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THE PREVALENCE OF CYANOTOXINS IN SOUTHERN CALIFORNIA WATERBODIES BASED ON SCREENING ASSESSMENTS AND REGIONAL MONITORING PROGRAMS

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EXECUTIVE SUMMARY

Harmful cyanobacteria blooms (cyanoHABs) have gained national attention in recent years due to the global increase in frequency of blooms. CyanoHABs cause a large number of water quality issues such as impairment of recreational uses, reduced aesthetics, lower dissolved oxygen concentrations, and taste and odor problems in drinking water, however, the production of toxins (called cyanotoxins), is the most concerning. Cyanotoxins cause illness and mortality in humans, domestic pets, wildlife and livestock. As such, there is a growing recognition that water quality programs should include these biological contaminants and cyanotoxins should be considered in ecological and human health risk assessments (Chapman, 2015, Brooks et al., 2016). Cyanotoxins have been listed as the highest priority analytes for inclusion in ambient monitoring programs by both USGS and EPA. Health advisory thresholds have also been developed by EPA for drinking water and by the State of California for recreational exposures. Despite the growing recognition of this risk, the extent and magnitude of cyanoHABs and cyanotoxin prevalence is poorly characterized in California, particularly in the heavily populated area of Southern California. The purpose of this report is to summarize the prevalence and extent of cyanotoxins, and in some areas, cyanobacteria, across a wide variety of aquatic habitats. The report consists of three main chapters: Chapter 1 provides an introduction and background on cyanoHABs; Chapter 2 summarizes the wadeable streams statewide assessment for cyanobacteria taxa and cyanotoxins; and Chapter 3 focuses on the extent of cyanotoxins in lentic waterbodies in Southern California, and several intensive studies conducted in San Diego.

All of the sites surveyed for cyanotoxins are shown in Figure 1. Microcystins, one type of cyanotoxin, were detectable and present in all of the waterbody types surveyed and across all land use types. Where possible, the toxin concentrations were color-coded to visualize if they were above or below the California recreational health advisory thresholds (0.8 µg L^{-1}, OEHHA 2012). Sample types for which there is no applicable health advisory threshold are shown in yellow and comprise wadeable stream samples of benthic algae and passive sampling devices (Solid Phase Adsorption Toxin Tracking, SPATT). SPATT was used as a screening assessment tool that provided insight into the overall cyanotoxin prevalence in the waterbodies.

