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# CYANOTOXIN AND CYANOBACTERIA MONITORING IN LAKE ELSINORE AND CANYON LAKE
## 2015-2017


Prepared by:

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# Cyanotoxin and Cyanobacteria Monitoring in Lake Elsinore and Canyon Lake

# 2015-2017

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## EXECUTIVE SUMMARY

Harmful cyanobacteria blooms (cyanoHABs) have gained national attention in recent years due to the global increase in frequency, severity and spatial extent of blooms. CyanoHABs cause many water quality issues and can cause illness and mortality in humans, domestic pets, wildlife and livestock. As such, there is a growing recognition that water quality programs should include these biological contaminants, and cyanotoxins should be considered in ecological and human health risk assessments (Chapman, 2015, Brooks et al., 2016). Health advisory thresholds have been developed by EPA for drinking water and draft recreational water quality criteria have been proposed. California established health-based trigger thresholds to protect human and canine health in recreational waterbodies for three cyanotoxins, microcystins, cylindrospermopsin and anatoxin-a. There are 3 human health trigger thresholds established for recreational waters, including the Caution Trigger, the Warning Tier I trigger and the Danger Tier II trigger.

The goals of the current study were to provide an assessment of cyanobacteria and cyanotoxins present in Lake Elsinore and Canyon Lake. The specific objectives were to (1) determine if cyanotoxins are routinely present and if so, determine if concentrations exceed human and canine health trigger thresholds, (2) determine the potential toxin-producing cyanobacteria taxa routinely present, (3) determine if cyanotoxin monitoring is warranted on a routine and frequent basis to protect the health of humans, wildlife, domestic pets and the beneficial uses of the lakes

*The results from **Lake Elsinore** indicate the following findings:*

- **Cyanotoxins were chronically detected at relatively high concentrations that frequently exceeded California human and canine health trigger thresholds for recreational waterbodies**
  - These are currently the highest recorded concentrations from Southern California lakes
- **Multiple cyanotoxins were frequently detected simultaneously;** the health risks and consequences are unknown for co-occurring toxin exposure because health trigger thresholds are based on exposure to a *single* cyanotoxin
- **Cyanobacteria taxonomic identifications indicated a high risk for multiple cyanotoxins to be routinely produced and co-occur in Lake Elsinore**
- **DNA barcoding was a useful tool to identify cyanobacteria and determine relative abundance**

*The results from **Canyon Lake** indicate the following findings:*

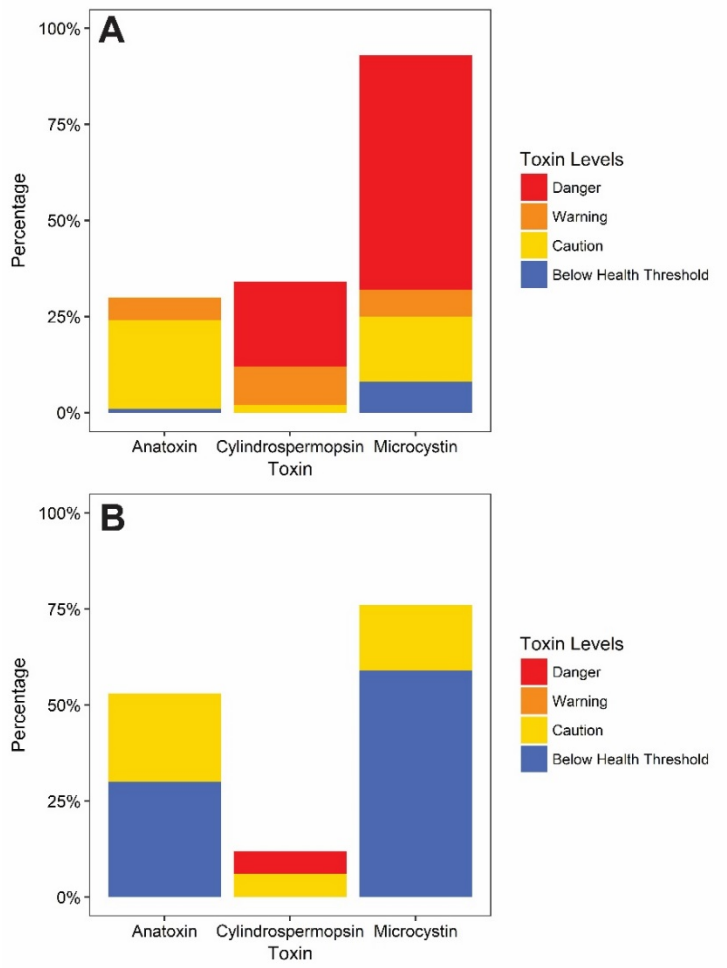
- **Microcystins were chronically detected at lower concentrations that occasionally exceeded California human health trigger thresholds**
  - There is a high risk of health consequences from chronic exposure to microcystins
- **Cylindrospermopsin and anatoxin-a were detected half of the time and only occasionally exceeded CA health trigger thresholds**
- **Cyanobacteria taxonomic identifications indicated a high risk for multiple cyanotoxins to be routinely produced and co-occur in Canyon Lake**

*Based on the results of this assessment program, the following is recommended:* **Cyanotoxins should be frequently and routinely monitored in both Lake Elsinore and Canyon Lake.** The dominance of

cyanobacteria, the ubiquitous and persistent detection of cyanotoxins, and the exceedance of CA human and canine health trigger thresholds in these highly frequented recreational lakes, indicate routine and frequent monitoring of cyanotoxins should be implemented to protect public health as well as wildlife and domestic pets.

**Figure ES1. The cyanotoxin results from (A) Lake Elsinore and (B) Canyon Lake from 2015 – 2017.**

The percentage of toxin samples that exceeded each health threshold are shown in the bar plots. The percent of samples that were positive but below human health trigger thresholds (blue), above human health trigger thresholds, yellow, orange and red, corresponding to Caution, Warning and Danger thresholds, respectively).



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## INTRODUCTION

Cyanobacteria are photosynthetic prokaryotes that have existed naturally for billions of years (Summons et al. 1999, Schopf 2000), inhabiting a wide variety of aquatic environments, including freshwater, brackish and marine ecosystems and can form dense blooms (Paerl 1988, Paerl and Fulton 2006, Whitton 2012). Many cyanobacteria are capable of producing toxins, referred to as cyanotoxins, which have gained national attention in recent years due to the global increase in frequency and duration of toxic blooms (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 environmental factors such as nutrient overenrichment and eutrophication, increased temperature, salinity, water residence time, vertical stratification and pH, many of which will likely be exacerbated with climate change (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).

Cyanotoxins represent a significant risk for humans, livestock, pets, and wildlife, causing illness and mortality (Edwards et al. 1992, van Halderen et al. 1995, Mez et al. 1997, Pouria et al. 1998, Carmichael et al. 2001, Backer et al. 2008, Stewart et al. 2008, Wood et al. 2010, Li et al. 2011, Backer et al. 2013, Trevino-Garrison et al. 2015). The acute poisoning symptoms in humans and animals include nausea, vomiting, diarrhea, cough, sore throat, rash and liver damage (Li et al. 2011, Backer et al. 2013, Weirich et al. 2014, Trevino-Garrison et al. 2015). Illness and mortality related to cyanotoxin poisoning in pets and livestock is likely significantly under-reported (Wood et al. 2010, Backer et al. 2013, Trevino-Garrison et al. 2015). The routes of human exposure to cyanotoxins include ingestion, inhalation and dermal contact. Ingestion pathways include consumption of contaminated shellfish or drinking water, as well as consumption of vegetables that are irrigated with contaminated water (Mohamed et al. 2009). Inhalation of aerosolized cyanotoxins can be a significant exposure route during recreational activities in bloom-ridden lakes (Backer et al. 2008, 2009, Levesque et al. 2014). Finally, many studies have documented the accumulation of cyanotoxins in aquatic and terrestrial organisms including freshwater and saltwater mussels, farmed crustaceans, corals, fish, zooplankton, crabs, oysters, clams, snails, and ducks (Williams et al. 1997, Amorim and Vasconcelos 1999, Matsunaga et al. 1999, Vasconcelos et al. 2001, Malbrouk and Kestemont 2006, Zimba et al. 2006, Richardson et al. 2007, Lehman et al. 2010, Miller et al. 2010). The accumulation and biomagnification of cyanotoxins into food webs is the main mechanism that causes illness and mortality in both terrestrial and aquatic wildlife, and represents a poorly understood exposure pathway in humans (see reviews by Stewart et al. 2008, Havens 2008).

Cyanotoxins produced in these freshwater systems have been shown to have effects far downstream of their biological origin, in marine ecosystems. A recent mass mortality of over 30 marine sea otters in Monterey Bay was due to microcystin intoxication from ingestion of contaminated shellfish (Miller et al. 2010). Microcystin was produced in Pinto Lake, a eutrophic water body that experiences frequent cyanobacterial blooms and drains to Monterey Bay via a 15-km segment of the Pájaro River (Miller et al. 2010, Kudela 2011). Watershed studies of Monterey Bay have shown that this downstream transport of microcystins is a persistent and prevalent issue throughout the watershed (Gibble and Kudela 2014). These studies underscore an important role of rivers as conduits that can transport intact toxins from inland waters to downstream marine environments.

