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Multiple co-occurring and persistently detected cyanotoxins and associated cyanobacteria in adjacent California lakes

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.toxicon.2020.12.019.

Ethical statement

The authors adhered to Elsevier's publishing ethics policy and ethical guidelines for journal publication.

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Abstract

The global proliferation of toxin producing cyanobacterial blooms has been attributed to a wide variety of environmental factors with nutrient pollution, increased temperatures, and drought being three of the most significant. The current study is the first formal assessment of cyanotoxins in two impaired lakes, Canyon Lake and Lake Elsinore, in southern California that have a history of cyanobacterial blooms producing high biomass as measured by chl-a. Cyanotoxins in Lake Elsinore were detected at concentrations that persistently exceeded California recreational health thresholds, whereas Canyon Lake experienced persistent concentrations that only occasionally exceeded health thresholds. The study results are the highest recorded concentrations of microcystins, anatoxin-a, and cylindrospermopsin detected in southern California lakes. Concentrations exceeded health thresholds that caused both lakes to be closed for recreational activities. Cyanobacterial identifications indicated a high risk for the presence of potentially toxic genera and agreed with the cyanotoxin results that indicated frequent detection of multiple cyanotoxins simultaneously. A statistically significant correlation was observed between chlorophyll-a (chl-a) and microcystin concentrations for Lake Elsinore but not Canyon Lake, and chl-a was not a good indicator of cylindrospermopsin, anatoxin-a, or nodularin. Therefore, chl-a was not a viable screening indicator of cyanotoxin risk in these lakes. The study results indicate potential acute and chronic risk of exposure to cyanotoxins in these lakes and supports the need for future monitoring efforts to help minimize human and domestic pet exposure and to better understand potential effects to wildlife. The frequent co-occurrence of complex cyanotoxin mixtures further complicates the risk assessment process for these lakes given uncertainty in the toxicology of mixtures.

Keywords

Cyanotoxins; Cyanobacteria; SPATT

1. Introduction

Toxin-producing cyanobacterial blooms have increased globally in frequency, duration, and geographic extent (Carmichael, 2008; Hudnell and Dortch, 2008; Paerl and Huisman, 2009; O’Neil et al., 2012; Paerl and Paul, 2012; Paerl and Otten, 2013; Quiblier et al., 2013; Hudon et al., 2014; Wood et al., 2014). These increases have been attributed to a wide variety of factors such as eutrophication and increases in temperature, salinity, water residence time, water column stratification, and pH, many of which will likely be exacerbated with greater periods of drought (Paerl, 1988; Paerl and Fulton, 2006; Carmichael, 2008; Paerl and Huisman, 2009; Paerl et al., 2011; O’Neil et al., 2012; Paerl and Paul, 2012; Paerl and Otten, 2013; Lehman et al., 2017). Many of these factors covary and are ultimately intertwined, but generally eutrophication and increased temperatures are

considered the most important factors contributing to the global increase in cyanobacterial harmful algal blooms (cyanoHABs) (Heisler et al., 2008; Paerl and Huisman, 2009; O'Neil et al., 2012; Paerl and Otten, 2013; Rigosi et al., 2014; Paerl et al., 2018).

Cyanotoxins have caused illness and mortality in humans, livestock, pets, and wildlife (Edwards et al., 1992; van Halderen et al., 1995; Mez et al., 1997; Pouria et al., 1998; Carmichael, 2001; Backer et al., 2008, 2013; Stewart et al., 2008; Wood et al., 2010; Li et al., 2011; Trevino--Garrison et al., 2015; Vidal et al., 2017), even though cyanotoxin poisoning in pets and livestock has been shown to be substantially under-reported (Wood et al., 2010; Backer et al., 2013; Trevino-Garrison et al., 2015). Cyanotoxins produced in inland drained or drainage lakes can have far reaching effects downstream from their origin. Cyanotoxin transport from such lakes has caused effects in marine environments such as the mortality of over 30 threatened marine California sea otters (*Enhydra lutris*) and cyanotoxin bioaccumulation in marine shellfish (Miller et al., 2010; Kudela, 2011; Gibble and Kudela, 2014; Preece et al., 2015, 2017; Gibble et al., 2016; Peacock et al., 2018). Microcystins can be retained in California mussels (*Mytilus californianus*) for up to 8 weeks after exposure but oysters (*Crassostrea* sp.) purged microcystins relatively quickly compared to mussels (Gibble et al., 2016).

Due to associated adverse health effects and the growing recognition that toxin-producing cyanobacterial blooms can severely affect water quality (Chapman, 2015; Brooks et al., 2017), many regulatory and health agencies have established guidance or health advisory thresholds. The World Health Organization (WHO) established guidance values for the probability of acute health effects from recreational activities based on the number of cyanobacteria cells, the concentration of microcystin-LR, and the concentration chlorophyll-*a* (chl-a) (Chorus and Bartram, 1999). The WHO guidance levels are categorized into low, medium, or high probability of adverse health effects. The low category has guidance levels of <20,000 cyanobacteria cells mL^{-1}, <10 μg L^{-1} of microcystin-LR, or <10 μg L^{-1} of chl-a. The medium category has levels of 20,000–100,000 cells mL^{-1}, 10–20 μg L^{-1} microcystin-LR, or 10–50 μg L^{-1} of chl-a. The high probability category has levels of 100,000–10,000,000 cells mL^{-1}, 20–2000 μg L^{-1} microcystin-LR, and 50–5000 μg L^{-1} of chl-a. The U.S. Environmental Protection Agency recently established health advisory thresholds based on cyanotoxin concentrations in drinking water (https://www.epa.gov/cyanohabs/epa-drinking-water-health-advisories-cyanotoxins), and human health recreational ambient water quality criteria and swimming advisories for two cyanotoxins, microcystins and cylindrospermopsin, as shown in Table 1 (https://www.epa.gov/sites/production/files/2019-05/documents/hh-rec-criteria-habs-document-2019.pdf). In turn, California established recreational health-based exposure thresholds to protect human and canine health for three cyanotoxins: microcystins, cylindrospermopsin and anatoxin-a (Table 1) (CCHAB Network, 2020; OEHHA, 2012). The Caution Action Trigger requires public notification of a bloom without restriction of recreational activities. Exceedance of the Warning Tier I threshold requires restrictions on some recreational activities (such as swimming) while exceedance of the Danger Tier II Threshold results in recommendations to avoid all recreational activities and water contact.

Despite the recognition that nutrient over-enrichment is an important factor causing cyanobacterial blooms, most lakes in California, including impaired lakes with a history of high chl-*a* biomass and cyanobacterial blooms, are not routinely monitored for cyanobacteria and cyanotoxins. In southern California, Lake Elsinore is a natural freshwater lake that has been on the Federal Water Pollution Control Act of 1972 (Clean Water Act) Section 303(d) list of impaired waterbodies since 1994 (https://www.waterboards.ca.gov/santaana/water_issues/programs/tmdl/docs/303d/1994_303d.pdf) and is highly used for recreation. Canyon Lake, constructed approximately 2 miles upstream from Lake Elsinore, has also been on the 303(d) list since 1994. The goals of the current study were to: (1) determine if cyanotoxins were routinely present and if toxin concentrations exceeded human and canine health trigger thresholds thereby posing a public health issue, (2) assess cyanobacteria community composition and through culture isolation, identify potentially toxin-producing species, and (3) determine if chl-*a* could be used as an indicator of cyanotoxin threshold exceedance. This is the first formal assessment of cyanotoxins and cyanobacterial community composition to be conducted in these highly impaired eutrophic lakes.

2. Materials and methods

2.1. Study area

Lake Elsinore and Canyon Lake are located approximately 60 miles southeast of Los Angeles, in Riverside County, California (Fig. 1).

Both serve a variety of beneficial uses including recreation, aquatic and wildlife habitat, and municipal and domestic water supply (Canyon Lake). Lake Elsinore is a large (≈3000 acres), relatively shallow (≈20 feet) natural freshwater lake while Canyon Lake was constructed in 1928 and is surrounded by a housing community and is smaller (<450 acres). Both Lake Elsinore and Canyon Lake were listed as impaired under the Clean Water Act Section 303(d), due to excessive levels of nutrients. Lake Elsinore was additionally listed for low dissolved oxygen, sedimentation, and toxicity.

2.2. Discrete and time-weighted sample collection

A combination of discrete (grab) samples and passive samplers (time-weighted) were used to assess both lakes. For Canyon Lake, the sampling area focused on the east arm of the lake due to historical high chl-a concentrations that accumulate in that area and visual observations of blooms reported by homeowners. Canyon Lake is a privately-owned lake and only boats owned by property owners or the Property Owners Association Boat Operations are allowed access to the lake. During the study, the Property Owners Association provided access to a boat in 2015 and late in 2016. In early 2016, boat access was not provided, therefore sample collection was limited to a single land-based location at the eastern end of the east arm of the lake (Fig. 1). In Lake Elsinore, the sampling area focused on the highly used recreational area of La Laguna Beach (Fig. 1) because the study goal was to determine if cyanotoxins posed a health issue for humans and domestic pets. It was not feasible to revisit the same site for every sampling event in Lake Elsinore due to the extreme drought in California that caused the water levels to recede, and due

to extensive cyanobacterial mats that covered much of the lake surface water during 2016. Additionally, afternoon winds moved the cyanobacterial mats around the lake; therefore, it was not possible to revisit the same site locations for water sample collection. Surface water grab samples were collected by boat survey within the La Laguna area, and a YSI 6600 data sonde was used to measure chl-a to determine if there were large differences in chl-a concentrations throughout the recreational area. Surface scum and foam samples were collected when present and were only observed at the La Laguna recreational beach in Lake Elsinore, at the northwest shoreline shown in Fig. 1. Samples at both lakes were collected monthly in 2015 from July through October, bi-monthly or every 2 weeks in 2016 from May through October, and there were two collection dates in 2017 (August 21, 2017 and September 06, 2017).

