Diversity and Prevalence of Cyanobacteria and Cyanotoxins in Los Angeles Region Recreational Lakes and Reservoirs

Jayme Smith

Dana Shultz

Alle Lie

Susanna Theroux

SOUTHERN CALIFORNIA COASTAL WATER RESEARCH PROJECT

Technical Report 1309

Diversity and Prevalence of Cyanobacteria and Cyanotoxins in Los Angeles Region Recreational Lakes and Reservoirs

Jayme Smith, Dana Shultz, Alle Lie, and Susanna Theroux

Southern California Coastal Water Research Project, Costa Mesa, CA

February 2023

Technical Report 1309

Acknowledgements

The authors thank the staff of United Water Conservation District (Lake Piru), Los Angeles Sanitation (Machado Lake, Echo Park Lake, Balboa Park Lake, Lincoln Park Lake), Los Angeles County Department of Parks and Recreation (Legg Lake, Castaic Lake, Castaic Lagoon, Puddingstone Reservoir), Los Angeles County Public Works (Puddingstone Reservoir) Department of Water Resources (Pyramid Lake), Los Angeles Department of Water and Power (Lower Stone Canyon), AquaBio Environmental Technologies, Inc. (Malibou Lake), and Casitas Municipal Water District (Lake Casitas) for sampling assistance. The authors also thank the following individuals for assistance: Cody Fees, Raeann Iler, Annie Holt, Rosaly Castorena, Hayden Franciscus (field sampling effort), Emma Kocik, Caris Park (laboratory support), Syd Kotar, Bowen Du and Wenjian Lao (chemistry sampling and sample processing), Raphe Kudela, and Kendra Hayashi (SPATT sampler analyses). The State of California Surface Water Ambient Monitoring Program (SWAMP) Los Angeles region and the Southern California Coastal Water Research Project provided funding.

Prepared for:

Los Angeles Regional Water Quality Control Board, as a deliverable for the

“Ambient Assessment of Freshwater Harmful Algal Blooms Species and Toxins in Region 4 Lakes”

(Agreement No. 19-078-270-1)

This report should be cited as:

Smith, J., D. Shultz, A. Lie, and S. Theroux. 2023. Diversity and Prevalence of Cyanobacteria and Cyanotoxins in Los Angeles Region Recreational Lakes and Reservoirs. Technical Report 1309. Southern California Coastal Water Research Project. Costa Mesa, CA.

Keywords: Harmful algal blooms; cyanotoxins; cyanobacteria; recreation; lakes; reservoirs

EXECUTIVE SUMMARY

Reports of cyanobacterial harmful algal blooms (cyanoHABs) are increasing in California's surface waters. CyanoHABs pose a significant risk to human, wildlife, livestock, and pet health due to the consumption or exposure to cyanotoxins biosynthesized by multiple cyanobacterial species. Cyanobacteria species can produce a diversity of cyanotoxins of concern, including microcystins, cylindrospermopsin, and anatoxin-a, posing a challenge to monitoring programs that may not have a priori information on potential cyanotoxin hazards in a given system. The apparent increase in cyanoHAB are attributed to an array of environmental drivers, notably increases in nutrient concentrations, change in hydrology, and water temperatures, which are being exacerbated by climate change. These environmental factors cause an accumulation of algal organic matter, termed eutrophication. A lake's chlorophyll-a and nutrient concentrations can be used to determine its trophic status, from oligotrophic to hypereutrophic. Trophic status has been linked directly to an increase in toxic cyanoHABs.

In alignment with the priorities outlined in the SWAMP Freshwater HAB (FHAB) Program's Strategy document (Smith et al. 2021), the Los Angeles Regional Water Quality Control Board (LARWQCB) prioritized monitoring cyanoHABs and eutrophication in the lakes and reservoirs in the region. In the current study, we aimed to address key knowledge gaps about the pervasiveness of cyanotoxins and cyanobacteria species distributions in lakes and reservoirs within the Los Angeles-Ventura region. The goals of this study were to 1) determine if cyanotoxins were routinely present, 2) assess cyanobacteria community composition and diversity, particularly of toxigenic taxa, and 3) explore the effects of trophic status on cyanobacterial community structure and the presence of cyanotoxins. To achieve these goals, we conducted a regional survey of 17 waterbodies in the summer of 2020, with an intensification of monitoring in 3 waterbodies in the summer of 2021. These approaches were used in tandem to identify the lakes and reservoirs in the region where there are elevated risks of cyanoHABs over both space and time.

Our approach layered multiple indicators of both trophic state and cyanoHAB risk, including water nutrient analyses coupled with toxin analyses and DNA metabarcode sequencing. In total, we observed cyanotoxins in 65% of the 17 lakes, with microcystins being detected in multiple waterbodies, while anatoxin-a and cylindrospermopsin were each only observed in a single waterbody. We detected microcystin and anatoxin-a concentrations that exceeded the California Cyanobacteria and Harmful Algal Bloom (CCHAB) Network Caution Trigger Level in 4 of the 8 waterbodies with detectable toxins. Putative toxin producing cyanobacterial genera were present throughout the region and were observed in all lakes but Lake Balboa. Between 60% - 80% were either eutrophic or hypereutrophic, depending on the indicator used (chlorophyll-a, total nitrogen (TN), or total phosphorus (TP)). Trophic status had a significant structuring effect on cyanobacterial communities in hypereutrophic lakes, which were quite distinctive compared to communities in lower trophic state levels. While cyanotoxins were present across lakes of all trophic states, they were more commonly detected in eutrophic and hypereutrophic lakes and were also typically higher in concentrations.

Lakes Piru, Machado, and Legg were revisited for more temporally intensive sampling in the summer of 2021. Microcystins were detected in all three lakes using Solid Phase Adsorption Toxin Tracking (SPATT) samplers and were present in discrete water grab samples from Machado and Legg Lakes. The highest and most persistent microcystin levels were detected in Machado Lake, where microcystins were detected in water grab samples during four of the seven sampling events. In two of these instances, concentrations exceeding the microcystins Caution Trigger Level were observed. Anatoxin-a was also detected concurrently to microcystins during one of the visits. At Legg Lake, microcystins were observed in all SPATT samplers, but only detected in water grab samples during a single visit and concentrations were below recreational health trigger levels. Microcystins were detected in SPATT samplers in Lake Piru but never detected in water grab samples. Toxin producing cyanobacteria were common in all three lakes, but each lake had distinctive communities of toxigenic taxa. The three lakes were also distinctive in terms of trophic status as indicated by nutrients or chlorophyll-a concentrations. Machado Lake was hypereutrophic throughout the season and exceeded its Total Maximum Daily Loads (TMDL) targets for chlorophyll-a, TN, and TP throughout the summer. Legg Lake transitioned from a eutrophic to hypereutrophic condition, also exceeding the TMDL targets for chlorophyll-a and total nutrients. Lastly, Lake Piru transitioned from a mesotrophic to eutrophic state over the summer period.

The results of this work highlight the near ubiquitous presence of toxigenic cyanobacteria taxa in lakes and reservoirs across the Los Angeles-Ventura region. It also highlights the pervasiveness of waterbodies in eutrophic and hypereutrophic conditions. Together, this means that many waterbodies in this region are primed for the development of high biomass blooms of cyanotoxin capable of cyanotoxin production. Our study also highlighted the application of two distinctive sampling designs to address questions of regional and temporal variation in cyanoHABs and eutrophication. The regional survey design allowed for the overall trophic conditions of lakes and reservoirs in the region and how they related to potential cyanoHAB risks. This revealed the prevalence of eutrophication and the potential risks for cyanoHABs in the region. Routine, biweekly monitoring allowed for the observation of the variation in cyanotoxin levels and cyanobacterial community composition, therefore highlighting the differing risks for recreational health that occur throughout the summer. The combination of sampling designs highlighted the need for routine sampling to protect recreational uses in several waterbodies, particularly more at-risk eutrophic and hypereutrophic waterbodies.

Caveats in the scientific findings, and research recommendations to address these uncertainties include:

The approaches employed in this study do not allow for the clear identification of which species within a community are producing toxins. We recommend applying molecular ‘omics approaches to identify which cyanoHAB taxa have the genetic potential to produce cyanotoxins and identify when these taxa turn on (and off) their toxin production genes.

Phytoplankton community assessments in this study focused exclusively on cyanobacterial taxa; however, broader assessments of cyanobacterial and eukaryotic algal diversity in the region’s lakes could reveal a fuller understanding of overall HAB risks and dynamics.

Other science recommendations that can inform future management actions include:

- Formalize a partner lakes and reservoir monitoring program to (a) better characterize risk to beneficial uses, given the temporally limited sampling, with a suite of indicators consistent with forthcoming Water Board biostimulatory policy, and (b) providing training to ensure adequate HAB event response;
- Conduct a pilot project to screen for eutrophication status via remotely sensed satellite data to further prioritize in situ monitoring; and
- Conduct desktop environmental drivers assessment and data gaps analysis to proactively identify the initial set of recommendations for further management of eutrophication and identify site-specific data gaps.

Table of Contents

List of Figures

List of Tables

INTRODUCTION

The extent and magnitude of cyanobacterial harmful algal blooms (cyanoHABs) appear to be increasing in surface waters in both the United States and globally (Carmichael 2008; Huisman et al. 2018). CyanoHABs pose a significant risk to human, wildlife, livestock, and pets due to the production of toxins by many different cyanobacterial species (Backer et al. 2008; Edwards et al. 1992; Mez et al. 1997; Pouria et al. 1998; Trevino-Garrison et al. 2015; Wood et al. 2010). Collectively, the toxins produced by cyanobacteria are referred to as cyanotoxins and represent multiple classes of toxins including microcystins, cylindrospermopsin, and anatoxin-a. In recent years, beneficial uses of U.S. surface and drinking waters have faced negative impacts related to cyanotoxins including 'do not drink' orders issued in major cities such as Toldeo, Ohio (Steffen et al. 2017) and Salem, Oregon (Davis et al. 2019), and an increasing number of domestic animal deaths linked to recreational exposures (Backer et al. 2013; Puschner et al. 2008; Stewart et al. 2008).

Due to the growing awareness of the threats associated with cyanoHABs, efforts have been made on the national, state, and local levels to better understand these events, but data documenting the occurrence and severity of toxic events nationally is lacking (Brooks et al. 2016; Brooks et al. 2017; Carmichael and Boyer 2016). Nationally, the U.S. Environmental Protection Agency (EPA) has integrated cyanoHAB indicators into the National Lakes Assesment (NLA) starting in 2007, and have reported 76% of lakes were dominated by cyanobacterial genera (Loftin et al. 2016). On the whole, there is a lack of routine ambient monitoring program for cyanoHABs in many parts of the United States. While some local agencies, states, and tribes have ambient monitoring programs, many are relegated to responding to public reports of bloom events due to a lack of dedicated resources (Brooks et al. 2017; Carmichael and Boyer 2016).

Reports of cyanoHABs in California's lakes and reservoirs have increased in recent years. Statewide, there are an increasing number of waterbodies in California that have recurrent toxic cyanobacteria blooms including the Klamath River watershed, Clear Lake, Pinto Lake, lower Sacramento and San Joaquin Rivers and Delta, Lake Elsinore, and several East San Francisco Bay Area lakes. Many of these systems are eutrophic or hypereutrophic, have calm and/or stratified water columns, plenty of irradiance, and warm water temperatures (O'Neil et al. 2012; Paerl and Huisman 2008; Paerl and Paul 2012; Xu et al. 2010). In southern California, the presence of cyanotoxins in lentic and lotic freshwater systems has been documented in multiple locations (Fetscher et al. 2015; Howard et al. 2021). Studies have detected multiple types of cyanotoxins (including microcystins, anatoxin-a, cylindrospermopsin) at concerning levels, and found cyanobacterial communities dominated by potentially toxic taxa. Nonetheless, a majority of studies on cyanotoxins and cyanobacteria in lakes and reservoirs of Southern California are generally done on a lake-by-lake basis, in response to problems with cyanobacterial overgrowth.

The apparent increase in cyanoHAB are attributed to an array of environmental drivers, notably increases in waterbody eutrophication and water temperatures (Paerl and Huisman 2008), which are anticipated to be exasterbated by climate change (Brooks et al. 2017; Paerl and Paul 2012). These

environmental factors cause an accumulation of algal organic matter, termed eutrophication (Nixon 1995). A lake's chlorophyll-a and nutrient concentrations can be used to determine its trophic status, from oligotrophic to hypereutrophic. Trophic status has been linked directly to an increase in toxic cyanoHABs. Synoptic observations of cyanoHABs and trophic state indicators have underscored the relationship between increased occurrence of cyanoHABs in nutrient enriched conditions and have even resulted in the development of stressor-response models that generate recommended numeric targets for nutrients to minimize the risk of excessive biomass and cyanotoxins (Yuan and Pollard 2015, 2017). The CalEPA is working to develop numeric biostimulatory thresholds to better manage eutrophication and cyanoHABs in lakes and reservoirs, since the management of nutrient pollution and eutrophication is one of the primary mechanisms available to managers to work toward the reduction of cyanoHAB events. These thresholds will likely leverage these emergent modeling tools to identify nutrient criteria that will minimize the risk of cyanoHABs.

Several key knowledge gaps exist in southern California that make it difficult to assess cyanoHAB risks, drivers, and management options in the region. First, while assessments of cyanotoxin prevalence have been made previously in the Los Angeles-Ventura region, they generally have been made in response to public reports and have rarely included ancillary measurements beyond cyanotoxins. Second, there are few studies that characterize the cyanobacterial diversity in the lakes and reservoirs in the Los Angeles-Ventura region, thus there is an incomplete understanding of the diversity of toxigenic taxa over both space and time within the region. Lastly, the systematic assessment of the water quality, trophic status, and cyanoHAB indicators of lakes and reservoirs in the Los Angeles-Ventura region of southern California is lacking.

In the present study, the overarching goals of this study were to: 1) determine if cyanotoxins were routinely present, 2) assess cyanobacteria community composition and diversity, particularly of toxigenic taxa, and 3) explore the effects of trophic status on cyanobacterial community structure and the presence of cyanotoxins. Two complimentary field surveys approaches were used: 1) a temporally limited regional survey and 2) a temporally intensive monitoring of select lakes, both during the summer season. These approaches were used in tandem to identify the lakes and reservoirs in the region where there are elevated risks of cyanoHABs over both space and time. This is the first paired assessment of cyanotoxins and cyanobacterial community composition with water quality metrics to be conducted in many of these waterbodies.

METHODS

Study Design and Station Selection for the Regional Survey

In 2020, 58 sampling stations across 17 lakes and reservoirs throughout the Los Angeles Regional Water Quality Control Board (LARWQCB) regional bounds were sampled between August 24, 2020 through September 23, 2020 (Figure 1, Table 1). Waterbodies were considered for inclusion in this study if they held designated recreational uses (REC1 and REC2). The final selection of 17 waterbodies was curated to include a mix of large and small waterbodies in both rural and urban locations.