At both the federal level and within California, there is a growing recognition of the health and beneficial use risk of cyanotoxins. Due to this growing recognition that HABs can severely impact water quality and should be routinely monitored (Chapman 2015, Brooks et al. 2016), the U.S. Environmental Protection Agency has recently released health advisory thresholds for cyanotoxins in drinking water, has listed three cyanotoxins on the Contaminant Candidate List 3 (CCL3) (U.S. Environmental Protection Agency 2010) and has drafted proposed human health recreational ambient water quality criteria and/or swimming advisories for 2 cyanotoxins (https://www.epa.gov/wqc/draft-human-health-recreational-ambient-water-quality-criteria-andor-swimming-advisories). USGS has recently prioritized 12 cyanotoxins as Tier 1, or highest priority for inclusion in ambient water monitoring in the US (an additional 3 were listed at intermediate or low priority) (Olsen et al. 2013). California established health-based exposure thresholds to protect human and canine health in recreational waterbodies for three cyanotoxins, microcystins, cylindrospermopsin and anatoxin-a (OEHHA, 2012). There are separate thresholds for water, fish, and cyanobacterial mat matrices. In 2016, there was an amendment to the OEHHA voluntary guidance that established 3 human health trigger thresholds for recreational waters. These thresholds are the following:

**Table 1. Cyanobacteria harmful algal bloom triggers for recreational waters (all concentrations are listed in μg $L^{-1}$).**

| | Caution Trigger Level | Warning Tier I* | Danger Tier II* |
|---|---|---|---|
| Microcystins | 0.8 | 6 | 20 |
| Anatoxin-a | Detection | 20 | 90 |
| Cylindrospermopsin | 1 | 4 | 17 |

http://www.mywaterquality.ca.gov/monitoring_council/cyanohab_network/docs/triggers.pdf\.
*The CA Danger Tier II trigger thresholds recommends "people, pets and livestock should stay out of the water and away from water spray" and the Warning trigger threshold states " swimming is not recommended and that pets and livestock should be kept away from the water" (http://www.mywaterquality.ca.gov/monitoring_council/cyanohab_network/docs/appendix_a.pdf).

These recreational health advisory thresholds were created in response to recurring hotspots of toxic blooms in the Klamath River watershed, Clear Lake, Pinto Lake, Sacramento and San Joaquin River Delta, Lake Elsinore, and East San Francisco Bay Area lakes. Additionally, Copco and Iron Gate Reservoirs, the Klamath River, and Pinto Lake were placed on the State's 303d list due to impairment caused by microcystins, the first such listing in the State.

Preliminary samples collected in response to visible cyanobacterial blooms in May 2014 detected multiple cyanotoxins in Lakes Elsinore, Menifee and Canyon. Lake Elsinore samples exceeded CA recreational health trigger thresholds for humans for microcystin and cylindrospermopsin (Table 1). The concentrations detected were 1.2 μg/L and 4.1 μg/L respectively. *Cylindrospermopsis spp*. dominated the community composition and *Anabaena spp*. was also observed (both potential toxin producers). Canyon Lake had 3 cyanotoxins present, microcystins, 0.3 μg/L, cylindrospermopsin, 2.7 μg/L and anatoxin-a, 4.6 μg/L simultaneously, and 2 were above health trigger thresholds for recreational waters (Table 1). The goals of the current study were to expand on these results and provide a more comprehensive assessment of cyanobacteria and cyanotoxins present in the lakes. The specific objectives were to:

- Determine if cyanotoxins are routinely present and if so, determine if concentrations exceed human health trigger thresholds
- Determine the potential toxin producing cyanobacteria taxa routinely present
- Determine if cyanotoxin monitoring is warranted on a routine basis to protect human and wildlife health and the beneficial uses of the lakes

## MATERIALS AND METHODS

### Discrete Sample Collection

Discrete surface water samples were collected based on where the highest biomass had accumulated in the lake. This was determined by conducting boat surveys on the City of Lake Elsinore operations boat. A YSI sonde was deployed at the side of the boat to determine the location of highest chlorophyll-a in the highest recreational area of the lake (northwest side of the lake). Scum and foam samples were collected when present, usually at the northwest shoreline shown in Figure 1 (La Laguna Recreational Beach). Samples were collected monthly in 2015 from July through October, bi-monthly or every 2 weeks in 2016, and there were two collection dates in 2017 (21 August 2017 and 06 September 2017). For Canyon Lake, a similar approach was employed that focused on the east arm of the lake based on historical chlorophyll-a data illustrating high biomass in that arm of the lake.

Discrete surface water samples were collected in a 1-L glass bottle and then individual samples were collected for cyanotoxin, chlorophyll-a and DNA barcoding analysis. Whole water cyanotoxin samples were collected in 250-mls glass jars in 2016 and 2017 and frozen immediately in the field, and stored at -20$^{o}$C. The 2015 samples were collected in 100-mls HPDE plastic bottles, not glass, and stored as described above.

Chlorophyll-a (chl-a) samples were collected in 2016 and 2017 at Lake Elsinore and in 2016 at Canyon Lake. Chl-a samples were filtered onto 25mm Whatman GF/F filters (GE Whatman, Marlborough, MA, USA), frozen immediately, stored at -80$^{o}$C and analyzed within 2 weeks of collection.

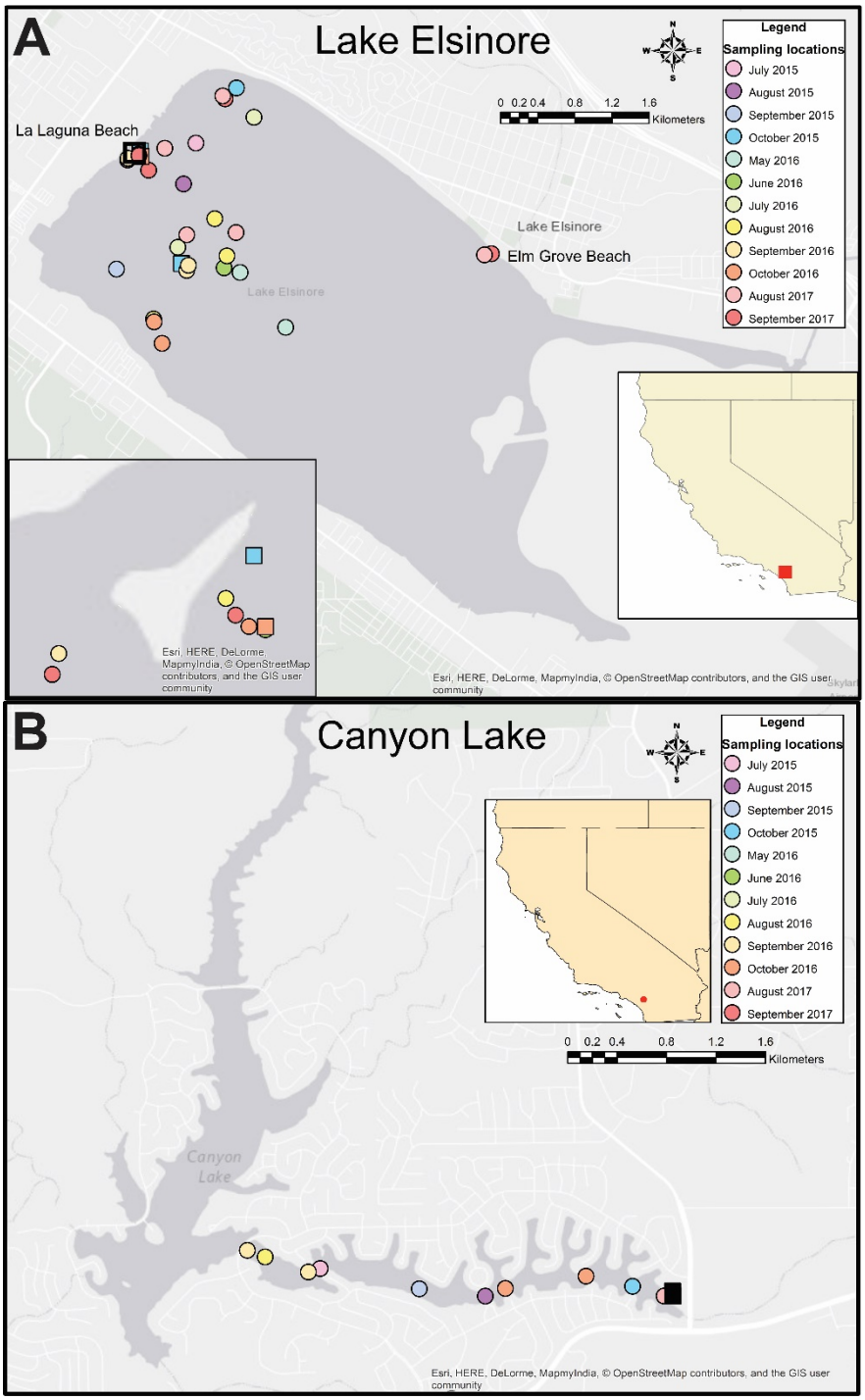
DNA barcoding samples were collected from Lake Elsinore in 2016 for 16S rRNA gene sequencing and community analysis as part of a pilot study to compare with microscopy samples. These samples were filtered onto a 0.2 um Whatman Nucleopore polycarbonate filter (GE Healthcare Life Sciences, Buckinghamshire, UK) and submerged in bead solution storage buffer (Mo Bio Laboratories, Inc., Carlsbad, CA) before being stored at -80°C. Before extraction, filters were thawed at 4°C. DNA extractions were performed with the PowerLyzer PowerSoil DNA isolation kit (Mo Bio Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. DNA yield was assessed with the Nanodrop 8000 (Thermo Scientific, Wilmington, DE, USA).

Discrete samples for cyanobacterial identification and relative abundance via microscopy were collected simultaneously with the other discrete samples in a 1.5-L HPDE bottle, placed inside a cooler, stored in an incubator overnight at the Southern California Coastal Water Research Project and analyzed live the following day.

Passive sampling devices, Solid Phase Adsorption Toxin Tracking, SPATT (MacKenzie et al., 2004, Lane et al., 2010, Kudela, 2011), were deployed continuously between site visits and provided time-integrated dissolved toxin samples of cyanotoxin presence. SPATT were deployed in 1 location of each lake in 2015

and 2016 (but none deployed in 2017), determined by the accessibility in high recreational use areas and ease of deployment, under a floating dock (see Figure 1 for locations).

**Figure 1. Map of study area and sampling locations at Lake Elsinore (A) and Canyon Lake (B).** SPATT sampling locations are shown by the black and colored squares and discrete sample locations are shown by the circles. Colors indicate collection timepoints.



