

Cyanotoxins in inland lakes of the United States: Occurrence and potential recreational health risks in the EPA National Lakes Assessment 2007

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ARTICLE INFO

Article history:

Received 1 September 2015

Received in revised form 31 March 2016

Accepted 5 April 2016

Available online 26 May 2016

Keywords:

Cyanotoxins

Microcystins

Cylindrospermopsins

Saxitoxins

Cyanobacteria

ABSTRACT

A large nation-wide survey of cyanotoxins (1161 lakes) in the United States (U.S.) was conducted during the EPA National Lakes Assessment 2007. Cyanotoxin data were compared with cyanobacteria abundance- and chlorophyll-based World Health Organization (WHO) thresholds and mouse toxicity data to evaluate potential recreational risks. Cylindrospermopsins, microcystins, and saxitoxins were detected (ELISA) in 4.0, 32, and 7.7% of samples with mean concentrations of 0.56, 3.0, and 0.061 µg/L, respectively (detections only). Co-occurrence of the three cyanotoxin classes was rare (0.32%) when at least one toxin was detected. Cyanobacteria were present and dominant in 98 and 76% of samples, respectively. Potential anatoxin-, cylindrospermopsin-, microcystin-, and saxitoxin-producing cyanobacteria occurred in 81, 67, 95, and 79% of samples, respectively. Anatoxin-a and nodularin-R were detected (LC/MS/MS) in 15 and 3.7% samples ($n = 27$). The WHO moderate and high risk thresholds for microcystins, cyanobacteria abundance, and total chlorophyll were exceeded in 1.1, 27, and 44% of samples, respectively. Complete agreement by all three WHO microcystin metrics occurred in 27% of samples. This suggests that WHO microcystin metrics based on total chlorophyll and cyanobacterial abundance can overestimate microcystin risk when compared to WHO microcystin thresholds. The lack of parity among the WHO thresholds was expected since chlorophyll is common amongst all phytoplankton and not all cyanobacteria produce microcystins.

Published by Elsevier B.V.

1. Introduction

Cyanotoxins, produced by cyanobacteria, are increasingly perceived as a global water-quality issue that has grown in scope and persistence, with linkages to land-use, land cover, weather patterns, hydrology, and biogeochemistry (Chorus and Bartram, 1999; Paerl and Scott, 2010). Potential exposure routes to cyanotoxins include contact, ingestion or inhalation during recreational activities, contaminated drinking water, crops

irrigated by contaminated water, and consumption of fish, shellfish, and algal supplements (Chorus and Bartram, 1999; Corbel et al., 2013; Heussner et al., 2012). Cyanobacterial harmful algal blooms (cyanoHABs) have been implicated in human and animal illness and death in over 50 countries and at least 41 U.S. States (Graham et al., 2009; Backer et al., 2015). The economic impacts of freshwater harmful algal blooms (HAB) have not been systematically estimated; however, impacts from eutrophication, one consequence of which is HABs, have been estimated to be \$2.2 billion (US dollars) annually (Dodds et al., 2009; USEPA, 2015).

Anatoxins are a group of at least 7 natural neurotoxins produced by a range of nitrogen fixing and non-nitrogen fixing, filamentous cyanobacteria including *Anabaena* sp., *Aphanizomenon* sp., *Cylindrospermum* sp., *Chrysosporum* sp., *Cuspidothrix* sp., *Dolichospermum* sp., *Oscillatoria* sp./*Planktothrix* sp., *Phormidium* sp., *Pseudoanabaena* sp., and *Raphidiopsis* sp. (Wonnacott and

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Gallagher, 2006; Osswald et al., 2007; Rubio et al., 2014; Jiang et al., 2015). Anatoxins are acetylcholine agonists affecting neurotransmission, with the potential to cause mammalian respiratory failure (Rubio et al., 2014; Wonnacott and Gallagher, 2006). Mouse toxicology studies have reported lethal dose for 50% (LD_{50}) of test subjects via intraperitoneal (i.p.) injection with anatoxins ranging from 200 to 375 μg anatoxin/kg of bodyweight (bw) (Chorus and Bartram, 1999).

Cylindrospermopsins are a group of at least 3 natural alkaloid toxins produced by *Anabaena* sp., *Aphanizomenon* sp., *Chrysosporum* sp., *Cylindrospermopsis* sp., *Lyngbya* sp., *Raphidiopsis* sp., and *Umezakia* sp. (Stüken et al., 2009; de la Cruz et al., 2013). Cylindrospermopsins are cytotoxic, but have also been indicated as having genotoxic, hepatotoxic, and possible carcinogenic effects (de la Cruz et al., 2013). Mouse toxicology studies have reported LD_{50} 's via i.p. injection for cylindrospermopsins as 2100 μg cylindrospermopsin/kg bw for acute toxicity (24 h exposure) and 200 μg cylindrospermopsin/kg bw after 5 days cumulative exposure, respectively (Ohtani et al., 1992) (with further data summarized Chorus and Bartram, 1999; de la Cruz et al., 2013).

Microcystins are a group of at least 80 natural toxins produced by many cyanobacteria genera including filamentous forms like *Anabaena* sp. and *Oscillatoria* sp./*Planktothrix* sp., colonial forms like *Microcystis* sp., and picoplankton forms like *Synechococcus* sp. and *Synechocystis* sp. Among the filamentous forms, microcystins have been shown to be produced by both nitrogen fixers as well as non-nitrogen fixers (Chorus and Bartram, 1999). While a range of toxicity endpoints have been reported for microcystins, they are most commonly reported as hepatotoxic and possible carcinogens as tumor promoters (Chorus and Bartram, 1999; WHO, 2010). Mouse bioassays have reported LD_{50} 's by i.p. injection for microcystins as ranging from 50 to >1200 μg microcystin/kg bw with most values between 50 and 300 μg microcystin/kg bw (Chorus and Bartram, 1999).

Nodularins are a group of cyclic pentapeptide cyanotoxins similar in chemical structure, toxicity, and mode of action to microcystins. They also possess the (2S,3S,8S,9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (ADDa) functional group common to the microcystin class of cyanotoxins (Chorus and Bartram, 1999; Chen et al., 2013). There are at least 12 known nodularins (Namikoshi et al., 1994; Carmichael, 1997; Mazur-Marzec et al., 2006; Chen et al., 2013) of which nodularin-R is the most common (Chen et al., 2013). *Nodularia* sp. is currently the only known aquatic nodularin producer and are known to predominately occur in brackish waters. This class of toxins is not expected to typically occur in "freshwater" inland settings, however there are cases where increased salinity occurs in inland waters due to drought, geology, and land-use practices. Mouse toxicology studies have reported LD_{50} 's via i.p. injection for nodularins ranging from 30 to >2000 μg nodularin/kg bw (Rinehart et al., 1994; Chen et al., 2013).

Saxitoxins are a group of at least 58 neurotoxins most commonly associated with paralytic shellfish poisoning (PSP) after consumption of seafood from marine environments (Wiese et al., 2010). Saxitoxins occurrence in freshwaters has been understudied, and there are several known producers amongst the freshwater cyanobacteria and include *Anabaena* sp., *Aphanizomenon* sp., *Cylindrospermopsis* sp., *Lyngbya* sp., *Oscillatoria/Planktothrix* sp., and *Planktolyngbya* sp. Saxitoxins are among the most potent group of natural mammalian neurotoxins known (Wiese et al., 2010; Cusick and Sayler, 2013). This class of toxins acts as calcium, potassium, and sodium channel blockers. Mouse toxicology studies have reported LD_{50} 's via i.p. injection with saxitoxins ranging from 1 to 10 μg saxitoxin/kg bw (summarized Chorus and Bartram, 1999; Deeds et al., 2008; Botana et al., 2010). Saxitoxin analogue toxicity has been reported to range from equivalent to

less than 1% compared to the toxicity of saxitoxin (Wiese et al., 2010).

Cyanotoxins occur with varying frequency, environmental persistence, and impact on humans, companion animals, livestock, and ecosystems (Chorus and Bartram, 1999; de la Cruz et al., 2013; Beaver et al., 2014; Hilborn and Beasley, 2015). Most studies have focused on microcystins; however, there are several other classes of cyanotoxins that can have adverse environmental health effects including anatoxins, cylindrospermopsins, nodularins, and saxitoxins. The US Centers for Disease Control and Prevention (CDC) Harmful Algal Bloom-related Illness Surveillance System (HABISS) study was conducted from 2007 through 2011, with 15 U.S. states contributing cyanotoxin data, and human and animal illness reports (Backer et al., 2015). Out of 3194 reported freshwater events, anatoxin-a (0–500 $\mu\text{g/L}$), microcystin (0–700 $\mu\text{g/L}$), and saxitoxin (concentration range not given) detections occurred in 7.6, 82, and 9.3% of events, respectively. Fifty-seven percent of canine poisonings ($n = 67$) were fatal, with anatoxin-a and microcystins implicated in 18 and 4% of cases, respectively. Of the 176 reported freshwater human exposures to cyanobacteria that resulted in illness, 27 case reports had associated cyanotoxin data. Anatoxin-a, cylindrospermopsins, and microcystins were measured in 81, 4, and 15%, respectively, of cases linked to human illness. Of note, 94% of cases were connected with routine monitoring efforts compared with event-response. Separate studies have also demonstrated that human cyanotoxin exposure in freshwater settings can occur from toxin aerosolization (Cheng et al., 2007; Backer et al., 2010). Microcystins have also been detected in human blood serum after exposure (Soares et al., 2006; Yuan et al., 2006; Chen et al., 2009). Vulnerable populations can be more susceptible to cyanotoxin-associated illnesses, and death may result in extreme cases such as dialysis using improperly treated water (Pouria et al., 1998; Azevedo et al., 2002; de la Cruz et al., 2013; Hilborn et al., 2013, 2014).