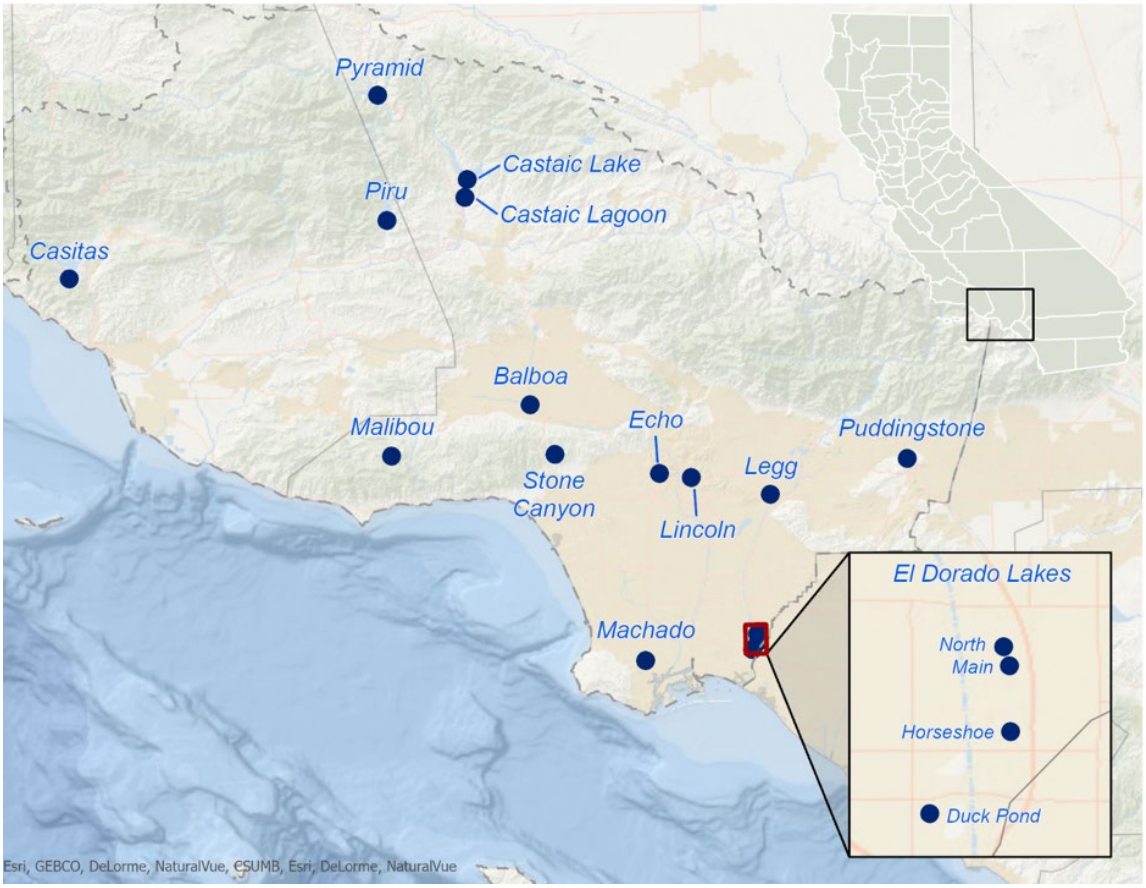

Figure 1. Seventeen waterbodies in Los Angeles and Ventura counties that were visited in the summer of 2020. The extent of Los Angeles Regional Water Quality Control Board (LARWQCB) is denoted by the dashed grey line. The inset on the lower right shows the location of the El Dorado Lakes, and the inset on the upper right shows where the LARWQCB is within the state of California.

Table 1. List of lakes and sampling effort in the summer 2020 regional survey. This includes lake names (Waterbody), lake area in hectares (ha), designated recreational 1 (Rec1) and recreational use 2 (Rec2) uses (E = established , P = potential), number of shore and index stations, if Solid Phase Adsorption Toxin Tracking passive samplers (SPATT) were deployed, and the date of visits for each waterbody.

Waterbody	Area (ha)	Rec1	Rec2	Number of shore stations	Number of index stations	SPATT	Date of Visit 1	Date of Visit 2
El Dorado Lakes - Horseshoe Lake	1	E	E	1	0	No	9/1/2020	9/15/2020
El Dorado Lakes - Duck Pond	1	E	E	1	0	No	9/1/2020	9/15/2020
Lincoln Lake	2	P	E	1	0	Yes	9/1/2020	9/15/2020
El Dorado Lakes - North Lake	2	E	E	1	0	No	9/1/2020	9/15/2020
Echo Lake	5	P	E	1	0	Yes	8/24/2020	9/8/2020
El Dorado Lakes - Main Lake	7	E	E	1	0	No	9/1/2020	9/15/2020
Legg Lake – North	10	E	E	1	1	No	9/3/2020	9/18/2020
Lake Balboa	11	Not any	Not any	1	1	Yes	8/27/2020	9/9/2020
Malibou Lake	16	E	E	2	1	Yes	8/26/2020	9/11/2020
Machado Lake	18	E	E	1	1	Yes	8/24/2020	9/8/2020
Stone Canyon Reservoir	49	P	E	1	0	Yes	8/27/2020	9/22/2020
Castaic Lagoon	74	E	E	1	1	Yes	9/4/2020	9/21/2020

Waterbody	Area (ha)	Rec1	Rec2	Number of shore stations	Number of index stations	SPATT	Date of Visit 1	Date of Visit 2
Puddingstone Reservoir	98	E	E	1	1	Yes	9/3/2020	9/18/2020
Piru Lake	494	E	E	2	1	Yes	8/25/2020	9/10/2020
Pyramid Lake	600	E	E	1	1	Yes	8/25/2020	9/21/2020
Lake Casitas	700	P	E	1	1	Yes	9/4/2020	9/23/2020
Castaic Lake	923	E	E	1	1	Yes	9/4/2020	9/21/2020

Each of the 17 waterbodies selected was sampled twice – once before Labor Day (9/7/2020) and once after Labor Day. At each sampling station, we took field measurements and collected water samples for water quality, cyanotoxin and cyanobacterial community measurements. Solid Phase Adsorptive Toxin Trackers (SPATT), which are passive sampling devices used to provide time-integrated data on the presence of cyanotoxins, were also deployed at waterbodies when possible. At least one shore station was sampled at every waterbody, and when possible, an index station near the center and/or deepest section of the waterbody was also sampled. Ten of the waterbodies were successfully sampled at index locations, and SPATT samplers were deployed successfully at twelve waterbodies (Table 1).

Study Design and Station Selection for Intensive Sampling

SCCWRP worked with the Los Angeles Regional Water Quality Control Board (LARWQCB) to select three waterbodies of interest for routine sampling to occur during the summer of 2021. The three lakes selected for routine sampling were Machado Lake, Legg Lake-North (hereafter referred to as Legg Lake), and Lake Piru. Each of these three lakes were sampled every other week beginning in mid-June 2021 and ending early September 2021 for a total of 7 visits to each lake (Figure 2, Table 2).

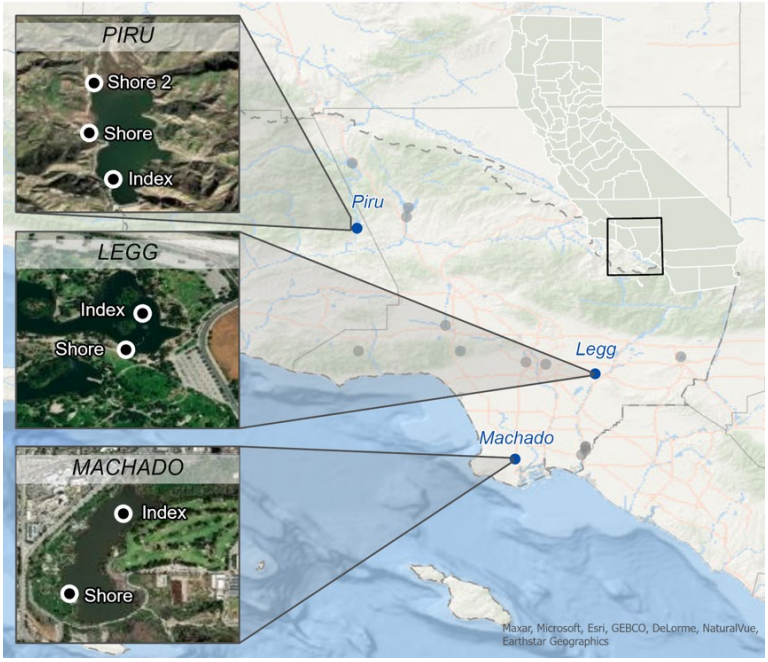

Figure 2. Three waterbodies selected by the Los Angeles Regional Water Quality Control Board (LARWQCB) for biweekly sampling in the summer 2021 routine monitoring (blue labeled points). Area indicated by the dashed gray line represents LARWQCB boundary. Gray points are waterbodies sampled in the summer 2020 regional survey. The inset on the upper right shows where the LARWQCB is within the state of California.

Machado Lake is a small urban lake (~16 hectares, ~1-meter average depth) located in the Harbor City neighborhood of Los Angeles, California within Ken Malloy Harbor Regional Park. Similar to Machado Lake, Legg Lake is a small urban lake (~10 hectares, ~2-meter average depth). The lake is located in South El Monte, California within the Whittier Narrows Recreational Area. Lake Piru Reservoir (hereafter Lake Piru) is a dammed reservoir located in the Los Padres National Forest, California. Unlike Machado Lake and Legg Lake, Lake Piru is located in a relatively undeveloped watershed and is comparatively large (~500 hectares) and deep (40-meter max depth). Lake Piru is dammed by the Santa Felicia Dam where water releases are made to support downstream wildlife habitat and migration, hydroelectric power generation, groundwater management, and flood control.

The lakes serve a variety of beneficial uses including recreation (Machado Lake: catch-and-release fishing; Legg Lake: fishing, paddle boating; Piru Lake: contact recreation, fishing, and boating) and aquatic and wildlife habitat. Both Machado Lake and Legg Lake are listed as impaired under the Clean Water Act Section 303(d), due to excessive levels of nutrients, toxic chemicals, and trash, and Total Maximum Daily Loads (TMDLs) have been established to address these impairments.

Machado Lake was sampled at two stations. The first was a shore station at the Ken Malloy Harbor Regional Park boat launch and the second was an offshore index station located at a Los Angeles City monitoring buoy to the northeast of the shore station. A SPATT sampler was deployed from the buoy at the index station for the duration of the study at Machado Lake. At Legg Lake, one shore station was sampled at the southeast shore near the kayak rentals. The offshore index station was located north of that area at what was assumed to be the deepest part of the lake. A SPATT sampler was deployed on a submerged log at the northern shoreline, about 50 meters from the index station, which was the closest location the SPATT sample could be safely secured. At Lake Piru, a total of three stations were sampled. The first was a shore station located at the public marina, the second was a shore station located at the Juan Fernandez boat launch, and the last was an offshore index station just north of the dam. A SPATT sampler was deployed at a Quagga Mussel monitoring buoy located at this station. Continuous temperature measurements were collected at the passive sampler deployment locations using HOBO 64K Pendant temperature data loggers.

Table 2. Description of the sampling efforts, including the number of shore and index stations, first and last visit dates, and total number of visits for each of the three waterbodies sampled in the summer 2021 routine monitoring survey.

Waterbody	Number of shore stations	Number of index stations	Date of First Visit	Date of Last Visit	Number of Visits
Machado Lake	1	1	6/14/2021	9/7/2021	7
Piru Lake	2	1	6/15/2021	9/8/2021	7
Legg Lake	1	1	6/16/2021	9/9/2021	7

Field Measurements and Water Collection

For all sampling events, a YSI Professional Plus Quatro was used to measure temperature (°C). Temperature measures were taken at the surface of shore stations and at the surface and 1-meter depth at index stations. At the index stations, a tube-sampler was used to collect a depth-integrated sample extending from the surface to twice the secchi depth. Discrete water samples were collected in 1-L amber glass bottles and kept cool and in the dark until further processing. Processing was conducted as soon as possible onsite.

Water Quality Measurements

Water samples for the analysis of total nitrogen (TN) and total phosphorus (TP) were collected by aliquoting whole water from the discrete grab into a single 60-mL HDPE bottle. Samples were collected at every station on every visit at each lake in both 2020 and 2021. In 2021, TN and TP samples from the marina shore station and index station during visit 7 at Lake Piru (9/8/2021) were lost. Samples were kept on ice, and frozen at -20°C immediately upon return to the laboratory. Samples were analyzed at the University of Maryland Center for Environmental Science Nutrient Analytical Services Laboratory. TP was analyzed using EPA Method 365.1 Determination of Phosphorus by Semi-Automated Colorimetry. TP minimum detection limit (MDL) was 0.0015 mg/L and reporting limit (RL) was 0.0045 mg/L. TN was analyzed using EPA Method 353.2 Determination of Nitrate-Nitrite Nitrogen by Automated Colorimetry. TN MDL was 0.05 mg/L and RL was 0.15 mg/L.

Chlorophyll-a samples were collected by syringe filtering water from the discrete grab onto a 25-mm 0.7 µm GF/F filter in a Swinnex filter holder. Duplicate samples were collected at every station, and filter volumes ranged from 10-60 mL depending on biomass. Filters were stored in borosilicate glass culture tubes and frozen at -80°C immediately upon return to the laboratory. Filters were extracted in 4 mL of 90% acetone for 18-24 hours in the freezer (-80°C). Acetone extracts were analyzed using a Turner

Designs Trilogy fluorometer using the non-acidification module. Chlorophyll-a results are reported as the average of the duplicate samples.

Cyanotoxin Measurements

Discrete water samples

Discrete water samples for the analysis of microcystins, anatoxin-a, and cylindrospermposin were collected into 20-mL scintillation vials. Samples were kept on ice, and frozen at -20°C immediately upon return to the laboratory. To extract total toxins from water samples, a cell lysing procedure of three freeze and thaw cycles was followed. Anatoxin-a required a preservative in order to remain stable through the cell lysing procedure. For all samples collected in 2020, samples were homogenized and split into two scintillation vials during the first thaw: (1) 9 mL sample water + 1 mL preservative for anatoxin-a measurements, (2) remaining sample water for microcystin and cylindrospermopsin measurements. In 2021, water samples were collected into a 20-mL scintillation vial containing Eurofins Abraxis anatoxin-a preservative at a 1:10 preservative: sample ratio in the field.

The three toxin classes of interest were measured using Eurofin Abraxis ELISA assays. Microcystins were measured using Eurofins Abraxis Product No. 520011 (MDL = 0.15 µg/L), cylindrospermopsin was measured using Eurofins Abraxis Product No. 522011 (MDL = 0.05 µg/L) and anatoxin-a was measured using Eurofins Abraxis Product No. 520060 (MDL = 0.15 µg/L). Assay instructions were followed for each toxin class, the plates were read on a BioTek Synergy H1 Hybrid Reader using Gen5 software, and concentrations were calculated using the Eurofins Abraxis Solver.

SPATT passive samplers

SPATT samplers were deployed at least 1 meter below the surface at each lake. SPATT samplers were constructed according to the procedure outlined in Howard et al. (2018). In brief, 3 g of HP20 resin was secured in between two pieces of 100 µm mesh using an embroidery hoop. SPATT samplers were deployed from a secure structure at each lake and retrieved at a later date. In 2020, deployment periods ranged from 13 to 27 days (Table 1). In 2021, SPATT samplers were deployed and replaced during subsequent visits for the duration of the study period at 14-day intervals. At retrieval, SPATT samplers were stored dry in Ziploc bags and kept on ice. SPATT samplers were stored in a -20°C freezer immediately upon return to the laboratory.

SPATT samplers were extracted and measured via liquid chromatography/mass spectrometry (LC-MS) as described in Kudela (2011). Briefly, resin in the SPATT sampler was transferred to a chromatography column for three extractions. Extract 1 was eluted with 10 mL of 50% MeOH with 2% formic acid (v/v), while Extract 2 and Extract 3 were each eluded with 20 mL of 50% MeOH. Samples were stored in the dark at 4°C until analysis on the LC-MS. All samples were analyzed for the following cyanotoxins: anatoxin-a, homoanatoxin-a, cylindrospermopsin, nodularin-R, and 8 congeners of microcystin (MC-LA, MC-LF, MC-LR, MC-dmLR, MC-LY, MC-RR, MC-WR, and MC-YR). All extracts were analyzed via LC-MS with ESI and selected ion monitoring (SIM) on an Agilent 6130 with a Phenomenex Kinetix (100 x 2.1) C18 column. The method was adapted from Mekebri et al. (2009) with minor modifications to account for the choice of column and LC-MS/SIM instead of tandem mass spectrometry (Kudela 2011). Briefly, a

mobile phase gradient was employed with solvent A consisting of water and solvent B consisting of acetonitrile acidified with 0.1% formic acid. Analysis included replicates and matrix-additions, with quantification based on external standards. The detection limit for SPATT samplers analyses was 0.05 ng g^{-1} for all congeners. The percent recovery (Kudela 2011) was ~58-100% for each derivative using a standardized recovery method.

Cyanobacterial DNA Sequencing and Analysis

Cyanobacterial community DNA samples were collected in triplicate by syringe filtering sample water onto a 47 mm 0.45 µm mixed cellulose ester membrane filter in an acid washed Swinnex filter holder. The volume of water filtered ranged from 10-240 mL depending on observed biomass during each sampling event. Filters were stored in 2-mL polypropylene screw cap tubes. In 2020, filters were preserved with 1 mL of Qiagen DNeasy PowerSoil PowerBead Solution (Qiagen, Venlo, NE) due to longer field days. In both years, filters were stored on ice after collection and frozen at -80°C immediately upon return to the laboratory.