All grab samples (including whole water, scum, and foam samples) were collected following the State of California Surface Water Ambient Monitoring Program (SWAMP) Standard Operating Procedures for cyanotoxins (available for download via https://drive.google.com/file/d/0B40pxPC5g-D0T01OVUx4amhDaVk/view). Surface whole water grab samples were collected in 1-L glass bottles, homogenized, and then sub-samples were aliquoted for cyanotoxins, chl-a, and 16S rRNA gene sequencing analyses. Cyanotoxin samples were collected in 250-mL glass jars in 2016 and 2017, frozen immediately in the field, and stored at –20 °C. The 2015 cyanotoxin samples were collected in 100-mL high-density polypropylene (HPDE) plastic bottles, frozen immediately in the field and stored at –20 °C. Samples for the analysis of chl-a were filtered onto 25-mm Whatman GF/F filters (GE Whatman, Marlborough, Massachusetts, USA), frozen immediately, stored at –80 °C and analyzed by fluorometry within 2 weeks of collection. The 16S rRNA gene sequencing and community analysis samples were collected from Lake Elsinore only in 2016 and were filtered onto a 0.2-μm Whatman Nuclepore polycarbonate filter (GE Healthcare Life Sciences, Buckinghamshire, UK) and submerged in bead solution storage buffer (Mo Bio Laboratories, Inc., Carlsbad, California) before being stored at –80 °C. Before extraction, filters were thawed at 4 °C, and DNA extractions were performed with the PowerLyzer PowerSoil DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, California) according to the manufacturer's instructions. The Nanodrop 8000 (Thermo Scientific, Wilmington, Delaware, USA) was used to assess DNA yield.

Discrete grab samples for cyanobacterial identification and relative abundance via microscopy were collected simultaneously with the other discrete samples in a 1.5-L HDPE bottle and stored in an incubator overnight at the Southern California Coastal Water Research Project, at 21 °C and an irradiance of 150 μmol photons m^{-2} s^{-1} (12:12 light:dark cycle). Samples were analyzed live the following day.

Passive sampling devices, called Solid Phase Adsorption Toxin Tracking, SPATT (MacKenzie et al., 2004; Lane et al., 2010; Kudela, 2011, 2017), provided time-weighted dissolved cyanotoxin measurements. SPATT were deployed in one location of the lake in 2015 and 2016 determined by the accessibility and ease of deployment, usually under a floating dock (see Fig. 1 for locations in both lakes). SPATT were continuously deployed, and one SPATT sampler was retrieved at each sampling timepoint at which time a new SPATT sampler was deployed. SPATT were used as a complement to traditional water grab

samples to elucidate toxin dynamics by revealing the persistence of toxins while alleviating the limitations of temporal and spatial resolution associated with traditional water grab samples. SPATT samplers were constructed using Diaion HP20 resin (Mitsubishi Chemical Corporation) following standard operating procedures as described in Kudela (2017) and deployed monthly in 2015 and for 2-week timeperiods in 2016. The results are reported as ng of toxin per 1 g of resin (ng g^{-1}).

2.3. Cyanotoxin analysis

2.3.1. Discrete water sample cyanotoxin analysis—Discrete grab samples were extracted, processed and analyzed for cyanotoxins at the University of California, Santa Cruz, California, according to methods described in Mekebri et al. (2009) and Kudela (2011). Samples were stored frozen until extraction and analyzed by liquid chromatography/mass spectrometry (LCMS) with electrospray ionization (ESI) with selected ion monitoring (SIM) on an Agilent 6130 instrument equipped with a Phenomenex Kinetex C18 column (microcystins and nodularin) or Agilent Polaris-Ether C18 column (anatoxin-a and cylindrospermopsin).

Four microcystin congeners (MC-LA, MC-LR, MC-RR, MC-YR) were analyzed with the modifications described in Miller et al. (2010) and Kudela (2011). The 2017 sample analysis also included an additional congener, MC-LF. Method detection limits for all microcystins and nodularin were 0.10 ng mL^{-1}. Anatoxin-a and cylindrospermopsin were analyzed according to EPA Method 545 modified to account for differences in the analytical equipment (U.S. EPA, 2015). Samples from 2015 were also analyzed for anatoxin-a using a Cogent diamond hydride column. Water samples were processed for anatoxin-a by sonicating water and syringe-filtering (using a 0.2-μm Teflon filter) with direct-injection of 20 μL of sample. The analytical method followed Cogent KnowledgeBase article AA-00807 using anatoxin-a dissolved in 7:93 MeOH:H_20 with 0.1% formic acid (National Research Center Canada) as the reference. Cogent, AA-00807, was accessed at http://kb.mtc-usa.com/article/AA-00807/0/Anatoxin-a-ANTX-A.html. Method detection limits for anatoxin-a and cylindrospermopsin were 0.05 ng mL^{-1} and 0.28 ng mL^{-1}, respectively. For all toxins, blanks were analyzed for every 10 samples, and standard curves were run at the beginning and end of each set of samples using 6–10 standards at 1–500 ng mL^{-1}. Standards were obtained from National Research Council Canada, Sigma-Aldrich, and Enzo Life Sciences. Matrix spike recoveries were completed with each sample run and exceeded 93% for all toxins.

Water samples were analyzed for saxitoxin using the BIOO Scientific MaxSignal™ Saxitoxin (PSP) Enzyme-Linked Immunosorbent Assay (ELISA) test kit (BIOO Scientific Corp., Austin, Texas, Cat. No. 1034), following the manufacturer's instructions for sample extraction and analysis.

Additional analyses were conducted on a subset of samples using UPLC® analyses performed with a Waters Acquity Ultra Performance LCMS system (UPLC/MS/MS; Waters Corporation, Massachusetts, USA) at Northern Kentucky University. The method used small particle technology and separation of several microcystins and other toxins in a single run by UPLC/MS/MS using a previously published method (Oehrle et al., 2017). The

separation of 11 congeners of microcystins and other toxins in a single run included in the analysis were MC-LR, MC-RR, MC-YR, MC-LA, MC-LF, MC-LY, MC-LW, MC-WR, MC-DAsp-LR, MC-DAsp-RR, MC-HTyR, as well as anatoxin-a and cylindrospermopsin. The triple quad mass spectrometer (MS/MS) used was a model TQS mass detector (Waters). The various toxins were detected via multiple reaction monitoring (MRM), which monitors a specific precursor ion and fragment ion (product ion) for each toxin. For all toxins the main fragment ion (loss of ADDA group, mass 135) was monitored as well as one additional fragment ion for confirmation. In addition, several toxins produced both singly and multiply charged molecular ions ($M+H^+$ or $M+2H^+$). In those cases, both were monitored and used for compound confirmation with the singly charged form being used for calibration. Calibration was done using standards for each toxin (purchased from Sigma-Aldrich or Alexis Chemical) with a 5-point curve from 0.5 to 500 ppb being generated. Typical correlation coefficients for the curve (r^2) of 0.99x were obtained where x had a value of 6 or better. The detection limits for all toxins in this analysis were <0.5 ppb. Samples were either run at full strength or with various dilutions (1–1, 1–20, 1–50 or 1–100 with deionized (DI) water) depending upon toxin levels detected and/or foreseen to allow the monitored levels to fall within the calibration curve range. UPLC separation was achieved on an Acquity UPLC HSS T3 column (100 mm × 2.1 mm, i.d., 1.8-μm particle size, 130 Å pore size, Waters) and maintained at 35 °C. A 12-min gradient using a 0.1% formic acid (aqueous) and 0.1% formic acid (acetonitrile) mobile phase was used at a flow rate of 0.45 mL/min. The acidified acetonitrile was initially run at 2% for 0.80 min, increased to 70% over 9 min, washed with 80% acetonitrile for 1 min and returned to 2% for 2 min prior to the next injection.

2.3.2. SPATT cyanotoxin analysis—SPATT samples were analyzed at the University of California, Santa Cruz for four microcystin congeners, MC-LA, MC-LR, MC-RR, and MC-YR, and nodularin-R by LCMS with electrospray ionization (ESI) with SIM on an Agilent 6130 with a Phenomenex Kinetex C18, 2.1 × 100 mm, 2.6-μm column. The method was adapted from Mekebri et al. (2009) with minor modifications to account for the choice of column and LCMS/SIM instead of tandem mass spectrometry (Kudela, 2011). Anatoxin-a and cylindrospermopsin were analyzed as described above. The samples were prepared as described in Kudela (2011). Analysis included replicates and matrix-additions, with the quantification based on external standards. The methodological detection limit for SPATT analyses was 0.05 ng g^{-1} for all congeners. The percent recovery was reported in Kudela (2011) and was ~58–100% for the congeners using a standardized recovery method, with MC-RR being lowest followed by MC-LR (~88%), MC-YR (~100%), and MC-LA (~100%). Nodularin-R and anatoxin-a were not evaluated previously, but laboratory analysis following the same methods provided recovery of 70 and 90%, respectively.