**Cyanotoxin Analysis**

Discrete cyanotoxin samples were analyzed at the University of California, Santa Cruz and were extracted and processed according to methods described in Mekebri et al. 2009 and Kudela 2011, for four microcystin congeners (MCY-LA, MCY-LR, MCY-RR, MCY-YR) and nodularin with the modifications described in (Miller et al. 2010, Kudela et al 2011). The 2017 sample analysis also included an additional congener, MCY-LF. Anatoxin-a and cylindrospermopsin were analyzed according to EPA 545 (https://19january2017snapshot.epa.gov/sites/production/files/2015-11/documents/epa_815-r-15-009_method_545.pdf). Briefly, samples were stored frozen until extraction and processing, and analyzed by liquid chromatography/mass spectrometry (LC-MS) with electrospray ionization (ESI) with selected ion monitoring (SIM) on an Agilent 6130 instrument equipped with a Phenomenex Kinetix C18 column (microcystins and nodularin) or Agilent Polaris-Ether C18 column (anatoxin-a and cylindrospermopsin). Blanks were analyzed for every 10 samples, and standard curves were run at the beginning and end of each set of samples. Matrix Spike recoveries were completed with each sample run. Cyanotoxin samples were processed by mixing with 100% MeOH (1:1; final concentration 50%) and processed by sonication.

Anatoxin-a samples were prepared by sonicating whole water and syringe-filtering (using a 0.2 µm Teflon filter) with direct-injection of 20 µL sample. The analytical method followed Cogent KnowledgeBase article AA-00807 using anatoxin-a dissolved in 7:93 MeOH:H20 with 0.1% formic acid (National Research Center Canada) as the reference. Cogent, AA-00807. Accessed at http://kb.mtc-usa.com/article/AA-00807/0/Anatoxin-a-ANTX-A.html.

Saxitoxin samples were analyzed using the BIOO Scientific MaxSignalTM Saxitoxin (PSP) ELISA test kit (BIOO Scientific Corp., Austin, TX, Cat. No. 1034). The manufacturer's instructions were followed for sample extraction and analysis.

Shoreline samples were collected from 2 locations at Lake Elsinore, Elm Grove Beach and La Laguna Beach, on 21 August 2017 and 06 September 2017 (Figure 1). Those samples were collected as described above in the Discrete Sample Collection section, but cyanotoxin analysis was conducted at Bend Genetics, LLC (Sacramento, CA) using Abraxis ADDA ELISA Kit PN520011 for microcystins, Abraxis ELISA kit, PN520060 for anatoxin-a, and Abraxis ELISA kit, PN622011 for cylindrospermopsin.

SPATT samples were analyzed at the University of California, Santa Cruz for four microcystin congeners (MCY-LA, MCY-LR, MCY-RR, MCY-YR) and nodularin by liquid chromatography/mass spectrometry (LC-MS) with electrospray ionization (ESI) with selected ion monitoring (SIM) on an Agilent 6130 with a Phenomenex Kinetix (100x2.10) C18 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 using the same LCMS. The samples were prepared as described in Kudela, 2011. Analysis included replicates and matrix-additions, with the quantification based on external standards. The Method Detection Limit was 0.05 ng $g^{-1}$ for all congeners. Percent recovery is reported in Kudela, 2011, and was ~58-100% for the congeners using a standardized recovery method, with MCY-RR being lowest followed by MCY-LR (~88%), MCY-YR (~100%), and MCY-LA (~100%).

### Cyanobacteria Identification by Microscopy and Genetic Barcoding

*Relative Abundance by Microscopy*: Potential toxin-producing cyanobacteria were identified to the genus level (species when possible). Briefly, samples were homogenized by successive inversions and an aliquot was poured into 20mL tissue culture dishes, settled overnight and viewed under an Olympus CKX41 inverted microscope, at the University of Southern California. Each cyanobacteria genus identified was assigned a relative abundance code based on the percentage of the community composition of the sample. Relative abundance codes are as follows: dominant (D) (>50% of the community composition), Abundant (A) (25-49% of the community composition), Common (C) (10-24% of the community composition), Present (P) (1-9% of the community composition), and Rare (R) (<1% of the community composition).

*DNA Barcoding:* The V4 region of the 16S rRNA gene was sequenced according to the Earth Microbiome Project (EMP) recommended protocols, using universal using universal Bacteria/Archaea primers 515 F/806 R (Caporaso et al., 2011; 2012). Paired-end 2 × 250 basepair sequencing from barcoded amplicon products was performed at Laragen, Inc (Culver City, CA, USA) on an Illumina MiSeq platform.

Raw Illumnina DNA sequences were demultiplexed and all sequences were removed if they contained >1 mismatch to the barcode sequence. Sequences were further processed using the Illumina MiSeq Recorder software to remove adapter, barcode, and primer sequences. DNA sequences were further processed using QIIME v1.9.1 (Caporaso et al., 2010), forward and reverse DNA reads were merged into contigs, singleton reads were removed, chimeric sequences were removed, reads were discarded with ambiguous 'N' base calls, and files were converted into fasta format. De novo, furthest-neighbor operational taxonomic units (OTU) were assembled and representative sequences chosen from UCLUST's OTU clustering algorithm as implemented in QIIME with a maximum sequence identity difference of 3% (corresponding to OTU identity threshold of 97%) (Edgar, 2010). The most abundance sequence in each OUT cluster was chosen as a representative sequence. The taxonomic assignment of the representative OUT sequences was determined using UCLUST in QIIME against the SILVA v128 taxonomy database (Yilmaz et al., 2013). All samples were rarified down to the lowest total reads of the sample set (45,000). To better understand community structure, we performed Analysis of Similarity (ANOSIM) analyses as implemented in the R package *vegan* (Oksanen et al., 2013) using Bray-Curtis distances. We performed non-metric multidimensional scaling (NMDS) analysis and alpha diversity analyses using the R package *phyloseq* (McMurdie et al., 2013).

### Chlorophyll-a Analysis

Chlorophyll-a (chl-a) samples were collected in 2016 and 2017 and analyzed within 2 weeks of collection following EPA 445.0. Samples were extracted in 90% acetone for 24 hours at -20$^{o}$C in the dark and analyzed using a Turner Trilology fluorometer (Turner Designs, Sunnyvale, CA USA) at the Southern California Coastal Water Research Project.

## RESULTS

### Lake Elsinore Cyanotoxins

*Overall summary, 2015-2017:* Over all three years, 68% of water samples and 71% of SPATT samples indicated there were 2 or more cyanotoxins detected, and 37% and 7% of water and SPATT samples

indicated 3 or more cyanotoxins detected (Figure ES3). For all years, microcystin was detected in 92.7% of water samples, which ranged from not detected to 5,665 μg $L^{-1}$ and ranged from 36.5 – 45,300 μg $L^{-1}$ in foam and scum samples (Figure 2, Table 3). Of the positive water samples, 17% were at the caution health threshold (0.8 - 5.9 μg $L^{-1}$), 7% were at the warning health threshold (6 – 19.9 μg $L^{-1}$) and 61% were at the danger health threshold (≥20 μg $L^{-1}$). The SPATT sample results collected throughout 2015 and 2016 were all positive for microcystins (100% of samples detected microcystins) and ranged from 1.35 ng $g^{-1}$ to 845 ng $g^{-1}$ (Table 2). Anatoxin-a and cylindrospermopsin were detected in 29.3% and 34.1% of samples respectively, and 21.4% and 57.1% of SPATT samples respectively (Figure 2, Table 2). Anatoxin-a ranged from below detection to 0.5 μg $L^{-1}$ in water samples and ranged from 11.7 – 37 μg $L^{-1}$ in foam and scum samples. Of these samples, 23% were at the caution health threshold (detection - 19.9 μg $L^{-1}$) and 6% were at the warning threshold (20 - 89.9 μg $L^{-1}$). The SPATT results for anatoxin-a ranged from below detection to 299 ng $g^{-1}$. Cylindrospermopsin water sample results ranged from not detected to 21.2 μg $L^{-1}$ and 37 – 273 μg $L^{-1}$ from foam and scum samples (Figure 2, Table 3). Of these samples, 2.4% were at the caution health threshold (1 – 3.9 μg $L^{-1}$), 10% were at the warning health threshold (4 – 16.9 μg $L^{-1}$), and 22% were at the danger threshold (≥17 μg $L^{-1}$). The SPATT sample results ranged from not detected to 4,465 ng $g^{-1}$ (Figure 2, Table 2).

*2015 Results:* Water samples collected in 2015 indicated cyanotoxin concentrations were extremely low or not detected in Lake Elsinore. Microcystins were detected on July 29, 2015 (0.01 μg $L^{-1}$) but were not detected for the other 3 monthly sampling dates in August, September and October. However, the SPATT samplers indicated low concentrations of microcystin and ranged from 1.3 ng $g^{-1}$ – 40.5 ng $g^{-1}$ (Table 2, Figure 2). SPATT samplers were deployed, but not recovered from Lake Elsinore in July and August 2015. Anatoxin-a and cylindrospermopsin were not detected in any of the 4 water samples collected in 2015. There was one sample collected on September 16, 2015, that contained 1.5 μg $L^{-1}$ of saxitoxin. Nodularin was analyzed on one water sample collected on 14 October 2015 and was below the method detection limit.

**Table 2 Cyanotoxin results from all SPATT samples collected from Lake Elsinore (LE) and Canyon Lake (CL) in 2015.**

| Location | Date | MCY | ANA | CYL |
|---|---|---|---|---|
| LE | 18 September | 40.52 | <MDL | <MDL |
| LE | 14 October | 1.35 | <MDL | <MDL |
| LE | 14 October | 3.21 | 4.85 | <MDL |
| CL | 29 July | 22.80 | <MDL | <MDL |
| CL | 20 August | 33.66 | <MDL | <MDL |
| CL | 16 September | 9.95 | <MDL | <MDL |
| CL | 14 October | 4.50 | <MDL | <MDL |

Microcystins (MCY; includes -LR, -LA, -YR-, -RR congeners), Anatoxin-a (ANA), and Cylindrospermopsin (CYL) concentrations are in ng $g^{-1}$. Sample collection date represents retrieval date.