DNA extractions were done using the Qiagen DNeasy PowerSoil Kit (Qiagen, Venlo, NE) according to the manufacturer's instructions. DNA yield was assessed with a Nanodrop 8000 (Thermo Scientific, Wilmington, DE, USA). The cyanobacteria-specific DNA barcode primer set described in Monchamp et al. (2016) (CYB359F and CYB784R) was used to target the V4 and V5 regions of the 16S rRNA gene. An Illumina MiSeq platform using 2x250 reads was used for paired-end base pair sequencing from barcoded amplicon products at Laragen, Inc. (Culver City, CA, USA). Raw Illumina DNA sequences were demultiplexed and processed using the Illumina MiSeq Recorder software to remove the adapter, barcode, and primer sequences.

We processed DNA sequence data using QIIME2 2021.11 (Bolyen et al. 2019, http://qiime2.org). We generated Amplicon Sequence Variants (ASV) using DADA2 (Callahan et al. 2016) as implemented in QIIME2. We assigned taxonomy to the representative ASV sequences using BLAST in QIIME2 against cyanobacteria sequences culled from the SILVA v138 taxonomy database (Quast et al. 2012; Yilmaz et al. 2014) that had been modified with harmonized species names to align with the California Master Taxa List (https://swamp.waterboards.ca.gov/swamp_checker/Default.aspx). Cyanobacterial ASV relative abundances (RA) were calculated as the percentage of total reads per sample. RA for order, family, and genus level taxonomy were calculated. We performed manual BLAST searches using the NCBI BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to gain further insight into ASV sequence homology to available reference sequences from Genbank. The bioinformatic pipeline code is available online (https://github.com/SCCWRP). A manual BLAST search was performed on a subset of ASVs that did not have a taxonomic match and remained unassigned. These ASVs matched with mitochondrial/chloroplast sequences or otherwise unidentified cyanobacterial sequences and were therefore retained as 'Unassigned' for analyses. Singleton ASVs that were represented by only one read in one sample were removed.

Lake Trophic Status and Cyanotoxin Evaluation Thresholds

Lake trophic status was assessed using chlorophyll, TP, and TN as indicators (Table 3). The chlorophyll-a and TP values proposed in Carlson (1977) were used to assign trophic states of oligotrophic, mesotrophic, eutrophic, or hypereutrophic to waterbodies. Corresponding TN thresholds were derived using the modeling approaches described in Yuan and Pollard (2015). The approach used chlorophyll-a concentrations associated with the transition between each trophic state and dissolved organic carbon concentration estimates of 2 mg/L, 4 mg/L, and 6 mg/L to define the boundaries for mesotrophic, eutrophic, and hypereutrophic, respectively. TN concentrations below the mesotrophic line were classified as oligotrophic.

The California Cyanobacteria Harmful Algal Bloom (CCHAB) Network has developed recreational cyanotoxin trigger levels for water samples meant to be protective of human and domestic animal health for total microcystins, anatoxin-a, and cylindrospermopsin. The Caution Action Trigger requires public notification of a bloom that does not restrict 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. These trigger levels were used to evaluate observed cyanotoxin concentrations for water grab samples. The 'Danger Tier II Thresholds are 20 µg/L, 90 µg/L, and 17 µg/L, for microcystins, anatoxin-a, and cylindrospermopsin, respectively. The Warning Tier I thresholds are 6 µg/L, 20 µg/L, and 4 µg/L, for microcystins, anatoxin-a, and cylindrospermopsin, respectively. Lastly, the 'Caution Action Trigger levels are 0.8 µg/L, detection, and 1 µg/L, for microcystins, anatoxin-a, and cylindrospermopsin, respectively (CCHAB 2016). No analogous thresholds exist for SPATT passive samplers.

Statistical Analyses

All statistical analyses were performed using R statistical software (R Core Team 2013). Multivariate statistical approaches were employed to analyze differences among the cyanobacterial community structures in relation to environmental and trophic state variables. Nonmetric multidimensional scaling (nMDS) analysis using the Bray-Curtis dissimilarity metric was conducted using the vegan package in R to identify the influence of environmental factors (trophic status and temperature) on community structures. Analysis of similarity (ANOSIM) tests were used to test for significant dissimilarities in the community composition by categorical variables such as trophic status. Mantel tests were used to test for differences in communities based on continuous variables such as temperature.

Table 3. Ranges of concentrations of chlorophyll-a, total phosphorus (TP), and total nitrogen (TN) applied to define trophic status of lakes and reservoirs.

Trophic Category	Chlorophyll-a (µg/L)	TP (mg/L)	TN (mg/L)
Oligotrophic	<2.6	< 0.012	<0.15
Mesotrophic	2.6-7.3	0.012-0.024	0.15-0.32
Eutrophic	7.3-56	0.024-0.096	0.32-0.91
Hypereutrophic	> 56	>0.096	>0.91

RESULTS

Regional Physiochemical Properties and Trophic Status

Across the region, surface temperatures across all lake stations had a range of nearly 10°C (Figure 3). Temperatures dropped between the first and second visit for surface and subsurface measurements at all stations and all waterbodies. Temperatures ranged from 25.3-31.4°C during visit 1 and from 21.9-29.4°C during visit 2.

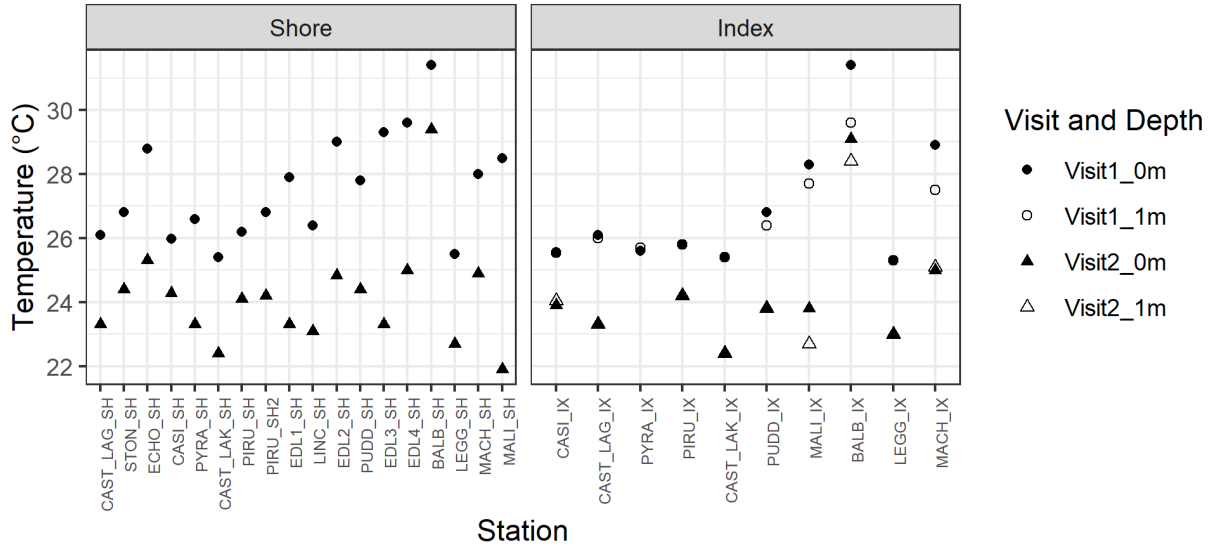

Figure 3. Temperature measured at the shore stations (left) and ten index stations (right) during two visits in the summer 2020 regional survey. The circles are observations collected from visit 1 and triangles are observations from visit 2. The closed symbols represent observations from the surface and open symbols represent observations at 1 meter depth. The labels on the x-axis indicate each lake visited, with shore stations indicated by an SH and index stations indicated by IX. Location are Castaic Lagoon (CAST_LAG), Stone Canyon Reservoir (STON), Echo Park Lake (ECHO), Lake Casitas (CASI), Pyramid Lake (PYRA), Castaic Lake (CAST_LAK), Lake Piru (PIRU), El Dorado Lakes-North Lake (EDL1), Lincoln Park Lake (LINC), El Dorado Lakes-Main Lake (EDL2), Puddingstone Reservoir (PUDD), El Dorado Lakes-Horseshoe Lake (EDL3), El Dorado Lakes-Duck Pond (EDL4), Lake Balboa (BALB), Legg Lake (LEGG), Machado Lake (MACH), and Malibou Lake (MALI).

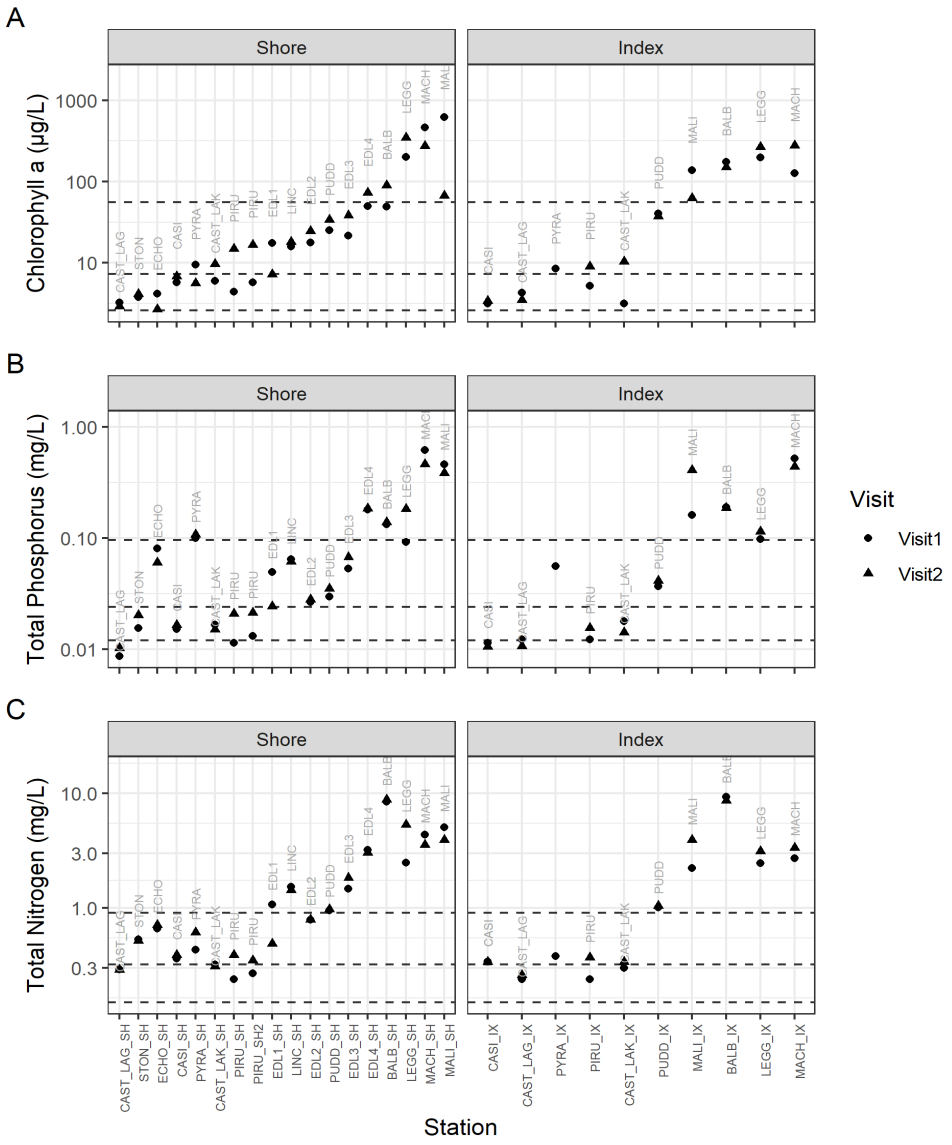

Figure 4. A) Chlorophyll-a, B) total phosphorus, and total nitrogen concentrations measured at the shore stations (left) and index stations (right) in the summer 2020 regional survey. The circles are observations collected from visit 1 and triangles are observations from visit 2. The labels on the x-axis indicate each lake visited, with shore stations indicated by an SH and index stations indicated by IX. Location are Castaic Lagoon (CAST_LAG), Stone Canyon Reservoir (STON), Echo Park Lake (ECHO), Lake Casitas (CASI), Pyramid Lake (PYRA), Castaic Lake (CAST_LAK), Lake Piru (PIRU), El Dorado Lakes-North Lake (EDL1), Lincoln Park Lake (LINC), El Dorado Lakes-Main Lake (EDL2), Puddingstone Reservoir (PUDD), El Dorado Lakes-Horseshoe Lake (EDL3), El Dorado Lakes-Duck Pond (EDL4), Lake Balboa (BALB), Legg Lake (LEGG), Machado Lake (MACH), and Malibou Lake (MALI). Data are ordered on the x-axis by increasing chlorophyll-a concentration. Dashed lines correspond to the mesotrophic (lowest dashed line line), eutrophic (middle dashed line), and hypereutrophic (upper dashed line) thresholds. Data are reported on a semi-log scale.

A majority of the lakes were either eutrophic or hypereutrophic, regardless of the indicator used. Considering the maximum measured chlorophyll-a at the ten lakes with index stations, two lakes were mesotrophic, four lakes were eutrophic, and four lakes were hypereutrophic (Table 3, Figure 4A). Observed chlorophyll-a concentrations spanned a wide range of 2.70- 623.18 µg/L for all samples. Chlorophyll-a concentrations were the highest at Malibou Lake (623.18 µg/L, shore station) and Machado Lake (278.73 µg/L) and were the lowest at Echo Park Lake (2.70 µg/L, shore station) and Castaic Lake (3.15 µg/L, index station).

Using the maximum measured TP concentrations at the ten lakes with index station samples as the indicator of trophic status, one lake could be classified as oligotrophic, three lakes as mesotrophic, two lakes as eutrophic, and four lakes as hypereutrophic (Table 3, Table 4, Figure 4B). TP concentrations ranged from 0.009-0.626 mg/L during this study. The highest TP concentrations were observed at Machado Lake with maximal concentrations of 0.612 mg/L and 0.516 mg/L observed at the shore and index stations, respectively. The lowest TP concentrations were observed at Castaic Lagoon (0.009 mg/L, shore station) and Lake Casitas (0.011 mg/L, index station).

Using TN concentrations as indicator, slightly different classifications were derived, in which one lake was classified as mesotrophic, four lakes as eutrophic, and five lakes as hypereutrophic (Tables 3- 4, Figure 4C). TN concentrations ranged from 0.24-9.29 mg/L. The highest TN concentrations were observed at Lake Balboa, with maximal concentrations of 8.83 mg/L mg/L and 9.29 mg/L mg/L observed at the shore and index stations, respectively. Lake Piru and Castaic Lagoon had the lowest concentrations of 0.24 mg/L at the shore and index stations of each lake, respectively. The mass ratio of TN:TP across waterbodies ranged from 4-64. The majority of waterbodies surveyed would be considered nitrogen enriched with TN:TP values greater than the Redfield ratio (7.22), which is commonly used to infer the balance between N and P (Appendix Figure A1).

Seasonal Variations in Physiochemical Lake Properties

The three lakes (Machado Lake, Piru Lake, and Legg Lake) routinely monitored over the summer of 2021 had somewhat distinctive temperatures. Legg Lake had the highest temperatures almost every day over the entire summer, while Piru Lake generally had the lowest (Figure 5A). Diel variations in temperatures were relatively stable for Legg Lake and Piru Lake, while observed temperature in Machado Lake were more variable. Overall, the three lakes had similar trends in temperature, in which the temperature steadily increased early summer, with a relatively sudden and atypical decrease in temperature in late July. Such decrease was observed in Legg Lake and Machado Lake, but less so in Piru Lake. Temperatures at the three lakes remained somewhat stable for the majority of August and began to decrease late August.

The three lakes were also distinctive in terms of trophic status as indicated by nutrients or chlorophyll-a concentrations (Figures 5B, C, and E). Machado Lake was hypereutrophic no matter the indicator used (i.e., Green lines above top dashed line if Figures 5B, C, and E). Legg Lake was considered eutrophic as

indicated by TP concentrations, hypereutrophic as indicated by TN concentrations, and went from eutrophic to hypereutrophic based on chlorophyll-a concentrations. Lastly, Piru Lake was in between mesotrophic, and eutrophic based on TP and TP concentrations, and increased from oligotrophic/mesotrophic status to eutrophic over the summer based on chlorophyll-a concentrations. There was also more spatial variation in TP concentrations at Piru Lake than the other two lakes, with samples from the index station and one shore station (SH) being lower and mostly confined in the range for mesotrophic systems, while the TP concentrations at the second shore station (SH_2) was higher and mostly considered as eutrophic (5 of 7 samples) over the summer.