2.3.3. Cyanobacteria cultures cyanotoxin analysis—Cyanobacteria cultures were isolated from environmental samples collected from Lake Elsinore and Canyon Lake between July and September 2016. Single cyanobacteria were isolated by micropipetting from a natural sample through a series of three washes in GF/F filtered and microwave sterilized source water, resulting in the isolation of a single cell or colony into a single well of a 24-well plate. The cyanobacteria were cultured using a BG-11 derivative and harvested

during stationary phase. The culture material was analyzed for cyanotoxins at the Organic Geochemistry Laboratory in the U.S. Geological Survey Kansas Water Science Center by LC/MS/MS including anatoxin-a, cylindrospermopsin, nodularin, and 10 congeners of microcystin (MC-HiLR, MC-HtYR, MC-LA, MC-LF, MC-LR, MC-LW, MC-LY, MC-RR, MC-WR, and MC-YR) adapted after a method reported in Loftin et al. (2016). Sample preparation included three sequential freeze/thaw cycles to lyse cells, filtration by 0.7-μm filter, and a mini-extraction of the filter using acidic methanol to improve recovery of the cyanotoxins. Analysis was performed using an Agilent 1260 Bioinert LC coupled with an Agilent 6460 Triple Quadrupole Mass Spectrometer. Separation was achieved using an Atlantis T3 Column C18, 3 × 150 mm, 3-μm column. Mobile phase A consisted of water, 0.1% formic acid, and 2 mM ammonium formate. Mobile phase B consisted of 50/50 (v/v) methanol/acetonitrile, 0.1% tetrahydrofuran, and 2 mM ammonium formate. Electrospray ionization was used to ionize analytes, and MRM was used to detect precursor and fragment ions for each analyte. At least one quantifier MRM and one qualifier MRM were used for each analyte. Calibration standards were prepared for each analyte using standards purchased from National Research Council Canada or Enzo Life Sciences from 0.01 to 5.00 μg L^{-1}. Stacked injection was used to simultaneously inject 94 μL of sample, standard, or blank along with 2 μL of simetone (internal standard, 100 μg L^{-1} solution), 2 μL of ethylenediaminetetraacetic acid (EDTA, complexing agent, 5 mM solution), and 2 μL of either DI water (for samples, blanks, and standards) or toxin mixture (for standard addition injections, 50 μg L^{-1} mixture). Results are available via ScienceBase (Donovan et al., 2020).

2.4. Cyanobacteria identification and analysis

2.4.1. Morphological identification—Cyanobacteria were identified to genus (species when possible) using an Olympus CKX41 inverted microscope (Olympus America, Center Valley, Pennsylvania, USA) at the University of Southern California according to Komárek (Komárek, 1992; Komárek and Komárková, 2002; 2003; 2004; Komárek and Zapomě;lová, 2007; Komárek et al., 2010). Briefly, samples were homogenized by successive inversions and an aliquot was poured into 20-mL tissue culture dishes and settled overnight. The relative abundance index (RAI), a qualitative index of the abundance of phytoplankton and cyanobacteria, was used to evaluate trends and community composition as described in Jester et al. (2009). The RAI was developed by the California Department of Public Health and has been used for marine HAB monitoring in California since at least 2000 (Jester et al., 2009). Each cyanobacterial genus and/or species was assigned a relative abundance code based on its numerical percentage of community composition of the sample. Relative abundance codes were as follows: dominant (D) (>50% of community composition), Abundant (A) (25–49% of community composition), Common (C) (10–24% of community composition), Present (P) (1–9% of community composition), and Rare (R) (<1% of community composition).

2.4.2. Cyanobacteria identification using DNA sequences—Amplicon library preparation and quantification of pooled libraries was performed at Laragen, Inc. (Culver City, California, USA). Briefly, the V4 region of the 16S rRNA gene was amplified using universal Bacteria/Archaea primers 515 F/806 R according to the Earth Microbiome Project (EMP) recommended protocols (Caporaso et al., 2011, 2012). Paired-end 2 × 250 basepair

sequencing from barcoded amplicon products was performed on an Illumina MiSeq platform (Illumina, San Diego, California, USA). Sequences were processed using the Illumina MiSeq Recorder software to remove adapter, barcode, and primer sequences.

Sequences were further processed using QIIME 2 2018.11 (Bolyen et al., 2018). Sequences were demultiplexed and quality filtered using the q2-demux plugin followed by denoising and chimera removal with DADA2 using q2-dada2 (Callahan et al., 2016). Taxonomy was assigned to resulting amplicon sequence variants (ASVs) using q2-feature-classifier (Bokulich et al., 2018) with classify-consensus-blast (Camacho et al., 2009; Edgar, 2010) against SILVA v138 taxonomy database (Yilmaz et al., 2014) prepared for use with QIIME 2 (Bokulich, n.d.; Bokulich et al., 2018). Analysis of Similarity (ANOSIM), permutational multivariate analysis of variance using distance matrices (adonis), and non-metric multidimensional scaling (NMDS) analyses were performed with R package *vegan* (McMurdy and Holmes, 2013;Oksanen et al., 2013) using Bray-Curtis distances to examine community structure across samples.

Taxonomic assignments for cyanobacteria ASVs are provided in Supplemental Table 3. Raw DNA sequences were deposited in Genbank under ProjectID PRJNA681629.

2.5. Chlorophyll-a analysis

Samples for the analysis of chl-a were collected in 2016 and 2017 and analyzed within 2 weeks of collection following EPA Method 445.0 (Arar and Collins, 1997). Samples were extracted in 90% acetone for 24 h at –20 °C in the dark and analyzed using a Turner Trilogy fluorometer (Turner Designs, Sunnyvale, California, USA) at the Southern California Coastal Water Research Project.

Logistic regression was used to determine if chl-a (log 10 transformed) was a significant predictor of cyanotoxins. Statistical packages of RStudio, version 1.1.423 were used and the significance was set at 0.05 for all statistical analysis.

3. Results

3.1. Lake Elsinore cyanotoxins

All cyanotoxin results are presented in Supplemental Table 1.

3.1.1. Multiple cyanotoxins—Two or more cyanotoxins were detected in 84% of samples, and three or more cyanotoxins were detected in 42% of samples when data from all three years were considered. In 2016 (when frequent monitoring occurred for 6 months), 92% and 53% of samples had two or more and three or more cyanotoxins detected, respectively (Fig. 2).

There were multiple toxins detected from both sample dates in 2017, three cyanotoxins from August 21, 2017 and two cyanotoxins from 09 Sept 2017. Only one date out of four sampling dates had multiple toxins in 2015, however fewer cyanotoxins were included in the analysis in that year.

3.1.2. Microcystins—For all three years combined, microcystins were detected in 93% of water, scum and foam samples; of those samples with toxin detected, 61% exceeded the Danger Tier II health threshold, 7% exceeded the Warning Tier I health threshold, and 17% exceeded the Caution Action health threshold (Fig. 3, Tables 1 and 2).

The concentrations from water samples ranged from below detection (bd) to 5,665 μg L^{-1}, and foam and scum samples ranged from 35 to 45,300 μg L^{-1} (Fig. 2, Tables 2 and 3, Supplementary Table 1). All SPATT samplers deployed continuously throughout the study in 2015 and 2016 detected microcystins ranging from 1.3 ng g^{-1} to 850 ng g^{-1} (Fig. 2 and Supplementary Table 1).

Microcystins were not detected in the monthly water samples collected in August, September, and October during 2015, however, SPATT results indicated low concentrations of microcystins ranging from 1.4 ng g^{-1} to 41 ng g^{-1} (Supplementary Table 1).

Microcystins were detected in all water, foam, scum, and SPATT samples collected during 2016 (Fig. 2 and S1, Tables 2 and 3, Supplementary Table 1). All water, foam and scum sample concentrations exceeded the California recreational health thresholds except for one collected on 09 May 2016 (0.6 μg L^{-1}). Water sample concentrations ranged from 0.6 to 5,665 μg L^{-1}, foam and scum samples ranged from 35 to 45,300 μg L^{-1}, and SPATT concentrations were 5.7–845 ng g^{-1}. The most common microcystin congener detected from water, scum, and foam samples (of the four congeners analyzed via LCMS) was MC-RR (91% of samples), followed by MC-LR (78%), MC-YR (65%), with MC-LA only detected in one sample from October 24, 2016 (Supplementary Figure S1A). SPATT results were similar, as MC-RR was the most common congener detected (91%), followed by MC-LR (82%), MC-YR (54%), with MC-LA only detected from the same date as the water samples as noted above (Supplementary Figure S1B). The most predominant microcystin congener in the subset of samples analyzed by UPLC/MS/MS for 11 microcystin congeners was MC-WR, followed by MC-RR. The congener, MC-WR, comprised 16–100% of the total concentration of microcystins, and MC-WR comprised more than 30% of the total microcystins in 80% of the samples (Table 3).