In 1999, the World Health Organization (WHO) evaluated human recreational exposure to microcystin-LR and established provisional guidance values based on a tolerable daily intake (TDI) of 0.04 $\mu\text{g/kg}$ bw to protect human health (Chorus and Bartram, 1999). The WHO provisional guidelines recommend the use of at least 1 of 3 criteria to assess recreational risk: (1) microcystin-LR concentration, (2) total chlorophyll, or (3) total cyanobacterial abundance. Many government entities use values similar to those set for microcystin-LR as a threshold for total microcystins or use cyanobacteria abundance (Graham et al., 2009; Chorus, 2012). In addition, a few U.S. states have put forward recreational thresholds for anatoxins, cylindrospermopsins, and saxitoxins. Values range from 1 to 90 $\mu\text{g/L}$ for anatoxins, 4 to 6 $\mu\text{g/L}$ for cylindrospermopsins, and 3 $\mu\text{g/L}$ for saxitoxins (Graham et al., 2009; Chorus, 2012). The disparity among guideline values for cyanotoxins is a direct effect of insufficient toxicological and epidemiological information.

Cyanotoxin occurrence was screened by three different enzyme-linked immunosorbent assays (ELISAs) for cylindrospermopsins, microcystins/nodularins, and saxitoxins in water samples collected from 1161 natural lakes and reservoirs for the U.S. Environmental Protection Agency (EPA) National Lakes Assessment between May and October 2007 (2007 NLA). Anatoxin-a and nodularin-R in addition to cylindrospermopsins, and microcystins were screened by liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) in 27 samples. Microcystin occurrence, total chlorophyll, and cyanobacteria abundance were evaluated based on WHO relative human health risk guidelines for microcystin exposure. The purpose of this study was to evaluate the national occurrence of cyanotoxins in lakes and reservoirs of the United States and provide information to aid management decisions related to cyanobacteria and associated toxins.

2. Materials and methods

2.1. Study design

The 2007 Survey of the Nation's Lakes (EPA, 2009) was a Generalized Random Tessellation Stratified (GRTS) design for a finite resource using reverse hierarchical ordering of selected lakes. There were 1161 natural lakes and reservoirs (hereafter lakes unless specified) sampled mid-lake at least once between May and October 2007. A small subset of 95 lakes were resampled during a second visit and data were combined with initial visit data as unique samples for this analysis. NOAA's National Climatic Dataset (NCDC, 2007) was used to describe general patterns in air temperature and precipitation during the sample collection season.

2.2. Sample collection and preservation

Integrated photic zone samples were collected using a 2 m length of polyvinyl chloride pipe (internal diameter = 3.2 cm) equipped with a ball valve. The vertical sampler was lowered to a depth of 2 m unless Secchi transparency indicated that photic depth was less than 2 m. In the event that photic zone was calculated to be shallower than 2 m, the sampler was lowered to the shallower depth. Multiple samples were composited in a 4 L cubitainer until sufficient volume was collected. Composite samples were split for microcystins (500 mL), chlorophyll-a (2 L), and phytoplankton (1 L) analyses in high density polyethylene bottles. Microcystin and chlorophyll-a samples were immediately stored on ice and phytoplankton samples were preserved with 10 mL of Lugol's Iodine solution. Chlorophyll-a samples were filtered on shore immediately following sample collection (EPA, 2009).

2.3. Cyanotoxin sample processing

Cyanotoxin samples were shipped frozen overnight to the U.S. Geological Survey (USGS) Organic Geochemistry Research Laboratory in Lawrence, KS. Samples were processed by three sequential freeze/thaw cycles to lyse intact cyanobacteria and release toxins into the dissolved phase (Loftin et al., 2008; Graham et al., 2010). Lysed sample aliquots were filtered with a 10 mL Norm-Ject polypropylene syringe (ThermoFisher Scientific, Waltham, MA, USA) coupled to a 0.45 μm PVDF Syringe filter (Millex[®]-HV, 33 mm diameter, Millipore Corp., Bellerica, MA) and then stored frozen (-20°C) until analysis.

2.4. Detection of cylindrospermopsins, microcystins, and saxitoxins by enzyme-linked immunosorbent assay (ELISA)

Lysed and filtered surface water samples were analyzed by three separate enzyme-linked immunosorbent assays acquired from Abraxis, LLC (Warminster, PA) for cylindrospermopsins, microcystins/nodularins, and saxitoxins. Methods have been previously reported (Graham et al., 2010; Loftin et al., 2015). Samples exceeding the highest calibration standard were diluted. The minimum reporting levels (MRL) were 0.05 $\mu\text{g/L}$ as cylindrospermopsin equivalents, 0.10 $\mu\text{g/L}$ as microcystin-LR equivalents, and 0.02 $\mu\text{g/L}$ as saxitoxin equivalents. Anatoxins were not measured by assay since no commercial assay existed at the time of this work. Approximately 20% of all samples were reanalyzed by ELISA as laboratory replicates and 10% as laboratory matrix spikes at one of 2 concentration levels (0.75 or 1.0 $\mu\text{g/L}$ of microcystin-LR). Laboratory quality control data are summarized in Loftin et al. (2015). Laboratory replicates and laboratory spiked matrix samples were considered acceptable at a relative standard

deviation (% RSD) of 28.3 (equivalent to $\pm 20\%$ of average or expected value) or less. More variation may be expected with field replicates due to difficulty with homogenizing intact cyanobacterial cells when splitting samples, therefore, field replicate samples were not reanalyzed if % RSD exceeded 28.3%, but rather accepted as inherent variability due to temporal/spatial variation.

2.5. Detection of cyanotoxins by liquid chromatography tandem mass spectrometry (LC/MS/MS)

A subset of 2.2% of samples were also analyzed by a direct inject, multiclass cyanotoxin liquid chromatography tandem mass spectrometry mass spectrometry (LC/MS/MS) method (Loftin et al., 2008; Graham et al., 2010) that included anatoxin-a (ANAA), cylindrospermopsin (CYLS), deoxycylindrospermopsin (DCYL), DMAC (domoic acid), lyngbyatoxin-a (LYGA), microcystin-LA (MCLA), microcystin-LF (MCLF), microcystin-LR (MCLR), microcystin-LW (MCLW), microcystin-LY (MCLY), microcystin-RR (MCRR), microcystin-YR (MCYR), nodularin-R (NODR), and okadaic acid (OKAC) with an estimated minimum reporting level of 0.010 $\mu\text{g/L}$ for each toxin. Laboratory replicates were analyzed for 5 of the 27 samples analyzed by LC/MS/MS with a % RSD of 28.3% considered as acceptable. The 2.2% subset was selected from across the nation to have approximately 50% of the samples as non-detections based on microcystin ELISA results (Loftin et al., 2015).

2.6. Determination of chlorophyll

EPA used a performance-based method for chlorophyll analysis as described in Rigosi et al. (2014). Briefly, a known volume of water was filtered through a 0.7 μm glass fiber filter, extracted in a 90% acetone:reagent grade water mixture, and measured fluorometrically. The MRL for the fluorometer was 3.0 $\mu\text{g/L}$ and when the volume filtered was accounted for, the method MRL was 0.10 $\mu\text{g/L}$. While this method can be specific for chlorophyll-a, there are known chlorophyll b and c interferences due to spectral overlap depending on phytoplankton taxonomic composition, therefore this analysis will be referred to as chlorophyll instead of chlorophyll-a (EPA, 1997, section 4.2).

2.7. Cyanobacteria enumeration and identification

Cyanobacteria were identified and enumerated as described in Beaulieu et al. (2013). Briefly, preserved samples were counted until 400 natural units were reached. The genera level was the lowest taxonomic level used in this analysis due to incomplete or inconsistent taxonomy at species level. Contrary to previous reports (Beaulieu et al., 2013), picoplankton were identified in some, but not all, samples. Since there was no means to correct for partial identification of picoplankton in the dataset, picoplankton have significance in the context of microcystin production, and dropping picoplankton from the analysis would decrease the accuracy and precision of the enumeration data; data were evaluated with and without picoplankton. Interpretation with and without picoplankton did not alter key findings; therefore, picoplankton were included in data analyses. Potential anatoxin, cylindrospermopsin, microcystin, and saxitoxin producers (Table 1) were identified and abundances were summed. Data interpretation was focused on occurrence and abundance because WHO microcystin recreational metrics do not use biovolume. All taxonomic names were used as provided to the genera level and no attempt was made to reclassify cyanobacteria into other genera based upon newer suggestions justified by polyphasic approaches (Palinska and Surosz, 2014; Komárek, 2016; Pinevich, 2015). Genetic resolution would be required or systematic identification of specific morphologic structures such

Table 1

Summary statistics of potential cyanotoxin producing cyanobacteria.

