Cyanotoxin occurrence in large rivers of the United States

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ABSTRACT
Cyanotoxins occur in rivers worldwide but are understudied in lotic ecosystems relative to lakes and reservoirs. We sampled 11 large river sites located throughout the United States during June–September 2017 to determine the occurrence of cyanobacteria with known cyanotoxin-producing strains, cyanotoxin synthetase genes, and cyanotoxins. Chlorophyll a concentrations ranged from oligotrophic to eutrophic (0.5–64.4 µg L⁻¹). Cyanobacteria were present in the algal communities of all rivers (82% of samples, n = 50) but rarely dominated the phytoplankton (0–52% of total abundance; mean = 8.8%). Pseudanabaena and Planktothrix occurred most often, and many (64%) of the cyanobacterial genera identified (n = 25) have known cyanotoxin-producing strains. Cyanotoxin synthetase genes occurred in all but one river. The mcyE and sxtA genes were most common, present in 73% of rivers and 44% and 40% of samples, respectively. The cyaA gene was less common (22% of samples) but occurred in 64% of rivers. The anaC gene was detected in one river (4% of samples). Anatoxin-a and microcystins were detected at low levels (0.10–0.38 µg L⁻¹) in 2 midcontinent rivers. Cylindrospermopsins and saxitoxins were not detected. Cyanobacteria, cyanotoxin synthetase genes, and cyanotoxins were present at low concentrations throughout this subset of US rivers. Eutrophic rivers located in the midcontinent region of the United States had the highest algal biomass, abundance of cyanotoxin synthetase genes, and cyanotoxin occurrence.

Introduction
Cyanobacteria, and the cyanotoxins they produce, are a global concern because of potential harm to human, ecological, and economic health. A perceived increase in the frequency and severity of cyanobacteria-related events has occurred in recent decades, and cyanobacteria are expected to pose challenges to inland water quality well into the future (Brooks et al. 2016). Cyanobacteria dominate phytoplankton communities more often in the quiescent waters of lentic environments than in lotic environments where flow-driven processes favor smaller, more rapidly growing algae (Reynolds and Descy 1996, Chételat et al. 2006). A global analysis by Smith (2003), however, showed that nutrient enrichment has led to increased cyanobacterial dominance in both lentic and lotic ecosystems.

Rivers are inherently connected to the physical, chemical, and biological processes of upstream aquatic ecosystems (Baker et al. 2016). Such interconnectivity results in lotic algal communities that are a mix of organisms derived within a site and from upstream benthic and planktonic sources (Reynolds and Descy 1996). Nutrients, as moderated by hydrologic conditions such as flow and flushing rate, also influence algal biomass and the structure of algal communities in lotic environments (Van Nieuwenhuyse and Jones 1996, Heiskary and Markus 2001, Chételat et al. 2006). Cyanobacteria and associated cyanotoxins in lotic environments may therefore develop within river reaches during favorable conditions (Al-Tebrineh et al. 2012a, Cha et al. 2017) or be transported downstream from upstream source areas (Preece et al. 2017).

Cyanotoxins associated with benthic and planktonic cyanobacteria occur in lotic ecosystems worldwide (de la Cruz et al. 2013, Preece et al. 2017, Aguilera et al. 2018) but most studies to date have focused on lentic ecosystems (Brooks et al. 2016). Cyanotoxins may accumulate in riparian food webs, even when cyanobacteria represent a
relatively small proportion of overall algal biomass (Moy et al. 2016), and cause harm hundreds of kilometers downstream from original source areas (Graham et al. 2012, Otten et al. 2015). Concerns such as these underscore the need for cyanotoxin research in lotic environments where cyanobacteria may not typically dominate the algal community. Because of the interconnectivity of lentic and lotic ecosystems, we hypothesized that cyanobacteria would be nearly ubiquitous in large rivers of the United States and occur more frequently than cyanotoxins, as observed in US lakes (Loftin et al. 2016b). We also hypothesized that cyanotoxin synthetase genes would occur more often than cyanotoxins but less often than cyanobacteria. To test these hypotheses, we measured cyanobacteria, cyanotoxin synthetase genes, and cyanotoxins at 11 large river sites throughout the contiguous United States during June–September 2017.