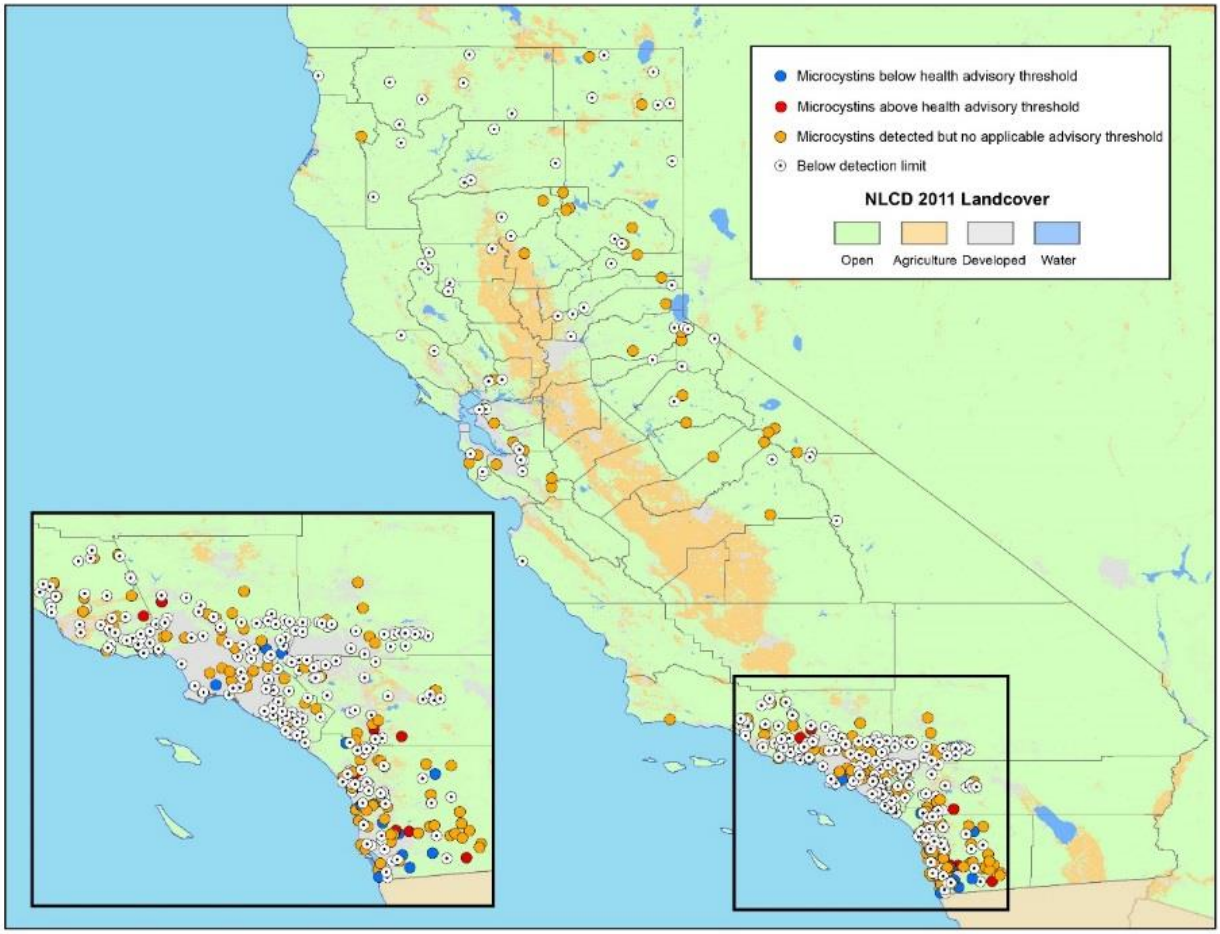

Figure 1. Map showing all of the sites surveyed for microcystins. The colors correspond to the concentration of microcystins detected in relation to the California health advisory threshold. Red circles exceed the threshold, blue circles are within the threshold, yellow circles are data for which there is no applicable threshold (either benthic algal samples or passive samplers), and white circles with a dot in the center were below the method detection limit.

Surveys of > 1,200 wadeable stream segments were conducted throughout California during the spring and summer of 2007 through 2013, revealing a high occurrence of potentially toxigenic benthic cyanobacteria. Benthic microcystins were detected in one-third of the sites, based primarily on one-time sampling, from 2011 to 2013. Sites where microcystins were detected spanned a variety of surrounding land-use types, from open space (i.e., undeveloped land) to heavily urbanized/agricultural. Other cyanotoxins, such as lyngbyatoxin, saxitoxins, and anatoxin-a, were also measured at subsets of sites, and were detected, albeit at lower rates than microcystins. Results of this study provide strong evidence that wadeable streams could be significant sources of cyanotoxin inputs to receiving waters, a finding that has implications for the management of drinking water, wildlife, and recreational resources, within both the streams themselves and in downstream rivers, lentic water bodies, and the ocean.

Microcystins were prevalent in all types of lentic waterbodies surveyed (depressional wetlands, lakes, reservoirs, estuaries and coastal lagoons) across the land-sea continuum in Southern California. The toxin concentrations were generally low across most lentic habitats, as only a small number of sites exceeded

recreational health thresholds for acute toxicity. However, SPATT results indicated that microcystins were prevalent throughout lentic waterbodies and that traditional grab samples underestimated the presence of microcystins. The persistence of detectable microcystins across years and seasons underscores the likelihood of a low-level, chronic risk through both direct exposure (drinking water and recreation) and indirect exposure via bioaccumulation of toxin to higher trophic levels in the food web. In contrast to these surveys, the San Diego bloom response survey of targeted lakes and reservoirs, detected high acute toxin concentrations that have implications for human, wildlife and domestic pet health and indicated a high level chronic risk.

Multiple cyanotoxins were detected simultaneously in some systems, indicating multiple stressors, the risk of which is uncertain because health thresholds are based on exposures to single toxins. Additionally, this study documented the first detection of cylindrospermopsin and anatoxin-a in Southern California waterbodies. A wide variety of potential toxin-producing cyanobacteria were identified, indicating a poor understanding of the HAB community dominating these waterbodies.

Based on the results of these assessment and monitoring programs are the following recommendations:

1) **Cyanotoxins should be included in ambient water quality monitoring and assessment programs.** Both USGS and EPA have recommended cyanotoxins to be high priority analytes in national water quality programs. Due to the dominance of cyanobacteria and the ubiquitous and persistent detection of microcystins in these heavily utilized aquatic habitats, more frequent monitoring should be considered to quantify this risk to health and beneficial uses.

    a. The sampling approach to characterize the risk of cyanotoxins should be carefully considered as the results from this study show that traditional contaminant assessment approaches consisting of one-time grab samples will underestimate toxin prevalence and will not provide insight on the persistence of cyanotoxins. Care should be taken to distinguish whether the objective is to characterize human versus ecosystem health risk to cyanotoxins, as the method of sampling differs, depending on the focus. Monitoring to assess human health risk should be more frequent than traditional chemical contaminant sampling since cyanotoxins are a biological contaminant, and therefore, the detection and concentrations can fluctuate on the order of days. Beach water quality monitoring programs aimed at protecting human health are a more appropriate approach for cyanotoxins.