Despite Machado Lake being hypereutrophic and having the highest TN concentrations over the entire summer, TN:TP ratios at Machado Lake were lowest at the start of the summer (Figure 5D). Values lower than the Redfield ratio value of 7.22 (as indicated by the dashed line in Figure 5D) imply P limitation while values above the Redfield ratio imply N limitation. Therefore, Machado Lake was P-limited at the beginning of summer 2021 and shifted to N-limited after visit 3 (7/12/2021). Such shifts corresponded to the appearance and persistence of cyanotoxins (Discussed in later sections; Figure 7). Legg Lake was N-limited throughout the summer, while Piru lake was generally N-limited except for one shore station sample (SH_2) from visit 5 (8/9/2021).

Chlorophyll-a concentrations at the three lakes had similar trends, with an overall increase from the beginning of the summer (visit 1) to the end (visit 7; Figure 5E). However, peak chlorophyll-a concentrations were typically observed in the middle of the summer (visit 4, in late July) for all three lakes. The peak in chlorophyll-a during visit 4 corresponded to a peak in TN concentrations (Figure 5C) and was particularly pronounced at Machado Lake.

Routine monitoring of Machado Lake and Legg Lake revealed exceedances TMDL targets. Machado Lake has a TMDL that was established in May 2008 (https://www.waterboards.ca.gov/water_issues/programs/tmdl/docs/machadolake/R08_006_machadolake_nutrient.pdf) with numeric targets for chlorophyll-a of 20 µg/L (monthly average), TN of 1.0 mg/L (monthly average) and TP of 0.1 mg/L (monthly average). Legg Lake has a TMDL that was established in March 2012 (https://19january2017snapshot.epa.gov/www3/region9/water/tmdl/la-lakes/LALakesTMDLsSection9LeggLakes.pdf) with numeric targets for chlorophyll-a of 20 µg/L (summer time (May – September) and annual average) , TN of 0.65 mg/L (summer time (May – September) and annual average), and TP of 0.065 mg/L (summer time (May – September) and annual average). Our results indicate that both lakes did not meet these targets for the duration of our study in 2021 (Figure 5).

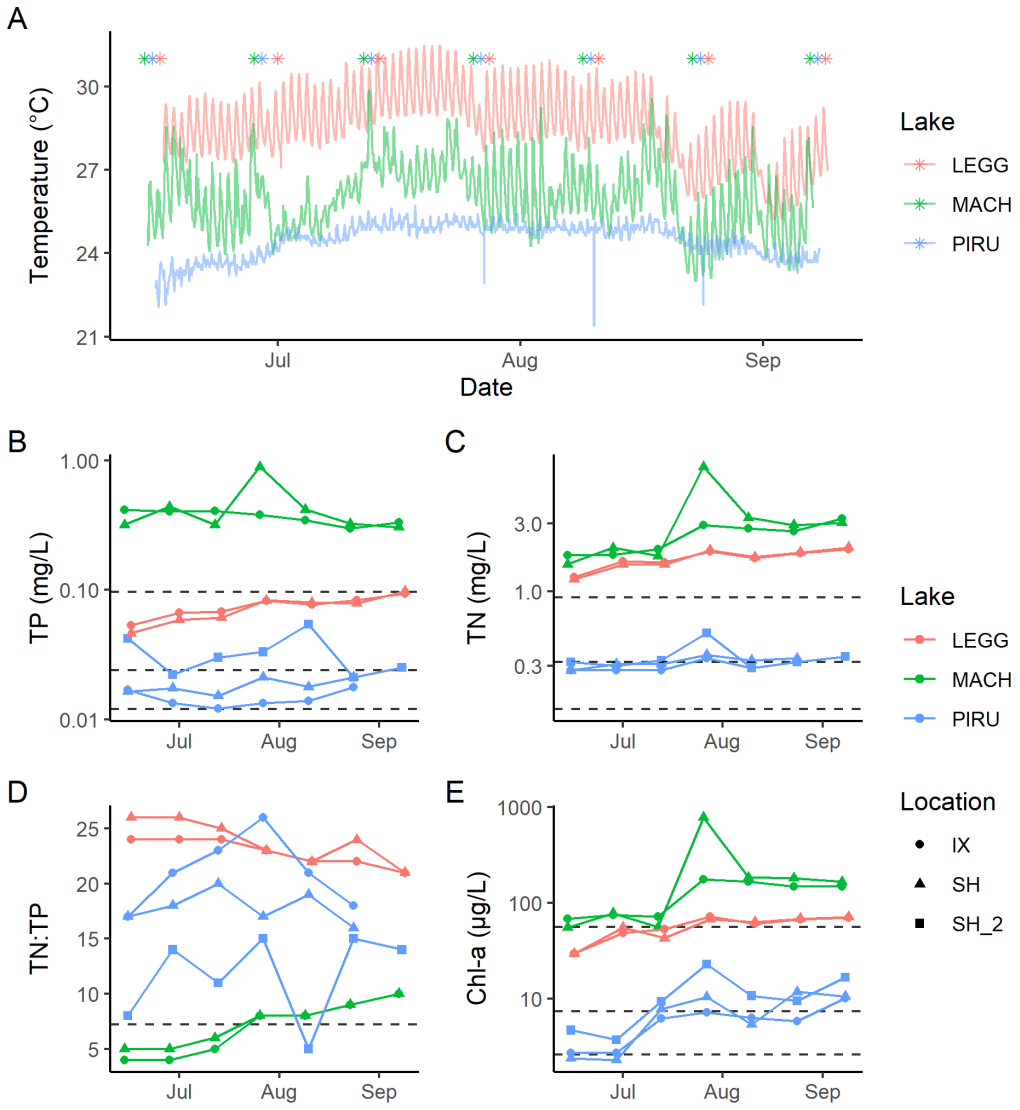

Figure 5. Water quality data collected from Machado Lake (MACH; green lines and symbols), Legg Lake (LEGG; red lines and symbols), and Piru Lake (PIRU; blue lines and symbols) in the summer 2021 sampled at shore stations (SH and SH_2) and index station (IX) at each lake. Panel A) shows continuously measured subsurface water temperature. Asterisks at the top of the panel indicate the dates of the seven sampling events. Panel B) shows total phosphorus concentrations (TP). Panel C) total nitrogen concentrations (TN). Panel D) shows mass:mass TN:TP ratios. Panel E) chlorophyll-a concentrations (Chl-a). Dashed lines in B), C), and E) denote the threshold for mesotrophic (bottom dashed line), eutrophic (middle dashed line), and hypereutrophic (top dashed line) trophic status of lakes. Dashed line in D) denotes the Redfield mass:mass ratio of 7.22. Data for TP, TN and Chl-a are reported on a semi-log scale.

Table 4. Eutrophication status of ten waterbodies with index stations sampled. Table includes the maximum index station chlorophyll-a and total phosphorus, Carlson Index eutrophication status based on those concentrations and maximum concentration of toxin detected in either shore or index stations.

Waterbody	Max Chl (µg/L)	Eutrophication Status (Chl)	Max TP (mg/L)	Eutrophication Status (TP)	Max TN (mg/L)	Eutrophication Status (TN)	Max Toxin (µg/L)	Toxin Type	CA trigger level
Machado Lake	278.7	Hypereutrophic	0.516	Hypereutrophic	4.34	Hypereutrophic	0.24	ATX	Caution
Legg Lake	267.2	Hypereutrophic	0.114	Hypereutrophic	5.34	Hypereutrophic	1.52	MCY	Caution
Lake Balboa	175.6	Hypereutrophic	0.189	Hypereutrophic	9.29	Hypereutrophic	bd	NA	NA
Malibou Lake	136.9	Hypereutrophic	0.408	Hypereutrophic	5.07	Hypereutrophic	1.7	MCY	Caution
Puddingstone Reservoir	40.6	Eutrophic	0.041	Eutrophic	1.04	Hypereutrophic	bd	NA	NA
Castaic Lake	10.3	Eutrophic	0.018	Mesotrophic	0.34	Eutrophic	0.67	MCY	None
Lake Piru	9.0	Eutrophic	0.016	Mesotrophic	0.39	Eutrophic	bd	NA	NA
Pyramid Lake	8.4	Eutrophic	0.056	Eutrophic	0.61	Eutrophic	0.26	MCY	None
Castaic Lagoon	4.3	Mesotrophic	0.012	Mesotrophic	0.29	Mesotrophic	bd	NA	NA
Lake Casitas	3.4	Mesotrophic	0.011	Oligotrophic	0.39	Eutrophic	0.19	CYL	None

Cyanotoxins Across the Region

Cyanotoxins were detected in discrete grabs or SPATT samples from 65% of the seventeen LARWQCB waterbodies sampled in 2020. Microcystins, cylindrospermopsin, and anatoxin-a were detected in discrete water samples collected in seven different lakes. Microcystins were detected in seven of the SPATT samplers deployed in twelve waterbodies. Four distinct congeners of microcystin (microcystin-RR, microcystin-YR, microcystin-LR, and microcystin-LA; out of 8 congeners detectable by the LC-MS analysis) were detected in SPATT samplers.

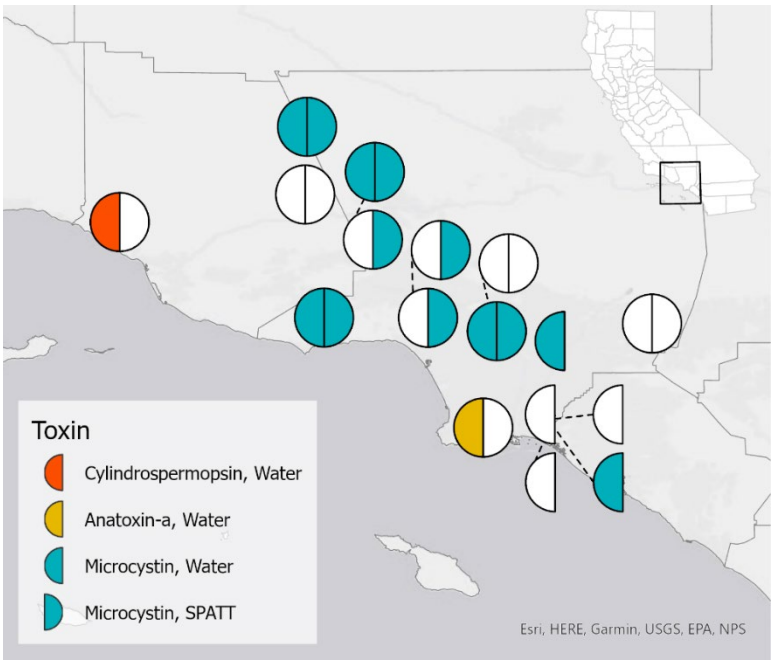

Figure 6. Cyanotoxin detection in the seventeen waterbodies from the summer 2020 regional survey. Toxins analyzed include microcystin (teal), cylindrospermopsin (orange), and anatoxin-a (gold). Samples included discrete water samples (left half circle) and SPATT samplers (right half circle) that were deployed at twelve lakes.

Microcystins were detected in 53% (9 of 17) of waterbodies sampled (Figure 6; Appendix Figure A2; Appendix Table A1). This included detection in discrete water samples from 35% (6 of 17; 0.17-3.20 µg/L) of waterbodies sampled and detection in SPATT samples from 58% (7 of 12) lakes where passive samplers were deployed. Of the six waterbodies with microcystin detects in discrete water grabs, four waterbodies had toxins exceeding the California Tier 1 Caution Level of 0.8 µg/L (Appendix Figure A1). No sample exceeded the California Tier 2 Warning Level of 6.0 µg/L in this study. Microcystin congeners -LA, -LR, -RR, and -YR were detected in SPATT samples, with total microcystin concentrations ranging from 1-145 ng/g resin (Appendix Table A2).

Anatoxin-a was only detected in discrete water grabs at Machado Lake. Anatoxin-a was detected in all samples (N=4) from this waterbody, ranging from 0.19-0.26 µg/L. These samples are above the California

Tier I caution level, which occurs at detection of anatoxin-a, but below the California Tier II warning level of 20 µg/L. SPATT samples were analyzed for anatoxin-a, but it was not detected in any of the SPATT samples.

Cylindrospermopsin was detected only in discrete water samples at Lake Casitas. Cylindrospermopsin was detected in all samples (N=4) from this waterbody, ranging from 0.11-0.19 µg/L. These samples are below the California Tier I caution level of 4.0 µg/L (Appendix Figure A2). SPATT samples were analyzed for cylindrospermopsin, but it was not detected in any of the SPATT samples.

Cyanotoxin Dynamics Over the Summer of 2021 in Three Waterbodies

Multiple cyanotoxin classes were observed in the discrete water samples collected at Machado Lake. Total microcystins were detected in all samples collected at Machado Lake between mid-July and early September 2021 (Figure 7; Appendix Table A3). Total microcystin concentrations exceeded the California Warning trigger level for total microcystins (0.8 µg/L) in both samples collected in the month of August (visits 5 and 6) at the shore station. Water concentrations at the index station never exceeded any of the trigger levels. Anatoxin-a was also detected in water samples, but only in early August (visit 5) at the index location. This observation exceeded the California Warning trigger level for total anatoxin-a (detection using a method with a detection limit of <0.1 µg/L). As in the discrete water samples, microcystins were detected in all SPATT samplers retrieved in the second half of the summer (Supplemental Table S4). Microcystin-LW was only observed in the SPATT sampler retrieved during visit 4, and then only microcystin-RR was detected in the SPATT samplers retrieved during the remaining visits in August and September. No other cyanotoxin classes were detected from the SPATT samplers in Machado Lake.

Microcystins were only detected in one of the discrete water samples collected at Legg Lake but were observed in all deployed SPATT samplers (Figure 7; Appendix Table A4). Total microcystins were detected in the water sample collected from the index station during visit 4 at a concentration of 0.16 µg/L, just above the method detection limit (Figure 7). This observation was concurrent with a peak in chlorophyll-a *a* concentration observed in the lake (Figure 7) Microcystins were detected in SPATT all samplers retrieved between June and September in Legg Lake (Appendix Table A4). Microcystin-RR was detected in all samplers, albeit in variable concentrations. Microcystin-LA was detected in samplers retrieved during every visit except for visit 5, which also had the lowest overall toxin concentrations. Microcystin-LR was only observed in the SPATT sampler retrieved during visit 3. No other cyanotoxin classes were detected from the SPATT samplers.

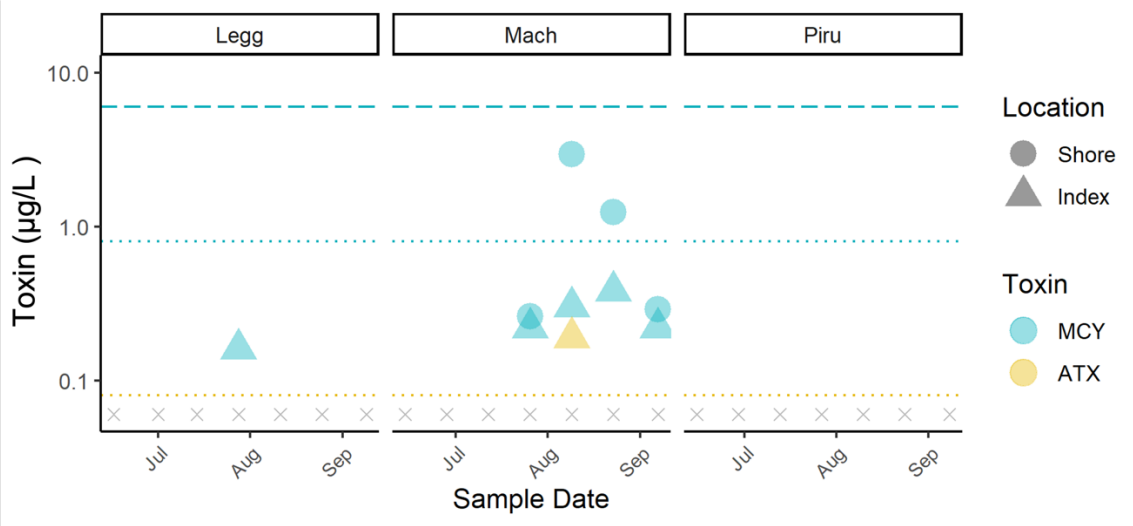

Figure 7. Cyanotoxin concentrations in discrete water samples from Machado Lake (MACH), Legg Lake (LEGG), and Piru Lake (PIRU) in the summer 2021 routine monitoring. Microcystins (MCY, teal symbols) and anatoxin-a (ATX, gold symbols) were both detected at Machado Lake. Teal dashed lines are California's Tier 1 Caution (0.8 µg/L) and Tier 2 Warning (6.0 µg/L) trigger levels for microcystins. The gold dashed line is California's Tier 1 Caution trigger level for anatoxin (detection at 0.15 µg/L). Toxin concentrations are reported on a log scale.