Microcystin concentrations also exceeded California recreational health thresholds in 2017, with concentrations ranging from 1.3 to 355 μg L^{-1} in water, foam, and scum samples (Table 2, Supplementary Table 1). The water samples collected on 21 August 2017 ranged from 1.3 to 149 μg L^{-1} and 6.9–355 μg L^{-1} on 06 Sept 2017. The congeners, MC-LR and MC-RR, were detected in all six samples collected on 21 August 2017 and in all three samples collected on 06 Sept. The MC-LA and MC-LF congeners were included in the analysis, however, neither of these congeners were detected in the 2017 samples.

3.1.3. Anatoxin-a—Anatoxin-a was detected in 30% of water, scum, and foam samples and 21% of SPATT samplers for all three years combined (Figs. 2 and 3, Table 2 and Supplementary Table 1). Water sample concentrations ranged from bd to 0.58 μg L^{-1} and foam and scum samples ranged from bd to 37 μg L^{-1} (Fig. 2, Table 2, Supplementary Table 1). The Danger Tier II human health threshold was never exceeded; however, the Warning

Tier I and Caution Action health thresholds were exceeded in 23% and 6% of samples, respectively (Fig. 3).

Anatoxin-a was not detected in any of the four water samples collected in 2015 but was detected in one SPATT sample retrieved on 14 Oct 2015 (4.9 ng g^{-1}). In 2016, anatoxin-a was detected in 26% of the water, foam, and scum samples and ranged from bd to 37 μg L^{-1} (Table 2, Supplementary Table 1). Only two SPATT samples in 2016 detected anatoxin-a, on 07 Aug and 14 Nov, and concentrations ranged from bd to 300 ng g^{-1} (Fig. 2B). Anatoxin-a exceeded the Caution Action health threshold on one sample collection date in 2017. Anatoxin-a was not detected in any of the samples collected in 2017.

3.1.4. Cylindrospermopsin—Cylindrospermopsin was detected in 35% of water, scum, and foam samples throughout all years combined, and exceeded the Danger Tier II health threshold in almost a quarter of samples, while an additional 10% and 2.4% of samples exceeded the Warning Tier I and Caution Action Trigger thresholds, respectively (Fig. 3 and Supplementary Table 1). The concentrations in water samples ranged from bd to 21 μg L^{-1} and foam and scum sample concentrations ranged from bd to 273 μg L^{-1} (Fig. 2A, Table 2, Supplementary Table 1). Cylindrospermopsin was detected in 57% of SPATT samples, and concentrations ranged from bd to 4,500 ng g^{-1} (Fig. 2B).

In 2015 cylindrospermopsin was not detected in any samples (Supplementary Table 1). By contrast, cylindrospermopsin was detected in 52% of water, foam, and scum samples collected in 2016, and concentrations ranged from bd to 273 μg L^{-1} (Fig. 2A, Table 2). SPATT detected cylindrospermopsin in 73% of samples collected in 2016 and concentrations ranged from bd to 4,466 ng g^{-1} (Fig. 2B). Cylindrospermopsin was detected from samples collected on 21 Aug 2017 and ranged from bd to 11 μg L^{-1} and was not detected in samples collected on 06 Sept 2017.

3.1.5. Other cyanotoxins (nodularin and saxitoxin)—For all three years combined, nodularin was detected in 22% of water, scum and foam samples and ranged from bd to 18 μg L^{-1} and was not detected in any of the SPATT samples (Supplementary Table 1). Nodularin was only analyzed for one water sample collected in 2015 on 14 Oct (result was below detection). In 2016, nodularin was analyzed on all samples collected, but was not detected from SPATT samples. Water samples ranged from bd to 0.90 μg L^{-1} and foam and scum samples ranged from bd to 19 μg L^{-1} (Table 2 and Supplementary Table 1). In 2017, nodularin was detected in one foam sample collected on 21 Aug 2017 (0.08 μg L^{-1}).

Saxitoxin was analyzed in water samples in 2016 (samples from May through August only) but was not included in 2017 analyses due to the lack of toxin detected in previous years. Saxitoxin was not detected in any water samples collected during 2016.

3.2. Lake Elsinore cyanobacterial community composition, identification of cyanotoxin producers and chlorophyll-a concentration

The results of microscopy-based determination of relative abundances (RAI) in Lake Elsinore indicated that the most abundant and dominant cyanobacterial genera were *Aphanizomenon*, *Aphanocapsa*, *Cylindrospermopsis*, *Planktothrix,* and *Microcystis* (Fig.

4). Commonly observed genera included *Merismopedia*, *Planktolyngbya, Aphanizomenon* cf. *flos-aquae, Pseudanabaena* and *Raphidiopsis*. In both years, the early season was characterized by abundant *Aphanizomenon* taxa. In 2016, the cyanobacterial community exhibited a distinct shift in species between 23 June and 20 July, with a *Planktothrix*-dominant community transitioning to a *Microcystis*-dominant community (Fig. 4B).

The DNA sequencing results from 2016 generally agreed with the microscopy analyses, revealing a *Planktothrix*-dominant cyanobacterial community in May and June (Fig. 5). The *Planktothrix* sequences were primarily from a single ASV that comprised approximately 24% of the total sequence reads in early May and matched *Planktothrix agardhii* reference sequences (Supplemental Table 3). Other abundant ASVs in early season samples were identified as *Sphaerospermopsis aphanizomenoides* (previously, *Aphanizomenon aphanizomenoides*), a potentially toxic cyanobacterium that thrives at high temperatures (Horecká and Komárek, 1979; (Zapomělová et al., 2011); Cires and Ballot, 2016). In agreement with the microscopy analyses, the DNA results revealed a shift on July 20, 2016 to a cyanobacterial population dominated by two *Microcystis* ASVs, that at their peak (August 29, 2016) comprised approximately >50% of the total microbial sequence reads. *Nodosilinea* and *Cyanobium* also comprised >1.0% of the microbial population at various points throughout the summer season, and *Cylindrospermosis* was abundant in late July through early September. Both the cyanobacteria and total microbial community were strongly structured by sampling month, and weakly associated with chl-a concentrations, which peaked in August, and cylindrospermopsin concentrations which peaked in July (Table 4).

Isolation of cyanobacteria from water samples collected between 12 July and September 16, 2016 resulted in cultured isolates of *Aphanizomenon, Cylindrospermopsis, Merismopedia, Microcystis aeruginosa, Planktolyngbya, Planktothrix*, *Phormidium*, and *Raphidiopsis* (Table 5). Two separate *Microcystis aeruginosa* cultures were positive for MC-LR, MC-RR, MC-WR, and MC-YR and one culture additionally had a detection of MC-HiLR. Individual microcystin concentrations, when detected, ranged from 0.24 to 500 μg L^{-1}. Anatoxin-a and cylindrospermopsin were not detected in any of these isolates.

Chl-a results from 2016 ranged from 25 to 1,100 μg L^{-1} with highest concentrations observed in August and September when concentrations exceeded 600 μg L^{-1} (Supplementary Figure S2). Chl-a results from 2017 were 140 μg L^{-1} on 21 Aug 2017 and ranged from 140 to 290 μg L^{-1} on 06 Sept 2017 (data not shown). Statistical analysis revealed that chl-a was a significant predictor of microcystins from water samples (p-value < 0.002) but not from SPATT samples (p-value < 0.1). Additionally, chl-a was not a significant predictor for cylindrospermopsin, anatoxin-a, or nodularin for either water or SPATT samples (p values > 0.05 for water samples and SPATT samples).

3.3. Canyon Lake cyanotoxins

All cyanotoxin results are presented in Supplemental Table 2.

3.3.1. Multiple cyanotoxins—Across all three years, 35% of water samples and 27% of SPATT samples detected two or more cyanotoxins and 6% and 7% of water and SPATT samples indicated three or more cyanotoxins, respectively (Fig. 2).

3.3.2. Microcystins—For all three years combined, microcystins were detected in 76% of water samples with concentrations ranging from bd to 1.6 μg L^{-1} (Figs. 2C and 3B and Supplementary Table 2) and in 57% of SPATT samples, with concentrations ranging from bd to 34 ng g^{-1} (Fig. 2D, Supplementary Table 2). Water samples collected during 2015 had undetectable microcystins in Canyon Lake. Microcystins detected in SPATT samples from Canyon Lake were consistently low in 2015, with concentrations ranging from 4.5 to 34 ng g^{-1} (Supplementary Table 2). All four microcystin congeners were detected from SPATT, with MC-RR detected in all samples, MC-LR, MC-YR and MC-LA in three, two, and one of the samples, respectively. In 2016, microcystins were detected in 90% of water samples and 36% of SPATT samples and concentrations ranged from bd to 1.6 μg L^{-1} (Fig. 2C) and bd to 1.5 ng g^{-1} (Fig. 2D) respectively. MC-RR was the only congener detected from SPATT. MC-RR was the predominant congener detected in 63% of water samples and MC-LR was detected in 36% of samples with MC-YR only detected in 1 sample, and MC-LA not detected. MC-RR was the only congener detected in the 2017 water samples collected on 22 Aug 2017 and 12 Sept 2017 and concentrations were 0.19 μg L^{-1} and 0.14 μg L^{-1}, respectively.