2) **Develop capacity to analyze, interpret and use passive sampling technologies in cyanotoxin monitoring.** Monitoring and assessment tools such as passive samplers (i.e. SPATT) were successfully used to determine prevalence of microcystins and should be incorporated to future ambient monitoring and assessment programs. These sampling devices are particularly useful at capturing ephemeral events that traditional grab samples do not capture, and provide a more comprehensive view of cyanotoxins in a waterbody or region. Currently, most analytical laboratories in California do not provide analytical services for SPATT samplers, despite the published methodology and similar instrumentation used for grab sample analysis (Lane et al. 2010, Kudela 2011). A technology training for State, County and Local laboratories is needed in order to accommodate the growing use of SPATT sampling throughout California.

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CHAPTER 1: INTRODUCTION

Cyanobacteria are photosynthetic prokaryotes that have existed naturally for billions of years (Summons et al. 1999, Schopf 2000), inhabiting a wide variety of aquatic environments, including freshwater, brackish and marine ecosystems and can form dense blooms (Paerl 1988, Paerl and Fulton 2006, Whitton 2012). Many cyanobacteria are capable of producing toxins, referred to as cyanotoxins, which have gained national attention in recent years due to the global increase in frequency and duration of toxic blooms (Carmichael 2008, Hudnell and Dortch 2008, Paerl and Huisman 2009, O'Neil et al. 2012, Paerl and Paul 2012, Paerl and Otten 2013, Quiblier et al. 2013, Hudon et al. 2014, Wood et al. 2014). These increases have been attributed to a wide variety of environmental factors such as nutrient overenrichment and eutrophication, increased temperature, salinity, water residence time, vertical stratification and pH, many of which will likely be exacerbated with climate change (Paerl 1988, Paerl 1996, Paerl and Fulton 2006, Carmichael 2008, Paerl and Huisman 2009, Paerl et al. 2011, O'Neil et al. 2012, Paerl and Paul 2012, Paerl and Otten 2013).

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

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

At both the federal level and within California, there is a growing recognition of the health and beneficial use risk of cyanotoxins. Due to this growing recognition that HABs can severely impact water quality and

should be routinely monitored (Chapman 2015, Brooks et al. 2016), the U.S. Environmental Protection Agency has recently released health advisory thresholds for cyanotoxins in drinking water and has listed three cyanotoxins on the Contaminant Candidate List 3 (CCL3) (U.S. Environmental Protection Agency 2010). USGS has recently prioritized 12 cyanotoxins as Tier 1, or highest priority for inclusion in ambient water monitoring in the US (an additional 3 were listed at intermediate or low priority) (Olsen et al. 2013). California established health-based exposure thresholds to protect human and canine health in recreational waterbodies for three cyanotoxins (see Table 1-1), (OEHHA 2012). These thresholds comprise water, fish, crust and mats, however, no thresholds have been established for benthic algal samples (Chapter 2) or for passive sampling devices such as SPATT (Chapter 3). These recreational health advisory thresholds were created in response to recurring hotspots of toxic blooms in the Klamath River watershed, Clear Lake, Pinto Lake, Sacramento and San Joaquin River Delta, Lake Elsinore, and East San Francisco Bay Area lakes. Additionally, Copco and Iron Gate Reservoirs, the Klamath River, and Pinto Lake were placed on the State's 303d list due to impairment caused by cyanotoxins, the first such listing in the State.

Despite the recognition of this growing threat, understanding of the extent and magnitude of the cyanoHAB threat in California is poorly characterized, particularly in heavily populated regions such as Southern California. One previous study documented the prevalence of *Microcystis spp*., a known producer of microcystins, in lentic waterbodies in California (Magrann et al. 2015). The purpose of this report is to summarize the extent and magnitude of cyanotoxins across a variety of aquatic habitats found across the landscape in California, from streams (statewide, Chapter 2), to lakes, depressional wetlands and coastal lagoons, as documented in several different studies in Southern California (Chapter 3).