*2016 Results:* In contrast to 2015, 2016 samples had relatively high concentrations of multiple cyanotoxins detected simultaneously that far exceeded the California recreational health advisory thresholds. Microcystins were detected in all water, foam, scum and SPATT samples collected in 2016 (Figure 2, Table 3). All microcystin concentrations detected from water, foam and scum samples exceeded the CA health thresholds except for one collected on 09 May 2016 (0.6 μg $L^{-1}$) and ranged from 0.6 – 45,300 μg $L^{-1}$. The caution threshold was exceeded for 21.7% of 2016 samples (see Table 1 for CA

health thresholds), 4% exceeded the warning health threshold and 65% exceeded the danger health threshold. The most common microcystin congener detected from water, scum and foam samples was MCY-RR (91% of samples), followed by MCY-LR (78%), MCY-YR (65%) and MCY-YR was only detected in 1 sample from 24 October 2016 (see Appendix A). SPATT sample results were similar, as MCY-RR was the most common congener detected (91%), followed by MCY-LR (82%), MCY-YR (54%), and MCY-LA was only detected from the same date as the water samples listed above. Anatoxin-a was detected in 26% of the water, foam and scum samples collected in 2016 and ranged from below detection to 37 µg $L^{-1}$ (Figure 2, Table 3). The caution threshold was exceeded in 22% of the samples, 4% exceeded warning threshold, and none exceeded the danger threshold. Only 2 SPATT samples detected anatoxin-a, on 06 August 2016 and 24 October 2016, and concentrations in 2016 ranged from not detected to 299 ng $g^{-1}$. Cylindrospermopsin was detected in 52% of water, grab and scum samples and concentrations ranged from below detection to 273 µg $L^{-1}$. Of these, 4% were at the caution health threshold, 9% were at the warning threshold, and 39% were at the danger threshold. The SPATT samplers detected cylindrospermopsin in 73% of samples from 2016 and concentrations ranged from below detection to 4,465 ng $g^{-1}$. Nodularin was detected in 22% of water, scum and foam samples and ranged from below detection to 18 µg $L^{-1}$ and was not detected in any of the SPATT sample results. Saxitoxin was analyzed from water samples collected in May through August 2016, but no saxitoxin was detected in any of these samples.

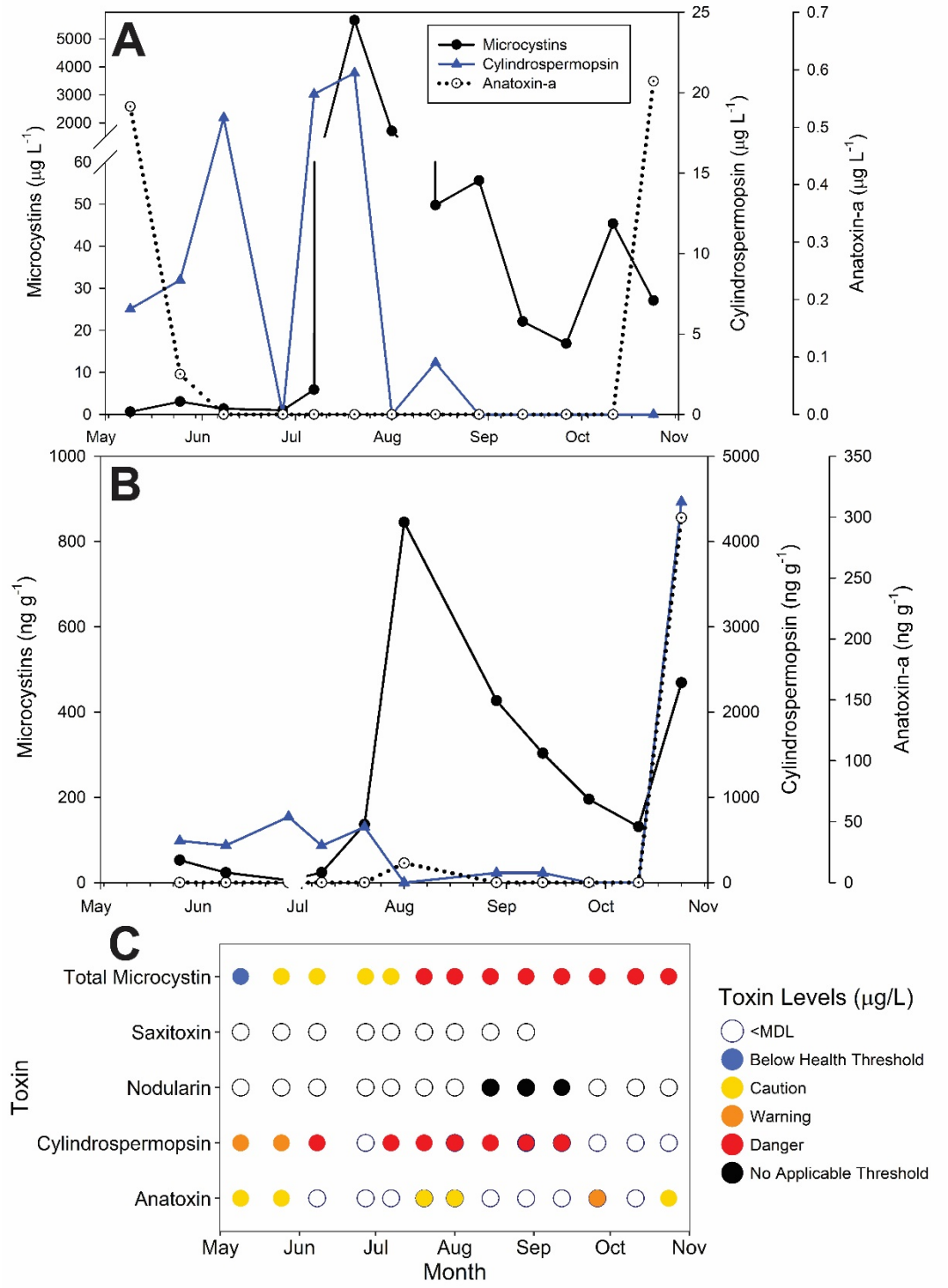
**Table 3 Table showing all scum and foam sample results for cyanotoxins collected from Lake Elsinore in 2016 and 2017.**

| Sample Collection Date | Type of sample | MCY | ANA | CYL | NOD |
|---|---|---|---|---|---|
| 20 July 2016 | Scum | **45,300** | **18.5** | **45** | <MDL |
| 01 August 2016 | Scum | **1,801** | **11.7** | **103** | <MDL |
| 15 August 2016 | Scum | **1,131** | <MDL | **181** | 10.2 |
| 15 August 2016 | Foam | **16,623** | <MDL | **112** | 1.7 |
| 29 August 2016 | Foam | **9,053** | <MDL | **37** | <MDL |
| 12 September 2016 | Foam | **10,955** | <MDL | **273** | 18.8 |
| 26 September 2016 | Scum | **9,634** | **37.1** | <MDL | <MDL |
| 11 October 2016 | Foam | **488** | <MDL | <MDL | <MDL |
| 21 August 2017 | Scum | **71** | <MDL | **5.6** | <MDL |
| 21 August 2017 | Foam | **93** | <MDL | **11** | 0.08 |

Microcystins (MCY; includes -LR, -LA, -YR-, -RR congeners), Anatoxin-a (ANA), Cylindrospermopsin (CYL) and Nodularin (NOD) concentrations are in µg $L^{-1}$. All concentrations for microcystins, anatoxin-a and cylindrospermopsin exceeded California recreational health thresholds for the Tier III Danger threshold and are shown in bold type. No health thresholds have been established for nodularin.

**Figure 2 Cyanotoxin concentrations detected in water samples (A) and SPATT samples (B) collected from Lake Elsinore 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 black dotted line and white circles. The toxins that exceeded California Recreational Health Thresholds are shown in color according to threshold (C).



*2017 Results:* The 2017 results indicated multiple, co-occurring cyanotoxins detected simultaneously, similar to 2016. All samples collected exceeded the health thresholds for microcystins, and concentrations ranged from 1.3 – 355 µg $L^{-1}$ from water, foam and scum samples (Table 3 foam and scum sample concentrations). The shoreline samples collected from Elm Grove Beach and La Laguna Beach ranged from 33 – 129 µg $L^{-1}$ on 21 August 2017 and 43 – 311 µg $L^{-1}$ on 06 September 2017. The water samples collected via boat ranged from 1.3 – 149 µg $L^{-1}$ on 21 August 2017 and 7 – 355 µg $L^{-1}$ on 06 September 2017. The congeners, MCY-LR and MCY-RR, were detected in all 6 samples collected on 21 August 2017 and in all 3 samples collected on 06 September 2017 and ranged from 0.1 – 20 µg $L^{-1}$ and 1 – 121 µg $L^{-1}$ respectively. MCY-YR was detected in 4 samples collected on 21 August 2017 and from 1 sample collected on 06 Sept 2017 and ranged from 2 – 76 µg $L^{-1}$ and 5 – 231 µg $L^{-1}$ respectively. The MCY-LA and MCY-LF congeners were included in the analysis, however, neither of these congeners were detected in any of the 2017 samples. Nodularin was detected in one foam sample collected on 21 August 2017 (0.08 µg $L^{-1}$). Anatoxin-a exceeded the caution health advisory on both sample collection dates in 2017. Shoreline samples ranged from 0.2-0.6 µg $L^{-1}$ and from below detection to 0.3 µg $L^{-1}$ from samples collected on 21 August 2017 and 06 September 2017, respectively. Anatoxin-a was not detected from any of the boat samples collected on 21 August 2017 and ranged from below detection to 0.3 µg $L^{-1}$ from samples collected on 06 September 2017. Cylindrospermopsin was not detected in any of the shoreline samples collected in 2017. The discrete samples collected from the boat ranged from below detection to 11 µg $L^{-1}$ collected on 21 August 2017, which exceeded the Warning Tier I health advisory and all samples collected on 06 September 2017 were below detection.

