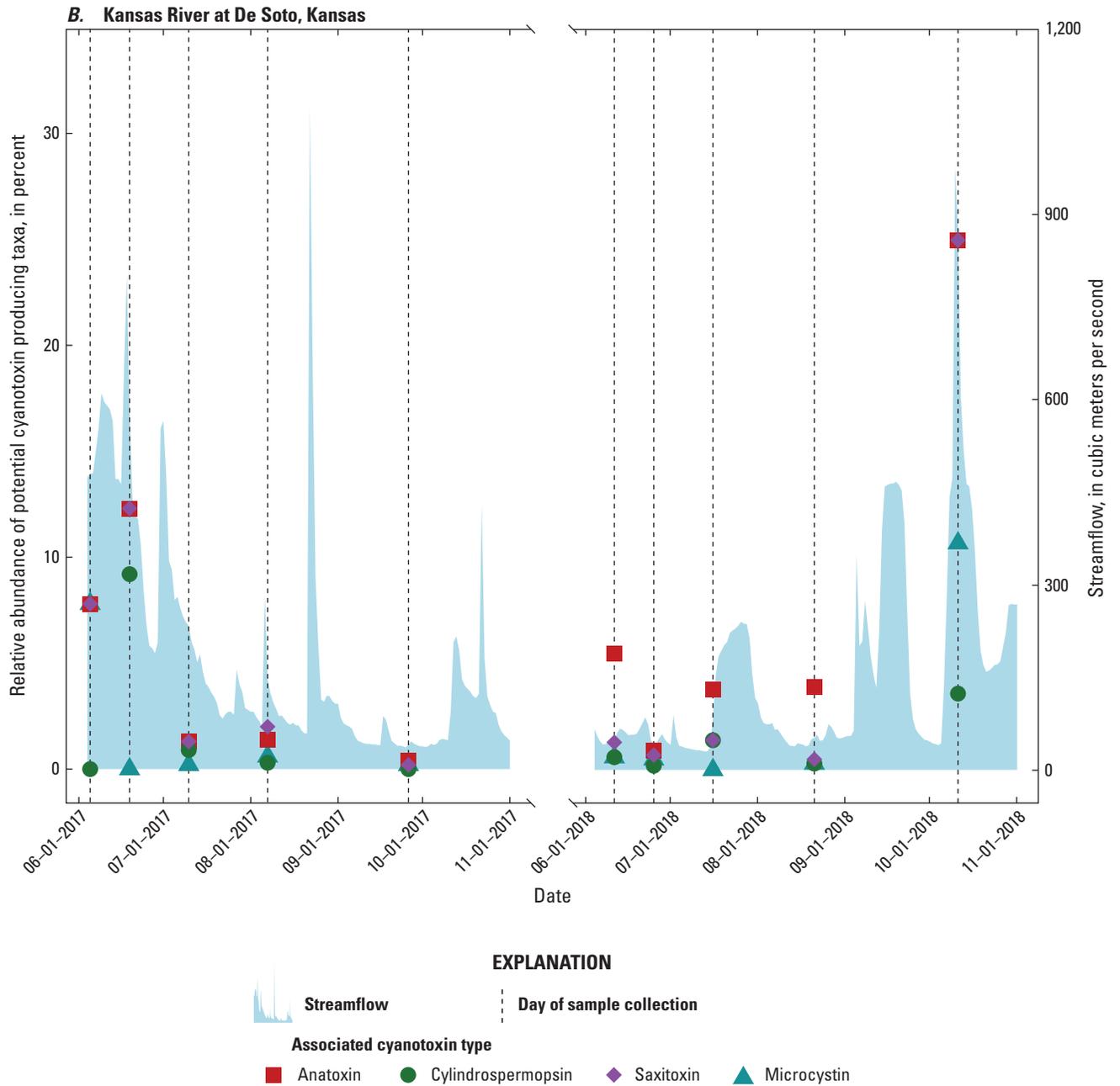


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