Study sites

All 11 study sites are large inland or coastal rivers routinely sampled as part of the United States Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Project and equipped with streamflow gauging stations (Deacon et al. 2015). These sites were selected to include a diverse range of drainage areas, streamflow conditions, and trophic states throughout the United States (Fig. 1, Table 1). All rivers have numerous dams within their watersheds and 4 have mainstem dams located within 5 km upstream from the study site (Table 1). For this analysis, midcontinent rivers were defined as those located west of the Mississippi River and east of the Rocky Mountains.

Methods

All rivers were sampled multiple times (n = 2–8) during June–September 2017 following NAWQA sample schedules and protocols (Deacon et al. 2015). Isokinetic sampling techniques that provide samples representative of stream conditions were used. In addition to the nutrient (total nitrogen and phosphorus) and suspended sediment samples regularly collected by NAWQA at these sites, we collected samples for the analysis of chlorophyll a (Chl-a), phytoplankton community composition, genes present in cyanotoxin synthetase gene clusters, and cyanotoxins. Samples for all analyses were processed from a single composite sample except cyanotoxin synthetase genes, which were collected as near-surface grabs at the centroid of flow. Chl-a (minimum reporting level [MRL]: 0.10 µg L$^{-1}$) was analyzed using US Environmental Protection Agency method 445.0 (Arar and Collins 1997). The volume of water filtered for Chl-a analysis depended on the amount of suspended material in the sample, between 56 and 250 mL in these samples. Phytoplankton samples were collected in 500 mL high-density polyethylene (HDPE) bottles and preserved with acidified Lugol’s iodine. Phytoplankton were concentrated by settling and enumerated

Figure 1. Location of rivers sampled and occurrence of cyanotoxins and cyanotoxin synthetase genes during June–September 2017.
by natural unit (cell, colony, or filament) to the lowest possible taxonomic level. Ideally, a minimum of 400 natural units were counted per sample (Rosen et al. 2018). Live samples were incubated in indirect sunlight for 8 weeks to help identify challenging taxa using features more readily observed in living organisms. Incubation also served as a qualitative indicator of the presence of taxa not abundant enough to be detected and enumerated using the standard microscopy technique.

Samples for genetic analyses were collected in autoclaved and bleached 500 mL polypropylene bottles. Genes present in anatoxin, cylindrospermopsin, microcystin, and saxitoxin synthetase gene clusters were analyzed by quantitative polymerase chain reaction (qPCR). Molecular assays targeted the anacC (anatoxin; Sabart et al. 2015; cyrA (cylindrospermopsin; Al-Tebrieh et al. 2012b); Dolichospermum-, Microcystis-, and Planktothrix-specific mcyE (microcystin; Rantala et al. 2006, Sipari et al. 2010); and sxtA (saxitoxin; Al-Tebrieh et al. 2012b) genes. Primer and probe information, as well as run conditions, can be found in each of the above cited references. Details of sample concentration, extraction, and qPCR methods are described in Stelzer et al. (2013). Plasmid standards for each assay were used to establish standard curves for quantification.

The copy number of each plasmid standard was calculated using the DNA concentration measured by the Qubit dsDNA High Sensitivity Assay (Life Technologies, Carlsbad, CA, USA) and the molecular weight of the plasmid. Standard curve information for this study and assay detection limits are listed in King et al. (2020a).