3.3.3. Anatoxin-a—Anatoxin-a was detected in 53% of water samples across all three years and ranged from bd to 9.3 μg L^{-1} (Figs. 2C and 3B, Supplementary Table 2). SPATT samples indicated anatoxin-a detected in 14% of samples and concentrations ranged from bd to 730 ng g^{-1} (Fig. 2D). Anatoxin-a was not detected in any of the water or SPATT samples collected in 2015 (Supplementary Table 2). In 2016, anatoxin-a was detected in 27% of water samples and concentrations ranged from bd to 9.4 μg L^{-1} and 18% of SPATT samples, ranging from bd to 735 ng g^{-1} (Fig. 2). The 22 Aug 2017 sample was 0.63 μg L^{-1} which exceeded the California Caution Action health threshold for anatoxin-a concentration (Table 1) and no anatoxin-a was detected in the 12 Sept 2017 sample.

3.3.4. Cylindrospermopsin—Cylindrospermopsin was detected in 12% of all water samples and in 46% of SPATT samples across all three years of sampling (Fig. 3B). Concentrations ranged from bd to 18 μg L^{-1} from water samples (Fig. 2C) and from bd to 141 ng g^{-1} from SPATT (Fig. 2D and Supplementary Table 2). Cylindrospermopsin was not detected in samples collected in 2015 and 2017.

3.3.5. Other cyanotoxins (nodularin and saxitoxin)—Nodularin was not analyzed in water samples collected during 2015 or 2017 but was analyzed in one SPATT sample collected on 14 Oct 2015, which was below detection. Samples collected in 2016 indicated nodularin was present in 1 SPATT sample collected on 10 Oct 2016 (0.12 ng g^{-1}) and was below detection in all water samples. Three samples collected on 07 July 2016, 20 July 2016 and 01 Aug 2016 were below detection for saxitoxin. Other samples collected in 2016 were not analyzed for saxitoxin (Supplementary Table 2).

3.4. Canyon Lake cyanobacterial community composition, identification of cyanotoxin producers and chlorophyll-a concentration

Dinoflagellates dominated the community composition during July 2015 only. Cyanobacteria were abundant August through October, and included the genera *Cylindrospermopsis* spp., *Limnothrix, Planktolyngbya*, and *Pseudoanabaena* (Fig. 6A). Chlorophytes dominated the community composition in May 2016, but the composition transitioned to cyanobacterial dominance in July with common genera including *Cylindrospermopsis, Limnothrix,* and *Planktolyngbya*. *Arthrospira* was abundant in July and early August, and the community composition transitioned in August to strong dominance by *Pseudanabaena* (Fig. 6B).

Isolation of cyanobacteria from water samples collected between 20 August and 12 September 2016 resulted in cultured isolates for *Cylindrospermopsis* and *Pseudanabaena*. No cyanotoxins were detected from these isolates (Table 5).

Statistical analysis revealed that chl-a was not a significant predictor of microcystins, cylindrospermopsin, anatoxin-a, or nodularin from water samples collected from Canyon Lake (p-values ranged from 0.1 to 0.6), nor from SPATT samples (p-values ranged from 0.3 to 0.6). The chl-a concentrations ranged from 2.5 to 25 μg L^{-1} in 2016 and the peak concentrations were 25 μg L^{-1} on 07 July 2016 and 23 μg L^{-1} on 24 Oct 2016 (Figure S2).

4. Discussion

4.1. Persistent cyanotoxin detections that frequently exceeded health thresholds in Lake Elsinore

Cyanotoxins were detected at concentrations that frequently exceeded California recreational health thresholds (Table 1) and were persistently detected throughout the study period. The cyanotoxin concentrations detected during this study are currently the highest recorded concentrations of microcystins, anatoxin-a, and cylindrospermopsin in southern California lakes and estuaries (Magrann et al., 2015; Howard et al., 2017; Tatters et al., 2019), and among the highest reported within California lakes (Kudela, 2011). The sea otter mortality event reported in Miller et al. (2010) documented microcystin concentrations in Pinto Lake of 2,900 μg L^{-1}, substantially lower than the maximum concentrations detected in Lake Elsinore.

Cylindrospermopsin has rarely been detected in California and the exceedance of California recreational health thresholds was unexpected, and further study would be beneficial to better characterize the temporal occurrence of this toxin and to investigate the sources of production (Loftin et al., 2016; Howard et al., 2017; Tatters et al., 2019).

Studies of the health implications of human exposure were beyond the focus of this assessment study and there was no formal system in California for reporting blooms or illness at the time. However, events such as those reported here have led to an illness response program and documentation system being formalized in California to investigate human, wildlife, and pet illness and mortality as a result of bloom exposure. The concentration of microcystins detected in Lake Elsinore exceeded concentrations that

have been reported to result in human illnesses. For example, microcystin concentrations in Uruguay that resulted in a family becoming ill, including acute liver failure requiring a liver transplant, had maximum concentrations ranging from 56 to 8,200 μg L^{-1} (Vidal et al., 2017). This range of concentrations is 5.4 to 800-fold lower than the maximum concentrations detected in Lake Elsinore in this study (5,665 to 45,300 μg L^{-1}).

Water, scum, foam, and SPATT samples collected from Lake Elsinore yielded predominance of the same microcystin congeners dominating the composition of total microcystins (Supplementary Figure S1). The subset of samples analyzed via UPLC/MS/MS, that included an additional seven MC congeners, indicated that MC-WR was a predominant congener detected in all samples (Table 3). This indicates the total concentration of microcystins from water, scum, foam, and SPATT samples analyzed via LCMS (that excluded MC-WR) was likely underestimated.

Cyanotoxin measurements from cultured isolates from environmental samples collected at Lake Elsinore on 16 Sept 2016 indicated that *Microcystis aeruginosa* was capable of the production of MC-LR, MC-RR, MC-WR, MC-YR and MC-HiLR (Table 5). The cyanobacteria producing cylindrospermopsin, anatoxin-a, and nodularin were not identified and could be a focus of future studies.

In addition to high concentrations detected in Lake Elsinore, cyanotoxins were also chronically detected in water and SPATT samples, across multiple months and seasons, similar to findings in other studies of California waters (Kudela, 2011; Gibble and Kudela 2014; Gibble et al., 2016; Howard et al., 2017; Peacock et al., 2018; Tatters et al., 2019). Microcystins exceeded one of the three California health thresholds in most samples and recreational swimming was not recommended 68% of the time because of recreational health threshold exceedance (Table 1). The recreational uses of Lake Elsinore were severely impacted due to both the exceedance of health thresholds and the chronic detections at the most accessible recreational locations indicating a high risk for immediate impacts to human, wildlife, and domestic pet health.

The Federal Water Pollution Control Act of 1972 (the Clean Water Act) requires the California Water Boards to assess and identify surface waters that do not meet water quality standards (Section 303(d) of the Clean Water Act). The assessment and impairment determination is governed by the Water Quality Control Policy for developing California's Clean Water Act Section 303(d) (https://www.waterboards.ca.gov/board_decisions/adopted_orders/resolutions/2015/020315_8_a mendment_clean_version.pdf). The current study results for Lake Elsinore could be included in the next assessment of cyanotoxin and cyanoHAB waterbody impairments similar to other waterbodies within California and the U.S. (North Coast Regional Water Quality Control Board, 2010; Michigan Department of Environmental Quality, 2017; Davis et al., 2019).

Future investigations are warranted to identify the environmental drivers of toxin production and community composition as well as to identify the species responsible for cylindrospermopsin, anatoxin-a, and nodularin production in Lake Elsinore.

4.2. Persistent cyanotoxin detections that occasionally exceeded health thresholds in Canyon Lake

In contrast to Lake Elsinore, microcystins were persistently detected in Canyon Lake at concentrations that only occasionally exceeded California recreational health thresholds. These findings indicate relatively low but persistent microcystin concentrations detected across multiple months and seasons. There have been reports of significant health consequences from chronic exposure to microcystins for human and wildlife globally (Bury et al., 1995; Wiegand et al., 2000; de Figueiredo et al., 2004; Jacquet et al., 2004; Malbrouk and Kestemont, 2006; Backer et al., 2008, 2009; Li et al., 2011, 2016; Trevino-Garrison et al., 2015). Cylindrospermopsin and anatoxin-a were detected half of the time and, similar to microcystins, only occasionally exceeded California recreational health trigger thresholds in Canyon Lake. The chronic risk of exposure to cyanotoxins in Canyon Lake supports the need for future monitoring in order to minimize the risk and better understand the potential impacts to humans, domestic pets, and wildlife.

4.3. Multiple cyanotoxins were frequently detected

Co-occurring cyanotoxins were frequently detected in both lakes investigated in this study (Fig. 2). The health consequences and risks from exposure to co-occurring cyanotoxins is poorly characterized because health thresholds are typically based on exposures to a single cyanotoxin (Table 1). Due to differing chemical structures and resulting inherent bioactivities, the various co-occurring classes of cyanotoxins may act as synergistic or antagonistic physiological stressors. A recent study has also shown that for MC-LR and anatoxin-a specifically, the response can be synergistic or antagonistic, depending on the test organism (Metcalf and Codd, 2020). The co-occurrence of multiple cyanotoxins from a single location has been documented in other studies, both within and outside the United States (Graham et al., 2010; Gkelis and Zaoutsos, 2014; Rodriquez et al., 2014; Sabart et al., 2015; Pekar et al., 2016), and in California (Howard et al., 2017; Tatters et al., 2017, 2019; Bouma-Gregson et al., 2018; Peacock et al., 2018). Studies characterizing the consequences of exposure to multiple cyanotoxins for human, wildlife, and ecological health would help address this gap in knowledge.