	Potential toxins produced ^a	% Occurrence (n = 1249)	Cell abundance (cells/mL)		
			Median	Mean	Maximum
All potential anatoxin producers	A	81	7.5E+02	4.6E+03	3.0E+05
All potential cylindrospermopsin producers	C	67	6.8E+02	6.9E+03	3.0E+05
All potential microcystin producers	M	95	3.7E+03	3.0E+04	5.0E+06
All potential nodularin producers	N	0.24	5.3E+02	2.6E+03	7.1E+03
All potential saxitoxin producers	S	79	8.8E+02	7.5E+03	3.0E+05
<i>Anabaena</i> sp.	A, C, M, S	55	3.5E+02	3.5E+03	2.1E+05
<i>Anabaenopsis</i> sp.	M	12	2.0E+02	1.4E+03	3.5E+04
<i>Aphanizomenon</i> sp.	A, C, S	28	7.6E+02	8.6E+03	3.0E+05
<i>Aphanocapsa</i> sp.	M	35	1.2E+02	1.4E+04	3.1E+06
<i>Coelosphaerium</i> sp.	M	14	9.6E+02	6.2E+03	4.6E+05
<i>Cylindrospermopsis</i> sp.	C, S	3.8	4.4E+02	2.4E+03	2.6E+04
<i>Cylindrospermum</i> sp.	A	3.1	2.8E+02	1.9E+03	3.4E+04
<i>Leptolyngbya</i> sp.	M	12	1.2E+03	4.4E+03	1.4E+05
<i>Limnothrix</i> sp.	M	6.8	5.8E+02	1.3E+04	4.2E+05
<i>Lyngbya</i> sp.	C, M, S	23	1.7E+02	1.9E+03	5.1E+04
<i>Microcystis</i> sp.	M	57	3.2E+03	2.9E+04	5.0E+06
<i>Nodularia</i> sp.	N	0.24	5.3E+02	2.6E+03	7.1E+03
<i>Oscillatoria</i> sp.	A, M, S	34	2.0E+02	2.0E+03	7.7E+04
<i>Phormidium</i> sp.	A, M	9.3	4.9E+02	6.0E+03	2.5E+05
<i>Planktolyngbya</i> sp.	M, S	8.2	3.1E+02	2.6E+03	7.3E+04
<i>Planktothrix</i> sp.	A, M, S	6.2	7.0E+01	2.8E+03	8.6E+04
<i>Pseudoanabaena</i> sp.	A, M	9.0	2.9E+02	1.8E+03	6.0E+04
<i>Oscillatoria</i> sp./ <i>Planktothrix</i> sp.	A, M, S	38	2.1E+02	2.3E+03	8.6E+04
<i>Raphidiopsis</i> sp.	A, C	12	7.3E+01	1.3E+03	7.6E+04
<i>Snowella</i> sp.	M	2.4	7.0E+02	1.9E+03	2.8E+04
<i>Synechococcus</i> sp. ^b	M	≥24	≥2.0E+02	≥4.5E+02	≥8.8E+03
<i>Synechocystis</i> sp. ^b	M	≥17	≥5.4E+02	≥2.6E+03	≥7.9E+04

^a A, potential anatoxin producer; C, potential cylindrospermopsin producer; M, potential microcystin producer; N, potential nodularin producer; S, potential saxitoxin producer.

^b The picoplankton, *Synechococcus* sp. and *Synechocystis* sp., were not measured by all analysts. Percent occurrence and cell abundance may be larger than reported.

as aerotopes to differentiate planktic and suspended benthic forms (Wacklin et al., 2009).

2.8. Data analyses

All summary statistics were conducted using Microsoft Excel and Origin Pro 2015 (Northampton, MA, USA). Nonparametric Mann–Whitney tests were conducted using Origin Pro 2015 to discern statistical significance between median cyanotoxin concentrations from manmade versus natural lakes. All mapping was conducted using ArcMap version 9.3 (ESRI Inc., Redlands, CA). All latitudes and longitudes were recorded in decimal degrees in NAD 83. All available data were used for analysis of detection frequency. Detections only were used to calculate summary statistics on concentrations to provide better context of representative exposure concentrations when toxins occurred. When multiple parameters were compared (e.g. toxins and cyanobacteria abundance), analysis was only conducted on samples that reported data for all parameters.

2.9. Assessment of relative probability of adverse human health effects due to microcystins

The WHO provisional guidance on probable adverse human health risks due to recreational microcystin exposure is based on thresholds for low, moderate, high, or very high risk of acute affects (Chorus and Bartram, 1999) using one of three metrics: microcystins-LR (low: <10 µg/L, moderate: <20 µg/L, high: <2000 µg/L, and very high: >2000 µg/L), chlorophyll-a (low: <10 µg/L, moderate: <50 µg/L, high: <5000 µg/L, and very high: >5000 µg/L), and cyanobacteria abundance (low: <20,000 cells/mL, moderate: <100,000 cells/mL, high: <10,000,000 cells/mL, and very high: >10,000,000 cells/mL). Microcystins-LR risk thresholds were

applied against the sum of measured microcysts for this analysis, as has become common practice (Chorus, 2012).

3. Results

3.1. Lake description and climate

Natural lakes (includes natural lakes with dams) represented 45% of lakes sampled and reservoirs represented 55%. Median lake area, perimeter, and depth at sampling point were 0.70 km², 5.6 km, and 5.9 m, respectively (Table SI-1). Maximum lake area, perimeter, and depth at sampling point, were 1700 km², 2800 km, and 97 m, respectively.

NOAA's National Climatic Data Center (NCDC, 2007) annually ranks states according to "record below normal", "much below normal", "below normal", "near normal", "above normal", "much above normal", and "record" above normal for a variety of climatic variables. Including temperature and precipitation, compared to the period of record (113 yrs as of 2007). In the United States, 2007 was the 10th warmest year on record at the time, with a national mean temperature of 12.4 °C which was 0.8 °C above the 1901–2000 mean. Mean precipitation during 2007, was variable, with the southeastern states and California having much below normal precipitation levels and North Carolina being the record driest. The central plains states and the northeastern tier states from Maine to Indiana had above normal or much above normal precipitation. All other states had near normal or below normal precipitation.

3.2. Occurrence of cyanotoxins in the contiguous 48 U.S. States by ELISA

Occurrence of cyanotoxins in the United States varied spatially by chemical class. Cylindrospermopsins were detected in 4.0% of

samples (Table SI-2, Loftin et al., 2015) and occurred most frequently in the midwestern and south central United States and parts of Florida with 74% ($n = 50$) of detections occurring east of -100° longitude and south of 40° latitude (Fig. 1). Maximum, mean, and median concentrations (detections only) were 4.4, 0.56, and $0.10 \mu\text{g/L}$. Microcystins were detected in 32% of samples (Table SI-2, Loftin et al., 2015) throughout the United States, but occurred most frequently in the upper midwest plains and Great Lakes areas with 91% of detections east of -105° longitude (Fig. 2), as described in EPA (2009) and Beaver et al. (2014). Maximum, mean, and median concentrations (detections only) were 230, 3.0, and $0.49 \mu\text{g/L}$. Saxitoxins were detected in 7.7% of samples (Table SI-2, Loftin et al., 2015) and occurred throughout the United States, but occurred more frequently in the northern half of the country; 82% of saxitoxin detections occurred North of 37° latitude with the remaining detections occurring predominately in Oklahoma and Florida (Fig. 3). Maximum, mean, and median concentrations (detections only) were 0.38, 0.061, and $0.030 \mu\text{g/L}$.

Co-occurrence was evaluated by examining the subset of samples with at least one toxin class detected. The three cyanotoxin classes co-occurred in only 4 samples (0.32%). Cylindrospermopsins co-occurred with microcystins and saxitoxins in 0.96 and 0.80% of samples, respectively. Saxitoxins co-occurred with microcystins in 5.0% of samples. There was no clear monotonic association in concentration of co-occurring cyanotoxin classes. Cylindrospermopsins did not co-occur with microcystins when concentrations of either class were greater than $4.5 \mu\text{g/L}$.

This study design does not lend itself to be a “true” temporal trends assessment. The results, however, are informative in that maximum detection frequencies for each toxin did not coincide with each other within even a 2 months period (Table SI-3). The frequency of cylindrospermopsin occurrence increased from May (0% of samples) through October (11% of samples). Cylindrospermopsin concentrations, however, did not follow the same seasonal pattern; mean concentrations in July and August were approximately half that of other months (Table SI-3).

Microcystins were not detected in May or October; however, the frequency of microcystin occurrence was generally similar during June–September (28–36% of samples). Mean microcystin concentrations were generally similar across months ($1 \mu\text{g/L}$), with the exception of August (mean $6.0 \mu\text{g/L}$). Saxitoxins, like microcystins, only occurred between June and September. Saxitoxins were detected most frequently in June and August (about 10% of samples), but the highest concentrations occurred in August and September (0.07–0.08 $\mu\text{g/L}$).

Cyanotoxin occurrence and concentration among natural versus manmade lakes were evaluated. Cylindrospermopsins occurred 3.5 times more frequently and mean, median, and maximum concentrations were about 2 times higher in manmade lakes compared with natural lakes (Table SI-2). Detection frequency for microcystin occurrence was similar (natural: 18% versus manmade: 14%), but mean, median, and maximum concentrations were nearly 3 times greater in natural compared with manmade lakes. Saxitoxin detection frequency was 3.8% in both lake types and only slightly greater mean, median, and maximum concentrations in manmade lakes which is likely within experimental error of the measurements. The Mann Whitney test of medians supports these assertions with p-values of 0.0033, 2.5E–7, and 0.20 for cylindrospermopsins (manmade lakes), microcystins (natural lakes), and saxitoxins (manmade lakes), respectively.

3.3. Cyanotoxin detection by liquid chromatography-triple quadrupole mass spectrometry

ELISAs exist for several of the more commonly measured toxins including anatoxin-a more recently (2015). As a screening technology independent verification is ideal and LC/MS/MS is well suited to provide analyte specific quantitation at environmentally relevant concentrations when suitable analytical standards exist. A subset of 27 samples (2.2%) was measured by LC/MS/MS to evaluate what other cyanotoxin classes are occurring in the Nation's waters (see Loftin et al., 2015, Appendix Table 10).