Cyanotoxin samples were collected in 250 mL HDPE bottles triple rinsed with native water before filling. Total cyanotoxins were measured by enzyme-linked immunosorbent assays (ELISA) and direct-inject multi-analyte liquid chromatography/tandem mass spectrometry (LC/MS/MS; Graham et al. 2010). All samples (n = 50) were analyzed for anatoxins (MRL: 0.15 µg L\(^{-1}\)), cylindrospermopsins (0.05 µg L\(^{-1}\)), microcystins (0.1 µg L\(^{-1}\); -adda specific), and saxitoxins (0.02 µg L\(^{-1}\)) using Abraxis (Warminster, PA, USA) ELISA kits. A subset of samples (n = 6) representing a range of sites and conditions were analyzed for anatoxin-a (MRL: 0.08 µg L\(^{-1}\)); cylindrospermopsin (0.1 µg L\(^{-1}\); microcystin-LA (0.1 µg L\(^{-1}\); -LF (0.1 µg L\(^{-1}\); -LR (0.1 µg L\(^{-1}\); -LW (0.1 µg L\(^{-1}\); -LY (0.1 µg L\(^{-1}\); -RR (0.08 µg L\(^{-1}\); -WR (0.3 µg L\(^{-1}\); -YR (0.1 µg L\(^{-1}\); -HiLR (0.1 µg L\(^{-1}\); and -HYR (0.1 µg L\(^{-1}\); and nodularin-R (0.05 µg L\(^{-1}\)) by LC/MS/MS. About 10% of all samples collected were concurrent field replicates. Additional details on

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**Table 1.** Number of samples, drainage area, number of major dams in the drainage area, and mean streamflow, nutrient, and suspended sediment concentrations at rivers sampled during June–September 2017. Sites highlighted in gray had mainstem dams located within 5 km upstream. For the purposes of this study, site location was defined as eastern (e), midcontinent (m), or western (w) United States.

| Site                                | USGS station number | Number of samples | Drainage area (km²) | Major dams* | Streamflow\(\text{m}^3\text{s}^{-1}\) | Total nitrogen\(\text{mg L}^{-1}\) | Total phosphorus\(\text{mg L}^{-1}\) | Suspended sediment\(\text{mg L}^{-1}\) |
|-------------------------------------|---------------------|------------------|---------------------|-------------|-----------------|-----------------|-----------------|-----------------|
| Connecticut River at Thompsonville, CT\(\text{e}\) | 01184000            | 7                | 25 019             | 77          | 349             | 0.541           | 0.035           | 6.7             |
| Delaware River at Trenton, NJ\(\text{f}\)    | 01463500            | 3                | 17 560             | 33          | 221             | 1.142           | 0.071           | 6.0             |
| Susquehanna River at Conowingo, MD\(\text{f}\) | 01578310            | 2                | 70 189             | 125         | 744             | 1.371           | 0.071           | 31.2            |
| Chattahoochee River near Whitesburg, GA\(\text{f}\) | 02338000            | 5                | 6294               | 46          | 77              | 2.893           | 0.166           | 163.0           |
| Ohio River at Cannellton, IN\(\text{f}\)       | 03303280            | 2                | 251 229            | 709         | 2233            | 1.740           | 0.159           | 63.0            |
| Mississippi River at Hastings, MN\(\text{f}\)  | 05331580            | 4                | 96 089             | 93          | 545             | 4.596           | 0.126           | 52.7            |
| Kansas River at De Soto, KS\(\text{f}\)       | 06892350            | 5                | 154 767            | 89          | 216             | 2.290           | 0.536           | 411.8           |
| Missouri River at Hermann, MO\(\text{f}\)     | 06934500            | 5                | 1 353 269          | 992         | 2712            | 2.555           | 0.348           | 260.3           |
| Trinity River below Freeport, CA\(\text{f}\)   | 08057410            | 8                | 16 260             | 97          | 59              | 6.096           | 0.783           | 107.3           |
| Sacramento River at Freeport, CA\(\text{f}\)   | 11447650            | 4                | 61 445             | 135         | 583             | 0.208           | 0.037           | 21.9            |
| Willamette River at Portland, OR\(\text{f}\)   | 14211720            | 5                | 29 008             | 37          | 365             | 0.550           | 0.054           | 6.3             |

* Number of major dams in the drainage area obtained from Wieczorek et al. (2018).

All study sites had USGS streamflow gauging stations. Streamflow data were downloaded from the USGS National Water Information System database (https://waterdata.usgs.gov/nwis) using the 8-digit USGS Station Number presented in the table and 15-minute data were used to calculate mean streamflow for the period June–September 2017.