4.4. Multiple potential cyanotoxin producing cyanobacteria identified

The cyanobacterial genera and species observed in this study indicated a high risk for the production and co-occurrence of multiple cyanotoxins in these ecosystems. Given the range of taxa observed, the detection of multiple cyanotoxins in this study is not surprising. Previous studies of cyanobacterial species identifications in Lake Elsinore also indicated the historical presence of multiple potential toxin-producing species identified in 2003 and 2010 (cyanotoxins were not analyzed) (Oza, 2003; Tobin, 2011). Overall, multiple cyanotoxin producers were present, and other cyanotoxins outside the scope of this work were potentially present based on cyanobacterial identifications including lyngbyatoxin-a, BMAA, homoanatoxin-a, neosaxitoxins.

The identification of cyanobacterial species using DNA barcode sequencing for taxonomic identification has been applied in the Sacramento-San Joaquin Delta and Clear Lake systems in California (Kurobe et al., 2013). Microscopy and DNA barcoding conducted

in this study were in relative agreement with respect to cyanobacterial genera/species detected and their relative abundances. There was good agreement with respect to broad community composition results between both methods indicating that DNA barcoding could be used on a routine basis to determine the relative abundance of dominant cyanobacterial taxa. Both approaches identified the shift in cyanobacterial taxa in mid-July, from *Planktothrix* to *Microcystis* dominance, during which time the microcystin concentrations simultaneously increased by one-thousand-fold. Additionally, DNA barcoding detected considerable microdiversity (beyond that possible by microscopy) and therefore may eventually be useful in distinguishing toxic species, or closely-related strains that differ in their capacity to produce toxins. These results highlight the importance of pursuing molecular identification for complex species assemblages. Developing a DNA-based library for toxic cyanobacteria could allow for the future development of genetic screening tools for biotoxin tracking and quantitative polymerase chain reaction (qPCR) assays. Additionally, metabarcode sequencing of associated microbial communities might also help inform the triggers and timing of cyanobacterial bloom events and toxin production. DNA barcoding offers a unique perspective that may prove useful for community characterization and cyanotoxin management.

4.5. Chlorophyll-a was not a viable screening indicator

Chl-a has been used as a screening indicator for the risk of microcystins in lakes and has been used to evaluate nutrient pollution and linked to management actions, such as reduced nitrogen and regulatory thresholds for total nitrogen (Kudela, 2011; Otten and Paerl, 2011; Rinta-Kanto et al., 2009; Yuan et al., 2014; Yuan and Pollard, 2015). The 2007 National Lake Assessment conducted by the U.S. Environmental Protection Agency found that chl-a overestimated microcystin risk when compared to the World Health Organization thresholds (Loftin et al., 2016). Additionally, the results from the current study indicate high frequency of occurrence of multiple cyanotoxins, and chl-a was not a good indicator of cylindrospermopsin, anatoxin-a, or nodularin. Therefore, chl-a was not a viable screening indicator for Lake Elsinore or Canyon Lake.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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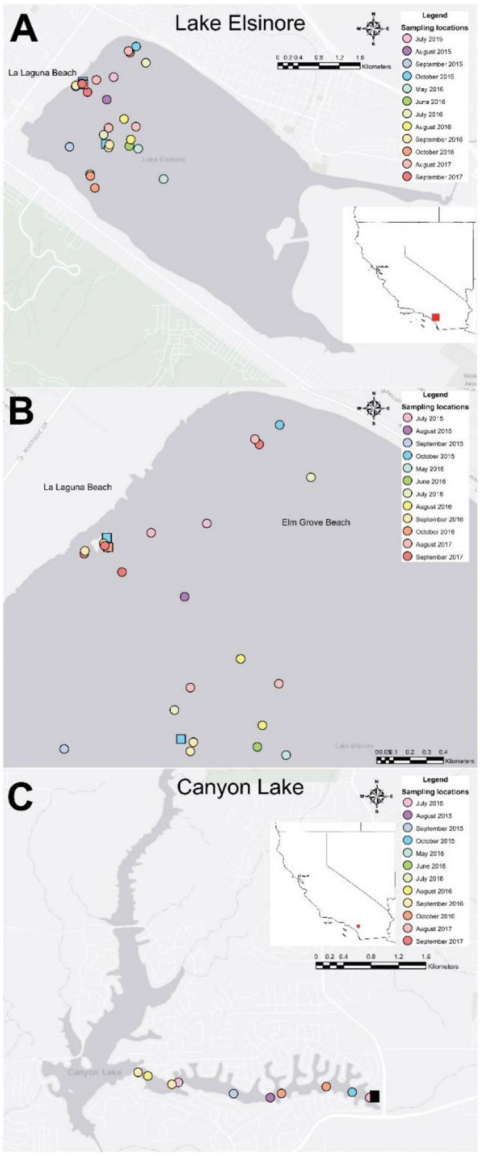

Fig. 1.

Map of study area and sampling locations at Lake Elsinore (A), an expanded view of the northwest part of Lake Elsinore (B) and Canyon Lake (C). Solid Phase Adsorption Toxin Tracking (SPATT) sampling locations are shown by the black squares. The discrete (grab) sample locations are shown by the colored circles where the colors indicate collection timepoints based on the legend in the figure.

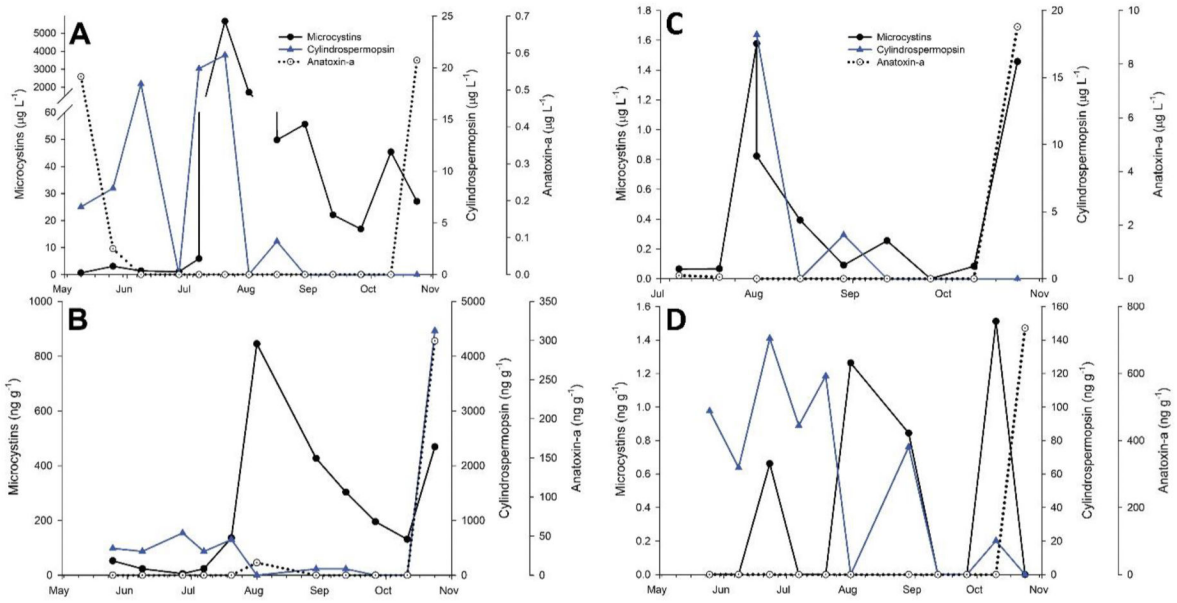

Fig. 2.

Cyanotoxin concentrations detected in (A) water samples and (B) SPATT samples collected from Lake Elsinore in 2016 and (C) water samples and (D) SPATT samples collected from Canyon Lake in 2016. Microcystins are shown by the solid black line and circles, cylindrospermopsin is shown by the blue line and triangles, and anatoxin-a results are shown by the dotted line and white circles. Note the break on the y-axis for microcystin results from water samples (A) and the different scales between (A) and (C).