Table 1-1. OEHHA Action Thresholds for cyanotoxins in California (from OEHHA, 2012)

	Microcystins (LA, LR, RR, and YR)	Anatoxin-a	Cylindro-spermopsin	Media (units)
Human recreational uses[1]	0.8	90	4	Water (µg/L)
Human fish consumption	10	5000	70	Fish (ng/g) ww[2]
Subchronic water intake (dog)[3]	2	100	10	Water (µg/L)
Subchronic crust and mat intake (dog)	0.01	0.3	0.04	Crusts and Mats (mg/kg) dw[4]
Acute water intake (dog)[5]	100	100	200	Water (µg/L)
Acute crust and mat intake (dog)	0.5	0.3	0.5	Crusts and Mats (mg/kg) dw[4]
Subchronic water intake (cattle)[6]	0.9	40	5	Water (µg/L)
Subchronic crust and mat intake (cattle)[6]	0.1	3	0.4	Crusts and Mats (mg/kg) dw[4]
Acute water intake (cattle)[6]	50	40	60	Water (µg/L)

[1]The most highly exposed of all the recreational users were 7- to 10-year-old swimmers. Boaters and water-skiers are less exposed and therefore protected by these action levels. This level should not be used to judge acceptability of drinking water concentrations.

[2]Wet weight (ww) or fresh weight

[3]Subchronic refers to exposure over multiple days

[4]Based on sample dry weight

[5]Acute refers to exposures in a single day

[6]Based on small breed dairy cows because their potential exposure to cyanotoxins is greatest

CHAPTER 2: WADEABLE STREAMS

Despite the fact that cyanobacteria are known to inhabit streams (Ward et al. 1985, Becker 1990, Dudley and D'Antonio 1991), little has been published regarding their potential for cyanotoxin production. Some exceptions include investigations in Spain (Aboal et al. 2002, 2005), which revealed microcystins in algal mats growing in shallow streams within calcareous catchments. Various studies on cyanobacterial mats (e.g., *Phormidium*) and the toxins they produce have also been conducted in New Zealand rivers (Heath et al. 2010, Harland et al. 2013). However, little work has been published for North American streams, particularly those that are "wadeable," which are defined in this study as stream segments that can be sampled by field crews wearing chest waders (i.e., estimated as measuring < 1 meter at its deepest). An important distinction of wadeable streams relative to other fresh water body types is that while the algal communities of lakes, ponds, lagoons, and large rivers are often dominated by phytoplankton, the major component of algal biomass in streams is typically benthic (Bellinger and Sigee 2010). These communities can occur as microalgae within the "biofilm" coating on stream substrata. They also comprise macroalgae that are attached to stream substrata or that have detached and floated to the water surface, as well as filamentous forms loosely entrained in aquatic vegetation or occurring as diffuse masses in slow-moving water. All of these communities may contain species that produce cyanotoxins. However, prior to this study, no characterization of the toxin concentrations associated with benthic cyanobacteria has ever been characterized in California wadeable streams.

This study sought to answer two key questions: 1) How abundant are potentially toxigenic benthic cyanobacteria in California wadeable streams? and 2) Are anthropogenic factors likely to influence the prevalence of these cyanobacteria, and/or cyanotoxin concentrations, in these systems? To begin addressing these questions, we present the geospatial distribution of potentially toxigenic benthic cyanobacteria based on samples composited across 150-m-long stream segments that span a variety of surrounding land-use types throughout California. In addition, the concentrations and frequency of detection of multiple cyanotoxins is reported, with an emphasis on microcystins. However, these concentrations cannot be put into context of health risk because there are no cyanotoxin health advisory thresholds established for benthic algal samples. To our knowledge, this is the first large-scale study to examine cyanotoxin concentrations in the wadeable stream benthic environment, accompanied by information on species-level cyanobacterial community composition.

Methods

Study area

California's stream network is approximately 280,000 km long and drains a large (424,000 km^2), diverse landscape. There are temperate rainforests in the northwest and deserts in the northeast and southeast, but the majority of the state has a semi-arid, Mediterranean climate (Omernik1987). California's geology is complex, with recently uplifted and poorly consolidated marine sediments in the Coast Ranges, alluvium in its broad internal valleys, granitic batholiths along the eastern border, and recent volcanic lithology in the northern mountains. The native landscapes of some regions of the state have been nearly completely converted to agricultural or urban land uses (e.g., the Central Valley, the San Francisco Bay area, and the South Coast,) (Sleeter et al. 2011).

Sampling scope and site selection

Algal community composition samples were collected via stream monitoring surveys during the spring-summer of 2007 to 2013, and cyanotoxin samples were collected from 2011 to 2013. The target

population for the surveys was perennial and non-perennial wadeable streams in California. The grand mean of depths across the sites sampled in the surveys was 12 cm (median = 10). The grand mean of wetted widths (i.e., the distance between the sides of the channel at the point where stream substrata are no longer surrounded by surface water) was 5.7 m (median = 4.1).