Nutrient and suspended sediment data were downloaded from the USGS National Water Quality Assessment Project Water Quality Summary database (https://cida.usgs.gov/quality/rivers/sites) and were used to calculate means for the period June–September 2017. All n for nutrients and suspended sediment are reflected in the table.
methods used and Chl-a, phytoplankton, cyanotoxin synthetase gene, and cyanotoxin data (including results from replicate samples) are available in King et al. (2020a, 2020b).

Nonparametric Spearman rank-correlation analysis was used to test for monotonic relations between the abundance of cyanobacterial genera with known cyanotoxin-producing strains and cyanotoxin synthetase genes. Spearman rank-correlation analyses ($p = 0.05$) were conducted using SigmaPlot 13.0. Relations among cyanobacteria, cyanotoxin synthetase genes, and cyanotoxins could not be tested because of the small number of cyanotoxin detections.

**Results and discussion**

**Algal biomass and cyanobacterial community composition**

During June–September 2017, mean streamflow in midcontinent rivers encompassed the entire range (59–2712 m$^3$ s$^{-1}$) of flows measured in this study. Nutrient and suspended sediment concentrations were generally highest in midcontinent rivers (Table 1). Chl-a concentrations ranged from 0.5 to 64.4 µg L$^{-1}$ (mean = 14.4 µg L$^{-1}$, $n = 49$). Mean and maximum Chl-a concentrations in midcontinent rivers were 3 to >20 times higher than rivers in other parts of the country (Fig. 2a). Nutrient and Chl-a concentrations were indicative of eutrophic conditions in midcontinent rivers and oligo-mesotrophic conditions in eastern and western rivers, except for 2 eastern rivers (Chattahoochee and Ohio) where nutrients were indicative of eutrophy and Chl-a was indicative of oligotrophy (Dodds et al. 1998).

Cyanobacteria were present in algal communities (82% of samples, $n = 50$) but dominant (>50% of total abundance) in only one sample (Fig. 2b). Cyanobacteria represented between 0% and 52% (mean = 8.8%) of the total phytoplankton community. In contrast to Chl-a, no clear regional patterns emerged in cyanobacterial contribution to the algal community, although the highest relative abundances were observed in a midcontinent river (Fig. 2b). We identified 25 cyanobacterial genera in this study. *Pseudanabaena* and *Planktothrix*, common cyanobacteria in riverine phytoplankton assemblages (Reynolds and Descy 1996), occurred 28–82% more often than other genera (Table 2). Most identified genera favor planktonic habitats (Casamatta and Hašler 2016), suggesting benthic entrainment was not a substantial source of cyanobacteria in these communities.

Of the cyanobacteria identified, 64% have known cyanotoxin-producing strains, including the 7 most

![Figure 2](image-url). Algal biomass and relative cyanobacterial abundance in eastern, midcontinent, and western United States rivers during June–September 2017; sites are arranged from low to high mean total phosphorus concentration (Table 1). (a) Algal biomass as chlorophyll $a$ ($n = 49$); (b) relative contribution of cyanobacteria to the total phytoplankton community ($n = 50$).
frequently occurring genera (Table 2). As such, cyanobacterial communities were typically dominated (mean = 91% of total cyanobacterial abundance, n = 41) by genera associated with cyanotoxin production. Genera with known anatoxin-α, microcystin-, and saxitoxin-producing strains occurred in most (100%, 91%, and 100%, respectively) rivers and were present in 70–80% of samples. Genera with known cylindrospermopsin-producing strains occurred less frequently, present in 48% of samples from 63% of rivers. Although relative abundance was typically much lower, occurrence of cyanobacteria with known cyanotoxin-producing strains in these rivers was similar to that observed in US lakes. Previous work showed that genera with anatoxin-α, microcystin-, and saxitoxin-producing strains were present in 80–95% of samples, and genera with cylindrospermopsin-producing strains were present in 67% (Loftin et al. 2016b). Given the connectivity of lentic and lotic environments through riverine networks, this similarity was not unexpected. Planktonic cyanobacteria in large rivers may originate in upstream impounded waters (Reynolds and Descy 1996), and all study sites had upstream reservoirs (Table 1) that may have influenced cyanobacterial community composition.