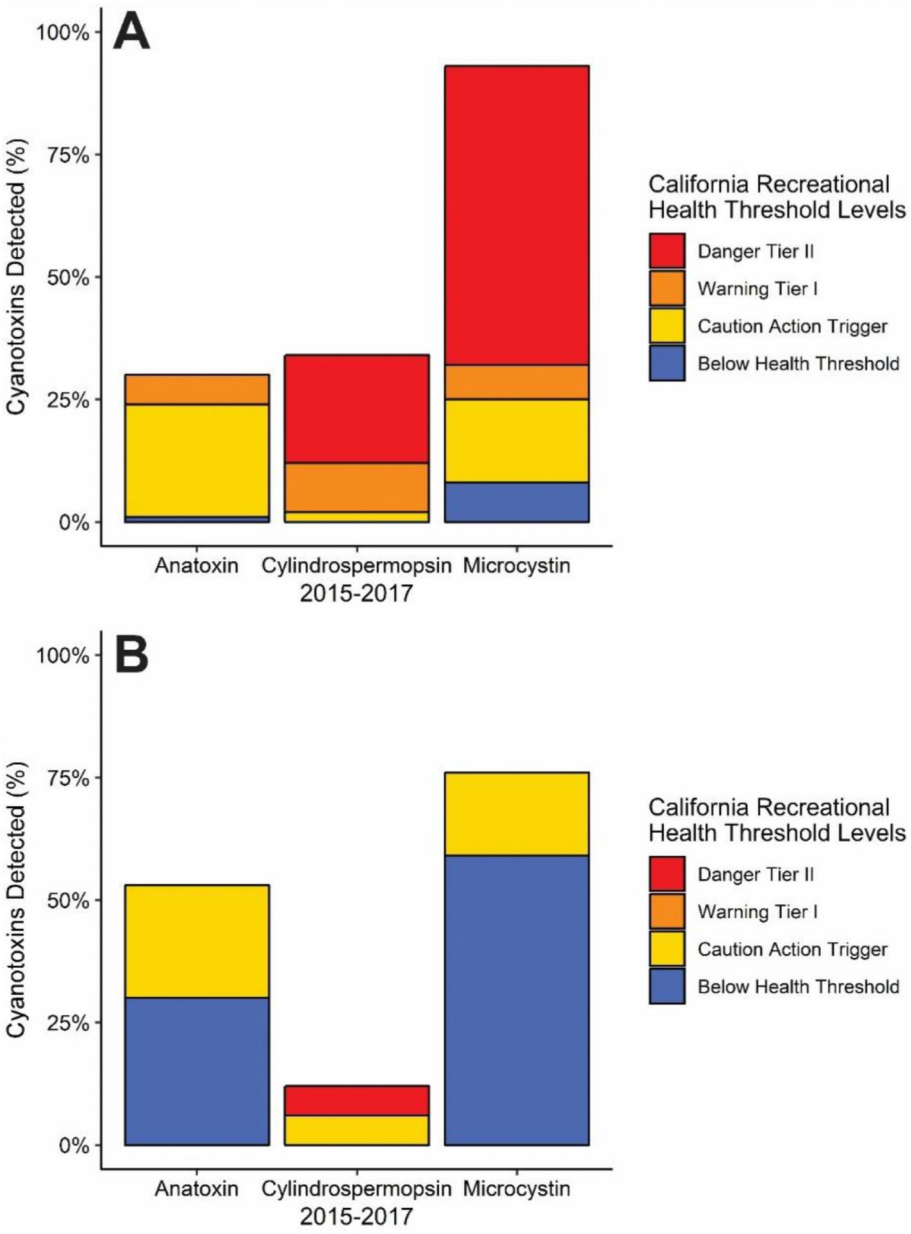

Fig. 3.

The percentage of cyanotoxin samples that were positive from (A) Lake Elsinore and (B) Canyon Lake from samples collected in 2015–2017. The percent of cyanotoxin samples that were positive but below California Recreational Health Thresholds are indicated in blue and samples above California Recreational Health Thresholds are indicated by yellow, orange, or red corresponding to the Caution Action Trigger, Warning Tier I, and Danger Tier II, respectively (Table 1). Samples that were below the methodological detection limit are excluded from the figure.

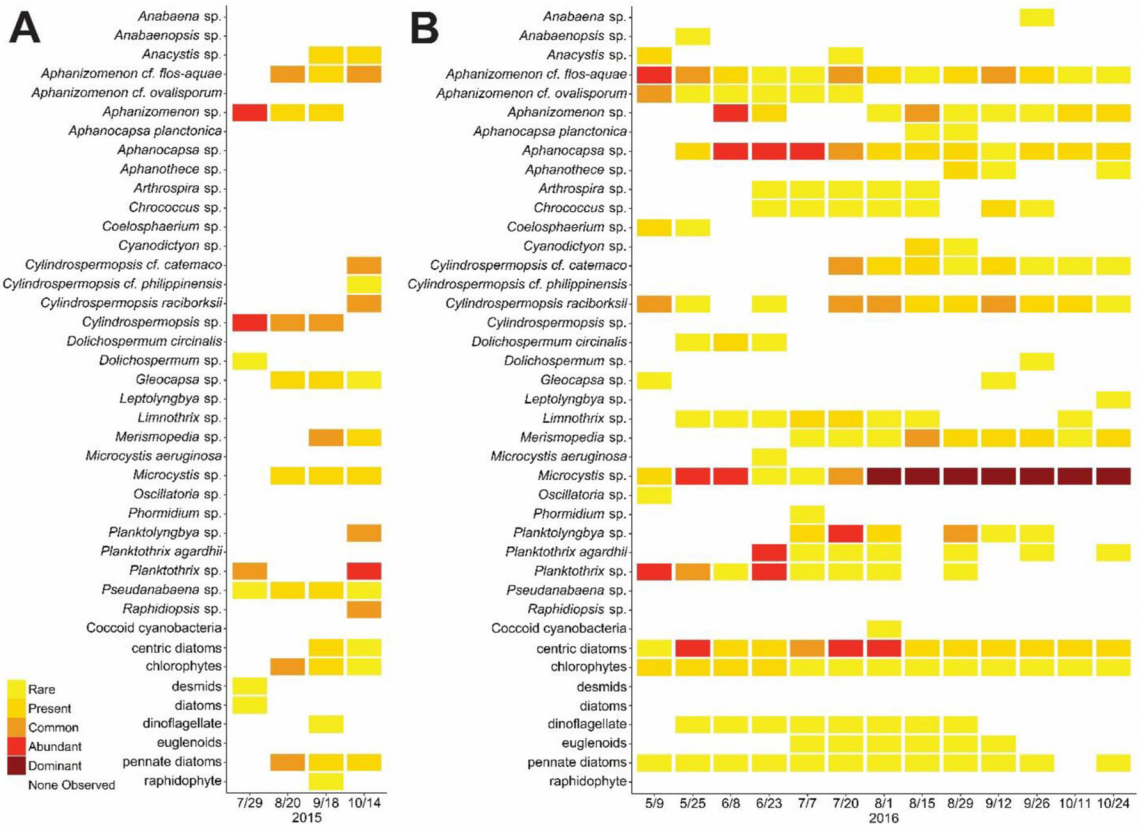

Fig. 4.

Relative abundance index of cyanobacteria, diatoms, and dinoflagellates from Lake Elsinore collected in (A) 2015 and (B) 2016. Relative abundance categories are the following percentages of the community composition: Rare <1%, Present 1 - <10%, Common 10 - <25%, Abundant 25-<50%, Dominant 50–100% and are indicated by different color shades in the figure. White spaces or a lack of color indicate no observations of the corresponding phytoplankton categories or genera indicated on the y-axis.

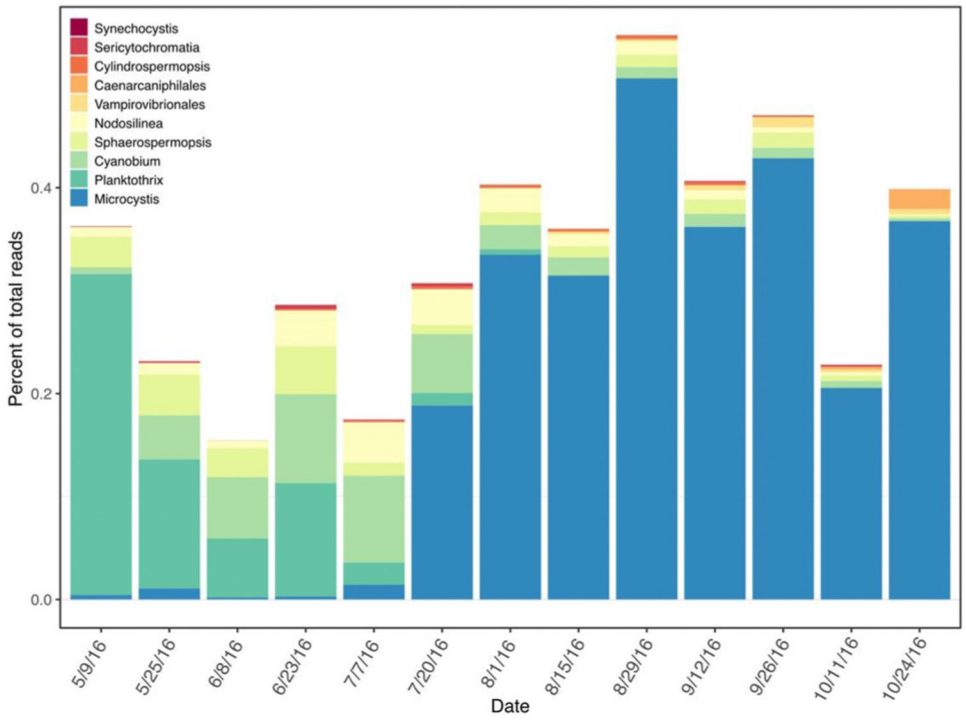

Fig. 5.

Relative abundances of cyanobacterial genera in surface water samples as a percentage of total 16S sequence reads throughout the Lake Elsinore 2016 season.