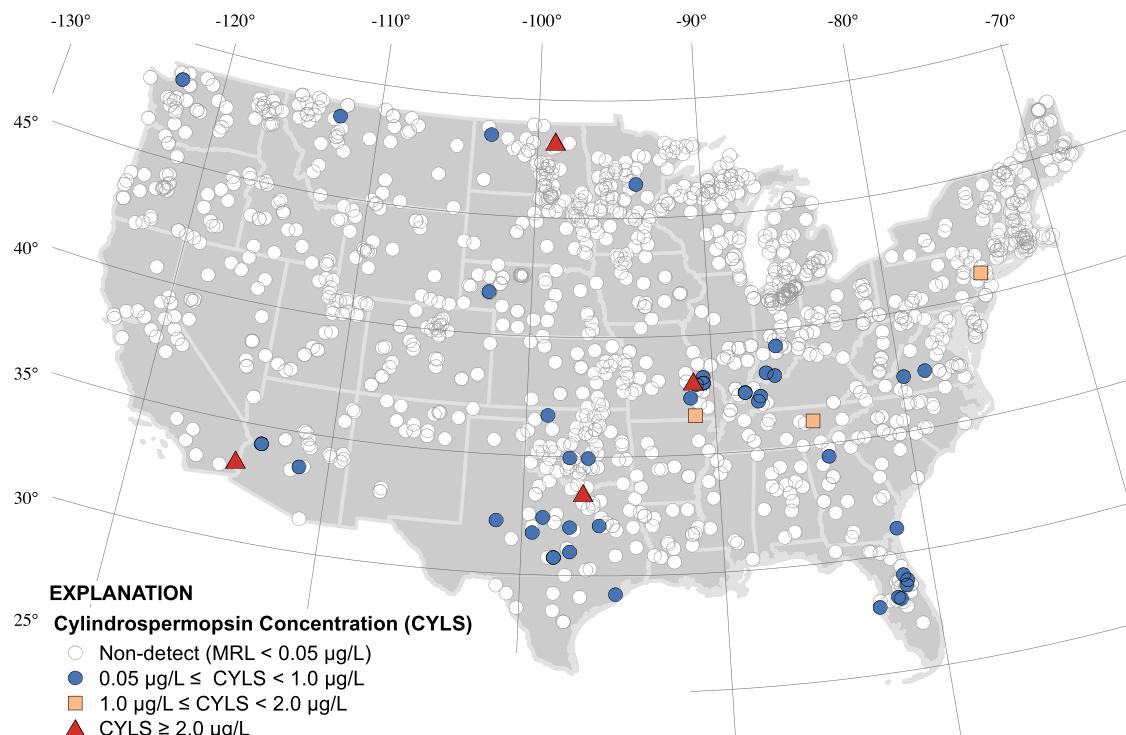


Fig. 1. United States occurrence of cylindrospermopsins in the contiguous 48 U.S. states. Map not shown to scale.

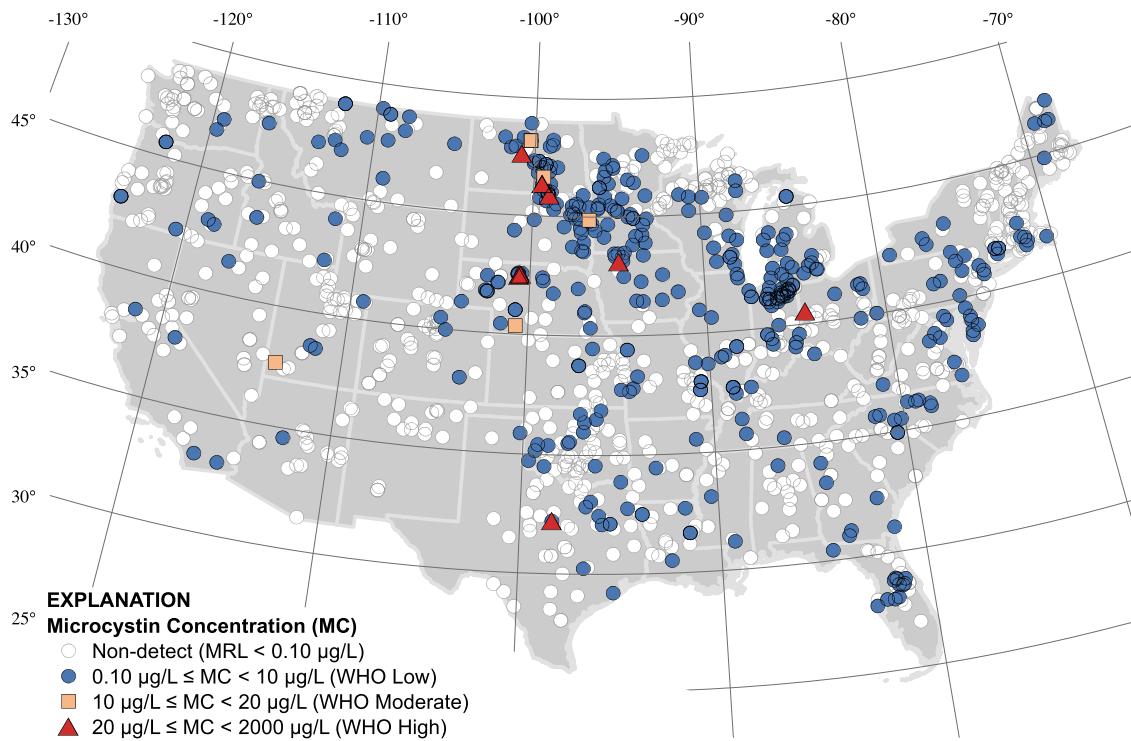


Fig. 2. United States occurrence of microcystins in the contiguous 48 U.S. states categorized by World Health Organization relative probable health risk. WHO low, moderate, and high refer to the relative human recreational health thresholds for microcystin exposure. Map not shown to scale.

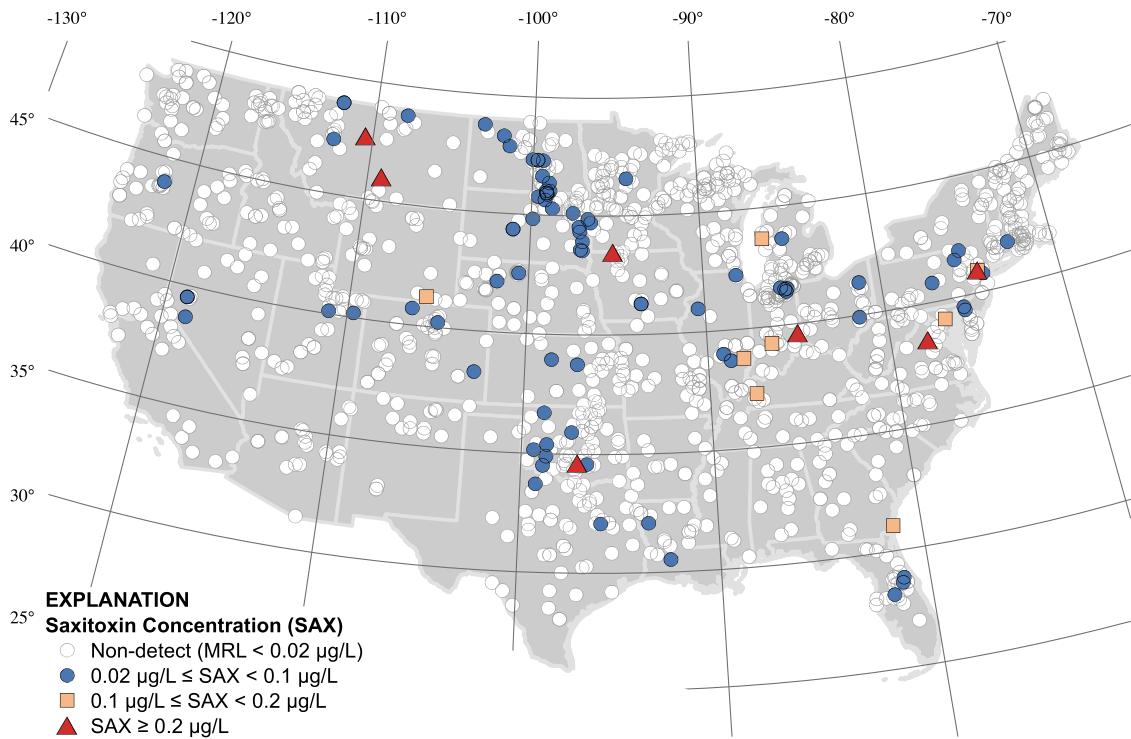


Fig. 3. United States occurrence of saxitoxins in the contiguous 48 U.S. states. Map not shown to scale.