For the community composition data, 1,565 sampling events occurred at 1,279 unique sites (see maps in Results). For the toxin data, which largely correspond to a subset of the sites with community-composition data, 413 samples were analyzed for total microcystins across 368 stream sites. A subset of these were also analyzed for a select group of other cyanotoxins, including saxitoxins, anatoxin-a, lyngbyatoxin, nodularin, and cylindrospermopsin.

The majority of sampling sites were selected "probabilistically" (Stevens and Olsen 2004), such that results (e.g., microcystin concentrations) from the surveys could be extrapolated to statewide estimates. The probability surveys were designed according to the methods described in Stevens and Olsen (2004), using the "SPSurvey" package (Kincaid and Olsen 2008) in the R language and environment for statistical computing (version 2.15.1, R Core Team 2012). SPSurvey employs an objective sampling-site-selection technique called "Generalized Random Tessellation Stratified" (GRTS, Stevens and Olsen 2004). The GRTS procedure results in a list of randomly selected, spatially balanced sampling sites, such that the resulting dataset can be used to generate regional condition estimates (e.g., in terms of microcystin concentrations) with known confidence limits.

Sample and data collection

A "multi-habitat method" method (Fetscher et al. 2009) was employed to identify and quantify benthic algae from 150-m-long stream segments (hereafter referred to as sampling "sites"). "Composite" samples were collected by isolating benthic specimens from a known surface area over a variety of stream substrata in proportion to their relative abundances in the stream and combining them. A fresh, "qualitative" sample was also collected by gathering an intact sample of all macroalgal types observed within the sampling site. By providing intact, unfixed specimens, the qualitative samples 1) aided laboratory identification of specimens in the quantitative sample that may have been fragmented in the course of collection (Fetscher et al. 2009, Stancheva et al. 2012), 2) were used, as needed, for isolation and culturing of specimens of interest, and 3) facilitated an assessment of overall macroalgal diversity in the stream segment sampled.

In addition to collecting benthic algae, percent areal cover of microalgae was recorded according to the methods of Fetscher et al. (2009). Microalgal cover was assessed based on the presence/thickness of the often slimy biofilm coating on stream substrata, the abundance of which was recorded by binning the coating into thickness categories (including zero thickness, for apparent absence of a biofilm) at 5 objectively determined points along each of 11 equidistantly distributed transects down the length of the stream segment (for a total of 105 points).

Taxonomic analyses of stream cyanobacterial samples

Macroalgae were processed separately from the microscopic algal fraction of each composite sample to allow proper qualitative and quantitative identification and enumeration of the soft-bodied (i.e., non-diatom) algae in the sample, including cyanobacteria (Stancheva et al. 2012). To distinguish between fractions, Sheath and Cole's (1992) definition of "macroalgae" was adopted. Specifically, "macroalgae" include large (macroscopic) specimens that are filamentous, colonial, tuft-forming, crustose, tissue-like, or coenocytic algae and cyanobacteria that have forms recognizable with the naked eye. Example genera

include *Nostoc*, *Rivularia*, *Cladophora*, *Oedogonium*, *Rhizoclonium*, *Batrachospermum*, *Lemanea*, *Spirogyra*, *Zygnema*, *Mougeotia*, and *Vaucheria*. See Sheath and Cole (1992) for definitions of forms.

Rather than homogenizing the entire original sample and using counting chambers (Lowe and Laliberte 1996, Stevenson and Bahls 1999), both algal fractions were processed separately to allow identification to the lowest possible taxonomic level (generally species), which was possible due to the high-quality preservation of macroalgal vegetative and reproductive structures, and the even distribution of microalgae on a standard microscope slide. Biovolumes measured in the laboratory were transformed into volume per area of stream bottom sampled ($\mu m^3 cm^{-2}$).

In addition to collecting the biovolume information for each recorded specimen, up to 100 epiphytes on the macroalgae were enumerated, and taxa present in the qualitative sample recorded. Identifications were based on the cyanobacteria taxonomic concept and nomenclature of Komárek and Anagnostidis (1999, 2005) and Komárek (2013). Refer to Appendix A for a more in-depth discussion of taxonomic standards employed in this analysis.