### Cyanotoxin synthetase genes

At least one of the measured cyanotoxin synthetase genes occurred in all rivers except one (Connecticut), and multiple cyanotoxin synthetase genes were detected in most rivers during June–September 2017 (Fig. 1). The *mcyE* and *sxtA* genes occurred most frequently, detected in 44% and 40% of samples (n = 50), respectively, from 8 rivers (73%). Although the *mcyE* and *sxtA* genes were widespread, occurrence (77% and 65% of detections, respectively) was highest in midcontinent rivers (Fig. 3a and d). In addition, maximum *mcyE* gene abundances were 8–154 times higher (among samples with detectable *mcyE* genes) in midcontinent rivers than elsewhere in the country and maximum *sxtA* gene abundances were 3–10 times higher. The *cyrA* gene was detected less frequently (22% of samples, n = 50) but occurred in over half (64%) of the rivers. No clear regional patterns were detected in *cyrA* occurrence or abundance (Fig. 3e). The *anaC* gene was detected only in the Mississippi River (4% of samples, n = 50).

The *mcyE* gene assays used in this study targeted 3 specific microcystin-producing genera: *Dolichospermum*, *Microcystis*, and *Planktothrix*. The *Dolichospermum*-specific *mcyE* gene was not detected, even though *Dolichospermum* occurred relatively frequently (40% of samples, 54% of rivers). The *Microcystis*-specific *mcyE* gene (40% of samples, 72% of rivers) occurred more frequently than the *Planktothrix*-specific *mcyE* gene (20% of samples, 36% of rivers). *Microcystis*-specific *mcyE* occurred throughout the United States, while *Planktothrix*-specific *mcyE* was observed only in midcontinent and eastern rivers (Fig. 3b–c). Globally, studies of the biogeography of cyanotoxin synthetase genes are rare, and, to the best of our knowledge, this is the first research on cyanotoxin synthetase gene occurrence to include such widely distributed study sites.

Spearman rank analysis indicated that the correlations between the abundance of cyanobacterial genera with known cyanotoxin-producing strains and cyanotoxin synthetase genes were positive but weak (all $r_s < 0.45$, all $p > 0.20$). Note, however, that the relatively widespread occurrence of the *Microcystis*-specific *mcyE* gene is in direct contrast to observed cyanobacterial communities where *Microcystis* was absent (Table 2). Although *Microcystis* was not detected in preserved samples, it was observed after incubation of live material from the 3 midcontinent rivers (Kansas, Mississippi, and Missouri) with the highest *Microcystis*-specific *mcyE* gene abundances (Fig. 3b). Therefore, *Microcystis* may have been present at abundances too low to be detected by traditional enumeration. Most samples with *cyrA* gene detections (82%, n = 11) did not show a corresponding occurrence of genera with known cylindrospermopsin-producing strains (Table 2).

### Table 2. Cyanobacterial genera present in rivers (n = 11) and samples (n = 50) during June–September 2017 and cyanotoxins known to be produced by some strains.

| Genera         | Rivers with genera present (%) | Samples with genera present (%) | Cyanotoxins* |
|----------------|-------------------------------|---------------------------------|--------------|
| *Pseudanabaena*| 91                            | 58                              | ATX, MC      |
| *Planktothrix* | 82                            | 40                              | ATX, MC, SAX |
| *Aphanocapsa*  | 54                            | 38                              | MC           |
| *Dolichospermum* | 54                       | 40                              | ATX, CYL, MC, SAX |
| *Cuspidiformis*| 45                            | 24                              | ATX, SAX     |
| *Limnothrix*   | 45                            | 20                              | SAX          |
| *Merismopedia* | 45                            | 32                              | MC           |
| *Eucapsis*     | 36                            | 14                              | —            |
| *Planktolyngbya* | 36                  | 20                              | —            |
| *Aphanizomenon*| 27                            | 14                              | ATX, CYL, MC, SAX |
| *Chroococcus*  | 27                            | 8                               | —            |
| *Dactylococcus*| 27                            | 14                              | —            |
| *Phormidium*   | 27                            | 8                               | ATX, MC, SAX |
| *Snowella*     | 27                            | 8                               | —            |
| *Anabaenopsis* | 18                            | 12                              | MC           |
| *Calothrix*    | 18                            | 12                              | MC           |
| *Cylindrospermopsis* | 18   | 20                              | CYL, SAX     |
| *Nostoc*       | 18                            | 4                               | MC           |
| *Anabaena*     | 9                             | 2                               | ATX, CYL, MC, SAX |
| *Cylindrospermum* | 9                     | 2                               | ATX, SAX     |
| *Komrophorion* | 9                             | 2                               | —            |
| *Romeria*      | 9                             | 12                              | —            |
| *Schizothrix*  | 9                             | 2                               | —            |
| *Synechococcus*| 9                             | 2                               | MC           |