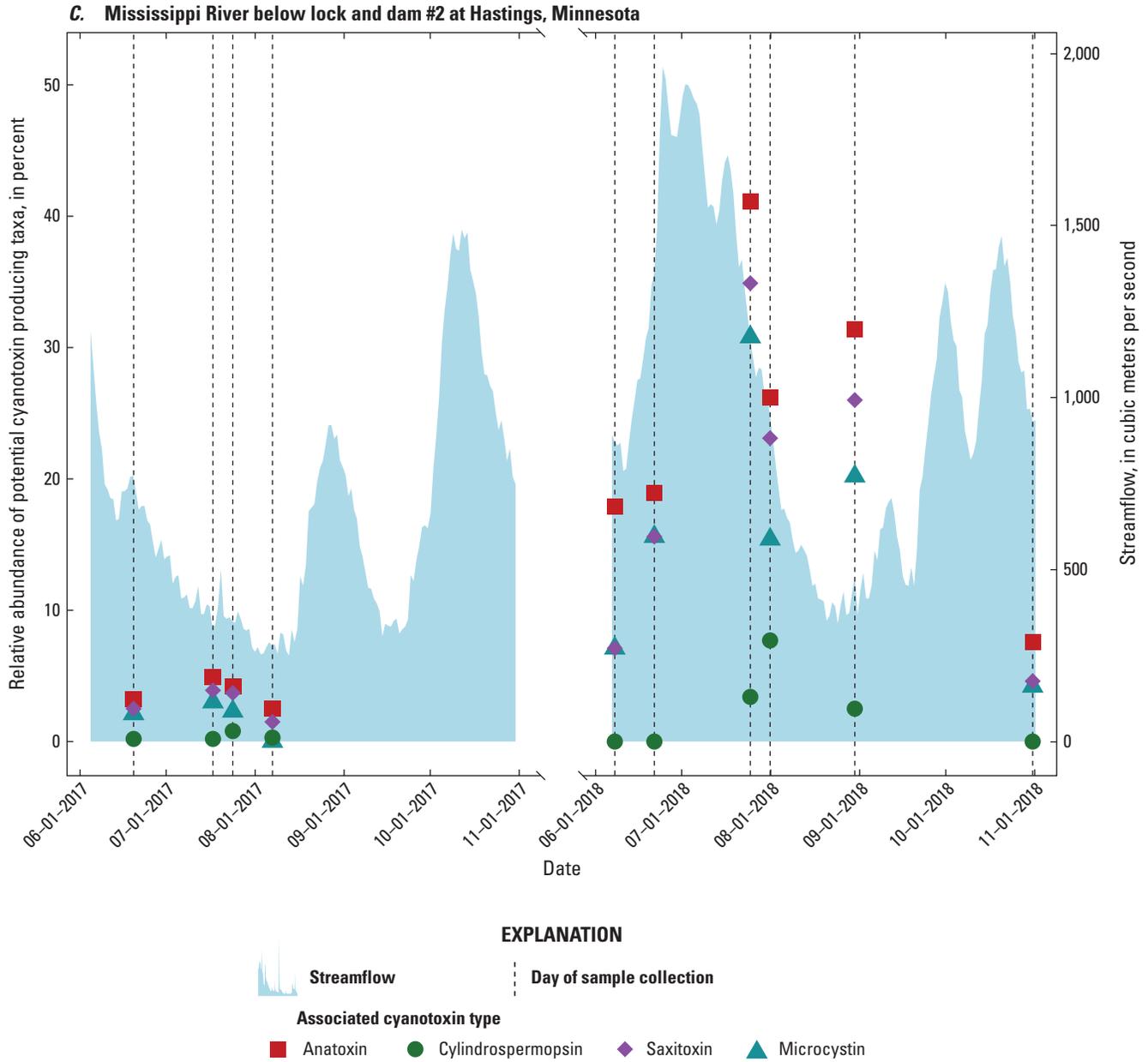