Laboratory analysis of chlorophyll *a* samples

To estimate the amount of algal biomass in sampling sites, aliquots were drawn from the composite sample, filtered on to Whatman™ GF/F (i.e., glass-fiber) filters, which have a nominal pore size of 0.7 µm, stored frozen (-20°C), and analyzed for chlorophyll *a* content using EPA method 445.0. Chlorophyll *a* concentrations measured in the laboratory were transformed into mass per area of stream bottom sampled ($mg\ m^{-2}$).

Laboratory analyses of cyanotoxin samples

At a subset of sites (19 in 2011, 98 in 2012 and 251 in 2013), aliquots were also drawn from the composite samples for the determination of cell-bound cyanotoxin concentrations. A known volume of composite sample was filtered on to Whatman™ GF/F filters and stored frozen (-20°C) until analysis. Samples collected in 2011 were analyzed for four microcystin congeners (MCY-LA, MCY -LR, MCY -RR, MCY -YR) and anatoxin-a by liquid chromatography–mass spectrometry (LC-MS). Those collected in 2012 were analyzed for the same four congeners either by LC-MS (to yield results for each congener separately), or (for the four in aggregate) by enzyme-linked immunosorbent assay (ELISA). In addition, a subset of the samples was analyzed for saxitoxins (by ELISA) and/or for lyngbyatoxin, anatoxin-a, cylindrospermopsin, and nodularin (by LC-MS). All 2013 samples were analyzed for the four microcystin congeners by ELISA. Note that, hereafter, "total microcystins" refers to the combined values for the four microcystin congeners listed above, whether they are the ELISA results or summed results from the LC-MS analyses.

Microcystins were analyzed by ELISA using the Envirologix QuantiPlate™ kit (Envirologix, Portland, ME, Cat. No. EP 022, as described in Kudela 2011). The BIOO Scientific MaxSignal™ Saxitoxin (PSP) test kit (BIOO Scientific Corp., Austin, TX, Cat. No. 1034) was used for saxitoxin analysis. Prior to analysis, the sample-containing filters were extracted in 3 mL of Milli-Q™ water, sonicated for 30 seconds to ensure cell disruption, and centrifuged for 10 min at 2,147 g (as described in Seubert et al. 2014). The extract was then analyzed according to the manufacturer's instructions for both toxins.

For the samples analyzed by LC-MS, electrospray ionization (ESI) with selected ion monitoring (SIM) on an Agilent 6130 Phenomenex Kinetix™ C18 column was employed. This 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).

Generating regional estimates of the prevalence of toxigenic taxa and microcystin production

To estimate the frequency of occurrence of potentially toxigenic cyanobacterial taxa and levels of microcystin production across California streams, data from the probability-survey sampling sites were used to generate descriptive statistics for data distributions and cumulative distribution functions (Kincaid and Olsen 2008). A cumulative distribution function depicts the estimated probability distribution of a given measured value (e.g., benthic microcystin concentrations) relative to the cumulative proportion of the geographic unit of interest (e.g., percent of stream kilometers in the state). Estimates were calculated using the Horvitz-Thompson estimator (1952), which is a weighted average of sample values, where weights are adjusted according to the spatial relationship among sites. Confidence intervals were based on local neighborhood variance estimators (Stevens and Olsen 2003), which assumes that samples located close together tend to be more alike than samples that are far apart. Estimates were generated using the "SPSurvey" package (Kincaid and Olsen 2008) in R. All graphics presented here were prepared with the R package, "ggplot2" (Wickham 2009).

Exploring land-use relationships

Potential anthropogenic influences on 1) the distribution of toxigenic taxa and 2) levels of benthic microcystin production were explored by looking at relationships with the proportion of coarse-resolution land-use types ("agricultural" vs. "developed" vs. undeveloped "open space") within three buffers of varying radii (10 km, 5 km, and 500 m) centered on each sampling site. Note that in this context, "open space" does not necessarily connote "pristine." In addition, Mantel tests (Mantel 1967) were used to assess spatial autocorrelation of results among sites. This information is useful for generating hypotheses about potential anthropogenic vs. natural drivers of benthic microcystin production.

In preparation for analysis, microcystin concentration data were log-transformed and latitude/longitude were converted to Equidistant, Cylindric Map Projection coordinates using the "SPSurvey" package (Kincaid and Olsen 2008) in R. Euclidean distance matrices were then calculated for each variable using the "ecodist" package (Goslee and Urban 2007) and Mantel tests were performed using the "ade4" package (Dray and Dufour 2007).

Identifying candidate microcystin producers in California streams

Indicator species analysis (Dufrêne and Legendre 1997) was used to inform inferences about potential microcystin-producing taxa in the sampling sites. The analysis was carried out using PC-ORD v6 software (McCune and Grace 2002) on genus presence-absence data. Significance levels for each genus' group membership assignment were generated via Monte Carlo methods.