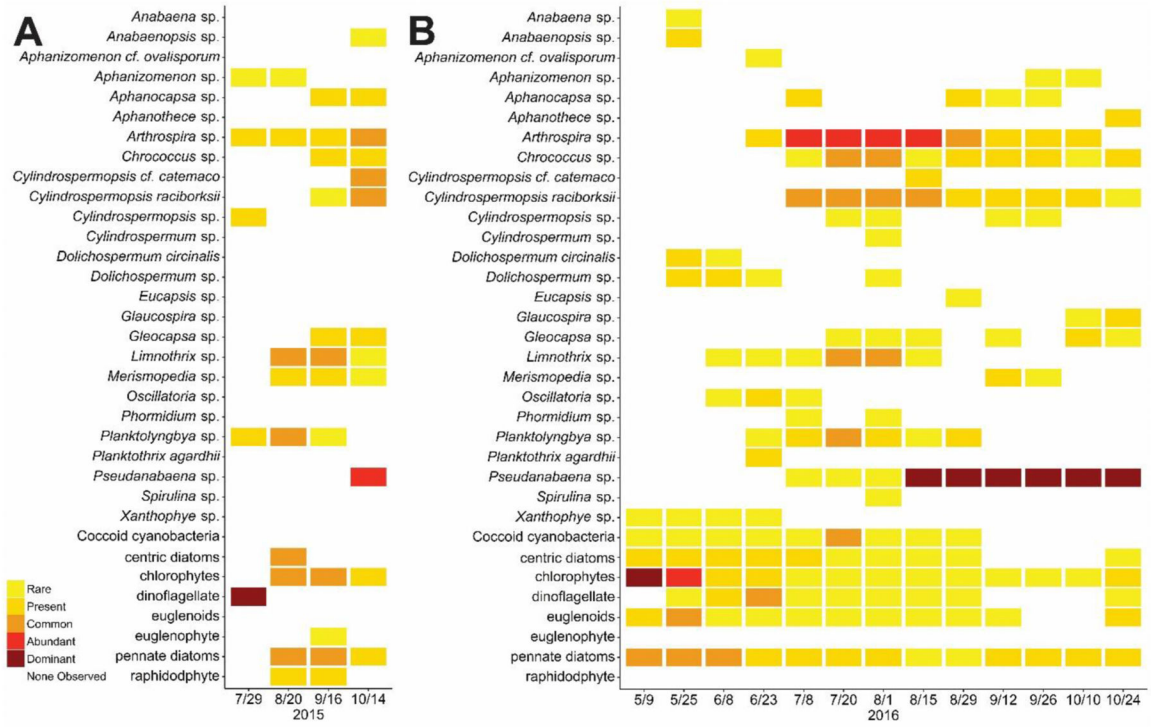

Fig. 6.

Relative abundance index of cyanobacteria, diatoms, and dinoflagellates from Canyon Lake collected in (A) 2015 and (B) 2016. Relative abundance categories are the following percentages of the community composition: Rare <1%, Present 1 - <10%, Common 10 - <25%, Abundant 25-<50%, Dominant 50–100% and are indicated by different color shades in the figure. White spaces or a lack of color indicate no observations of the corresponding phytoplankton categories or genera indicated on the y-axis.

Table 1

United States Environmental Protection Agency and California cyanotoxin criteria and health thresholds (in μg L^{-1}).

	Microcystins	Anatoxin-a	Cylindrospermopsin
U.S. EPA Recreational Criteria[a]	8.0	None	15
U.S. EPA Drinking Water Advisory[b,d]	0.3	None	0.7
U.S. EPA Drinking Water Advisory[b,e]	1.6	None	3.0
California Caution Action Trigger[c]	0.8	Detection	1.0
California Warning Tier I[c]	6.0	20	4.0
California Danger Tier II[c]	20	90	17

[a] https://www.epa.gov/sites/production/files/2019-05/documents/hh-rec-criteria-habs-document-2019.pdf

[b] https://www.epa.gov/cyanohabs/epa-drinking-water-health-advisories-cyanotoxins.

[c] California Cyanobacteria Harmful Algal Bloom Network, 2016 (My Water Quality: California Harmful Algal Blooms (HABs)).

[d] Infants and young children.

[e] School aged children and adults.

Table 2

Results from scum and foam samples analyzed for cyanotoxins collected from Lake Elsinore in 2016 and 2017. Microcystins (MC, congeners included are MC-LR, MC-LA, MC-YR, MC-RR), Anatoxin-a (ANA), Cylindrospermopsin (CYL) and Nodularin (NOD) are reported in μg L^{-1} and were analyzed via LCMS. Samples analyzed via UPLC/MS/MS included additional MC congeners (MC-LF, MC-LY, MC-LW, MC-WR, DAsp-LR, DAsp-3RR, HTyR) and are indicated by the asterisk. The concentrations that exceeded California's Tier III Danger health threshold are in bold type (no threshold currently exists for nodularin). Microcystin and cylindrospermopsin concentrations that exceeded United States Environmental Protection Agency recreational criteria are italicized.

Sample Collection Date	Type of sample	MC	ANA	CYL	NOD
20 July 2016	Scum	***45,300***	**18**	***46***	bd
25 July 2016[a]	Scum	***12,000***	bd	bd	NA
01 Aug 2016	Scum	***1802***	**12**	***103***	bd
15 Aug 2016	Scum	***1132***	bd	***182***	10
15 Aug 2016	Foam	***16,623***	bd	***113***	1.7
18 Aug 2016[a]	Scum	***35***	bd	bd	NA
28 Aug 2016[a]	Scum	***12,000***	bd	bd	NA
29 Aug 2016	Foam	***9053***	bd	***37***	bd
12 Sept 2016	Foam	***10,955***	bd	***273***	19
26 Sept 2016	Scum	***9634***	**37**	bd	bd
11 Oct 2016	Foam	***489***	bd	bd	bd
21 Aug 2017	Scum	***71***	bd	**5.6**	bd
21 Aug 2017	Foam	***93***	bd	**11**	0.08

NA = not analyzed; bd = below detection.

[a]Samples analyzed via UPLC/MS/MS and include additional MC congeners (MC-LF, MC-LY, MC-LW, MC-WR, DAsp-LR, DAsp-3RR, HTyR).

Table 3

Results from Lake Elsinore water samples collected in 2016 analyzed by UPLC/MS/MS. The congeners included in analysis were MC-LR, MC-RR, MC-YR, MC-LA, MC-LF, MC-LY, MC-LW, MC-WR, DAsp-LR, DAsp-3RR, HTyR; however, only those for which toxin was detected are shown. Results are in μg L^{-1} and sample depths are either surface (S), depth integrated composite sample (DI), and shoreline at La Laguna Beach (LLS).

Sample Collection Date	Sample Depth	LR	RR	YR	WR	Dasp-3RR	Total MC
25 July	DI	2.9	4.8	1.2	12	bd	21
18 Aug	LLS	<0.50	0.60	0.50	4.9	bd	6.0
18 Aug	S	bd	<0.50	bd	4.8	bd	4.8
18 Aug	DI	bd	<0.50	0.4	4.7	bd	5.0
29 Aug	S	bd	2.5	<0.50	2.0	bd	4.5
29 Aug	DI	bd	1.7	0.8	1.4	bd	3.1
29 Aug	S	bd	1.4	<0.50	1.2	<0.50	2.6
29 Aug	DI	bd	1.4	<0.50	1.0	<0.50	2.4
29 Aug	S	bd	50	4.7	27	<0.50	81
29 Aug	LLS	bd	490	91	130	8.6	730
29 Aug	LLS	bd	2100	183	430	bd	2700

bd = below detection.

Table 4

Statistical analyses of the DNA sequencing results. ANOSIM R statistic and significance (in parentheses). Permutational multivariate analysis of variance using distance matrices (adonis) F statistic and significance (in parentheses).

	All ASVs	Cyanobacteria ASVs	Test
By month	0.76 ($p < 0.001$)	0.65 ($p < 0.004$)	ANOSIM
Total microcystins	1.07 ($p < 0.354$)	1.29 ($p < 0.24$)	adonis
Cylindrospermopsin	3.02 ($p < 0.33$)	3.84 ($p < 0.036$)	adonis
Chlorophyll-*a*	3.47 ($p < 0.014$)	4.43 ($p < 0.024$)	adonis

Table 5

List of cyanobacteria cultures isolated from Lake Elsinore and Canyon Lake in 2016, the date of sample collection and concentration of microcystins (MC) detected in the established culture analyzed by LC/MS/MS as described in section 2.3.3. Other MC congeners, cylindrospermopsin, and anatoxin-a were not detected and are not shown.

Genus/Species	Lake	Culture ID	Date of Isolation	MC-LR	MC-RR	MC-WR	MC-YR	MC-HiLR
Cylindrospermopsis	Lake Elsinore	EL-EA	26 July	bd	bd	bd	bd	bd
Aphanizomenon	Lake Elsinore	LE9	15 Sept	bd	bd	bd	bd	bd
Phormidium	Lake Elsinore	P-EL	26 July	bd	bd	bd	bd	bd
Planktolyngbya	Lake Elsinore	EL-E	26 July	bd	bd	bd	bd	bd
Microcystis aeruginosa	Lake Elsinore	N2-EL	23 Aug	1.72	448	506	100	0.52
Merismopedia	Lake Elsinore	M-EL	23 Aug	bd	bd	bd	bd	bd
Microcystis aeruginosa	Lake Elsinore	G-EL	16 Sept	0.24	153	19.5	13.8	bd
Planktothrix	Lake Elsinore	A4	26 July	bd	bd	bd	bd	bd
Raphidiopsis	Lake Elsinore	NAT9	23 Aug	bd	bd	bd	bd	bd
Pseudanabaena	Canyon Lake	PL-EL	26 Aug	bd	bd	bd	bd	bd
Cylindrospermopsis	Canyon Lake	Canyon A3	12 Sept	bd	bd	bd	bd	bd

bd = below detection.