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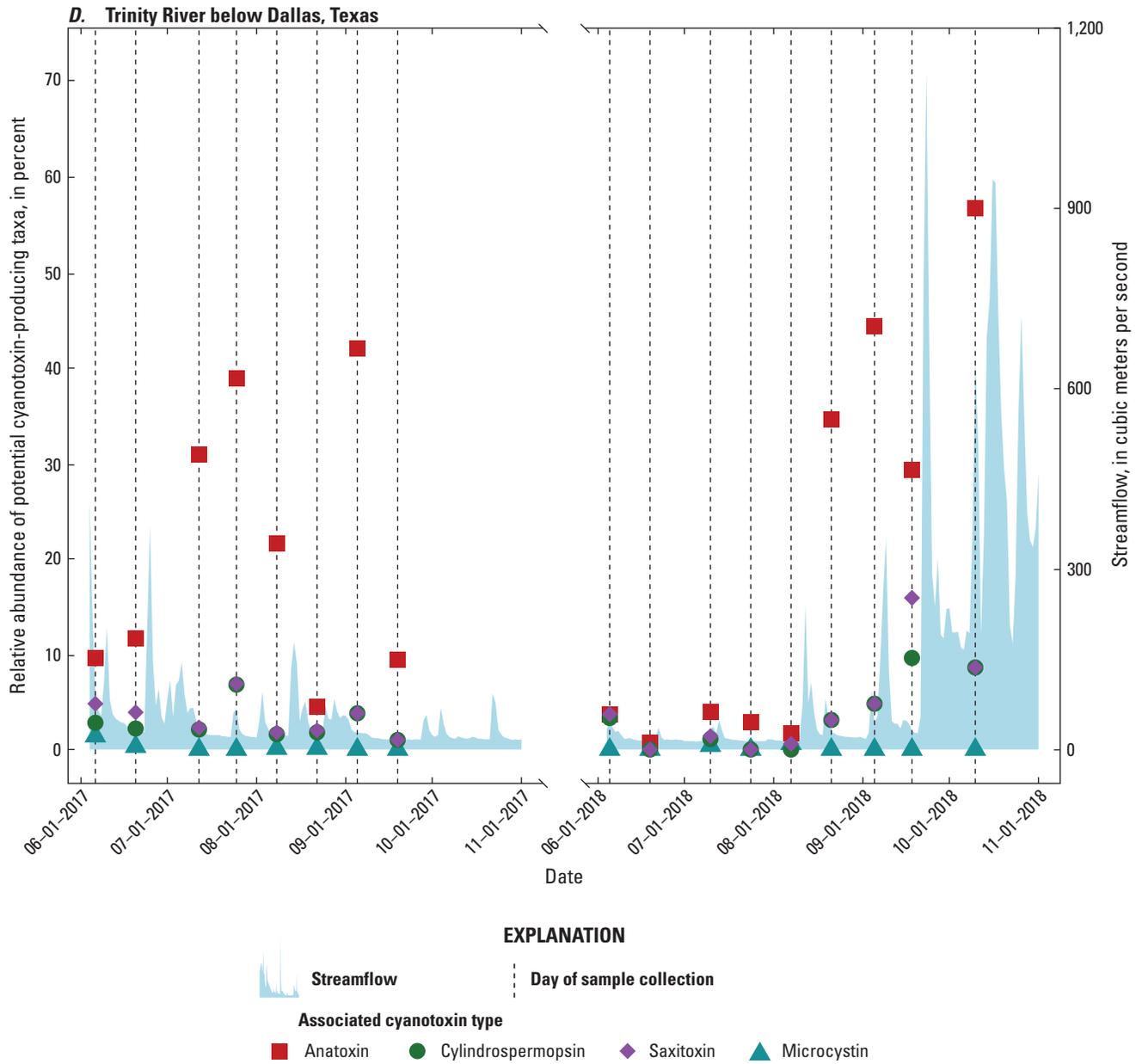


Figure 5. —Continued

temperature, light and nutrients—as moderated by hydrologic conditions—in shaping the structure of riverine cyanobacterial communities (fig. 4). Higher percent occurrence of cyanobacteria, genes, and cyanotoxins among rivers (fig. 2) than among samples (fig. 3) indicated that these constituents can be widespread, but display a high amount of temporal variability. Frequent sample collection is likely required to fully characterize occurrence of these constituents at individual sites. The current study was a pilot-scale effort that leveraged an existing study designed to meet objectives unrelated to cyanobacterial dynamics in large rivers. Therefore, rigorous spatiotemporal, seasonal, and statistical analyses of factors associated with cyanobacterial community dynamics were not tenable. Results from this pilot effort can, however, be used to inform studies designed to address critical research needs. Microcystins are the most commonly reported cyanotoxin class worldwide (Preece and others, 2017) and have been the subject of a substantial body of research. Other cyanotoxins may occasionally be present in lotic systems (Graham and others, 2020), and should also be the subject of focused research efforts given that we do not yet understand general occurrence patterns or know what kind of health risks they may (or may not) pose. Most cyanobacteria studies in large U.S. rivers have been conducted in response to events (Graham and others, 2012; Otten and others, 2015; Rosen and others, 2017) or have captured only ambient conditions (Heiskary and Markus, 2001; Chételat and others, 2006; Giblin and Gerrish, 2020). However, understanding cyanobacterial dynamics and the factors leading to potentially harmful events requires lotic studies designed to capture ambient conditions, periods of cyanobacterial dominance, and events such as extreme cyanobacterial overgrowth. Advances in our knowledge of the influence of upstream physical, chemical, and biological processes on the structure and function of lotic cyanobacterial communities (Reynolds and Descy, 1996; Baker and others, 2016; Cha and others, 2016; Giblin and Gerrish, 2020); downstream fate and transport of cyanobacteria, genes, and cyanotoxins (Graham and others, 2012; Otten and others, 2015; Preece and others 2017); and the role of key environmental influences (for example, T, TP, TN, SSC, and water residence time) at regional and local scales (Smith, 2003; Chételat and others, 2006; Cha and others, 2017; Xia and others, 2020) are required to develop reliable early indicators, mechanistic and empirical models, and mitigation strategies.