Results

Geospatial distribution of toxigenic cyanobacteria

Twenty-two cyanobacterial genera reported in the literature as possessing toxin-producing members (hereafter referred to as "toxigenic genera"), including 19 species known to possess toxin-producing strains (i.e., "toxigenic species"), were observed across the sampling sites during spring-summer monitoring surveys conducted from 2007 to 2013 (Table 2-1). *Leptolyngbya, Phormidium, Nostoc,* and *Anabaena* were the most frequently encountered toxigenic genera, and tended to exhibit some of the highest estimated biovolumes (among the toxigenic taxa) across sites (Table 2-2). Furthermore, three of

the six toxigenic species with the highest "prevalence index" (i.e., the product of the number of sites where the species was observed and its mean biovolume across sites) were *Nostoc* species (Appendix A).

Ninety-three percent of sites supported one or more toxigenic taxa during at least one sampling event. Of these, 25% supported species-level taxa known to be capable of cyanotoxin production. The subset of toxigenic taxa that are specifically microcystin-producers were nearly as common: Ninety-two percent of sites supported toxigenic genera, and of these, 16% supported toxigenic species. Toxigenic taxa were found throughout the state (Figure 2-1). Most sites where such taxa were recorded supported one or two such genera, but some had as many as eight in a single sampling event. No spatial autocorrelation in the distribution of toxigenic taxa was evident: Mantel's r was 0.017 ($p = 0.18$) for cyanotoxin-producing taxa in general, and 0.020 ($p = 0.12$) for microcystin producers.

Based on the probability surveys, 90% of stream kilometers statewide are estimated to support toxigenic (Table 2-1) genera (with a 95% confidence interval of 81 - 99%), and 23% are estimated to support toxigenic species (confidence interval: 9 - 36%). Fourteen percent of stream kilometers are estimated to support microcystin-producing species (confidence interval: 0 - 27%).

Patterns of cyanotoxin detection

Of the 368 sites sampled for total-microcystins analysis, 33% tested positive during at least one sampling event. Overall, the distribution of microcystin concentrations in stream benthos was highly skewed toward the low end, with the median falling below detection limits, the 75th percentile at 7 μg m^{-2}, and a mean of 46 μg m^{-2}. Although uncommon, high concentrations were observed in a few sites, with the maximum exceeding the mean by >10-fold, at 4,767 μg m^{-2}. There are no health advisory thresholds established for benthic algal samples, therefore, the health risk associated with these microcystin concentrations cannot be assessed.

Samples from a subset of 35 stream sites were tested for four individual microcystin (MCY) congeners over the course of 2011-2012. The most commonly encountered was MCY–LA, which was detected at 43% of the sites and exhibited a maximum concentration of 75.4 μg m^{-2}. The next most common (MCY–LR) was detected at 37% of sites, with a maximum concentration of 2.5 μg m^{-2}, followed by MCY–YR and MCY–RR, both of which were detected at 6% of sites and had maximum concentrations of 3.1 and 2.5 μg m^{-2}, respectively.

Based on the probability surveys, the percentage of stream kilometers statewide that are estimated to harbor microcystins at some point during spring-summer is 34% (confidence interval: 7 - 60%). Based on the cumulative distribution function (Figure 2-2), approximately 30% of California wadeable stream kilometers are estimated to harbor > 25 μg m^{-2} of benthic microcystins, and approximately 5% harbor > 300 μg m^{-2}.

After microcystins, the class of cyanotoxin most frequently detected in stream benthos was lyngbyatoxin, which was present at 21% of sites (N=14 samples collected, maximum concentration = 7.2 μg m^{-2}). Saxitoxin and anatoxin-a were detected much less frequently, at 7% (N=99) and 3% (N=33) of sites, respectively. The maximum observed concentration was 0.2 μg m^{-2} for saxitoxin and 12.1 μg m^{-2} for anatoxin-a (representing the sole instance of detection of that toxin). Tests for cylindrospermopsin and nodularin were conducted for a total of 14 stream sites, but neither was detected.

No spatial bias to the location of microcystin detections was readily apparent. The only possible exception was northwestern California, where relatively few samples were positive for the toxin (Figure 2-3).

Particularly high concentrations were observed in montane streams, especially in the Sierra Nevada (the highest-concentration in the data set overall was detected in the Lake Tahoe Basin) and parts of Southern California. Weak spatial autocorrelation among sampling sites in terms of microcystin concentrations was evident (Mantel's $r = 0.08$, $p = 0.02$).