*ATX = anatoxins; CYL = cylindrospermopsins; MC = microcystins; SAX = saxitoxin; — = no known cyanotoxin producing strains; information in this table based on Graham et al. (2008) and Bernard et al. (2017).
By comparison, genera with known cyanotoxin-producing strains were present in most samples with anaC (100%, n = 2), Planktothrix-specific mcyE (60%, n = 10), and sxtA (85%, n = 20) gene detections.

Cyanobacteria were not dominant members of the algal community in this study, and abundance of observed gene copies were typically <100 mL⁻¹ (Fig. 3). The relatively high sensitivity of qPCR analysis may have facilitated the detection of cyanobacteria containing the measured cyanotoxin synthetase genes, even when rare (Pacheco et al. 2016), as observed for Microcystis. However, differences between the cyanobacterial community and gene occurrence also may have been a result of the different sampling approaches used for these analyses (composite and near-surface grabs, respectively) or other factors, such as the presence of nonviable DNA in the environment or small unknown cyanobacteria that carry the measured genes. Substantial deviations also may occur when comparing cyanobacterial abundance and qPCR gene abundance because of the errors associated with both approaches (see review by Pacheco et al. 2016).

**Cyanotoxins**

Anatoxin-a and microcystins were detected at low levels in 2 midcontinent rivers. Anatoxins were not detected by ELISA. However, in the subset of samples analyzed by LC/MS/MS (n = 6), anatoxin-a was detected once (17% of measured samples) in the Mississippi River (0.1 µg L⁻¹; Fig. 1). Detection by LC/MS/MS (MRL: 0.10 µg L⁻¹) but not ELISA (MRL: 0.15 µg L⁻¹) was likely because of the lower MRL by LC/MS/MS. Microcystins (0.18–0.38 µg L⁻¹) were detected by ELISA in 3 samples (6%) from 2 rivers (18%), the Kansas and Mississippi (Fig. 1). Microcystin analysis by LC/MS/MS confirmed ELISA results. Cylindrospermopsins and saxitoxins were not detected during this study. Regional-scale studies of cyanotoxins associated with planktonic cyanobacteria in large rivers are rare, and most studies have focused on microcystins during cyanobacterial-dominated events. These studies show that microcystins are common in large rivers worldwide, and concentrations several orders of magnitude higher than observed in
this study may occur when cyanobacteria are abundant (Graham et al. 2012, Otten et al. 2015, Preece et al. 2017). Such studies on anatoxins, cylindrospermopsins, and saxitoxins in rivers are sparse and focused on cyanobacterial-dominated events. Anatoxin-a has been measured in planktonic samples from rivers in Argentina (Aguilera et al. 2018), Australia (John et al. 2019), and Russia (Stepanova et al. 2018) at concentrations up to 4 µg L\(^{-1}\). To the best of our knowledge, this is the first report of anatoxin-a in a large US river. Cylindrospermopsin and saxitoxin were not detected in this study but have been reported from other rivers in the United States (de la Cruz et al. 2013), Argentina (Aguilera et al. 2018), and Australia (Al-Tebrineh et al. 2012a).