Summary

Most cyanobacteria research in the United States has been conducted in lakes and reservoirs, where excess cyanobacterial biomass and cyanotoxins often affect recreational and drinking-water use because of human health concerns. Less is known about cyanobacterial occurrence and dynamics in large river systems. The U.S. Geological Survey National Water Quality Assessment Project collected water quality samples

during 2017–19 at 11 large river sites located throughout the conterminous United States to better understand cyanobacterial occurrence in rivers. The purpose of this report is to describe (1) cyanobacteria, cyanotoxin synthetase gene, and cyanotoxin occurrence during the first 2 years (2017 and 2018) of the pilot study, (2) concordance between cyanobacteria, cyanotoxin synthetase gene, and cyanotoxin occurrence, and (3) relations between cyanobacteria, cyanotoxin synthetase genes, cyanotoxins, and selected environmental variables.

Study sites were selected to represent a range of environmental conditions. All sites were sampled during summer and fall of 2017 and 2018 based on using National Water Quality Assessment sampling schedules and protocols. Water temperature, total nitrogen, total phosphorus, suspended sediment, and chlorophyll *a* samples were collected along with samples to assess phytoplankton community composition, genes present in cyanotoxin synthetase gene clusters (*anaC*, *cyrA*, *mcyE*, and *sxtA*), and cyanotoxins (anatoxins, cylindrospermopsins, microcystins, and saxitoxins).

The current study was a pilot-scale effort that leveraged an existing study designed to meet objectives unrelated to cyanobacterial dynamics in large rivers. Therefore, rigorous spatiotemporal, seasonal, and statistical analyses of factors associated with cyanobacterial community dynamics were not tenable. Nonetheless, results from this pilot effort can be used to inform future studies designed to address cyanobacterial dynamics in large rivers.

Cyanobacterial taxa with the potential to produce cyanotoxins occurred in all rivers. In 2017 and 2018, percent occurrence of potential anatoxin- and saxitoxin-producing taxa in all samples was greater than the percent occurrence of potential cylindrospermopsin- and microcystin-producing taxa. Among rivers, the *anaC* gene occurred about 2.5 times less often (36 percent of rivers) than other measured genes (90–100 percent of rivers). Differences in the percent occurrence of each gene were more pronounced among samples than among rivers. Overall, the *anaC* gene occurred (8 percent of samples) about 3 times less often than the *cyrA* gene (29 percent of samples) and about 7 times less often than the *sxtA* (51 percent of samples) and the *mcyE* gene (58 percent of samples). Microcystins were the only cyanotoxin detected during 2017 and 2018, detected in 36 percent of rivers and 7 percent of samples.

Concordance between the occurrence of cyanobacteria, genes, and cyanotoxins varied by cyanotoxin type and which set of comparisons were being considered. In general, cyanobacteria occurred more often than genes, and genes occurred more often than cyanotoxins. Between 20 and 91 percent of the time, cyanobacteria were present without the gene (genetic potential for cyanotoxin production) and between 88 and 100 percent of the time genes were present without the cyanotoxin.

Chlorophyll *a* was positively associated with nutrients and temperature and negatively associated with streamflow, reflecting the broad relations between these key drivers and phytoplankton biomass observed in lotic ecosystems

worldwide. Relative cyanobacteria abundance was positively associated with nutrients, streamflow, and suspended sediment, although only some associations were significant. The positive associations between cyanobacteria and streamflow and suspended sediment counter the general expectation that cyanobacteria are less abundant when residence times are short and light availability is decreased. However, increases in relative abundance was often associated with an abrupt increase in streamflow. These observations suggest the importance of upstream source areas on the occurrence of cyanobacteria in these rivers. Study findings demonstrate that cyanobacteria, genes, and cyanotoxins are present in large U.S. rivers under ambient conditions and illustrate the likely effect of downstream transport and flushing on relative contribution of cyanobacteria to the overall phytoplankton community. Additionally, the results agree with existing literature that support the importance of water temperature, light and nutrients—as moderated by hydrologic conditions—in shaping the structure of riverine cyanobacterial communities.