**Lake Elsinore Cyanobacterial Community Composition and Chlorophyll-a**

Lake Elsinore surface water cyanobacteria abundance from 2015 relative abundance results indicated the most abundant (25-50% of community composition) cyanobacteria genera were *Aphanizomenon*, *Cylindrospermopsis*, and *Planktothrix* (Figure 3A). The commonly observed (10-25% of community composition) genera included (in addition to those listed above) *Merismopedia*, *Planktolyngbya* and *Raphidiopsis*. The 2016 results exhibited a distinct shift in dominant species between early and late July as shown by both the microscopy relative abundance results (Figure 3B) and by the DNA barcoding (16S rRNA gene sequencing and community analysis) results (Figure 4A).

**Figure 3 Relative abundance 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 (R) <1%, Present (P) 1 - <10%, Common (C) 10 - <25%, Abundant (A) 25-<50%, Dominant (D) 50-100% and these are represented by different colors ranging from bright yellow to red from lowest to highest abundance. The exact colors for each category are in the legend at the bottom of the figure.

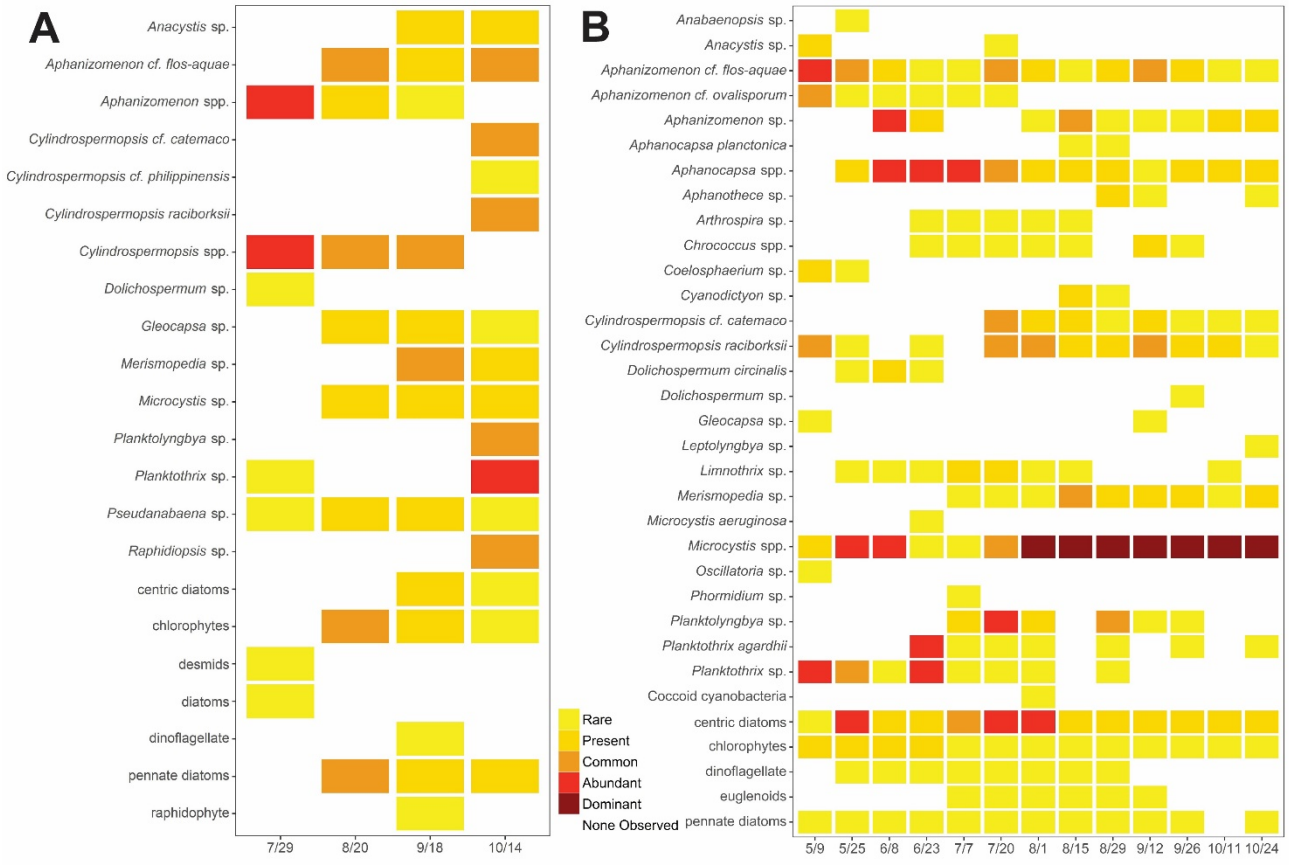
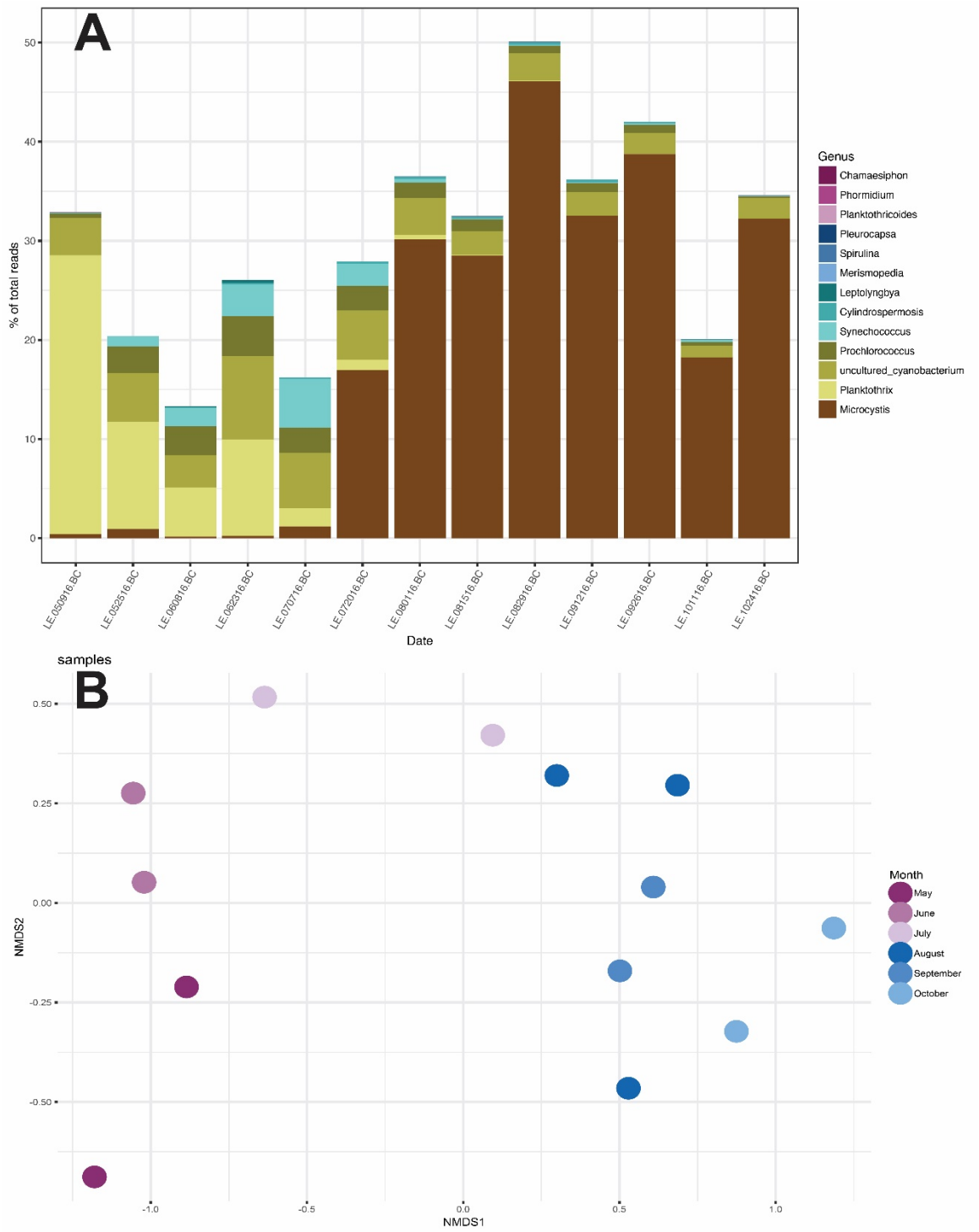


**Figure 4 Surface water cyanobacterial genera relative abundance as measured by DNA barcoding.** The relative abundance is presented as a percentage of total sequence reads throughout the Lake Elsinore 2016 season (A). NMDS of surface water cyanobacteria sequence reads, with chlorophyll a and toxin concentrations. Stress = 0.055 (B).




May, June, and early July 2016 cyanobacteria populations were comprised mostly of *Planktothrix* species, and on some dates *Aphanocapsa* and *Aphanizomenon* as well. The *Planktothrix* was primarily

from a single operational taxonomic unit (OUT) that comprised approximately 27% of the total microbial sequence reads in early May. In mid-July, there was a shift in the most abundant cyanobacterium as indicated by both the microscopy (Figure 3) and the DNA barcoding results (Figure 4). These samples were dominated by a single *Microcystis* OTU that at its peak (August 29, 2016) comprised approximately 45% of the total microbial sequence reads. Putatively-toxic genera *Leptolyngbya* and *Cylindrospermosis* were both present in the lake although at levels < 1% of the total microbial population according to the sequencing results and were rare (<1% of community composition) and common (10-25% of community composition) respectively as indicated by relative abundance results. Both the cyanobacterial community and total microbial community structure saw a decrease in alpha diversity beginning in July, synchronously with the increase in *Microcystis* dominance. Likewise, both the cyanobacteria and total microbial community were strongly structured by sampling month (ANOSIM, Table 4), and weakly associated with chl-a concentrations (PERMANOVA, Table 4).