Anatoxin-a, cylindrospermopsin, 5 microcystin congeners, and nodularin-R were detected by LC/MS/MS. ANAA was detected in 15% of samples and was detected with microcystins in 3 of 4 samples positive for microcystins, co-occurred with CYLS in a sample with no microcystin detections, and did not co-occur with saxitoxins. CYLS was detected in 7.1% of samples and did not co-occur with microcystin (Loftin et al., 2015, App. 10). MCLA, MCLR,

MCLY, MCRR, MCYR, and NODR were detected by LC/MS/MS in 13, 38, 19, 22, 26, and 3% of samples, respectively. MCLR was the most frequently occurring microcystin congener and also had the largest mean concentration (8.5 µg/L). NODR was only detected in 1 sample and had a concentration of 0.023 µg/L. The maximum number of microcystin congeners detected within an individual sample was 5 of the 7 measured congeners. Sixty-four percent

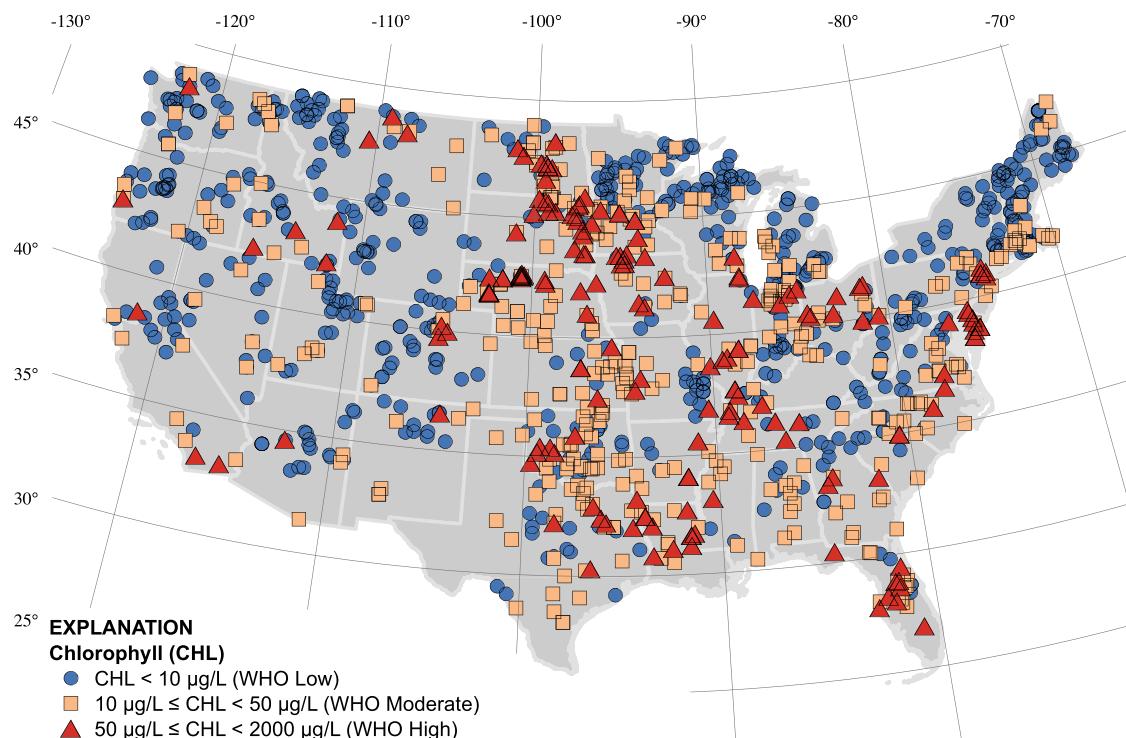


Fig. 4. United States occurrence of chlorophyll-*a* in the contiguous 48 U.S. states categorized by World Health Organization relative probable health risk due to microcystins. WHO low, moderate, and high refer to the relative human recreational health thresholds for microcystin exposure. Map not shown to scale.

($n = 9$ of 14) of samples positive for microcystins by LC/MS/MS had only 2 or 3 congeners measured. Increasing summed microcystin concentration was not necessarily an indicator of increasing congener number, but samples $\geq 6.3 \mu\text{g/L}$ in this subset of samples always had at least 2 congeners.

3.4. Chlorophyll in the contiguous 48 U.S. States

Ninety-nine percent of samples had measurable chlorophyll (Fig. 4). Concentrations ranged from <0.10 to $940 \mu\text{g/L}$ (mean: $29 \mu\text{g/L}$; median $7.6 \mu\text{g/L}$) and is consistent with other work (Jones and Bachman, 1976; Graham et al., 2004; Phillips et al., 2008). Chlorophyll concentrations $>10 \mu\text{g/L}$ (79% of all samples) predominately occurred east of -100° longitude (e.g. including and east of midwest states). Seventy percent of the western U.S. samples and 85% of samples from New England states had concentrations $<10 \mu\text{g/L}$ and nearly all samples were below $50 \mu\text{g/L}$ (91% of samples west of -100° longitude and 100% of New England samples). Chlorophyll values have been reported in lakes from other studies to range from <1 up to $546 \mu\text{g/L}$ in the central and upper midwestern regions of the United States in Graham et al. (2004). Likewise chlorophyll in 1138 European lakes ranged from <1 up to approximately $300 \mu\text{g/L}$ (Phillips et al., 2008).

3.5. Occurrence of phytoplankton with emphasis on cyanobacteria in the contiguous 48 U.S. States

Overall phytoplankton abundance ranged from 3 to $5.0\text{E}6$ cells/mL with a mean abundance of $4.0\text{E}4$ cells/mL. Cyanobacteria (Cyanophyta) were present in 98% of samples (Fig. 5) and dominated the phytoplankton community (greater than 50% of total phytoplankton abundance) in 76% of samples. A total of 54 different cyanobacterial genera were identified, 22 of which have been identified as potential cyanotoxin producers. Potential anatoxin-, cylindrospermopsin-, microcystin-, nodularin- and saxitoxin-producing cyanobacteria occurred in 81, 67, 95, 0.24,

and 79%, of samples respectively (Table 1), and highest monthly mean and maximum abundances were in July, August, and September (Table SI-4).

Microcystis sp. a genera known for microcystin production, was the most commonly detected cyanobacteria genera (57% of samples) and had the highest mean and maximum abundance ($2.9\text{E}4$ and $5.0\text{E}6$ cells/mL, respectively) of all cyanobacteria genera (Table 1). Several cyanobacteria genera were also relatively common throughout the United States and include *Anabaena* sp. (55% of samples), *Chroococcus* sp. (48%), *Aphanocapsa* sp. (35%), *Oscillatoria* sp. (34%), and *Aphanizomenon* sp. (28%) (Fig. SI-1).

3.6. Potential anatoxin-producing cyanobacteria

Potential anatoxin producers occurred in 81% of samples with mean, median, and maximum abundance values of $4.6\text{E}3$, $7.5\text{E}2$, and $3.0\text{E}5$ cells/mL, respectively (Table 1). The most frequently occurring potential anatoxin producer, *Anabaena* sp., was present in 55% of samples. The cyanobacteria genera with the highest abundances was *Aphanizomenon* sp. with a mean of $8.6\text{E}3$ and median of $7.6\text{E}2$ cells/mL (Table 1; Fig. SI-2).

3.7. Potential cylindrospermopsin-producing cyanobacteria and comparison with cylindrospermopsin concentrations

Potential cylindrospermopsin producers occurred in 67% of samples with mean, median, and maximum abundance values of $6.8\text{E}2$, $6.9\text{E}3$, and $3.0\text{E}5$ cells/mL, respectively. The most frequently occurring potential producer was *Anabaena* sp. detected in 55% of samples, but *Aphanizomenon* sp. tended to have the greater median ($7.6\text{E}2$ cells/mL), mean ($8.6\text{E}3$ cells/mL), and maximum ($3.0\text{E}5$ cells/mL) abundance values (Table 1). The least common potential cylindrospermopsin producer was *Cylindrospermopsis* sp. detected in only 3.8% of samples. Of the 50 samples with detectable cylindrospermopsin by ELISA, 7 did not have known potential producers present; cylindrospermopsin concentrations in these

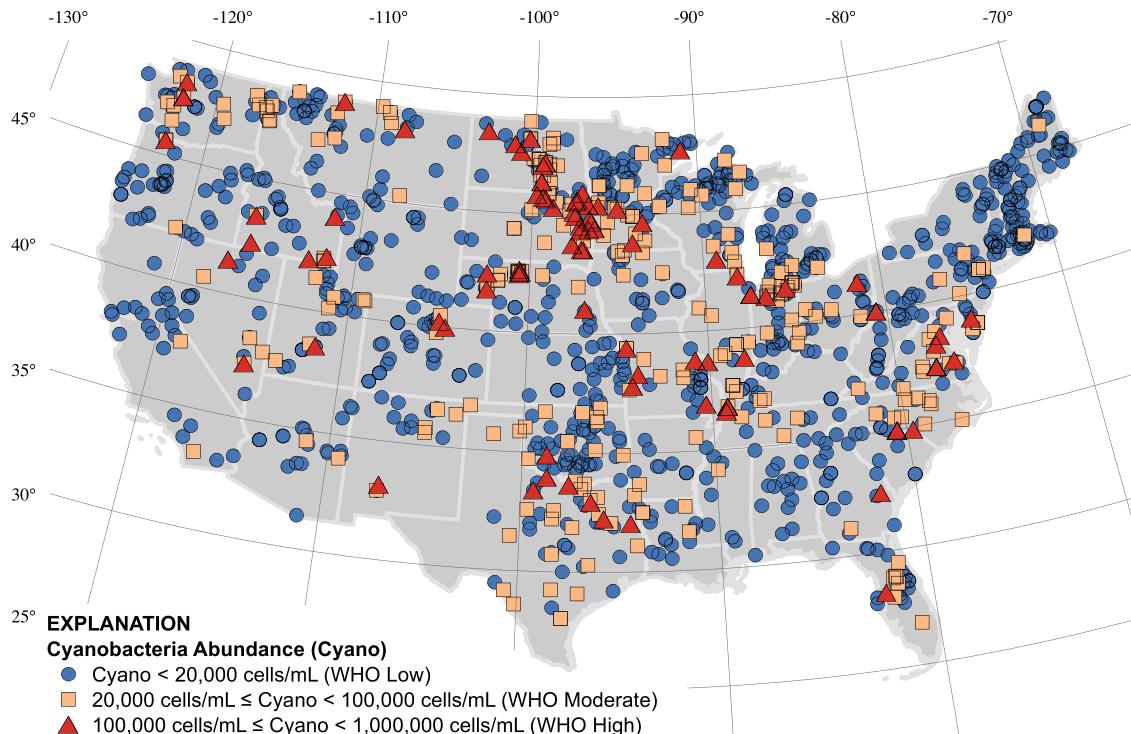


Fig. 5. Cyanobacteria abundance in the contiguous 48 U.S. states categorized by World Health Organization relative probable health risk due to microcystins. WHO low, moderate, and high refer to the relative human recreational health thresholds for microcystin exposure. Map not shown to scale.

samples ranged from 0.05 to 1.5 µg/L. Cyanobacteria and phytoplankton abundance in samples without known cylindrospermopsin producers were low (less than 3400 and 5400 cells/mL, respectively). Two cyanobacteria genera, *Anabaena* sp. and *Aphanizomenon* sp., combined represented 100% of the total potential cylindrospermopsin-producer cell abundance in 47% of samples with detectable cylindrospermopsins, and were dominant in 60% of samples. Five of the remaining 43 samples (12%) positive for cylindrospermopsin, were explained by the presence of *Cylindrospermopsis* sp. in 4 of those samples (that had associated phytoplankton data available) with concentrations ranging from 0.05 to 0.71 µg/L.