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Selected References

- Al-Tebrineh, J., Merrick, C., Ryan, D., Humpage, A., Bowling, L., and Neilan, B., 2012a, Community composition, toxigenicity, and environmental conditions during a cyanobacterial bloom occurring along 1,100 kilometers of the Murray River: *Applied and Environmental Microbiology*, v. 78, no. 1, p. 263–272. [Also available at <https://doi.org/10.1128/AEM.05587-11>.]
- Al-Tebrineh, J., Pearson, L.A., Yasar, S.A., and Neilan, B.A., 2012b, A multiplex qPCR targeting hepato- and neurotoxic cyanobacteria of global significance: *Harmful Algae*, v. 15, p. 19–25. [Also available at <https://doi.org/10.1016/j.hal.2011.11.001>.]
- Arar, E.J., and Collins, G.B., 1997, Method 445.0—*In vitro* determination of chlorophyll *a* and pheophytin *a* in marine and freshwater algae by fluorescence (rev. 1.2): U.S. Environmental Protection Agency, 22 p. [Also available at https://cfpub.epa.gov/si/si_public_file_download.cfm?p_download_id=525244&Lab=NERL.]
- Baker, M.A., Arp, C.D., Goodman, K.J., Marcarelli, A.M., and Wurtsbaugh, W.A., 2016, Stream-lake interaction—Understanding coupled hydro-ecological systems, chap. 7 of Jones, J.B., and Stanley, E.H., eds., *Stream ecosystems in a changing environment*: Amsterdam, Academic Press, p. 321–348. [Also available at <https://doi.org/10.1016/B978-0-12-405890-3.00007-5>.]
- Beaver, J.R., Manis, E.E., Loftin, K.S., Graham, J.L., Pollard, A.I., and Mitchell, R.M., 2014, Land use patterns, ecoregion, and microcystin relationships in U.S. lakes and reservoirs—A preliminary evaluation: *Harmful Algae*, v. 36, p. 57–62. [Also available at <https://doi.org/10.1016/j.hal.2014.03.005>.]
- Bernard, C., Ballot, A., Thomazeau, S., Maloufi, S., Furey, A., Mankiewicz-Boczek, J., Pawlik-Skowrońska, B., Capelli, C., and Salmaso, N., 2017, Cyanobacteria associated with the production of cyanotoxins, app. 2 of Meriluoto, J., Spoof, L., and Codd, G.A., eds., *Handbook of cyanobacterial monitoring and cyanotoxin analysis*: West Sussex, United Kingdom, John Wiley & Sons, Ltd, p. 501–525. [Also available at <https://doi.org/10.1002/9781119068761.app2>.]
- Bouma-Gregson, K., Olm, M.R., Probst, A.J., Anantharaman, K., Power, M.E., and Banfield, J.F., 2019, Impacts of microbial assemblage and environmental conditions on the distribution of anatoxin-a producing cyanobacteria within a river network: *The ISME Journal*, v. 13, no. 6, p. 1618–1634. [Also available at <https://doi.org/10.1038/s41396-019-0374-3>.]