**Table 4. Results of statistical analyses of genetic barcoding results.**

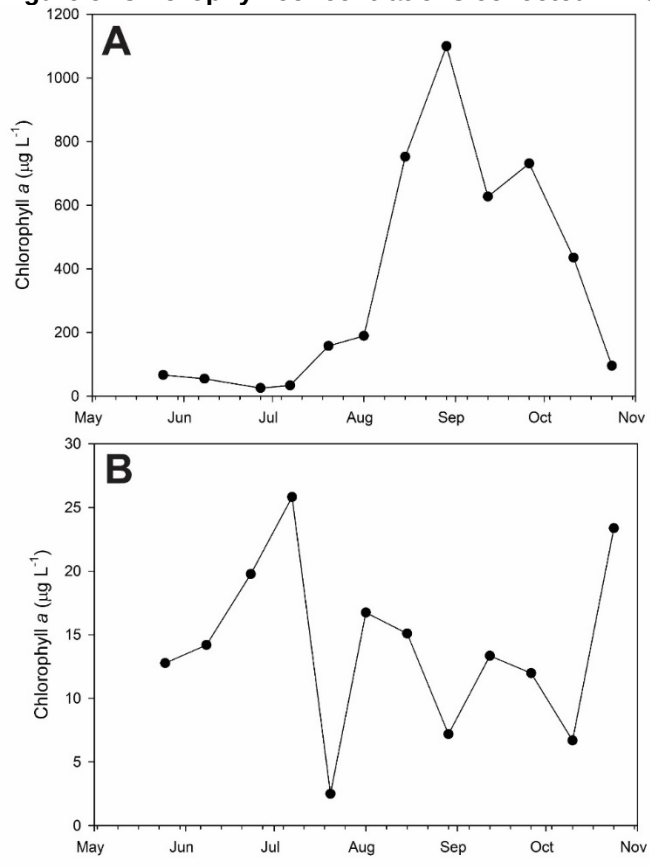
| | All OTUs | Cyanobacteria OTUs | Test |
|---|---|---|---|
| **By month** | 0.7714 ($p < 0.001$) | 0.6214 ($p < 0.001$) | ANOSIM |
| **Total microcystins** | 0.94286 ($p < 0.49$) | 0.80186 ($p < 0.64$) | PERMANOVA |
| **Cylindrospermopsin** | 1.7546 ($p < 0.07$) | 2.7508 ($p < 0.04$) | PERMANOVA |
| **Chlorophyll a** | 2.0162 ($p < 0.05$) | 3.7668 ($p < 0.02$) | PERMANOVA |

ANOSIM R statistic and significance in parentheses. PERMANOVA F statistic and significance in parentheses.

Previous studies of cyanobacteria species identification in 2003 and 2010 in Lake Elsinore indicate similar results, since potential toxin producing species identified in 2003 and 2010 included *Cylindrospermopsis raciborskii, Cylindrospermopsis c.f. catemaco, Aphanizomenon, Pseudanabaena limnetica, Pseudanabaena c.f. acicularis, Pseudanabaena catenata, and Planktothrix agardhii* (Oza, 2003, Tobin, 2011).

Chl-a results from 2016 ranged from 25 – 1,099 µg $L^{-1}$ with the peak on 29 August 2016, and highest concentrations (exceeding 600 µg $L^{-1}$) in August and September (Figure 5). Chl-a results from 2017 were 137 µg $L^{-1}$ on 21 August 2017 and ranged from 136 – 295 µg $L^{-1}$ on 06 September 2017. All chl-a concentrations measured were above the 25 µg $L^{-1}$ target threshold for 2020 (http://www.sawpa.org/wp-content/uploads/2012/05/Lake-Elsinore-and-Canyon-Lake-2015-2016-TMDL-Monitoring-Report_FINAL_Text_rev081616.pdf).

**Figure 5: Chlorophyll concentrations collected in 2016 at (A) Lake Elsinore and (B) Canyon Lake.**




**Canyon Lake Cyanotoxin Results**

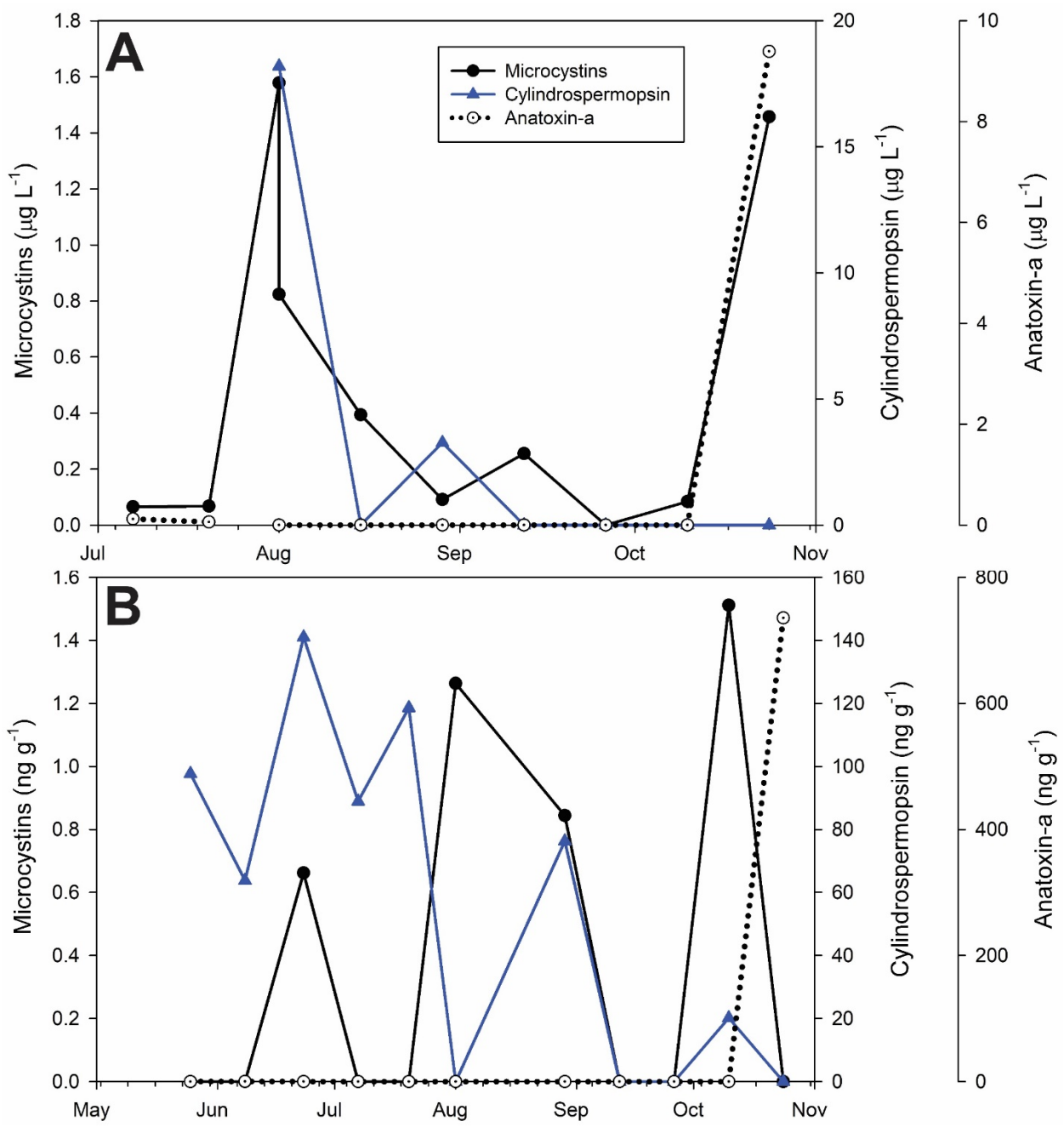
*Overall summary, 2015-2017:* Over all three years, 35% of water samples and 27% of SPATT samples indicated there were 2 or more cyanotoxins detected and 6% and 7% of water and SPATT samples indicated 3 or more cyanotoxins detected. For all years, microcystin was detected in 76% of water samples, which ranged from not detected to 1.5 μg $L^{-1}$ (Figure 6). Of the positive water samples, 17% exceeded Caution health threshold and none exceeded the Warning Tier 1 and Danger Tier II health thresholds (Table 1). Microcystins were detected in 57% of SPATT samples, and ranged from below detection to 33 ng $g^{-1}$ (Figure 6 shows 2016 data, Table 2 shows 2015 data). Anatoxin-a was detected in 53% of water samples and ranged from below detection to 9.4 μg $L^{-1}$. Water sample concentrations exceeded the caution health threshold in 23% of water samples and the warning and danger thresholds were never exceeded. Anatoxin-a was detected in 14% of SPATT samples and concentrations ranged from below detection to 735 ng $g^{-1}$. Cylindrospermopsin was detected in 12% of all water samples and ranged from below detection to 18 μg $L^{-1}$. The caution health threshold was exceeded in 6% of samples, and the other 6% exceeded the danger threshold (none exceeded the warning threshold). Cylindrospermopsin was detected in 50% of SPATT samples and ranged from below detection to 141 ng $g^{-1}$. Nodularin was not detected in the water samples, and was only detected in one SPATT sample collected in 2016 (was not analyzed in 2015 samples), 0.1 ng $g^{-1}$.