3.8. Potential microcystin-producing cyanobacteria and comparison with microcystin concentrations

Potential microcystin producers occurred in 95% of samples, and mean, median, and maximum concentrations were 3.0E4, 3.7E3, and 5.0E6, respectively (Table 1). Of the 405 samples with detectable microcystins, 5 had no cyanobacteria present and 6 had no known potential microcystin producers. Microcystin concentrations in samples lacking known potential producers ranged from 0.10 to 1.2 µg/L. Potential microcystin producers were the dominant member of the cyanobacteria community in 80% of samples, and the dominant member of the phytoplankton community in 57%. Two cyanobacteria genera, *Microcystis* sp. (57%) and *Anabaena* sp. (55%), were the most frequently occurring potential microcystin-producing cyanobacteria (Table 1), followed by *Aphanocapsa* sp. (35%) and *Oscillatoria* sp. (34%). The pico-plankton, *Synechococcus* sp. and *Synchocystis* sp. were identified in ≥24 and 17% of samples, respectively.

3.9. Potential nodularin-producing cyanobacteria

A potential nodularin-producer, *Nodularia* sp., was present in 3 samples (2 from North Dakota and 1 from South Dakota), with abundances ranging from 509 to 7090 cells/mL. Other known

potential nodularin producers such as *Nostoc* sp. were not present in any of the samples collected for this study. One of the three samples with *Nodularia* sp. had detectable microcystins/nodularins (12 µg/L) and saxitoxins (0.03 µg/L) as measured by ELISA. None of these samples were analyzed by LC/MS/MS. There was a separate Texas site with NODR (0.023 µg/L) detected by LC/MS/MS; microcystins were not detected by LC/MS/MS or ELISA in this sample. Though typically considered a brackish-water toxin, NODR has occasionally been detected in inland freshwaters (Beattie et al., 2000; Graham et al., 2010).

Conductivity of the 3 samples positive for *Nodularia* sp. ranged from 2140 to 9379 µS/cm, which equates to an estimated salinity range of 1.2–5.4 PSU (practical salinity units). The sample positive for nodularin-R by LC/MS/MS had an estimated salinity of 0.14 PSU. The area around this site in Texas appeared to have had nearly 2.5 times the normal precipitation from May 3 through July 26, 2007 (sample was collected July 26, 2007) compared to average precipitation between 1981 and 2010 for the same dates (NCDC, 2007) with most precipitation received prior to July 10, 2007.

It was not expected that *Nodularia* sp. would be common in this study because sites were typically excluded from this study if they could not be classified as freshwater. Salinity was elevated at or above 2.0 at 38 sites (3% of samples) ranging from 2.0 to 34 (Fig. SI-3 shows average salinities for samples ≥2.0). One to three elevated salinity sites were located in Colorado, Georgia, Kansas, Montana, Nebraska, Nevada, Oklahoma, Texas, Washington, and Wyoming. Of note, South Dakota and North Dakota had 6 and 17 elevated salinity sites, respectively. Many of these sites possessed conditions conducive to *Nodularia* sp. growth.

3.10. Potential saxitoxin-producing cyanobacteria

Potential saxitoxin producers occurred in 79% of samples and mean, median, and maximum abundances were 7.5E3, 8.8E2, and 3.0E5, respectively (Table 1). Of the 96 samples with detectable saxitoxins, 23 did not have known potential producers present. In

Table 2

Comparison of percentage of samples categorized by WHO relative probable health risk for microcystin exposure.

WHO relative health threshold	Percentage of samples (%) by category			
	Microcystin (n = 1250)	Chlorophyll-a (n = 1234)	Cyanobacteria abundance (n = 1247)	Potential microcystin producing cyanobacteria (n = 1247)
Low	99	55	73	80
Moderate	0.40	30	20	15
High	0.72	14	6.8	4.6
Very High	0.0	0.0	0.0	0.0

n, number of total samples.

samples with detectable saxitoxin and lacking known potential producers, concentrations ranged from 0.070 to 0.26 µg/L. Concentrations from 5 of 23 samples ranged from 0.11 to 0.26 µg/L. Samples with detectable saxitoxins typically had at least one potential saxitoxin producer present such as *Anabaena* sp., *Aphanizomenon* sp., *Oscillatoria* sp., or *Lyngbya* sp. and was usually the dominant genera in samples with detectable saxitoxins. One hundred percent dominance of *Anabaena* sp. was observed compared to other potential saxitoxins producers in 10 of 73 samples. Saxitoxin concentrations ≥0.12 µg/L always had 2–4 potential producers present. Most of these samples were dominated by *Anabaena* sp., *Aphanizomenon* sp., and *Oscillatoria* sp. Of note, *Cylindrospermopsis* sp. was dominant in the sample with the largest saxitoxin concentration.

3.11. Recreational assessment of human health risk from microcystin exposure based on World Health Organization guidelines for microcystins, chlorophyll, and cyanobacteria abundance

Based on WHO guidelines for microcystin, 99% of samples indicated a low risk, 0.40% a moderate risk, and 0.72% a high risk (Table 2). By comparison, based on cyanobacterial abundance and chlorophyll, 20 and 30% of samples indicated moderate risk, respectively and 6.8 and 14% high risk, respectively. Therefore, as the specificity of the metric used to indicate risk decreased (specificity: microcystin > cyanobacteria abundance > chlorophyll), the proportion of samples indicative of moderate and high risk increased (number of samples with elevated risk: microcystin < cyanobacteria abundance < chlorophyll). Based on the direct

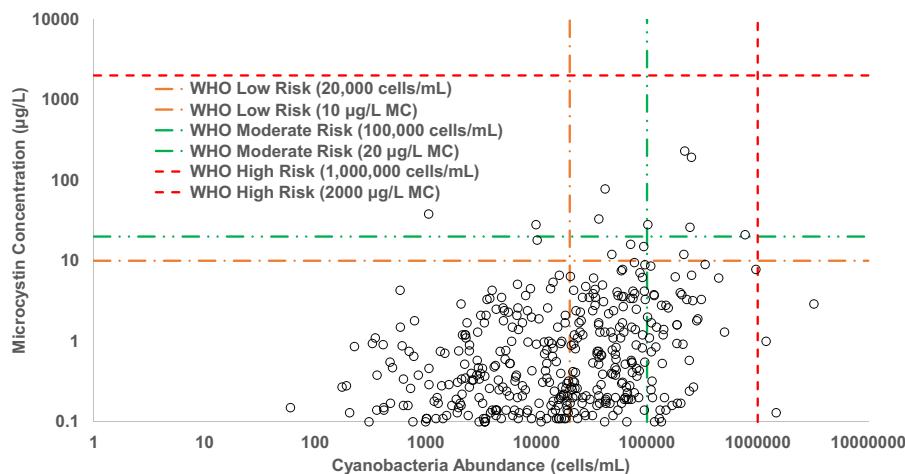


Fig. 6. Microcystin concentrations compared with chlorophyll as a function of WHO adverse probable health risk guidelines.

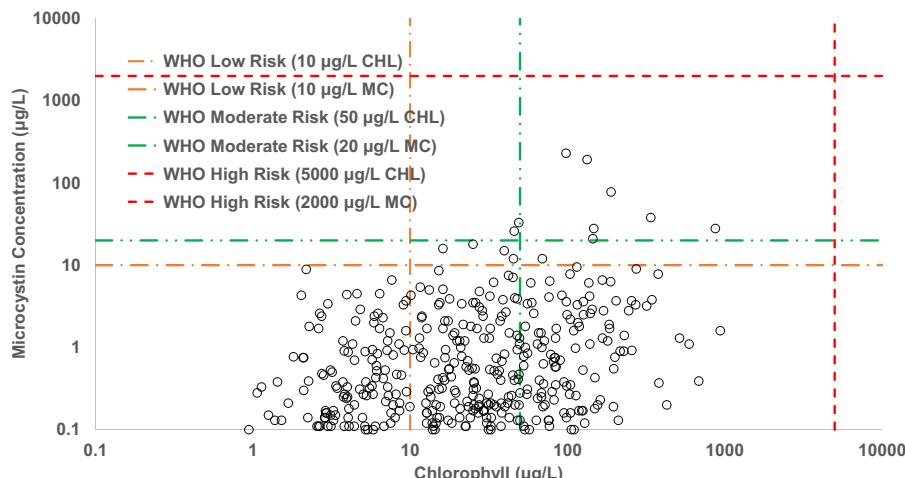


Fig. 7. Microcystin concentrations compared with cyanobacteria abundance as a function of WHO adverse probable health risk guidelines.