- Brooks, B.W., Lazorchak, J.M., Howard, M.D.A., Johnson, M.-V.V., Morton, S.L., Perkins, D.A.K., Reavie, E.D., Scott, G.I., Smith, S.A., and Steevens, J.A., 2016, Are harmful algal blooms becoming the greatest inland water quality threat to public health and aquatic ecosystems?: *Environmental Toxicology and Chemistry*, v. 35, no. 1, p. 6–13. [Also available at <https://doi.org/10.1002/etc.3220>.]
- Cha, Y.K., Soon Park, S., Won Lee, H., and Stow, C.A., 2016, A Bayesian hierarchical approach to model seasonal algal variability along an upstream to downstream river gradient: *Water Resources Research*, v. 52, no. 1, p. 348–357. [Also available at <https://doi.org/10.1002/2015WR017327>.]
- Cha, Y.K., Cho, K.H., Lee, H., Kang, T., and Kim, J.A., 2017, The relative importance of water temperature and residence time in predicting cyanobacteria abundance in regulated rivers: *Water Research*, v. 124, p. 11–19. [Also available at <https://doi.org/10.1016/j.watres.2017.07.040>.]
- Chételat, J., Pick, F.R., and Hamilton, P.B., 2006, Potomoplankton size structure and taxonomic composition—Influence of river size and nutrient concentrations: *Limnology and Oceanography*, v. 51, no. 1, part 2, p. 681–689. [Also available at https://doi.org/10.4319/lo.2006.51.1_part_2.0681.]
- Fetscher, A.E., Howard, M.D.A., Stancheva, R., Kudela, R.M., Stein, E.D., Sutula, M.A., Busse, L.B., and Sheath, R.G., 2015, Wadeable streams as widespread sources of benthic cyanotoxins in California, USA: *Harmful Algae*, v. 49, p. 105–116. [Also available at <https://doi.org/10.1016/j.hal.2015.09.002>.]
- Foss, A.J., Butt, J., Fuller, S., Cieslik, K., Aubel, M.T., and Wertz, T., 2017, Nodularin from benthic freshwater periphyton and implications for trophic transfer: *Toxicon*, v. 140, p. 45–59. [Also available at <https://doi.org/10.1016/j.toxicon.2017.10.023>.]
- Francy, D.S., Graham, J.L., Stelzer, E.A., Ecker, C.D., Brady, A.M.G., Struffolino, P., and Loftin, K.A., 2015, Water quality, cyanobacteria, and environmental factors and their relations to microcystin concentrations for use in predictive models at Ohio Lake Erie and inland lake recreational sites, 2013–14: U.S. Geological Survey Scientific Investigations Report 2015–5120, 58 p. [Also available at <https://doi.org/10.3133/sir20155120>.]
- Giblin, S.M., and Gerrish, G.A., 2020, Environmental factors controlling phytoplankton dynamics in a large floodplain river with emphasis on cyanobacteria: *River Research and Applications*, v. 36, no. 7, p. 1137–1150, accessed August 22, 2020, at <https://doi.org/10.1002/rra.3658>.
- Graham, J.L., Dubrovsky, N.M., Foster, G.M., King, L.R., Loftin, K.A., Rosen, B.H., and Stelzer, E.A., 2020, Cyanotoxin occurrence in large rivers of the United States: *Inland Waters*, v. 10, no. 1, p. 109–117. [Also available at <https://doi.org/10.1080/20442041.2019.1700749>.]
- Graham, J.L., Jones, J.R., Jones, S.B., Downing, J.A., and Clevenger, T.E., 2004, Environmental factors influencing microcystin distribution and concentration in the Midwestern United States: *Water Research*, v. 38, no. 20, p. 4395–4404. [Also available at <https://doi.org/10.1016/j.watres.2004.08.004>.]
- Graham, J.L., Loftin, K.A., Meyer, M.T., and Ziegler, A.C., 2010, Cyanotoxin mixtures and taste-and-odor compounds in cyanobacterial blooms from the Midwestern United States: *Environmental Science & Technology*, v. 44, no. 19, p. 7361–7368. [Also available at <https://doi.org/10.1021/es1008938>.]
- Graham, J.L., Loftin, K.A., Ziegler, A.C., and Meyer, M.T., 2008, Cyanobacteria in lakes and reservoirs—Toxin and taste-and-odor sampling guidelines: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A7.5, 65 p. [Also available at <https://doi.org/10.3133/twri09A7.5>.]
- Graham, J.L., Rosen, B.H., Loftin, K.A., and Stelzer, E.A., 2021a, Cyanotoxin, chlorophyll-a, and cyanobacterial toxin genetic data for samples collected at eleven large river sites throughout the United States, June through October 2018: U.S. Geological Survey data release, <https://doi.org/10.5066/P98RPC1E>.
- Graham, J.L., Rosen, B.H., Loftin, K.A., and Stelzer, E.A., 2021b, Phytoplankton data for samples collected at eleven large river sites throughout the United States, June through October 2018: U.S. Geological Survey data release, <https://doi.org/10.5066/P9N4Q9HG>.
- Graham, J.L., Ziegler, A.C., Loving, B.L., and Loftin, K.A., 2012, Fate and transport of cyanobacteria and associated toxins and taste-and-odor compounds from upstream reservoir releases in the Kansas River, Kansas, September and October 2011: U.S. Geological Survey Scientific Investigations Report 2012–5129, 65 p. [Also available at <https://doi.org/10.3133/sir20125129>.]
- Harris, K.A., Thacker, C., Ballard, D., and Syndercombe Court, D., 2006, The effect of cleaning agents on the DNA analysis of blood stains deposited on different substrates: *International Congress Series* v. 1288, p. 589–591. [Also available at <https://doi.org/10.1016/j.ics.2005.09.171>.]