Microcystins were not detected in any of the discrete water samples collected at Lake Piru, but were observed in SPATT samplers (Figure 7, Supplemental Table S4). Microcystins were detected in SPATT samplers retrieved in June and early July (visits 2 and 3) and in samples retrieved in late August and early September (visits 6 and 7). Interestingly, no microcystins were detected in samplers deployed between mid-July and early August (visits 4 and 5) concurrent with an observed spike in chlorophyll-a concentration (Figure 5E). Unlike the other lakes, only one congener, microcystin-LA, was detected. No other cyanotoxins were observed in the summer of 2021 in Lake Piru (Supplemental Table S4).

Regional Cyanobacterial Community Diversity and Identification of Cyanotoxin Producers

A high degree of cyanobacterial diversity was observed across the lakes in the region. The 57 samples collected from 17 waterbodies yielded 2,903 ASVs. Six orders, 22 families, and 45 genera were taxonomically identified (Supplemental Figure S3). The most frequent high abundance genera across samples were Synechococcus with relative abundance (RA) > 10% in 37 samples from 12 waterbodies, Microcystis with RA > 10% in 11 samples from 5 waterbodies, Iningainema with RA > 10% in 7 samples from 2 waterbodies, Dolichospermum with RA > 10% in 6 samples from two waterbodies, and Sphaerospermopsis with RA > 10% in 4 samples from 1 waterbody. Of those taxa, Dolichospermum, Microcystis, and Sphaerospermopsis each had a sample where they had RA > 95%.

Of the 45 identified genera, 21 were determined to be potential toxin producers (Smith et al. 2021; Figure 6A). Genera with potential to produce microcystin (15 genera), nodularin (3 genera), cylindrospermopsin (6 genera), anatoxins (7 genera), saxitoxins (7 genera), guanitoxin (1 genus), lyngbyatoxin (1 genus), and aplysiaoxin (1 genus) were identified (Figure 8B). Four waterbodies had samples with a single cyanobacterial genus dominating the community: Malibou (Dolichospermum), Legg (Microcystis), Machado (Sphaerospermopsis), and Castaic Lake (Synechococcus). Genera that comprised 50-100% of ASV's in a given sample were Synechococcus, Sphaerospermopsis, Microcystis, Dolichospermum. While just under half (47%) of genera identified throughout LARWQCB were determined to be toxin producers, toxin producers overwhelmingly made up the majority of identified cyanobacteria (Figure 8C). RA of toxin producers ranged from <1-99%, while RA of genera with no known toxin production ranged from <1-4%. The remaining percent is composed of unidentified ASVs.

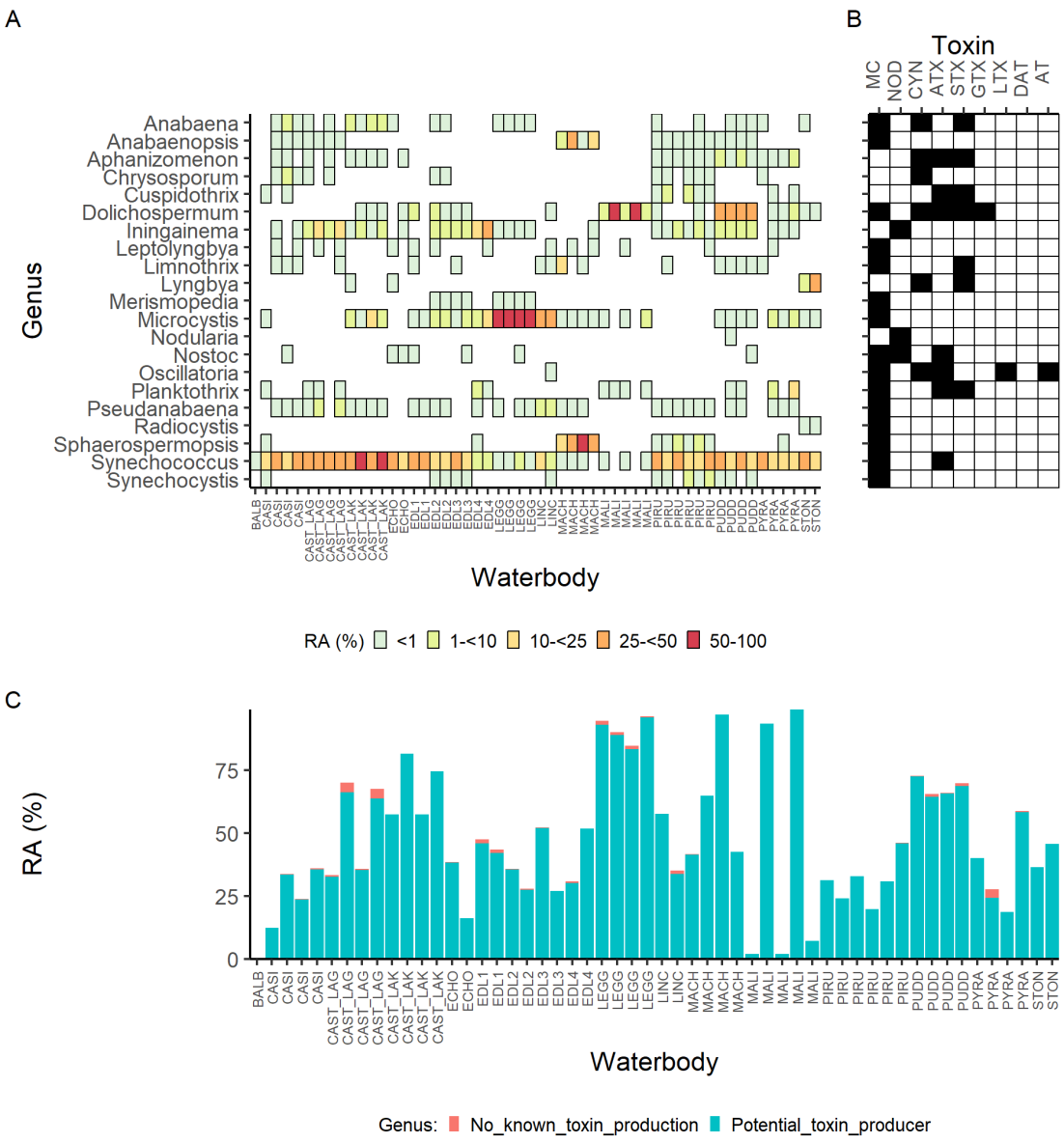

Figure 8. Relative abundance (RA) index of putatively toxigenic cyanobacteria genera as a percentage of total cyanobacterial ASVs in the summer 2020 regional survey, B) toxin production associated with each genus based on Smith et al. (2021), and C) RA of putatively toxic genera (teal) and genera with no known toxin production (orange). Toxins included in B are microcystin (MC), nodularin (nod), cylindrospermopsin (CYN), anatoxin (ATX), saxitoxin (STX), guanitoxin (GTX), lyngbyatoxin (LTX), debromoaplysiatoxin (DAT), aplysiaoxin (AT). Location on the x-axis in panels A and C are as follows: Location are Castaic Lagoon (CAST_LAG), Stone Canyon Reservoir (STON), Echo Park Lake (ECHO), Lake Casitas (CASI), Pyramid Lake (PYRA), Castaic Lake (CAST_LAK), Lake Piru (PIRU), El Dorado Lakes-North Lake (EDL1), Lincoln Park Lake (LINC), El Dorado Lakes-Main Lake (EDL2), Puddingstone Reservoir (PUDD), El Dorado Lakes-Horseshoe Lake (EDL3), El Dorado Lakes-Duck Pond (EDL4), Lake Balboa (BALB), Legg Lake (LEGG), Machado Lake (MACH), and Malibou Lake (MALI).

Cyanobacterial Community Dynamics Over Time in Three Routinely Monitored Waterbodies

A total of 20 distinct genera were taxonomically identified in Machado Lake over the course of the summer of 2021 (Appendix Table A5). Of these, *Anabaenopsis, Cuspidothrix, Dolichospermum, Microcystis, Sphaerospermopsis*, and *Synechococcus* were all detected at RA > 5% within the lake during the study period (Figure 9A). A large proportion of ASVs were unassigned (typically between 47%-95%). Twelve of the cyanobacterial genera detected in Machado Lake were putative toxin producers (Smith et al. 2021; Figure 9B). A majority of these taxa were potential microcystin producers, while a smaller proportion were putative producers of nodularin, anatoxins, saxitoxins, cylindrospermopsin or guanitoxin (Figure 9B). Although a large proportion of ASVs were unassigned, a majority of the ASVs with taxonomic assignments were potential toxin producing taxa and taxa with no reported toxin production were always present at RA < 3% (Figure 9C).

The most abundant toxin producing genera were *Sphaerospermopsis* and *Microcystis* in Machado Lake in summer 2021*,* both with RA exceeding 25% at different times in the summer (Figure 9A)*.* A notable increase in the RA of *Sphaerospermopsis* was observed on visit 4 (7/26/2021) at both the shore and index stations. This shift coincided with the initial detection of microcystins within the lake. Interestingly, a shift in community dominance was observed in the following visit in early August, where the community shifted towards the dominance of *Microcystis.* This shift was more extreme at the shore station with RA exceeding 50% (Figure 9A)*,* and more subtle at the index station where RA were roughly 2%. This shift occurred in tandem with the highest observation of microcystins at the shore station (2.96 µg/L; Figure 7; Appendix Table A3) and the detection of both microcystins and anatoxin-a at the index station. *Microcystis* remained the most dominant toxigenic taxa at the shore station in late August, but RA reduced to roughly 15%, while RA of *Microcystis* at the index station was 2%. Microcystins were still detected at both stations, but concentrations decreased slightly. A more substantial community shift was observed by the final visit to Machado Lake in early September, with an increased dominance of *Sphaerospermopsis* (Shore: 13%; Index: 9%), particularly at the shore station, as well as a rise in *Anabaenopsis* RA (Shore: 17%; Index: 18%).

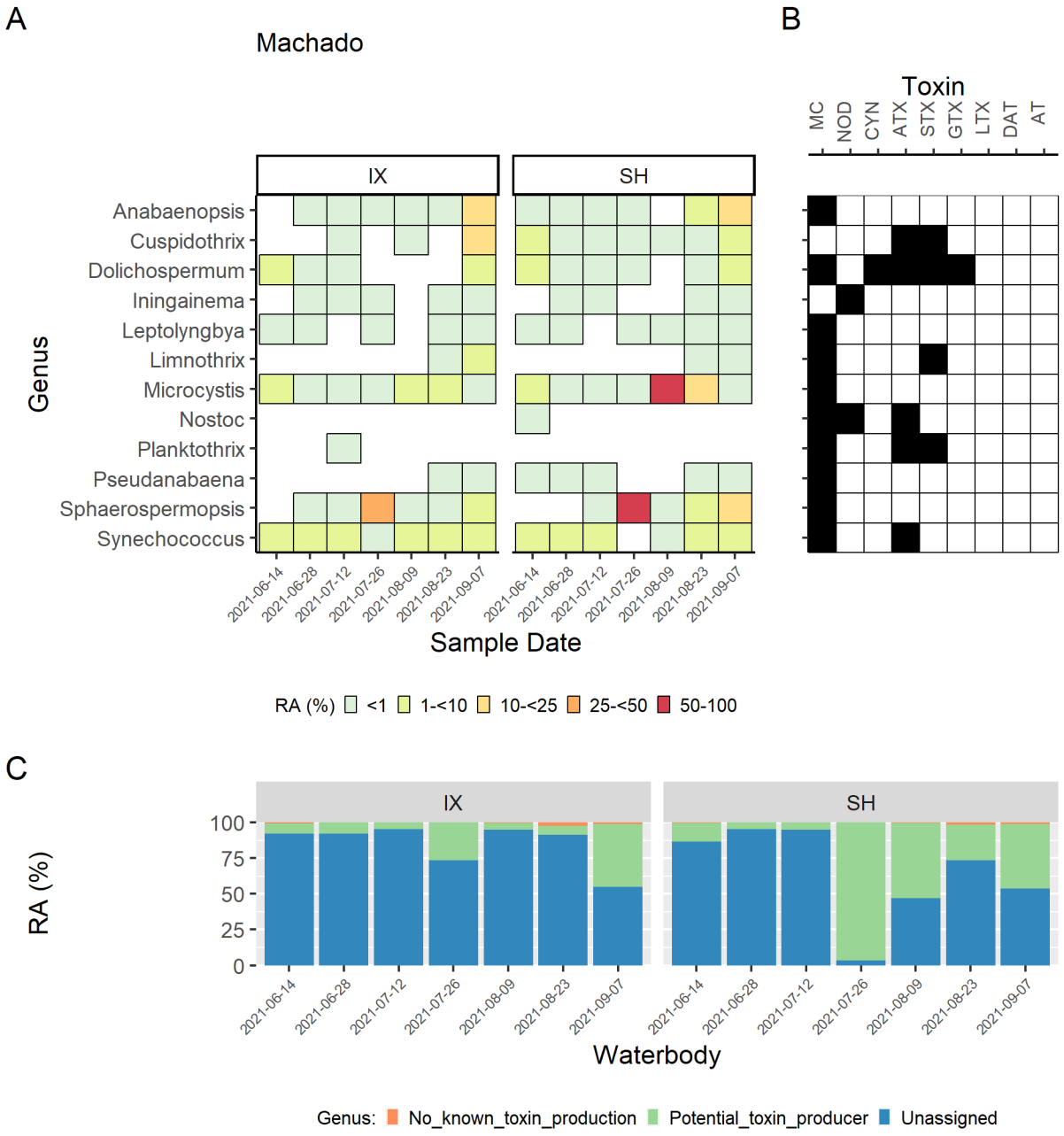

Figure 9. A) Relative abundance (RA) index of putatively toxigenic cyanobacteria genera in Machado Lake as a percentage of total cyanobacterial Amplicon Sequence Variants (ASVs) in the summer 2021 routine monitoring, B) toxin production associated with each genus based on Smith et al. (2021), and C) RA of putatively toxic genera (green), genera with no known toxin production (orange), and sequences that were unassigned (blue). Toxins included in B are microcystin (MC), nodularin (nod), cylindrospermopsin (CYN), anatoxin (ATX), saxitoxin (STX), guanitoxin (GTX), lyngbyatoxin (LTX), debromoaplysiatoxin (DAT), aplysiaoxin (AT).