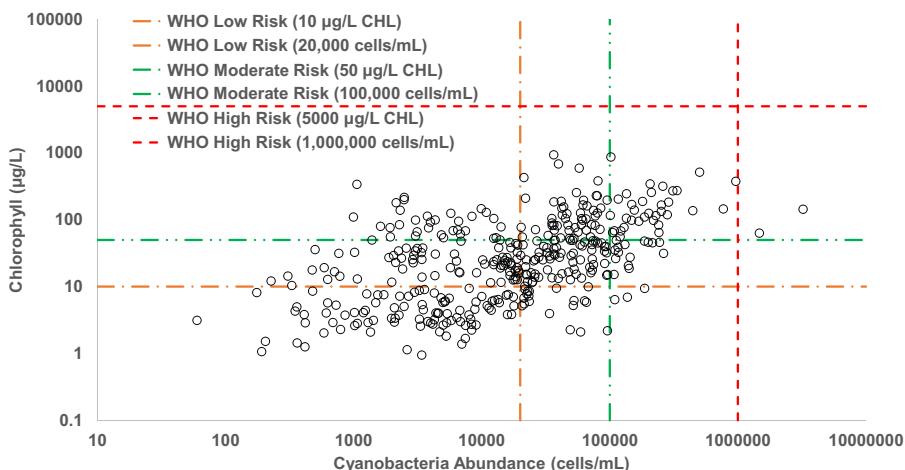


Fig. 8. Chlorophyll concentrations compared with cyanobacteria abundance as a function of WHO adverse probable health risk guidelines.

microcystin measurement metric, only 1.1% of samples were indicative of moderate to high risk, compared to 27% of samples based on cyanobacterial abundance (Fig. 6), and 44% of samples based on chlorophyll (Fig. 7), indicative of overall algal biomass. Fig. 8 shows that for samples positive for microcystins, but categorized by WHO chlorophyll and cyanobacteria abundance thresholds for low, moderate, and high risk, 24, 18, and 8.1% of samples showed agreement between WHO thresholds with chlorophyll biased high compared with cyanobacteria abundance. Overall, agreement among all 3 risk assessment metrics only occurred in 27% of samples with 24, 0.51, and 2.3% of samples accounted for in the WHO low, moderate, and high thresholds, respectively. Refinement of the cyanobacteria abundance metric to include only potential microcystin producing cyanobacteria slightly improved the relationship (Fig. SI-4), but over 20% of the samples were still classified as moderate to high risk, compared to 1.1% based on the microcystin metric (Table 2). When samples positive for microcystin only were examined (e.g. non-detects excluded), categorization of risk by all three WHO metrics showed agreement in only 50% of samples, despite a mean cyanobacteria dominance of 80% in the sample subset. Comparison of the chlorophyll and cyanobacteria abundance metrics in samples without detectable microcystin (e.g. microcystin detections excluded) showed agreement in 71% of samples.

4. Discussion

The 2007 NLA study begins to put cyanoHAB issues into a national context for lakes and reservoirs of the United States. Cyanobacterial harmful algal blooms are dynamic in their occurrence, persistence, and magnitude, and cyanotoxin occurrence is expected to vary with respect to physical, chemical, and biological conditions. This 2007 dataset provides a snapshot of cyanotoxin occurrence in lakes and reservoirs in the United States. Study findings indicate that: (1) microcystins are still the most commonly detected cyanotoxins, but ELISA and LC/MS/MS analyses indicate that more work is needed to characterize the occurrence of other understudied cyanotoxin classes; (2) cyanotoxins were detected in nearly every US state sampled, (3) cyanotoxin risk assessment is complex, (4) microcystin risk estimates based on World Health Organization guidelines varied depending on the metric used (microcystins, cyanobacteria abundance, or chlorophyll) even though potential microcystin-producing cyanobacteria were dominant in most samples, (5) risk assessment by microcystin alone was not necessarily protective regarding exposure risk to other cyanotoxin classes.

4.1. Cyanotoxins were detected in nearly every U.S. State sampled

Thirty-eight percent of lakes and 92% of states had detectable cyanotoxins during the 2007 NLA, clearly indicating that cyanoHABs are a national issue in the United States. Potential cyanotoxin producers were present in all 48 states sampled. Anecdotal and documented reports of cyanoHAB-related illnesses throughout the Nation demonstrate that there are clear human and animal health risks (Backer et al., 2015). Those states without cyanotoxin detections during the 2007 NLA (New Hampshire, New Mexico, South Carolina, and Vermont) have had cyanotoxin detections and/or suspected poisonings noted in previous studies (Graham et al., 2009; Journey et al., 2010; Carey et al., 2012).

Previous investigations (Yilmaz et al., 2008; Graham et al., 2009; Williams et al., 2009; Graham et al., 2010) have documented cylindrospermopsin occurrences in Florida, the midwestern U.S. and New York (Boyer, 2007). Boyer et al. (2007) reported cylindrospermopsins were detected rarely in New York (1.6%) contrasted with 22% in Florida (Williams et al., 2009). Cylindrospermopsin was reported in Missouri in 2009 with 14% ($n = 36$) of lakes having detectable concentrations by ELISA with all concentrations less than 1.0 µg/L (Graham and Jones, 2009). In a study conducted in 2006 in the midwestern U.S., cylindrospermopsins were detected in 8.7% ($n = 23$) of lakes with one detection each in Iowa and Kansas and concentrations did not exceed 0.14 µg/L (Graham et al., 2010).

Globally, *Cylindrospermopsis* sp. has been reported to be a widespread concern regarding production of cylindrospermopsins (Briand et al., 2004), but was only identified in 3.8% of samples in the 2007 NLA. Yilmaz et al. (2008) found that *Cylindrospermopsis* sp. strains isolated from Florida lacked the genetic capability to produce cylindrospermopsins and attributed cylindrospermopsin production to *Aphanizomenon* sp. or *Anabaena* sp. This evidence, combined with research showing that American strains of *Cylindrospermopsis* sp. are genetically distinct from African, Australian, and European strains (Dyble et al., 2002; Gugger et al., 2005) where cylindrospermopsin occurrence is more prevalent, suggests that *Cylindrospermopsis* sp. was likely not widely responsible for cylindrospermopsin occurrence in 2007 given the lack of association of *Cylindrospermopsis* sp. co-occurrence with cylindrospermopsin in the 2007 NLA. Poniedzialek et al. (2015) recently reported that both cylindrospermopsin and non-cylindrospermopsin producing *Cylindrospermopsis raciborskii* strains isolated from European waters possessed toxic metabolites that induced human white blood cell apoptosis and necrosis after 1 h of exposure. This finding necessitates investigation to determine if North American strains possess the ability to produce these unidentified toxins.

Microcystin occurred less frequently in the Pacific northwest region than expected, given historical reports of toxin occurrence and human illness back to 1976 (Jacoby and Kann, 2007; Trainer and Hardy, 2015). Human illnesses related to microcystin and anatoxin-a have been reported in the state of Washington since the mid-1970s (Trainer and Hardy, 2015). Fastner et al. (1999) summarized global microcystin concentration range from <1 to 1300 µg/L in Canada, Finland, Germany, Japan, Portugal, and Thailand. Microcystins have also been measured in Australia, Canada, Denmark, France, Germany, Japan, Portugal, South Africa, and the United Kingdom having microcystin concentrations ranging from <1 to 7100 µg microcystin per g of dry weight biomass. There have been a few other detections of NODR in freshwaters with no apparent producer present (Graham et al., 2010), suggesting there may be other, currently unknown NODR producers or perhaps a terrestrial source is possible from inflow events (Gehringer et al., 2012).

Saxitoxins have not been reported as frequently in freshwaters as microcystins, or even cylindrospermopsins, in part because they have not been analyzed for often. A study from Spain (Wörmer et al., 2011) detected saxitoxins in 5 of 41 (12%) freshwater lakes with saxitoxin concentrations ranging from 0.01 to 4.99 µg/L. Haas and Henriksen (2000) found 8 of 96 (8.3%) lakes and ponds in Denmark were positive for saxitoxins in samples collected with saxitoxin concentrations ranging from 14.7 to 219 µg/g dry weight (DW) of phytoplankton. Saxitoxins were detected in 17% ($n = 23$) of near-shore, targeted cyanobacteria accumulation samples in a study conducted in Iowa, Kansas, Minnesota, and Missouri with concentrations ranging from 0.02 to 0.19 µg/L (Graham et al., 2010). Concentrations have been reported as high as 1000 µg/L in a study of Finnish lakes (Rapala et al., 2005). Additional work is warranted in freshwater systems to identify other saxitoxin analogues as well. Data summarized in Chorus and Bartram (1999) indicate that samples of *Aphanizomenon flos-aquae* found in New Hampshire and *Cylindrospermopsis raciborskii* found in Brazil have been positive for both saxitoxin and neosaxitoxin. At least 12 different saxitoxins have been identified in *Anabaena circinalis* from Australia and *Lyngbya wollei* from a reservoir in Alabama had 9 saxitoxin analogues identified (summarized in Chorus and Bartram, 1999).