- Heiskary, S., and Markus, H., 2001, Establishing relationships among nutrient concentrations, phytoplankton abundance, and biochemical oxygen demand in Minnesota, USA, rivers: *Lake and Reservoir Management*, v. 17, no. 4, p. 251–262. [Also available at <https://doi.org/10.1080/07438140109354134>.]
- Helsel, D.R., Hirsch, R.M., Ryberg, K.R., Archfield, S.A., and Gilroy, E.J., 2020, Statistical methods in water resources: U.S. Geological Survey Techniques and Methods, book 4, chap. A3, 458 p. [Also available at <https://doi.org/10.3133/tm4A3>. Supersedes U.S. Geological Survey Techniques of Water-Resources Investigations, book 4, chap. A3, version 1.1.]
- King, L.R., Rosen, B.H., Graham, J.L., Dubrovsky, N.M., Foster, G.M., Loftin, K.A., and Stelzer, E.A., 2020a, Cyanotoxin, chlorophyll-a, and cyanobacterial toxin genetic data for samples collected at eleven large river sites throughout the United States, June through September 2017: U.S. Geological Survey data release, <https://doi.org/10.5066/P9TID1VX>.
- King, L.R., Rosen, B.H., Graham, J.L., Dubrovsky, N.M., Foster, G.M., Loftin, K.A., and Stelzer, E.A., 2020b, Phytoplankton data for samples collected at eleven large river sites throughout the United States, June through September 2017: U.S. Geological Survey data release, <https://doi.org/10.5066/P9EYP85Z>.
- King, L.R., Rosen, B.H., Graham, J.L., Dubrovsky, N.M., Foster, G.M., Loftin, K.A., and Stelzer, E.A., 2020c, Phytoplankton tally sheet, including photomicrographs, for samples collected at eleven large river sites throughout the United States, June through September 2017: U.S. Geological Survey data release, <https://doi.org/10.5066/P9KKN921>.
- Knowlton, M.F., and Jones, J.R., 2000, Seston, light, nutrients, and chlorophyll in the lower Missouri River, 1994–1998: *Journal of Freshwater Ecology*, v. 15, no. 3, p. 283–297. [Also available at <https://doi.org/10.1080/02705060.2000.9663747>.]
- Lee, C.J., and Henderson, R.J., 2020, Tracking water-quality in U.S. streams and rivers: U.S. Geological Survey National Water Quality Network website, accessed August 18, 2020, at <https://nrtwq.usgs.gov/nwqn>.
- Loftin, K.A., Clark, J.M., Journey, C.A., Kolpin, D.W., Van Metre, P.C., Carlisle, D., and Bradley, P.M., 2016b, Spatial and temporal variation in microcystin occurrence in Wadeable streams in the southeastern United States: *Environmental Toxicology and Chemistry*, v. 35, no. 9, p. 2281–2287. [Also available at <https://doi.org/10.1002/etc.3391>.]
- Loftin, K.A., Graham, J.L., Hilborn, E.D., Lehmann, S.C., Meyer, M.T., Dietze, J.A., and Griffith, C.B., 2016a, Cyanotoxins in inland lakes of the United States—Occurrence and potential recreational health risks in the EPA National Lakes Assessment 2007: *Harmful Algae*, v. 56, p. 77–90. [Also available at <https://doi.org/10.1016/j.hal.2016.04.001>.]
- Loftin, K.A., Meyer, M.T., Rubio, F., Kamp, L., Humphries, E., and Whereat, E., 2008, Comparison of two cell lysis procedures for recovery of microcystins in water samples from Silver Lake in Dover, Delaware, with microcystin producing cyanobacterial accumulations: U.S. Geological Survey Open-File Report 2008–1341, 9 p. [Also available at <https://doi.org/10.3133/ofr20081341>.]
- Matson, P.G., Boyer, G.L., Bridgeman, T.B., Bullerjahn, G.S., Kane, D.D., McKay, R.M.L., McKindles, K.M., Raymond, H.A., Snyder, B.K., Stumpf, R.P., and Davis, T.W., 2020, Physical drivers facilitating a toxigenic cyanobacterial bloom in a major Great Lakes tributary: *Limnology and Oceanography*, v. 65, no. 12, p. 2866–2882, accessed August 20, 2020, at <https://doi.org/10.1002/lno.11558>.
- Ohio River Valley Sanitation Commission [ORSANCO], 2020, Harmful algae blooms: Ohio River Valley Water Sanitation Commission web page, accessed June 22, 2020, at <http://www.orsanco.org/data/harmful-algae-bloom-data>.
- Otten, T.G., Crosswell, J.R., Mackey, S., and Dreher, T.W., 2015, Application of molecular tools for microbial source tracking and public health risk assessment of a *Microcystis* bloom traversing 300 km of the Klamath River: *Harmful Algae*, v. 46, p. 71–81. [Also available at <https://doi.org/10.1016/j.hal.2015.05.007>.]
- Pacheco, A.B.F., Guedes, I.A., and Azevedo, S.M.F.O., 2016, Is qPCR a reliable indicator of cyanotoxin risk in freshwater?: *Toxins*, v. 8, no. 6, article 172, 26 p. [Also available at <https://doi.org/10.3390/toxins8060172>.]
- Preece, E.P., Hardy, F.J., Moore, B.C., and Bryan, M., 2017, A review of microcystin detections estuarine and marine waters—Environmental implications and human health risk: *Harmful Algae*, v. 61, p. 31–45. [Also available at <https://doi.org/10.1016/j.hal.2016.11.006>.]
- Price, C.V., and Maupin, M.A., 2014, Documentation for the U.S. Geological Survey Public-Supply Database (PSDB)—A database of permitted public-supply wells, surface-water intakes, and systems in the United States: U.S. Geological Survey Open-File Report 2014–1212, 22 p., accessed June 15th, 2021, at <https://doi.org/10.3133/ofr20141212>.