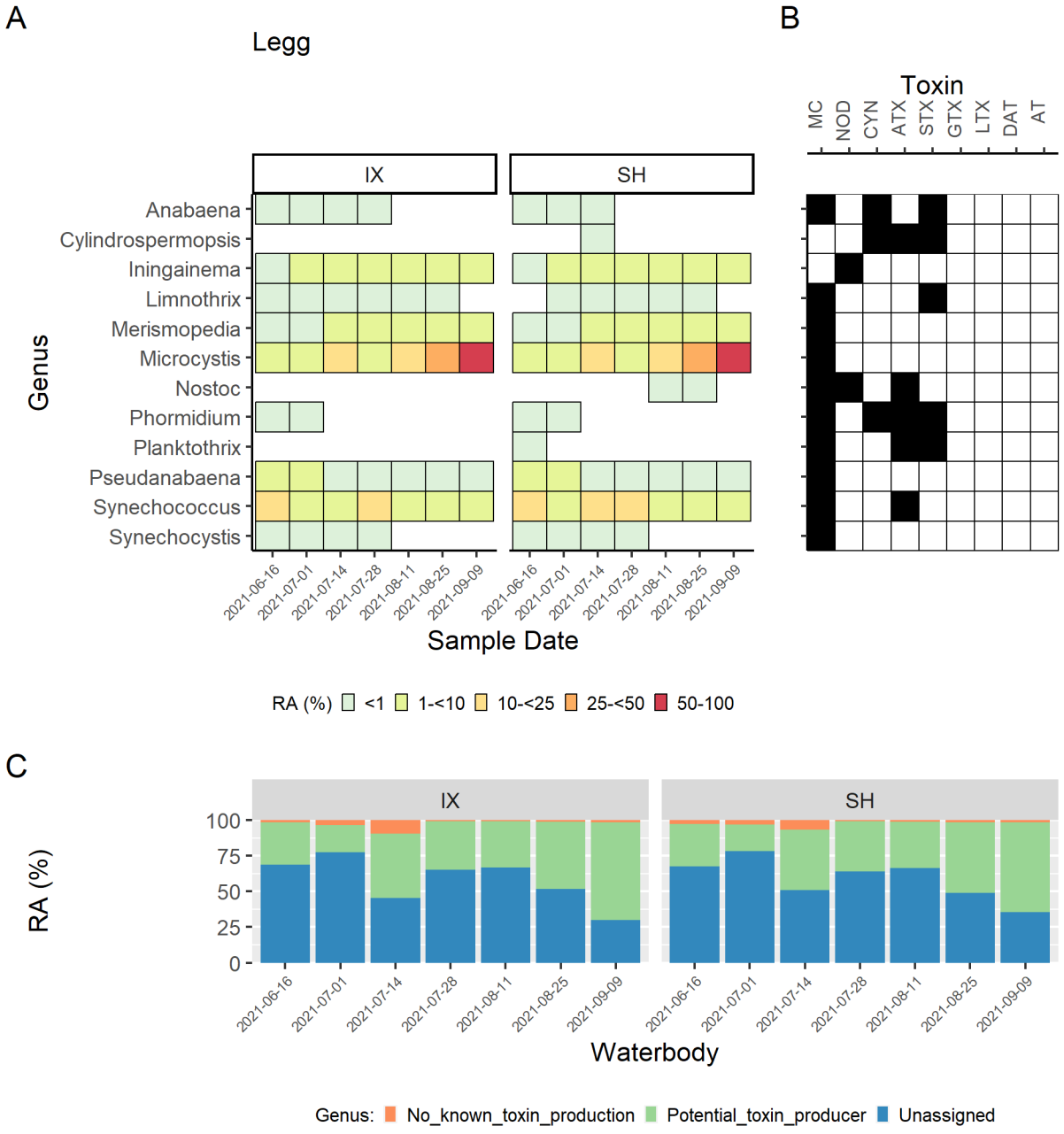

Figure 10. A) Relative abundance (RA) index of putatively toxigenic cyanobacteria genera in Legg Lake as a percentage of total cyanobacterial Amplicon Sequence Variants (ASVs) in the summer 2021 routine monitoring, B) toxin production associated with each genus based on Smith et al. (2021), and C) RA of putatively toxic genera (green), genera with no known toxin production (orange), and sequences that were unassigned (blue). Toxins included in B are microcystin (MC), nodularin (nod), cylindrospermopsin (CYN), anatoxin (ATX), saxitoxin (STX), guanitoxin (GTX), lyngbyatoxin (LTX), debromoaplysiatoxin (DAT), aplysiaoxin (AT).

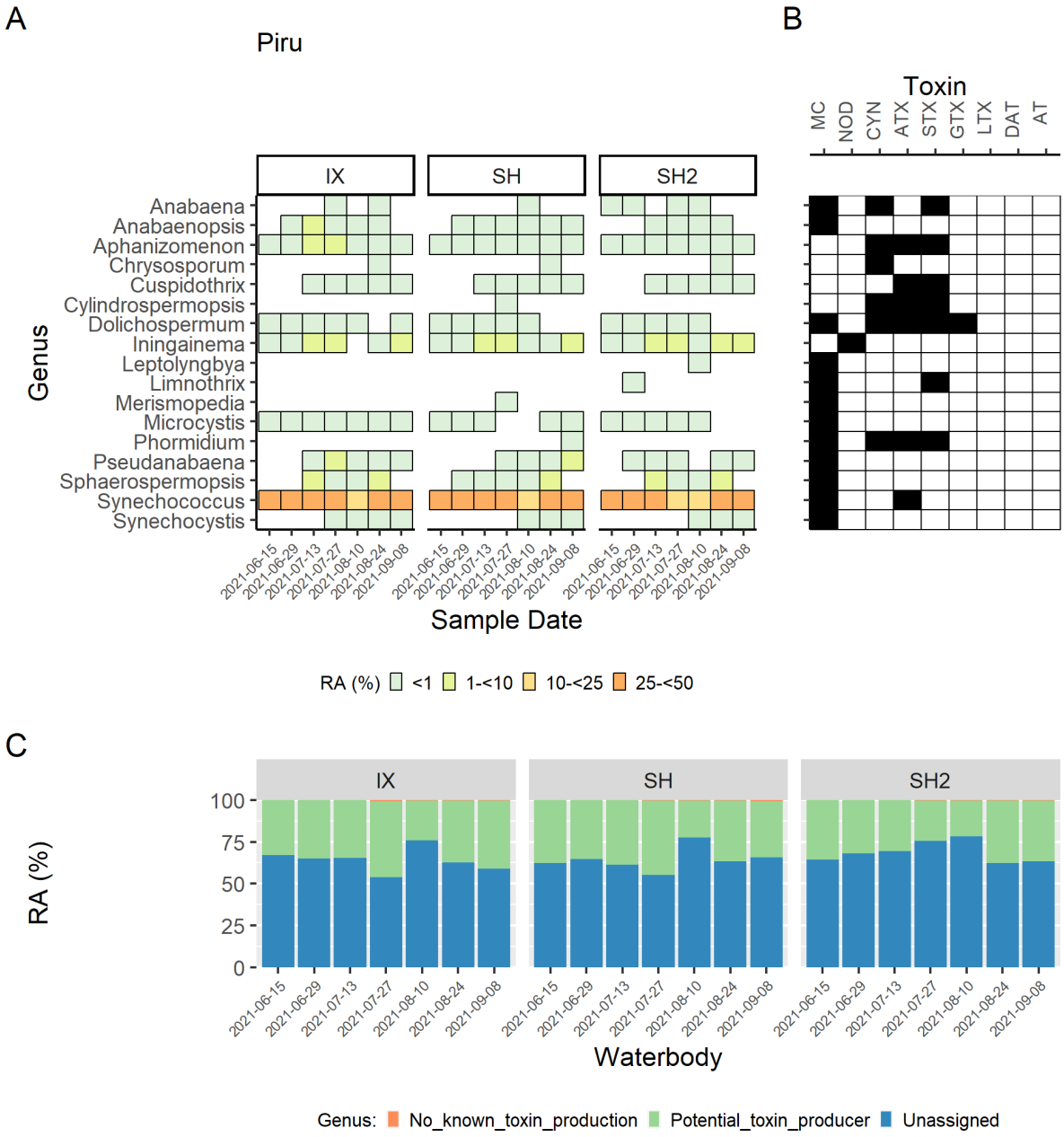

Figure 11. A) Relative abundance (RA) index of putatively toxigenic cyanobacteria genera in Lake Piru as a percentage of total cyanobacterial Amplicon Sequence Variants (ASVs) in the summer 2021 routine monitoring, B) toxin production associated with each genus based on Smith et al. (2021), and C) RA of putatively toxic genera (green), genera with no known toxin production (orange), and sequences that were unassigned (blue). Toxins included in B are microcystin (MC), nodularin (nod), cylindrospermopsin (CYN), anatoxin (ATX), saxitoxin (STX), guanitoxin (GTX), lyngbyatoxin (LTX), debromoaplysiatoxin (DAT), aplysiaoxin (AT).

Similar to Machado Lake, 20 cyanobacterial genera were taxonomically identified in Legg Lake (Appendix Table A5). Of these, *Inigainema, Mesrismopedia, Microcystis, Pseudanabaena, Romeria*, and *Synechococcus* were present at RA > 5% within the lake during the study period, while other taxa were present but at lower abundances (Figure 10A). Twelve of the cyanobacterial genera detected in Legg Lake were putative toxin producers (Smith et al. 2021; Figure 10A). A majority of these taxa were potential microcystin producers, while a smaller proportion were putative producers of other cyanotoxins (Figure 10B). Compared to Machado Lake, a smaller proportion of ASVs were unassigned, and a majority of the ASVs with taxonomic assignments were potential toxin producing taxa with RA ranging from <1-59% over the course of the summer (Figure 10C).

The most abundant toxin producing genera in Legg Lake were *Microcystis* and *Synechococcus* (Figure 10A). Over the course of the summer, the lake shifted towards a *Microcystis* dominated community with RA of this taxa exceeding 50% by the end of the summer. *Synechococcus* was a persistent member of the community as well, often exceeding RA of 10% in the first half of the summer. Interestingly, although microcystins were detected in SPATT samplers over the entire summer, the single detection of microcystin in water samples occurred at the index station during visit 4 (7/28/2021). This overlapped with a period where *Synechococcus* RA increased over 10% during both visits 3 (7/14/2021) and 4 and *Microcystis* abundances declined over that same period. As *Microcystis* abundances began to increase again following visit 4, microcystins continued to be detected in SPATT samplers, but in lower concentrations than in the earlier part of the summer.

A total of 24 distinct cyanobacterial genera were observed at Lake Piru (Appendix Table A5). *Synechococcus* was the most dominant taxa present at RA ranging from 20%-40% throughout the summer. Although a variety of other taxa were also present, they were always at RA less than 5%. Although at low RA (besides *Synechococcus*), 16 potential toxin producing genera were detected (Smith et al. 2021; Figures 11A & B). As with the other two lakes, a majority of these taxa were potential microcystin producers (Figure 11C).

Relationships Between Trophic State, Cyanobacterial Community Structure, and Cyanotoxins in The Regional Survey

In the 42 samples across the 10 waterbodies with index stations sampled, cyanobacterial community was most strongly structured by waterbody, but was also significantly structured by eutrophication status based on chlorophyll, TN, and TP concentrations (Table 5). Interestingly, communities from hypereutrophic lakes clustered distinctly from lakes of lower status categories, suggesting distinctive cyanobacterial communities are common under these conditions (Figure 12B-D). Several toxigenic taxa were over enriched in hypereutrophic lakes, including *Dolichospermum*, *Microcystis*, and *Sphaerospermopsis* (Appendix Table A6).

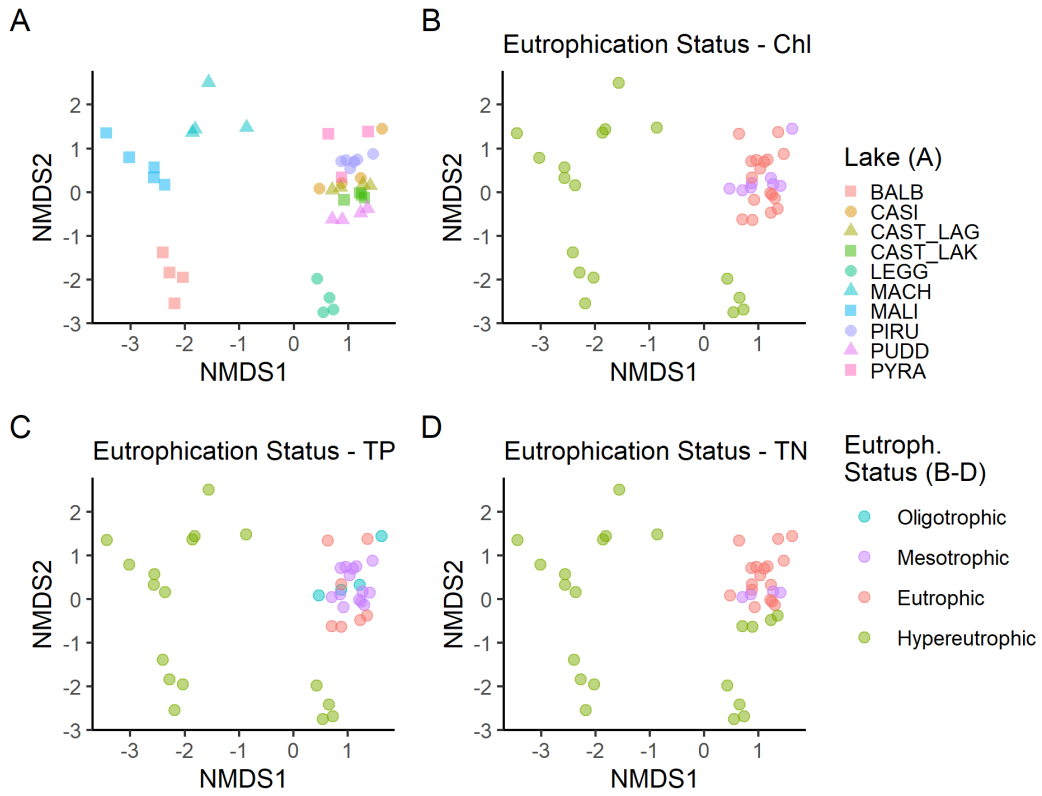

Figure 12. Non-metric Multidimensional Scaling plot of the ten waterbodies with index stations in the summer 2020 regional survey. In panel A, the colors indicate grouping by A) waterbody with Castaic Lagoon (CAST_LAG, olive triangle), Lake Casitas (CASI, gold circle), Pyramid Lake (PYRA, pink square), Castaic Lake (CAST_LAK, green square), Lake Piru (PIRU, purple circle), Puddingstone Reservoir (PUDD, pink triangle), Lake Balboa (BALB, red square), Machado Lake (MACH, blue triangle), and Malibou Lake (MALI, blue square). In panels B-D, the colors indicate eutrophication status, oligotrophic (blue circle), mesotrophic (purple circle), eutrophic (red circle), and hypereutrophic (green circle), based on concentrations of B) chlorophyll-a (Chl), C) total phosphorus (TP), and D) total nitrogen (TN).

Table 5. ANOSIM analysis of parameters related to cyanobacterial community inferred from amplicon sequence variants (ASVs) in the summer 2020 regional survey. All lakes were included in the analysis for relationship with toxin detection, while only samples from the ten lakes with index stations (Index Lakes) were included in the analysis for relationship between cyanobacterial community and eutrophication status. ANOSIM R statistic and significance (in parentheses) are reported.

Input Data	Parameter	Parameter Categories	ANOSIM Result
All lakes	Lake	Lake name (17 lakes, 57 samples)	0.87 (p = 0.0001)
All lakes	Toxin P/A	Toxin Presence/Absence	0.16 (p = 0.0020)
All lakes	Toxin Type	Microcystin, Cylindrospermopsin, Anatoxin-a, none	0.23 (p = 0.0026)
Index Lakes	Lake	Lake name (10 lakes, 42 samples)	0.87 (p = 0.0001)
Index Lakes	Trophic Status: Chl	Mesotrophic, Eutrophic, Hypereutrophic	0.36 (p = 0.0001)
Index Lakes	Trophic Status: TP	Oligotrophic, Mesotrophic, Eutrophic, Hypereutrophic	0.31 (p = 0.0001)
Index Lakes	Trophic Status: TN	Mesotrophic, Eutrophic, Hypereutrophic	0.23 (p = 0.0005)

However, due to the prevalence of unassigned taxa, indicator species analysis showed that hypereutrophic lakes were most strongly enriched with unassigned ASVs (40 ASVs, max stat = 0.84; Appendix Table A6). Other over enriched taxa were *Romeria* and *Nodosilinea*, which are not known toxin producers. Cyanobacterial communities from lakes in eutrophic, mesotrophic and oligotrophic condition were also strongly over enriched in unassigned ASVs, however, they were also over enriched in *Synechococcus* (Appendix Table A6).

In 57 samples across 17 lakes, the cyanobacterial community was strongly structured by waterbody with a weak but significant relationship between community structure and toxin presence, whether all cyanotoxins were considered together or considered individually (Table 5). Indicator species analysis revealed samples with detected microcystin in water samples had cyanobacterial communities over enriched with *Microcystis* and *Pseudanabaena*, both of which are microcystin producers. Samples with

detection of cylindrospermopsin had cyanobacterial communities over enriched with *Synechococcus*, *Pseudanabaena*, *Chrysosporum*, and Anabaena, the latter two of which are cylindrospermopsin producers. Samples with detection of anatoxin-a were over enriched with *Sphaerospermopsis*, *Anabaenopsis*, *Romeria*, and *Limnothrix*, none of which are known anatoxin-a producers (Appendix Table A7).

Table 6. ANOSIM and Mantel analysis of parameters related to the cyanobacterial community inferred from amplicon sequence variants (ASVs) in the summer 2021 routine monitoring of Machado Lake, Legg Lake, and Lake Piru. Parameters tested included: visits, toxin presence/absence (Toxin P/A), temperature, chlorophyll-a concentrations (Chl-a), total phosphorus concentrations (TP), total nitrogen concentrations (TN), and total nitrogen:total phosphorus ratio (TN:TP). Parameters with significant effect (p-value < 0.05) on the cyanobacterial community are indicated with an asterisk (*). The parameter 'Visit' for Piru lake only includes visits 1 – 6 due to the loss of samples from visit 7.