Primary producers such as cyanobacteria exist in the photic zone prior to near-surface stranding due to loss of buoyancy control and subsequent accumulation caused by wind and wave action. Results from mid-lake, integrated photic zone (ambient samples) such as acquired in the 2007 NLA provide a contrast to studies focused on near-shore cyanobacteria accumulations. It is known that toxin occurrence and concentration can be much higher in near-shore cyanobacteria accumulations (Lindon and Heiskary, 2009; Graham et al., 2010) and therefore ambient sampling efforts can produce the minimum risk associated with cyanoHABs in comparison. Mid-lake, integrated photic zone sampling is important for assessing cyanotoxin exposure during recreational activities that could aerosolize cyanotoxins (e.g. skiing and boating), drinking water intake vulnerability, and ecological impacts such as a loss of species diversity, toxicity, and impaired food web dynamics. Sampling near-shore accumulations is still critical to understanding recreational exposure risks at swimming beaches and is done by many states and also has ecological implications (Chorus and Bartram, 1999; Graham et al., 2009).

4.2. Cyanotoxin risk assessment is complicated

The potential for co-occurrence of multiple cyanotoxin classes, disparity between the presence of potential toxin producers and toxin occurrence, and seasonal changes in cyanobacterial community composition makes assessment quite difficult in the absence of mechanistic understanding regarding why and when toxins are

produced. Potential anatoxin, cylindrospermopsin, microcystin, and saxitoxin producers were prevalent throughout the nation in 2007. While potential producer presence does not automatically equate with cyanotoxin occurrence, it is known that a producer is necessary to make cyanotoxins. Conversely, the percent of the time that detected toxin corresponded with a known potential producer was 6.0, 34, and 9.7% for cylindrospermopsins, microcystins, and saxitoxins, respectively. While part of this mismatch can be characterized as dissolved-phase toxin transport from lysed cells, this also exemplifies the known disparity between toxin production and the presence of potential producers as identified by microscopy. There are clear implications for surrogate thresholds used in public health protections balanced against socioeconomic issues for microcystins and other cyanotoxin classes. Likewise, year to year variability in cyanotoxin occurrence is also a concern because this increases the difficulty of prediction and risk avoidance.

There are other classes of cyanotoxins that are not well studied, and are therefore not commonly measured such as aeruginosins, anabaenopeptins, other anatoxin analogues, anatoxin-a(s), cyano-peptilins, lyngbyatoxins, microcystins, oscillamides, and osillaginins (Sano and Kaya, 1997; Chorus and Bartram, 1999; Erhard et al., 1999; Sano et al., 2001; Graham et al., 2009; Welker et al., 2006; Chorus, 2012). In light of the staggering number of toxins, many without commercially available analytical standards or standards of sufficient quality, the prospect of risk assessment becomes staggering and costly, and further contributes to the overall lack of toxicity and epidemiology studies regarding human health. While scientifically there is a need for traditional analyte-by-analyte chemical approaches, effects-based screening methods supported by chemistry for interpretation will likely be a necessity in the future. This approach is already being developed for other classes of chemicals and is used in a more limited role for the more commonly studied cyanotoxins (Doyle et al., 2015).

4.3. Assessment of microcystins based on provisional WHO cyanobacteria abundance and chlorophyll guidance thresholds

Recreational risk estimated using the WHO guidance for cyanobacteria abundance and chlorophyll metrics was higher than risk estimates using the microcystin metric. Agreement by all 3 risk assessment metrics only occurred in 27% of samples with 24, 0.51, and 2.3% of samples accounted for in the WHO Low, Moderate, and High thresholds, respectively. Interestingly, of all samples that exceeded 100,000 cells/mL for cyanobacteria abundance ($n = 85$) only 65% had detectable levels of microcystin (>0.10 µg/L). When cyanobacteria were dominant, chlorophyll can be a good predictor of cyanobacteria (Forget et al., 2009; Ha et al., 2009; Hosikian et al., 2010), but not necessarily toxin occurrence. Some studies have noted strong correlations between microcystin and chlorophyll (Fastner et al., 1999), but this relation is dependent on dominance by microcystin-producing cyanobacteria. These relations form the basis for the WHO thresholds for cyanobacteria abundance and chlorophyll as predictors of potential microcystin exposure (Chorus and Bartram, 1999). Based on this understanding, it should be expected that microcystin exposure assessed with chlorophyll as a surrogate will provide a higher frequency of elevated warnings compared with cyanobacteria abundance and microcystin concentration (Table 2) when microcystin-producing cyanobacteria are not dominant.

Whether WHO risk assessments based on cyanobacteria or chlorophyll are perceived as overprotective depends on whether risk assessment balanced against socio-economics is narrowly focused on microcystins, or if other cyanotoxin classes less commonly measured are also considered a concern. Additionally, chlorophyll measurement, frequently used as an index for eutrophication, is still the most accessible option for early warning

of HABs and can be measured in the laboratory or with continuous in situ sensors and remotely sensed data. Even visual assessment of water color can be used by a well-educated public to give an instantaneous indicator warning of a potential HAB problem (Chorus and Bartram, 1999; Mattas-Curry et al., 2015).

4.4. Rationale for metric selection for cyanotoxin risk assessment

WHO provided a basis for assessment of microcystin risk based on microcystin, cyanobacteria, and chlorophyll metrics. At least in this data set, relations do not appear to consistently predict the same level of risk based on threshold levels for microcystin exposure. The benefits of cyanobacteria abundance, chlorophyll, and other useful relations covering all classes of toxins including those yet to be discovered should also be considered. While many entities do measure for microcystins, most do not currently (2016) assess the potential for other classes of toxins and are therefore defaulting to WHO microcystin guidelines to protect public health by proxy. This approach simplifies risk assessment, but monitoring samples that do not exceed an advisory threshold based on microcystin may have other cyanotoxins of potential environmental health concern present. Comparison of cylindrospermopsin and saxitoxin detections with WHO microcystin risk thresholds revealed that microcystin WHO thresholds can be a poor predictor of cylindrospermopsin and saxitoxin occurrence with 0% ($n = 50$) of cylindrospermopsin detections and only 3.1% ($n = 96$) of saxitoxin detections coinciding with a WHO microcystin threshold of moderate or high relative health risk. The presence of cylindrospermopsins or saxitoxins does not necessarily dictate increased risk, but in the absence of recreational health risk thresholds it is difficult to put these results in proper context.

4.5. Toxicity of cylindrospermopsins and saxitoxins

Only 4 samples exceeded 2.0 µg/L of cylindrospermopsin and 1 measurement exceeded the lowest U.S. state guidance value of 4.0 µg/L (California) out of 1249 samples. No samples exceeded even the lowest state saxitoxin threshold of 0.80 µg/L. These samples were all integrated photic zone samples and as such tend to be lower in both toxin and biomass compared to near-shore, targeted harmful algal bloom samples as has been shown by other studies (Lindon and Heiskary, 2009; Graham et al., 2010).

In this study, positive detections of cylindrospermopsins, microcystins, and saxitoxins did not have corresponding potential producers present in 14, 2.7, and 24% of samples, respectively. Separation of the toxin from the producer is commonly interpreted as coming from previously lysed planktonic cyanobacteria, however consideration should also be given to benthic and epiphytic forms. Given that visual warning of surface accumulations are commonly the first step in triggering a response to an event, attached algae represent a potential "unseen" risk where cyanotoxins may be transported large distances from their potential producers rendering cyanobacteria abundance and chlorophyll metrics ineffective in these cases. It is not clear what risk is associated with these types of events and the potential for cyanotoxin dilution during dissolved-phase transport. All of these challenges collectively make cyanotoxin risk assessment difficult and potentially expensive.

4.6. Options for rapid cyanotoxin assessment in the future

Public health protection is a balance between precaution, scientific understanding, competing risks, maximum resource utilization, and minimal socioeconomic impact. Direct measurement of a single, or a few, cyanotoxins in an event-based sampling scheme does little to prevent exposure to the potential pool of

cyanotoxins that may be present. It therefore becomes ever more important to relate early predictors to toxin production and relative risk. Technological advances have led to improvements for direct and indirect (surrogate) measures of cyanotoxins. Four areas showing promise for the future include real-time surrogate measures, biosensors, molecular techniques, and remote sensing (Singh et al., 2012; Moreira et al., 2014; Lunetta et al., 2015). While these techniques have been available for some time, validation at a national scale is still needed because matrix interferences vary from lake to lake just as limiting environmental factors that control phytoplankton succession and toxin production. Ultimately, use of all of these tools simultaneously should provide converging lines of evidence and therefore better understanding of cyanoHABs and perhaps better identification of early warning indicators when toxins will be produced.

Supporting information

All cyanotoxin data can be obtained via weblink at <http://pubs.er.usgs.gov/publication/ds929>. All other 2007 National Lake Assessment datasets can be obtained via weblink at http://water.epa.gov/type/lakes/NLA_data.cfm.

Acknowledgements

The USGS collaborated with EPA, and the U.S. states and tribes on the 2007 National Lakes Assessment to measure microcystins. USGS later expanded the scope of this study in collaboration with EPA to include measurement of cylindrospermopsins and saxitoxins to better understand the occurrence of cyanotoxin mixtures. Support was provided by the EPA Office of Wetlands, Oceans, and Watersheds (Interagency Agreement Number IA#DW1492215401), the EPA Office of Research and Development National Health and Environmental Effects Research Laboratory (IA#DW14958073), the USGS National Water Quality Assessment Program (NAWQA), and the USGS Toxic Substances Hydrology Program. The authors wish to acknowledge Bettie Kreakie, EPA and Barry Rosen, USGS for early reviews of the manuscript. The views expressed in this report by the EPA authors are those of the individual authors and do not necessarily reflect the views and policies of the U.S. Environmental Protection Agency. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.[CG]

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.hal.2016.04.001](https://doi.org/10.1016/j.hal.2016.04.001).

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