Anatoxin-a detection in the Mississippi River occurred concurrent with the highest observed abundance of anaC genes (number of copies: 110 mL\(^{-1}\)) and the presence of several cyanobacterial genera with known anatoxin-a producing strains (Caspidothrix, Dolichospermum, Planktothrix, and Pseudanabaena). Similarly, microcystin detection in the Kansas and Mississippi rivers occurred concurrent with the highest observed abundances of mcyE genes (copies: 400 and 21,710 mL\(^{-1}\), respectively). Cyanobacteria with microcystin-producing strains (Microcystis and/or Planktothrix) also were present, but rare (detected only in laboratory incubated samples for 2 of 3 samples), when microcystin was detected. The small number of cyanotoxin detections precludes a more rigorous analysis of the relation between cyanobacterial community composition, cyanotoxin synthetase genes, and cyanotoxins. Observed relations in other studies are inconsistent. In their review, Pacheco et al. (2016) concluded that while synthetase gene abundance provides insight about the genetic potential for cyanotoxin production within a cyanobacterial community, it is not a reliable indicator of cyanotoxin occurrence or concentration.

**Cyanotoxin occurrence in large rivers of the United States**

Cyanotoxin production is strain specific, and microscopic identification does not differentiate between toxin-producing and nontoxin-producing strains. Detection of cyanotoxin synthetase genes provides additional information about the genetic potential for cyanotoxin production within a cyanobacterial community that cannot be otherwise discerned (Pacheco et al. 2016). Cyanobacterial genera with known cyanotoxin-producing strains frequently occurred (48–80% of samples) in this subset of US rivers. Cyanotoxin synthetase genes occurred less frequently (4–44% of samples), suggesting the genetic potential for cyanotoxin production is not as widespread as indicated by taxonomic approaches. Microcystins are recognized as the most commonly occurring cyanotoxin worldwide (Preece et al. 2017), and we expected that the mcyE gene would occur most frequently in this study. The sxtA gene occurring at almost the same frequency as the mcyE gene was unexpected and is of interest given that little is known about saxitoxin occurrence in freshwaters. While measuring cyanotoxin synthetase genes provides a more detailed indicator of the potential for cyanotoxin production within a cyanobacterial community than algal taxonomy alone, the presence of cyanotoxin synthetase genes is not a definitive indicator of cyanotoxin presence or active cyanotoxin production (Pacheco et al. 2016). All river sites except one had detectable cyanotoxin synthetase genes at least once during this study (Fig. 1). However, cyanotoxins occurred infrequently (6–17% of samples) and were only detected in 2 rivers.

Cyanobacteria and cyanotoxin synthetase genes occurred across the trophic gradient represented by these rivers (Fig. 1, Table 1). However, occurrence and abundance of cyanotoxin synthetase genes and cyanotoxins were highest in the eutrophic midcontinent rivers (Fig. 3), which encompassed the full range of flows measured in this study. Higher occurrence in these midcontinent rivers may be a result of eutrophication-related shifts in algal community composition, either within the rivers or upstream reservoirs (Smith 2003). Cyanotoxins, and possibly synthetase genes, may persist and be transported over long distances without living cyanobacterial cells (Graham et al. 2012, Otten et al. 2015, Pacheco et al. 2016, Preece et al. 2017). Such spatio-temporal decoupling, and the interconnectivity of lentic and lotic ecosystems, pose challenges to understanding cyanotoxin dynamics in large rivers.

Cyanotoxins associated with planktonic cyanobacteria have been observed in rivers throughout the world, but extensive surface accumulations are less common than observed in lakes and reservoirs (Brooks et al. 2016, Preece et al. 2017). Given the perceived global increase in cyanotoxins, including in environments previously considered unlikely to support cyanotoxin-producing cyanobacteria such as oligotrophic lakes (Carey et al. 2012) and small streams (Loftin et al. 2016a), occurrence in large rivers may also increase. Results from this study indicate that cyanobacteria, cyanotoxin synthetase genes, and cyanotoxins were present at relatively low concentrations throughout this subset of US rivers. Future changes in streamflow regimes, temperature extremes, and water quality conditions may increase incidence of downstream transport or favor growth of cyanotoxin-producing cyanobacteria in large rivers (Smith 2003, Palmer et al. 2009, Brooks et al. 2016).
2016). Ongoing research will characterize the physico-chemical environment associated with cyanobacteria, cyanotoxin synthetase genes, and cyanotoxins in these rivers to better understand how changing conditions may result in increased occurrence.

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Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government. No potential conflict of interest was reported by the authors.

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