*2015 Results:* Water sample results collected in 2015 indicated microcystin concentrations were extremely low or not detected in Canyon lake. Microcystins were detected on July 29, 2015 (0.02 µg $L^{-1}$) but were not detected for the other 3 monthly sampling dates (August, September and October). The SPATT results from Canyon Lake indicated there was consistent low microcystin concentrations throughout the study period with concentrations ranging from 4.5-34 ng $g^{-1}$ (Table 2). Anatoxin-a and cylindrospermopsin were not detected in any of the 4 water samples or SPATT samples collected in 2015. Saxitoxin was detected from Canyon Lake water samples collected on July 29,2015 (1.8 µg $L^{-1}$) and September 16, 2015 (1.4 µg $L^{-1}$).

*2016 Results:* In 2016, microcystins were detected in 90% of water samples, and 27% of samples exceeded the caution health threshold, while no samples exceeded the warning and danger thresholds and concentrations ranged from not detected to 1.5 µg $L^{-1}$ (Figure 6). MCY-RR was the most common congener detected and ranged from not detected to 0.8 µg $L^{-1}$, MCY-LR ranged from below detection to 0.6 µg $L^{-1}$ and MCY-YR and MCY-LA were only detected in 1 sample on 24 October 2016 and 1 August 2016 respectively. Microcystins were detected from 36% of SPATT samples and concentrations ranged from below detection to 1.5 ng $g^{-1}$, all of which was MCY-RR, and none of the other congeners analyzed were detected. Anatoxin-a was detected in 27% of water samples and all of those exceeded the caution thresholds (none exceeded warning and danger thresholds), and concentrations ranged from below detection to 9.4 µg $L^{-1}$. Anatoxin-a was detected in 18% of SPATT samples and ranged from below detection to 735 ng $g^{-1}$. Cylindrospermopsin was detected in 18% of water samples, and ranged from below detection to 18 µg $L^{-1}$. The caution health threshold was exceeded in 9% of samples and the danger health threshold was also exceeded in 9% of health thresholds. SPATT samples detected cylindrospermopsin in 63% of samples and ranged from below detection to 141 ng $g^{-1}$. Nodularin was not detected from water samples but was detected in 1 SPATT sample at 0.1 ng $g^{-1}$. Saxitoxin was analyzed from 3 samples collected in July and August but no toxin was detected.

**Figure 6 Cyanotoxin concentrations detected in water samples (A) and SPATT samples (B) collected from Canyon Lake in 2016.**

Microcystins are shown by the solid black link and circles, cylindrospermopsin is shown by the blue line and triangles and anatoxin-a results are shown by the dotted line and white circles.

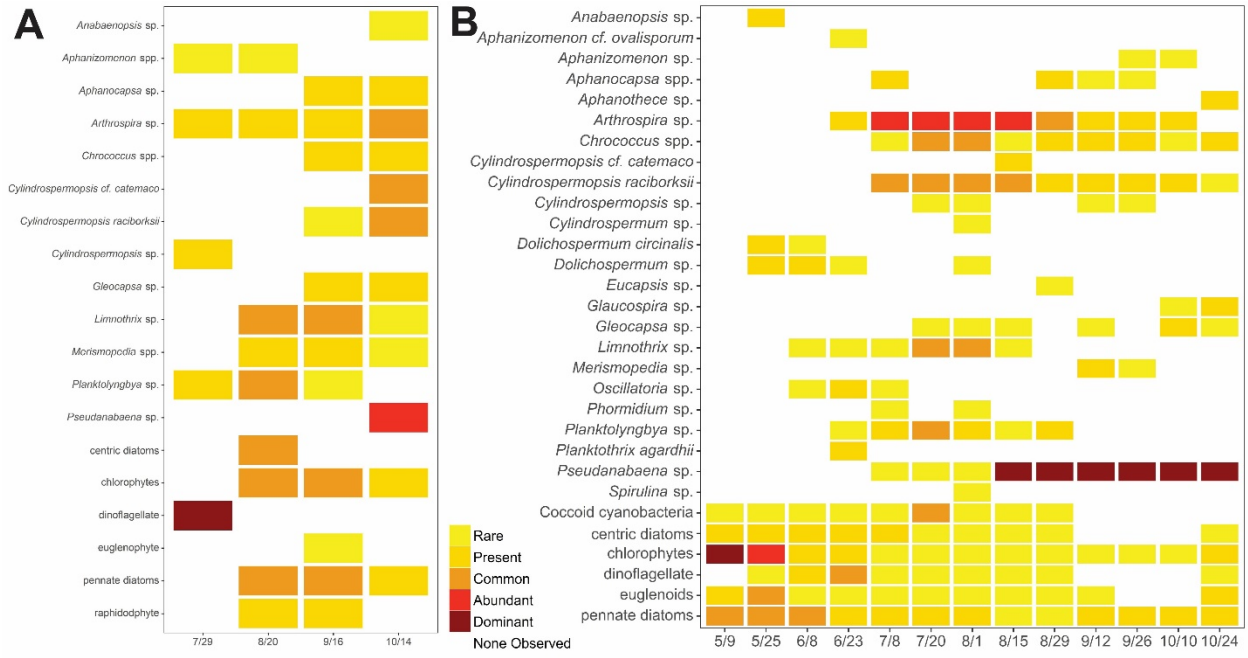



*2017 Results:* The 2017 sample results indicated microcystins at 0.1 µg $L^{-1}$, anatoxin-a ranged from below detection to 0.6 µg $L^{-1}$ and no cylindrospermopsin was detected. The 21 August 2017 sample exceeded the caution health threshold for anatoxin-a, but no other health thresholds were exceeded based on the 2 sample results collected in August and September 2017.

**Canyon Lake Cyanobacterial Community Composition and Chlorophyll-a**

Canyon Lake surface water cyanobacteria abundance from 2015 relative abundance results indicated dinoflagellates dominated the composition in July, and for the remaining field study period common cyanobacteria identified were *Cylindrospermopsis spp., Limnothrix,* and *Planktolyngbya* and *Pseudoanabaena* was abundant (Figure 7A). In 2016, chlorophytes dominated the community composition in May, but the composition transitioned to cyanobacteria dominance in July with common cyanobacteria identified including *Cylindrospermopsis spp., Limnothrix,* and *Planktolyngbya* (similar to 2015 results) and *Arthrospira* was abundant. In August, the community composition switched to clear dominance of *Pseudoanabaena* (Figure 7B).

**Figure 7 Relative abundance 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 (R) <1%, Present (P) 1 - <10%, Common (C) 10 - <25%, Abundant (A) 25-<50%, Dominant (D) 50-100% and these are represented by different colors ranging from bright yellow to red from lowest to highest abundance. The exact colors for each category are in the legend at the bottom of the figure.




The chl-a concentrations ranged from 2.5 – 25 µg $L^{-1}$ in 2016 and the peak concentrations were 25 µg $L^{-1}$ on 7 July 2016 and 23 µg $L^{-1}$ on 24 October 2016 (Figure 5). All dates except 7 July 2016 were below the target chl-a threshold for 2020 of 25 µg $L^{-1}$ (http://www.sawpa.org/wp-content/uploads/2012/05/Lake-Elsinore-and-Canyon-Lake-2015-2016-TMDL-Monitoring-Report_FINAL_Text_rev081616.pdf).

## DISCUSSION AND RECOMMENDATIONS

### *Lake Elsinore*

**Cyanotoxins were chronically detected at relatively high concentrations that frequently exceeded California health trigger thresholds.** The cyanotoxin concentrations detected during this study are currently the highest recorded concentrations of microcystins, anatoxin-a and cylindrospermopsin in southern California lentic waterbodies (Magrann et al., 2015, Howard et al., 2017), and among the highest reported within the State. There were human illnesses reported during the monitoring period. The concentrations of microcystins were higher in Lake Elsinore than in other studies resulting in human illness. 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 56-8200 μg $L^{-1}$ (Vidal et al., 2017). That is 5.4 to 800-fold lower than the maximum concentrations detected in Lake Elsinore (5,600 to 45,300 μg $L^{-1}$).

Microcystins were detected in 93% of water, scum and foam samples and 100% of SPATT samples. From the water, scum and foam samples, 61% exceeded the Danger Tier II health threshold (7% and 17% exceeded the Warning Tier I and Caution health thresholds respectively). Anatoxin-a was detected in 30% of water, scum and foam samples and 21% of SPATT samples. Water, scum and foam samples never exceeded the danger threshold but did exceed the Warning Tier I and Caution thresholds in 23% and 6% of samples respectively. Cylindrospermopsin was detected in 35% of water, scum and foam samples and 57% of SPATT samples. The Danger Tier II health threshold was exceeded in almost a quarter of water, scum and foam samples, while the Warning Tier I and Caution Trigger thresholds were exceeded in 10% and 2.4% of samples respectively. Figure 8 summarizes these results.

The results from this study suggest that 68% of the time, recreational swimming is not recommended based on CA guidance and health based trigger thresholds (OEHHA, 2012; http://www.mywaterquality.ca.gov/monitoring_council/cyanohab_network/docs/appendix_a.pdf). Therefore, the recreational beneficial uses of Lake Elsinore were severely impacted due to the high acute concentrations of cyanotoxins as well as the chronic detections at the most accessible recreational locations. The toxin results indicated a high risk for immediate impacts to human, wildlife and domestic pet health.

In addition to high acute concentrations detected, there were cyanotoxins were also chronically detected in water and SPATT samples, across multiple months and seasons, similar to findings in other CA studies (Kudela, 2011, Gibble and Kudela 2014, Gibble et al., 2016, Howard, et al., 2017, Peacock et al., 2018). Chronic exposure to microcystins can have human and wildlife health implications (Bury et al., 1995, Wiegand et al., 2000, de Figueiredo et al., 2004, Jacquet et al., 2004, Malbrouk et al., 2006, Backer et al., 2008, Backer et al., 2009, Li et al., 2011, Trevino-Garrison et al., 2015, Li et al., 2016) and can be transported into riparian food webs (Moy et al., 2016). While exposure studies were beyond the focus of this assessment study, there were human health illnesses reported during the monitoring period. Therefore, both acute and chronic exposure mechanisms are present in Lake Elsinore and both should be considered a predominant stressor with a high risk of human, wildlife and domestic pet health consequences.