Lake	Parameter	Test	Test Statistic	p-value
Machado Lake	Visit (1-7)*	ANOSIM	0.89	1.00E-04
Machado Lake	Toxin P/A*	ANOSIM	0.42	<0.01
Machado Lake	Temperature	Mantel	-0.18	0.91
Machado Lake	Chl-a*	Mantel	0.64	1.00E-04
Machado Lake	TP*	Mantel	0.4	0.01
Machado Lake	TN*	Mantel	0.69	1.00E-04
Machado Lake	TN:TP*	Mantel	0.48	1.00E-04
Legg Lake	Visit (1-7)*	ANOSIM	1	1.00E-04
Legg Lake	Temperature	Mantel	0.03	0.35
Legg Lake	Chl-a*	Mantel	0.66	1.00E-04
Legg Lake	TP*	Mantel	0.74	1.00E-04
Legg Lake	TN*	Mantel	0.72	1.00E-04
Legg Lake	TN:TP*	Mantel	0.37	0.01
Lake Piru	Visit (1-6)*	ANOSIM	1	1.00E-04
Lake Piru	Temperature*	Mantel	0.36	6.00E-04
Lake Piru	Chl-a*	Mantel	0.5	1.00E-04
Lake Piru	TP	Mantel	0.05	0.31
Lake Piru	TN*	Mantel	0.31	<0.01
Lake Piru	TN:TP	Mantel	-0.1	0.83

Parameters Related to Cyanobacterial Community Structure at the Three Routinely Monitored Lakes

While a number of factors had significant relationships with the cyanobacterial community at Machado Lake, Legg Lake, and Piru Lake in summer 2021, time had the strongest relationship for all three lakes (i.e., communities sampled during a certain visit would be more similar; Test statistics of 0.89 – 1 for the parameter 'Visits' in Table 6). This result is not surprising given that cyanobacteria have growth rates on the scale of days, and the cyanobacterial communities can have dramatic changes during the 2-week interval between sampling events. Chlorophyll-a, TN, and TP concentrations are parameters that are generally correlated (Refer to similar trends in Figures 5B, C, &E), and were all, along with the TN:TP ratio, significantly related to the cyanobacterial community structure at Machado Lake and Legg Lake. On the other hand, cyanobacterial community structure at Piru Lake was not related to TP concentrations or TN:TP ratio, but was, instead, significantly related to temperature.

DISCUSSION

In this study, we found that cyanotoxins and eutrophication was widely detected in Los Angeles-Ventura region lakes and reservoirs. Between 60% - 80% of the lakes where index sites were accessible were either eutrophic or hypereutrophic, depending on the indicator used (chlorophyll-a, total nitrogen (TN) or total phosphorus (TP)). When looking across the region, cyanotoxins were observed in 65% of the 17 lakes, with microcystins being detected in multiple waterbodies, while anatoxin-a and cylindrospermopsin were each only observed in a single waterbody.

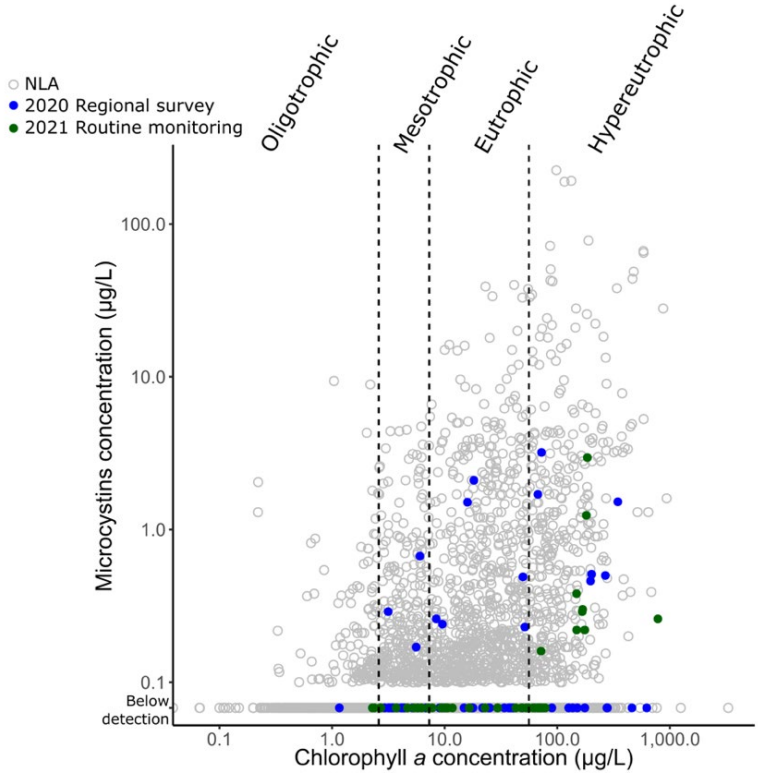

Figure 13. A comparison of the relationship between microcystins and chlorophyll a concentration between data from the US EPA National Lake Assessment program collected in 2007, 2012 and 2017 (NLA; gray open circles), summer 2020 regional survey (blue circles), and summer 2021 routine monitoring (dark green circles). Vertical dashed lines indicate the boundaries of various trophic status as indicated by chlorophyll-a concentrations (Table 3). Data are presented on a log scale.

When considering the entire population of lakes sampled in the 2020 regional survey, the median and range of cyanotoxin concentrations increased across the eutrophication gradient. Additionally, distinctive cyanobacteria communities emerged in lakes with increasing trophic status. The communities present in hypereutrophic lakes were very distinctive and were over enriched with several toxigenic taxa. Given the prevalence of toxigenic taxa, many of these lakes are primed for the development of a cyanoHAB events. Since these systems are replete with the essential nutrients for cyanobacterial growth, events can

develop with shifts in other conditions such as water column stratification, temperature, or nutrient ratios.

The findings of the 2020 regional survey can be placed into a national context by leveraging data from the the National Lakes Assessment (NLA). The NLA program conducts a probabalistic survey of lakes throughout the nation to assess eutrophication indicators, including the prevalence of cyanobacteria and microcystins, on an 5-year basis. Although we did not employ a probabalistic design in the present study, we can compare our paired chlorophyll and microcystin to the observations of this program (Figure 13). Several of the observations of chlorophyll-a in the Los Angeles-Ventura region were among some of the highest observed chlorophyll-a concentrations in the nation. The national dataset also highlights a similar pattern of increased range of observed microcystin concentrations with increasing trophic status, although the microcystin concentrations observed in 2020 are on the lower end of the full range of concentrations obsered nationally. This comparison also highlights that microcystins can be present in lakes in mesotrophic and oligotrophic conditions. In this study, cyanotoxins were also observed in some waterbodies of lower trophic status, such as Lake Casitas, highlighting that trophic status assessments should also include cyanoHAB indicators. Thus, trophic status is not the single factor driving the development of toxigenic cyanoHABs in any individual lake but can be an indication of increased risk for blooms.

Two different sampling designs were applied to address two different types of management questions in the present study. The regional survey of lakes conducted in 2020 was designed to understand the overall trophic conditions of lakes and reservoirs in the region and how they related to potential cyanoHAB risks. Here assessment over space allowed for the broader understanding of potential risks and general patterns in cyanoHABs in the region. However, this type of design did not provide good overall protection of recreational health (except for a brief snapshot in time), nor did it provide a careful assessment of lake specific drivers of cyanoHAB events. Routine monitoring, however, can better address these goals. The routine sampling of lakes in 2021 highlighted how the variation in cyanoHAB dynamics can result in differing risks for recreational health. In Machado Lake, for example, out of seven total sampling events, two visits revealed toxin concentrations above the California Caution Trigger Level, while the remaining five visits showed either the presence of toxins below recreational trigger levels or below methodological detection limits. The combination of sampling designs highlighted the need for routine sampling to protect recreational uses in several waterbodies, particularly more at-risk eutrophic, and hypereutrophic waterbodies.

The paired observations of cyanobacterial community composition, cyanotoxins and environmental conditions over time provided some potential insights into the drivers of toxin presence and persistence. The highest and most persistent microcystin levels were detected in Machado Lake, beginning in the second half of the summer. Of the three lakes, Machado Lake was the most nutrient enriched, with TN, TP, and biomass levels increasing throughout the summer. Through the examination of community composition in Machado Lake, some interesting transitions between toxigenic genera and changes in toxin levels were observed. During visit 4, there was an overall increase in TN at all three lakes during visit 4 (Figure 5C), but the spike in TN was particularly high for Machado Lake. This spike in TN also resulted in a shift from apparent nitrogen limitation to phosphorus limitation in the lake. During this

visit, the nitrogen fixing cyanobacterial genus, *Sphaerospermopsis* was among the most dominant members of the community, while *Microcystis,* which does not have the ability to fix nitrogen, was present but a lower relative abundance. The first detection of microcystins was also observed during this visit. A complete shift in community to *Microcystis* and notable increase in microcystins was observed during the following visit. It is possible that the presence and dominance of *Sphaerospermopsis* in the system introduced new nitrogen from the atmosphere into the water. Such priming of the system then allowed *Microcystis* to increase in abundance and eventually dominated the cyanobacterial community and had a potential impact on the observed toxin concentrations in the lake.

Scientific Data Gaps and Management Recommendations

The approaches employed in this study do not allow for the clear identification of which species within a community are producing toxins. For example, in Machado Lake, we can only infer which members of the community may have been producing toxin based on previous literature reports of toxin production. Deeper molecular characterization using 'omics approaches have the ability to specifically identify which cyanobacterial taxa in the community have the genetic potential to produce cyanotoxins as well as identifying when these taxa turn on (and off) their toxin production genes. When paired with observations of environmental conditions, this information can be linked to the specific conditions that trigger cyanotoxin production.

Phytoplankton community assessments in this study focused exclusively on cyanobacterial taxa, however broader assessments of cyanobacterial and eukaryotic algal diversity in the region's lakes could reveal a fuller understanding of overall HAB risks and dynamics. There are several emerging freshwater HAB issues caused by eukaryotic algae such as *Prymnesium parvum* (golden algae) that may emerge as California's climate continues to change and would be beneficial to assess the prevalence of these taxa regionally. In routine monitoring scenarios, full community assessment could lead to a deeper understanding of how biological relationships (competitive, mutualistic, etc.) among microbial species might control bloom dynamics and cyanotoxin production. In Lake Piru, there were changes in chlorophyll-a dynamics throughout the summer that were not necessarily explained by the assessment of cyanobacterial community and assessment of the eukaryotic algal community could have better explain these changes.

Other science recommendations that can inform future management actions include:

1. Formalize a partner lakes and reservoir monitoring program to
    a. better characterize risk to beneficial uses, given the temporally limited sampling, with a suite of indicators consistent with forthcoming Water Board biostimulatory policy
    b. provide training to ensure adequate HAB event response

2. Conduct a pilot project to screen for eutrophication status via remotely sensed satellite data to further prioritize in situ monitoring; and

3. Conduct desktop environmental drivers assessment and data gaps analysis to proactively identify the initial set of data needs and management recommendations to further mitigate eutrophication and risk of HABs.

References

Backer, C.L., Carmichael, W., Kirkpatrick, B., Williams, C., Irvin, M., Zhou, Y., Johnson, B.T., Nierenberg, K., Hill, R.V., Kieszak, M.S., Cheng, Y.-S. 2008. Recreational Exposure to Low Concentrations of Microcystins During an Algal Bloom in a Small Lake. Marine Drugs 6(2).

Backer, L.C., Landsberg, J.H., Miller, M., Keel, K., Taylor, T.K. 2013. Canine cyanotoxin poisonings in the United States (1920s–2012): Review of suspected and confirmed cases from three data sources. Toxins 5(9), 1597-1628.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature biotechnology 37(8), 852-857.

Brooks, B.W., Lazorchak, J.M., Howard, M.D.A., Johnson, M.-V.V., Morton, S.L., Perkins, D.A.K., Reavie, E.D., Scott, G.I., Smith, S.A., Steevens, J.A. 2016. Are harmful algal blooms becoming the greatest inland water quality threat to public health and aquatic ecosystems? Environmental Toxicology and Chemistry 35(1), 6-13.

Brooks, B.W., Lazorchak, J.M., Howard, M.D.A., Johnson, M.V.V., Morton, S.L., Perkins, D.A.K., Reavie, E.D., Scott, G.I., Smith, S.A., Steevens, J.A. 2017. In some places, in some cases, and at some times, harmful algal blooms are the greatest threat to inland water quality. Environ Toxicol Chem 36(5), 1125-1127.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nature methods 13(7), 581-583.

Carlson, R.E. 1977. A trophic state index for lakes 1. Limnology and oceanography 22(2), 361-369.

Carmichael, W. 2008. A world overview—One-hundred-twenty-seven years of research on toxic cyanobacteria—Where do we go from here?, Cyanobacterial harmful algal blooms: State of the science and research needs. Springer, pp. 105-125.

Carmichael, W.W. Boyer, G.L., 2016. Health impacts from cyanobacteria harmful algae blooms: Implications for the North American Great Lakes. Harmful algae 54, 194-212.

CCHAB. 2016. Appendix to the CCHAB Preliminary Changes to the Statewide Voluntary Guidance on CyanoHABs in Recreational Waters, January 2016. California Cyanobacterial and Harmful Algal Bloom Network.

Davis, T.W., Stumpf, R., Bullerjahn, G.S., McKay, R.M.L., Chaffin, J.D., Bridgeman, T.B., Winslow, C. 2019. Science meets policy: A framework for determining impairment designation criteria for large waterbodies affected by cyanobacterial harmful algal blooms. Harmful Algae 81, 59-64.

Edwards, C., Beattie, K.A., Scrimgeour, C.M., Codd, G.A. 1992. Identification of anatoxin-A in benthic cyanobacteria (blue-green algae) and in associated dog poisonings at Loch Insh, Scotland. Toxicon 30(10), 1165-1175.

Howard, M.D.A., Kudela, R.M., Caron, D.A., Smith, J., Hayashi, K. 2018. Standard Operating Procedure for Solid Phase Adsorption Toxin Testing (SPATT) Assemblage and Extraction of HAB Toxins.

Howard, M.D.A., Kudela, R.M., Hayashi, K., Tatters, A.O., Caron, D.A., Theroux, S., Oehrle, S., Roethler, M., Donovan, A., Loftin, K., Laughrey. Z. 2021. Multiple co-occurring and persistently detected cyanotoxins and associated cyanobacteria in adjacent California lakes. Toxicon 192:1-14

Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M., Visser, P.M. 2018. Cyanobacterial blooms. Nature Reviews Microbiology 16(8), 471-483.

Kudela, R.M. 2011. Characterization and deployment of Solid Phase Adsorption Toxin Tracking (SPATT) resin for monitoring of microcystins in fresh and saltwater. Harmful Algae 11, 117-125.

Loftin, K.A., Graham, J.L., Hilborn, E.D., Lehmann, S.C., Meyer, M.T., Dietze, J.E., Griffith, C.B. 2016. Cyanotoxins in inland lakes of the United States: Occurrence and potential recreational health risks in the EPA National Lakes Assessment 2007. Harmful Algae 56, 77-90.

Mekebri, A., Blondina, G., Crane, D. 2009. Method validation of microcystins in water and tissue by enhanced liquid chromatography tandem mass spectrometry. Journal of Chromatography a 1216(15), 3147-3155.

Mez, K., Beattie, K., Codd, G., Hanselmann, K., Hauser, B., Naegeli, H., Preisig, H. 1997. Identification of a microcystin in benthic cyanobacteria linked to cattle deaths on alpine pastures in Switzerland. European Journal of Phycology 32(2), 111-117.

Monchamp, M.-E., Walser, J.-C., Pomati, F., Spaak, P. 2016. Sedimentary DNA reveals cyanobacterial community diversity over 200 years in two perialpine lakes. Applied and Environmental Microbiology 82(21), 6472-6482.

O'Neil, J.M., Davis, T.W., Burford, M.A., Gobler, C.J. 2012. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. Harmful Algae 14, 313-334.

Paerl, H.W., Huisman, J. 2008. Blooms Like It Hot. Science 320(5872), 57.

Paerl, H.W., Paul, V.J. 2012. Climate change: Links to global expansion of harmful cyanobacteria. Water Res 46(5), 1349-1363.

Pouria, S., de Andrade, A., Barbosa, J., Cavalcanti, R.L., Barreto, V.T.S., Ward, C.J., Preiser, W., Poon, G.K., Neild, G.H., Codd, G.A. 1998. Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil. The Lancet 352(9121), 21-26.

Puschner, B., Hoff, B., Tor, E.R. 2008. Diagnosis of anatoxin-a poisoning in dogs from North America. J Vet Diagn Invest 20(1), 89-92.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O. 2012. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic acids research 41(D1), D590-D596.