- Rantala, A., Rajaniemi-Wacklin, P., Lyra, C., Lepistö, L., Rintala, J., Mankiewicz-Boczek, J., and Sivonen, K., 2006, Detection of microcystin-producing cyanobacteria in Finnish lakes with genus-specific microcystin synthetase gene E (*mcyE*) PCR and associations with environmental factors: *Applied and Environmental Microbiology*, v. 72, no. 9, p. 6101–6110. [Also available at <https://doi.org/10.1128/AEM.01058-06>.]
- Reynolds, C.S., and Descy, J.P., 1996, The production, biomass and structure of phytoplankton in large rivers: *Archiv für Hydrobiologie [renamed Fundamental and Applied Limnology]*, supplemental v. 113, no. 1–4, [Large Rivers v. 10, no. 1–4], p. 161–187.
- Rosen, B.H., Davis, T.W., Gobler, C.J., Kramer, B.J., and Loftin, K.A., 2017, Cyanobacteria of the 2016 Lake Okeechobee and Okeechobee Waterway harmful algal bloom: U.S. Geological Survey Open-File Report 2017–1054, 34 p. [Also available at <https://doi.org/10.3133/ofr20171054>.]
- Rosen, B.H., Loftin, K.A., Graham, J.L., Stahlhut, K.N., Riley, J.M., Johnston, B.D., and Senegal, S., 2018, Understanding the effect of salinity tolerance on cyanobacteria associated with a harmful algal bloom in Lake Okeechobee, Florida: U.S. Geological Survey Scientific Investigations Report 2018–5092, 32 p. [Also available at <https://doi.org/10.3133/sir20185092>.]
- Sabart, M., Crenn, K., Perrière, F., Abila, A., Lereboure, M., Colombet, J., Jousse, C., and Latour, D., 2015, Co-occurrence of microcystin and anatoxin-a in the freshwater lake Aydat (France)—Analytical and molecular approaches during a three-year survey: *Harmful Algae*, v. 48, p. 12–20. [Also available at <https://doi.org/10.1016/j.hal.2015.06.007>.]
- Sipari, H., Rantala-Ylinen, A., Jokela, J., Oksanen, I., and Sivonen, K., 2010, Development of a chip assay and quantitative PCR for detecting microcystin synthetase E gene expression: *Applied and Environmental Microbiology*, v. 76, no. 12, p. 3797–3805. [Also available at <https://doi.org/10.1128/AEM.00452-10>.]
- Smith, V.H., 2003, Eutrophication of freshwater and coastal marine ecosystems—A global problem: *Environmental Science and Pollution Research International*, v. 10, no. 2, p. 126–139. [Also available at <https://doi.org/10.1065/espr2002.12.142>.]
- Søballe, D.M., and Kimmel, B.L., 1987, A large-scale comparison of factors influencing phytoplankton abundance in rivers, lakes, and impoundments: *Ecology*, v. 68, no. 6, p. 1943–1954. [Also available at <https://doi.org/10.2307/1939885>.]
- Stelzer, E.A., Loftin, K.A., and Struffolino, P., 2013, Relations between DNA- and RNA-based molecular methods for cyanobacteria and microcystin concentration at Maumee Bay State Park Lakeside Beach, Oregon, Ohio, 2012: U.S. Geological Survey Scientific Investigations Report 2013–5189, 9 p. [Also available at <https://doi.org/10.3133/sir20135189>.]
- U.S. Environmental Protection Agency, [EPA], 2016, National wetland condition assessment 2011—A collaborative survey of the Nation’s wetlands: U.S. Environmental Protection Agency, EPA-843-R-15-005, 105 p. [Also available at https://www.epa.gov/sites/production/files/2016-05/documents/nwca_2011_public_report_20160510.pdf.]
- U.S. Geological Survey, 2021, USGS water data for the Nation: U.S. Geological Survey National Water Information System database, accessed June 16, 2021, at <https://doi.org/10.5066/F7P55KJN>.
- Van Nieuwenhuysse, E.E., and Jones, J.R., 1996, Phosphorus-chlorophyll relationship in temperate streams and its variation with stream catchment area: *Canadian Journal of Fisheries and Aquatic Sciences*, v. 53, no. 1, p. 99–105. [Also available at <https://doi.org/10.1139/f95-166>.]
- Wieczorek, M.E., Jackson, S.E., and Schwarz, G.E., 2018, Select attributes for NHDPlus version 2.1 reach catchments and modified network routed upstream watersheds for the conterminous United States: U.S. Geological Survey data release, accessed August 13, 2019, at <https://doi.org/10.5066/F7765D7V>.
- Xia, R., Wang, G., Zhang, Y., Yang, P., Yang, Z., Ding, S., Jia, X., Yang, C., Liu, C., Ma, S., Lin, J., Wang, X., Hou, X., Zhang, K., Gao, X., Duan, P., and Qian, C., 2020, River algal blooms are well predicted by antecedent environmental conditions: *Water Research*, v. 185, article 116221, 20 p. [Also available at <https://doi.org/10.1016/j.watres.2020.116221>.]
- Zar, J.H., 1999, *Biostatistical analysis* 4th ed.: New Jersey, Prentice-Hall Inc., 663 p.

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