**Multiple cyanotoxins were frequently detected simultaneously, the health risks and consequences are unknown for co-occurring toxin exposure.** 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*. These biotoxins have different mechanisms of toxicity that could have synergistic effects and act as different, but additive, physiological stressors. The study results indicate frequent detection of multiple cyanotoxins simultaneously. There were 2 or more cyanotoxins detected in 68% of water, scum and foam samples and 71% of SPATT samples (Figure 9). There were 3 or more cyanotoxins detected in 37% of water, scum and foam samples and 7% of SPATT samples. The co-occurrence of multiple cyanotoxins from a single location has been documented in other studies, both within and outside of the U.S. (Graham et al., 2010, Gkelis et al., 2014, Rodriquez et al., 2014, Sabart et al., 2015, Pekar et al., 2016) and in southern California (Howard et al., 2017, Tatters et al., 2017).

**Cyanobacteria genera and species identifications indicated a high risk for multiple cyanotoxins to be routinely produced and co-occur in these systems.** The initial study design was based on a tiered system to analyze toxins, based mostly on the presence of potential toxin-producing cyanobacteria genera and species. The results indicated multiple potential toxin-producing cyanobacteria observed in all samples. Therefore, the risk of cyanotoxin presence was high, and other cyanotoxins were potentially present (but not tested) including lyngbyatoxin, BMAA, homoanatoxin-a, neosaxitoxins.

**DNA barcoding is a useful tool to identify cyanobacteria and determine relative abundance.** DNA barcoding can be used to estimate the relative abundance of organisms in each sample and allows for high throughput of samples. The identification of cyanobacterial species using DNA barcode sequencing for taxonomic identification has been successful in the Sacramento-San Joaquin Delta and Clear Lake (Kurobe et al., 2013). The microscopy and DNA barcoding results from this study were effective in identifying cyanobacteria species abundances. There was good agreement of broad community composition results between both methods suggesting that DNA barcoding could be used on a routine basis to determine the relative abundance of cyanobacteria. Both measurements indicated that in May, June, and early July 2016 cyanobacteria populations were comprised mostly of *Planktothrix* spp. In mid-July, there was a shift in the dominant cyanobacterium to *Microcystis spp*. as indicated by both the microscopy and DNA barcoding results. The use of DNA barcoding will allow the development of a gene-specific screening tool for potential biotoxins and specific quantitative PCR assays using the sequences obtained in this study. Future studies should focus on the development of these tools for effective cyanobacteria and cyanotoxin management.

The combination of multiple potential cyanotoxin producing species identified, and the detection of multiple cyanotoxins simultaneously, highlights the need to monitor cyanotoxins in Lake Elsinore on a routine and frequent basis.

### *Canyon Lake*

**Microcystins were chronically detected at low concentrations that occasionally exceeded California health trigger thresholds.** Microcystins were detected in 76% of water samples, and 57% of SPATT samples. However, only 17% of water samples exceeded the Caution trigger threshold and no samples exceeded the Warning Tier I and Danger Tier II trigger thresholds (Figure 8). These results indicate low chronic and persistent microcystins present in Canyon Lake, across multiple months and seasons. As

stated above, there are health consequences that result from chronic exposure to microcystins for human and wildlife (Bury et al., 1995, Wiegand et al., 2000, de Figueiredo et al., 2004, Jacquet et al., 2004, Malbrouk et al., 2006, Backer et al., 2008, Backer et al., 2009, Trevino-Garrison et al., 2015) as well as documented transport into riparian food webs (Moy et al., 2016). Therefore, chronic exposure to microcystins should be considered a predominant stressor with a high risk of human, wildlife and domestic pet health consequences.

**Cylindrospermopsin and anatoxin-a were detected half of the time and only occasionally exceeded CA health trigger thresholds.** Cylindrospermopsin and anatoxin-a were detected less often in Canyon Lake compared with Lake Elsinore. Water samples detected cylindrospermopsin and anatoxin-a in 12% and 53% of samples respectively and SPATT samples detected these toxins in 50% and 14% respectively. Cylindrospermopsin exceeded the Danger Tier II trigger threshold in 6% of samples and the Caution trigger in 6% of samples while anatoxin-a exceeded the Caution trigger in 23% of water samples and no other trigger thresholds were exceeded.

**Cyanobacteria genera and species identifications indicated a high risk for multiple cyanotoxins to be routinely produced and co-occur in these systems.** The results indicated multiple potential toxin-producing cyanobacteria observed in most samples. There was a high risk of multiple cyanotoxins to be present and 35% of water samples and 27% of SPATT samples detected 2 or more cyanotoxins. Three or more cyanotoxins were detected in 6% of water samples and 7% of SPATT samples (Figure 9).

### *Recommendation*

The dominance of cyanobacteria and the ubiquitous and persistent detection of cyanotoxins in these highly frequented recreational lakes suggest that cyanotoxins should be included in routine and systematic monitoring programs to protect public health.

**Figure 8. The cyanotoxin results from(A) Lake Elsinore and (B) Canyon Lake from 2015 – 2017.**

The positive toxin samples are indicated by a colored circle, with the color corresponding to the CA health trigger thresholds (yellow, orange and red circles indicate Caution, Warning and Danger thresholds exceeded, respectively). There are currently no established thresholds for nodularin. Open circles indicate that no toxin was detected.

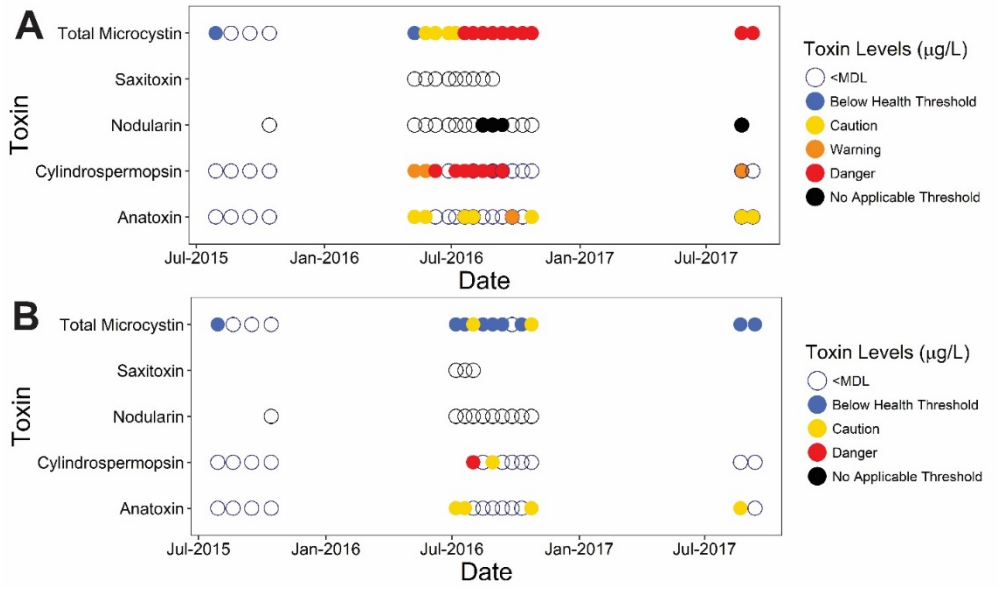
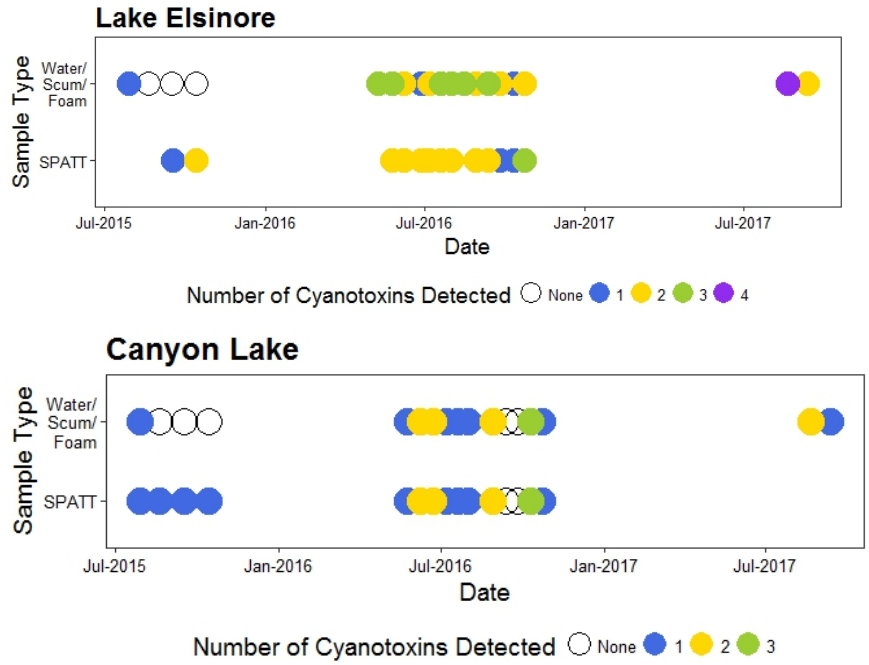



**Figure 9. The number of cyanotoxins detected simultaneously from Lake Elsinore (top panel) and Canyon Lake (bottom panel) from 2015 – 2017.** The top row in each plot shows results from water, scum and foam samples while the bottom row shows results from the passive samplers, called SPATT, that were deployed continuously and represent a time-integrated measurement.



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## APPENDICES

**Appendix A:** MCY congeners detected from whole water samples collected from Lake Elsinore (A) and Canyon Lake (B) in 2016. The toxins that exceeded California Recreational Health Thresholds are shown in color according to threshold (red for the Danger Tier II threshold, orange for the Warning Tier I threshold and yellow for the Caution threshold. Blue circles indicate microcystin detected below California Recreational Health Thresholds and open circles indicate no toxin detected.