Smith, J., Sutula, M., Bouma-Gregson, K., VanDyke, M. 2021. California State Water Boards Strategy to Develop and Implement a Freshwater Harmful Algal Bloom Monitoring Program. Southern California Coastal Water Research Project, Costa Mesa, CA.

Steffen, M.M., Davis, T.W., McKay, R.M.L., Bullerjahn, G.S., Krausfeldt, L.E., Stough, J.M.A., Neitzey, M.L., Gilbert, N.E., Boyer, G.L., Johengen, T.H., Gossiaux, D.C., Burtner, A.M., Palladino, D., Rowe, M.D., Dick, G.J., Meyer, K.A., Levy, S., Boone, B.E., Stumpf, R.P., Wynne, T.T., Zimba, P.V., Gutierrez, D., Wilhelm, S.W. 2017. Ecophysiological Examination of the Lake Erie Microcystis Bloom in 2014: Linkages between Biology and the Water Supply Shutdown of Toledo, OH. Environ Sci Technol 51(12), 6745-6755.

Stewart, I., Seawright, A.A., Shaw, G.R. 2008. Cyanobacterial poisoning in livestock, wild mammals and birds–an overview, In: Hudnell, H.K. (Ed.), Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. Springer, New York, NY, USA, pp. 613-637.

Trevino-Garrison, I., DeMent, J., Ahmed, S.F., Haines-Lieber, P., Langer, T., Ménager, H., Neff, J., Van der Merwe, D., Carney, E. 2015. Human Illnesses and Animal Deaths Associated with Freshwater Harmful Algal Blooms—Kansas. Toxins 7(2).

Wood, S.A., Heath, M.W., Holland, P.T., Munday, R., McGregor, G.B., Ryan, K.G. 2010. Identification of a benthic microcystin-producing filamentous cyanobacterium (*Oscillatoriales*) associated with a dog poisoning in New Zealand. Toxicon 55(4), 897-903.

Xu, H., Paerl, H.W., Qin, B., Zhu, G., Gaoa, G. 2010. Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. Limnology and Oceanography 55(1), 420-432.

Yilmaz, P., Parfrey, L.W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W., Glöckner, F.O. 2014. The SILVA and “all-species living tree project (LTP)” taxonomic frameworks. Nucleic acids research 42(D1), D643-D648.

Yuan, L.L., Pollard, A.I. 2015. Deriving nutrient targets to prevent excessive cyanobacterial densities in US lakes and reservoirs. Freshwater Biology 60(9), 1901-1916.

Yuan, L.L., Pollard, A.I. 2017. Using national-scale data to develop nutrient–microcystin relationships that guide management decisions. Environmental science & technology 51(12), 6972-6980.

APPENDIX A. SUPPLEMENTAL FIGURES AND TABLES

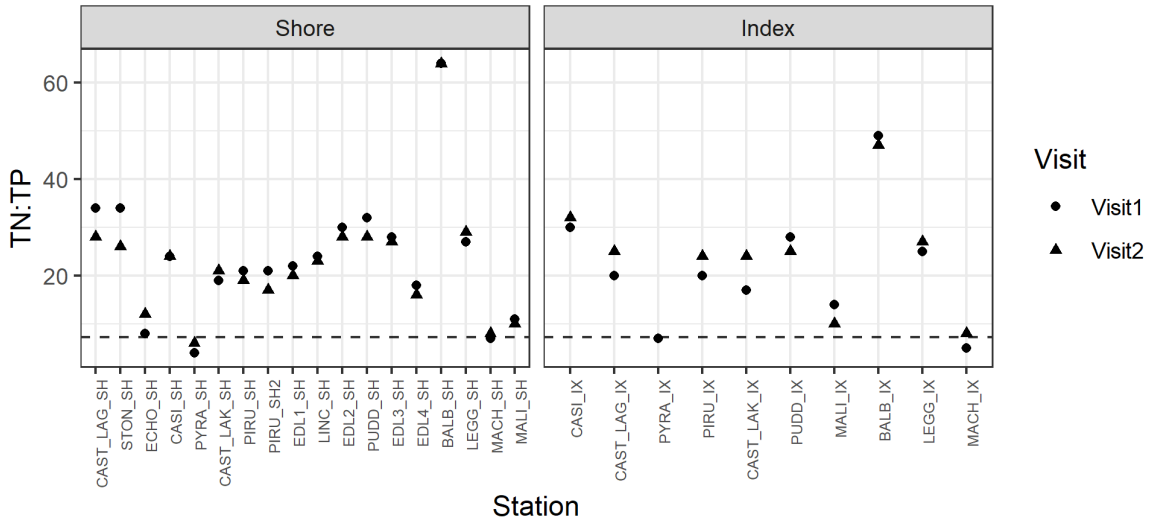

Figure A1. Total nitrogen to total phosphorus ratio (TN:TP) at the shore stations (left) and index stations (right) from the seventeen waterbodies in the summer 2020 regional survey. Dashed horizontal line denotes the Redfield ratio (7.22), which is the ratio commonly used to infer N and P balance. The labels on the x-axis indicate each lake visited, with shore stations indicated by an SH and index stations indicated by IX. Location are Castaic Lagoon (CAST_LAG), Stone Canyon Reservoir (STON), Echo Park Lake (ECHO), Lake Casitas (CASI), Pyramid Lake (PYRA), Castaic Lake (CAST_LAK), Lake Piru (PIRU), El Dorado Lakes-North Lake (EDL1), Lincoln Park Lake (LINC), El Dorado Lakes-Main Lake (EDL2), Puddingstone Reservoir (PUDD), El Dorado Lakes-Horseshoe Lake (EDL3), El Dorado Lakes-Duck Pond (EDL4), Lake Balboa (BALB), Legg Lake (LEGG), Machado Lake (MACH), and Malibou Lake (MALI).

Table A1. Microcystin (MC), Cylindrospermopsin (CYL), and Anatoxin-a (ATX) concentrations from discrete water samples from the summer 2020 regional survey. Samples were analyzed via ELISA and results are reported in µg/L. Concentrations exceeding California's Tier I Caution Health Threshold (0.8 µg/L for MC, 1.0 µg/L for CYL, detection at 0.15 µg/L for ATX) are in bold type.

Waterbody	Samples (N)	MC	CYL	ATX
El Dorado Lakes - Horseshoe	2	bd	bd	bd
El Dorado Lakes - Duck Pond	2	0.49-3.20	bd	bd
El Dorado Lakes - North	2	Bd	bd	bd
Lincoln Lake	2	1.51-2.10	bd	bd
Echo Lake	2	Bd	bd	bd
El Dorado Lakes - Main	2	Bd	bd	bd
Legg Lake - North	4	0.46-1.52	bd	bd
Lake Balboa	4	Bd	bd	bd
Malibou Lake	4	bd-1.70	bd	bd
Machado Lake	4	Bd	bd	0.19-0.26
Stone Canyon Reservoir	2	bd	bd	bd
Castaic Lagoon	4	bd	bd	bd
Puddingstone Reservoir	4	bd	bd	bd
Lake Piru	6	bd	bd	bd
Pyramid Lake	4	bd-0.26	bd	bd
Lake Casitas	4	bd	0.11-0.19	bd
Castaic Lake	4	bd-0.67	bd	bd

Table A2. Concentrations of microcystin (MC) congeners from SPATT samples collected at twelve waterbodies in the summer 2020 regional survey. MC congeners were measured with LC-MS and numbers are reported in ng/g. Congeners analyzed and detected were MC-RR, -YR, -LR, and -LA. Congeners that were included in the analysis but not detected include MC-WR, MC-LF, MC-dmLR, MC-LY, Anatoxin-a, H-Anatoxin, Cylindrospermopsin, and Nodularin-R.

Waterbody	MC-RR	MC-YR	MC-LR	MC-LA	Total MC
Castaic Lake	13.2	1.7	59.0	71.6	145.5
Pyramid Lake	108.2	bd	5.0	bd	113.1
Castaic Lagoon	9.1	9.7	51.8	30.5	101.0
Lincoln Lake	1.4	bd	bd	5.6	7.1
Lake Balboa	bd	bd	bd	4.0	4.0
Malibou Lake	3.1	bd	bd	bd	3.1
Stone Canyon Reservoir	bd	bd	bd	1.0	1.0
Echo Lake	bd	bd	bd	bd	bd
Machado Lake	bd	bd	bd	bd	bd
Puddingstone Reservoir	bd	bd	bd	bd	bd
Lake Piru	bd	bd	bd	bd	bd
Lake Casitas	bd	bd	bd	bd	bd

Table A3. Microcystin (MC), Cylindrospermopsin (CYL), and Anatoxin-a (ATX) concentrations at Machado Lake (Machado), Legg Lake (Legg), and Piru Lake (Piru) in the summer 2021 routine monitoring. Samples were analyzed via ELISA and results are reported in µg/L. Concentrations exceeding California's Tier I Caution Health Threshold (0.8 µg/L for MC, 1.0 µg/L for CYL, detection at 0.15 µg/L for ATX) are in bold type.

Waterbody	Visit	Date	MCY (SH)	MCY (IX)	ATX (SH)	ATX (IX)	CYL (SH)	CYL (IX)
Legg	1	6/16/2021	bd	bd	bd	bd	bd	bd
Legg	2	7/1/2021	bd	bd	bd	bd	bd	bd
Legg	2	7/14/2021	bd	bd	bd	bd	bd	bd
Legg	4	7/28/2021	bd	0.16	bd	bd	bd	bd
Legg	5	8/11/2021	bd	bd	bd	bd	bd	bd
Legg	6	8/25/2021	bd	bd	bd	bd	bd	bd
Legg	7	9/9/2021	bd	bd	bd	bd	bd	bd
Machado	1	6/14/2021	bd	bd	bd	bd	bd	bd
Machado	2	6/28/2021	bd	bd	bd	bd	bd	bd
Machado	2	7/12/2021	bd	bd	bd	bd	bd	bd
Machado	4	7/26/2021	0.26	0.22	bd	bd	bd	bd
Machado	5	8/9/2021	**2.96**	0.3	bd	**0.19**	bd	bd
Machado	6	8/23/2021	**1.24**	0.38	bd	bd	bd	bd
Machado	7	9/7/2021	0.29	0.22	bd	bd	bd	bd
Piru	1	6/15/2021	bd	bd	bd	bd	bd	bd
Piru	2	6/29/2021	bd	bd	bd	bd	bd	bd
Piru	2	7/13/2021	bd	bd	bd	bd	bd	bd
Piru	4	7/27/2021	bd	bd	bd	bd	bd	bd
Piru	5	8/10/2021	bd	bd	bd	bd	bd	bd
Piru	6	8/24/2021	bd	bd	bd	bd	bd	bd
Piru	7	9/8/2021	bd	bd	bd	bd	bd	Bd

Table A4. Concentrations of microcystin (MC) congeners from SPATT samples collected at Legg Lake, Machado Lake, and Lake Piru in the summer 2021 routine monitoring. Samples were analyzed via LC-MS and numbers are reported in ng/g. Congeners analyzed and detected were Microcystin-RR, -LR, -LA, and -WR. Congeners that were included in the analysis but not detected include MC-YR, MC-LF, MC-dmLR, MC-LY, Anatoxin-a, H-Anatoxin, Cylindrospermopsin, and Nodularin-R.

Waterbody	Deployment Date	Recovery Date	MC-RR	MC -LR	MC-LA	MC-WR	Total-MC
Legg Lake	6/16/2021	7/1/2021	2.5	bd	16.5	bd	19.0
Legg Lake	7/1/2021	7/14/2021	9.5	5.4	15.8	bd	30.7
Legg Lake	7/14/2021	7/28/2021	9.1	bd	19.9	bd	29.0
Legg Lake	7/28/2021	8/11/2021	3.8	bd	bd	bd	3.8
Legg Lake	8/11/2021	8/25/2021	1.8	bd	9.7	bd	11.5
Legg Lake	8/25/2021	9/9/2021	0.9	bd	4.8	bd	5.7
Machado Lake	6/14/2021	6/28/2021	bd	bd	bd	bd	bd
Machado Lake	6/28/2021	7/12/2021	bd	bd	bd	bd	bd
Machado Lake	7/12/2021	7/26/2021	bd	bd	bd	3.8	3.8
Machado Lake	7/26/2021	8/9/2021	1.0	bd	bd	bd	1.0
Machado Lake	8/9/2021	8/23/2021	1.9	bd	bd	bd	1.9
Machado Lake	8/23/2021	9/7/2021	2.4	bd	bd	bd	2.4
Lake Piru	6/15/2021	6/29/2021	bd	bd	2.0	bd	2.0
Lake Piru	6/29/2021	7/13/2021	bd	bd	2.6	bd	2.6
Lake Piru	7/13/2021	7/27/2021	bd	bd	bd	bd	bd

Waterbody	Deployment Date	Recovery Date	MC-RR	MC -LR	MC-LA	MC-WR	Total-MC
Lake Piru	7/27/2021	8/10/2021	bd	bd	bd	bd	bd
Lake Piru	8/10/2021	8/24/2021	bd	bd	5.5	bd	5.5
Lake Piru	8/24/2021	9/8/2021	bd	bd	4.5	bd	4.5

Table A5. The number of cyanobacterial orders, families, and genera identified in Machado Lake, Legg Lake, and Piru Lake in the summer 2021 routine monitoring.

Waterbody	Number of Orders	Number of Families	Number of Genera
Machado Lake	5	13	20
Legg Lake	5	15	20
Piru Lake	4	13	24

Table A6. Indicator species analysis comparing hypereutrophic lakes (as indicated by either chlorophyll-a, TN, and TP concentrations) and other lakes with lower trophic status ('Other') in the summer 2020 regional survey. Taxa significantly associated with samples from Hypereutrophic or Other lakes are listed. Only samples from the ten lakes with index stations were included in the analysis. Genera with an asterisk (*) indicate potential cyanotoxin producers. The number of amplicon sequence variants (ASVs) associated with each genus are shown. The indicator value index is a measure of the association between a given ASV and eutrophication with an index value of 1 indicating a high level of association.

Eutrophication Status	Genus	Number of ASVs	Maximum Indicator Value Index Statistic	Minimum Indicator Value Index Statistic
Hypereutrophic	Unassigned	40	0.84	0.42
Hypereutrophic	Romeria	2	0.587	0.42
Hypereutrophic	Dolichospermum*	4	0.485	0.42
Hypereutrophic	Microcystis*	5	0.485	0.485
Hypereutrophic	Nodosilinea	1	0.485	0.485
Hypereutrophic	Sphaerospermopsis*	3	0.485	0.485
Other	Unassigned	81	1	0.49
Other	Synechococcus*	25	0.957	0.49
Other	Aphanizomenon*	4	0.693	0.663
Other	Chrysosporum*	3	0.663	0.49
Other	Iningainema*	5	0.625	0.529
Other	Anabaena*	2	0.529	0.49
Other	Cuspidothrix*	2	0.529	0.529
Other	Dolichospermum*	2	0.529	0.529
Other	Pseudanabaena*	1	0.529	0.529
Other	Sphaerospermopsis*	1	0.49	0.49
Other	Synechocystis*	1	0.49	0.49

Table A7. Indicator species analysis comparing the samples with detectable anatoxin-a (ATX), cylindrospermopsin (CYL), and microcystin (MCY) in the summer 2020 regional survey. The genera significantly associated with samples containing ATX, CYL, and MCY are listed. Samples from all seventeen lakes the regional survey were included in the analysis. The number of amplicon sequence variants (ASVs) associated with each genus are shown. The indicator value index is a measure of the association between a given ASV and presence of a given toxin with an index value of 1 indicating a high level of association.

Focal Toxin	Genus	Number of ASVs	Maximum Indicator Value Index Statistic	Minimum Indicator Value Index Statistic	Focal toxin produced by genus
ATX	Sphaerospermopsis	8	1	0.707	No
ATX	Unassigned	27	1	0.483	Not applicable
ATX	Anabaenopsis	4	0.972	0.926	No
ATX	Romeria	1	0.765	0.765	No
ATX	Limnothrix	3	0.645	0.63	No
CYL	Unassigned	62	0.857	0.605	Not applicable
CYL	Synechococcus	13	0.844	0.667	No
CYL	Pseudanabaena	1	0.765	0.765	No
CYL	Chrysosporum	3	0.753	0.632	Yes
CYL	Anabaena	1	0.621	0.621	Yes
MCY	Microcystis	6	0.913	0.632	Yes
MCY	Unassigned	6	0.749	0.501	Not applicable
MCY	Pseudanabaena	2	0.516	0.516	Yes