Relationship of microcystin concentrations to coarse-resolution anthropogenic influences

No significant relationships were observed between land use surrounding the sampling sites and the frequency of microcystin detection (Figure 2-4), and there was no evidence of spatial autocorrelation in the tendency to produce microcystins (Mantel's $r = 0.004$, $p = 0.39$). However, where present, higher concentrations of the toxin tended to be associated with sampling sites in an undeveloped, open-space (as opposed to urbanized/agricultural) setting, regardless of the radius of the buffer around the site (Figure 2-5).

The greatest concentrations of microcystins occurred in high-elevation streams with high microalgal cover at the time of sampling. The possibility that the high concentrations of toxin in these streams was simply a by-product of overall higher algal biomass was eliminated by regressing percent microalgal cover on chlorophyll *a* concentration, and plotting the residuals against elevation (Figure 2-6). Even with the effect of overall algal biomass removed, higher microalgal cover was still associated with higher microcystin concentrations, among the higher-elevation sites.

The possibility that high-elevation/high-microalgal-cover conditions select for cyanobacterial taxa that can produce high levels of microcystins was explored via indicator species analysis (Dufrêne and Legendre 1997). Several cyanobacterial genera, including *Nostoc* and *Phormidium*, were significantly associated with high-elevation/high-microalgal-cover stream sites (Table 2-3), thus providing further evidence that they could be microcystin producers in California.

Discussion

Prevalence and distribution of toxigenic taxa and cyanotoxins

Study results indicate that potentially toxigenic benthic cyanobacteria inhabit the majority of California wadeable streams, and are widely distributed throughout the state. Microcystins were commonly detected within the stream benthos (during the spring-summer time frame), and while the other cyanotoxins measured were not detected as frequently (and were not sampled as comprehensively), the potential for these (and additional) toxins is high based on the results of the community-composition analysis. These findings challenge the conventional wisdom that only lentic water bodies and large rivers are susceptible to cyanotoxin-related impacts, and indicate that the risk of cyanotoxin export to downstream ecosystems is greater than previously thought. The health risk associated with these toxin concentrations cannot be assessed because health advisory thresholds have not been established for benthic algal samples.

While data are not available to identify conclusively which species are producing toxins, results of the study suggest some particularly common benthic cyanobacterial genera have been shown elsewhere (reviewed by Quiblier et al. 2013) to include toxigenic species. For example, *Nostoc* and *Phormidium* were each recorded in > 1/3 of stream sites surveyed, and indicator species analysis suggested that these genera are significantly associated with the types of sites (based on high elevation and high microalgal cover) where microcystin production was greatest in the study's data set (Figure 2-6). Because *Nostoc* and mats of *Phormidium/Oscillatoria/Lyngbya* are relatively straightforward genera to identify macroscopically in the field, information on their tendency to produce toxins in streams could eventually

help managers determine whether a toxic event might be underway or poised to occur. However, future, more definitive, steps to identify toxin producers in California will need to involve isolating likely specimens from the field and analyzing axenic tissues (i.e., those free of other contaminating organisms) individually, rather than in aggregate (as was done with the samples for this study).

Potential impacts of cyanotoxins within streams

Cyanotoxins could exert a variety of impacts within the local stream environment, in ways that have ramifications for monitoring and management of stream health. For example, they could be the cause of at least some instances of positive results from laboratory toxicity assays conducted as a part of monitoring surveys. Support for this phenomenon comes from studies showing toxic effects of cyanotoxin-containing extracts on invertebrates, such as cladocerans (Sotero-Santos et al., 2006, 2008, Okumura et al., 2007), which are often used as test organisms in water-column toxicity assays. Further support for this possibility comes from the finding, in Southern California coastal watersheds, that sublethal toxicity (in terms of depressed reproduction) was more extensive in streams within open-space settings (33%) than those in agricultural (30%) or urban (19%) settings (Mazor et al., 2015). Because undeveloped catchments are less likely to harbor anthropogenically derived toxins, the likelihood of naturally occurring toxins (such as cyanotoxins) triggering positive bioassay results is worth examining.

Gaining a better understanding of cyanotoxin effects on macroinvertebrate communities in California streams could also prove useful for understanding otherwise unexplainable causes of low biomonitoring index scores, and for determining whether any impacts on these communities are the result of natural phenomena, or whether they are exacerbated by human activities, and potentially responsive to corrective management actions. Aboal et al. (2002) found adverse effects of microcystins on stream benthic macroinvertebrates, and suggested that cyanobacterial biomass and/or pigments be measured to provide a context for interpreting index scores.

Numerous studies have demonstrated accumulation and biomagnification of cyanotoxins in aquatic food webs: specifically, in freshwater and saltwater mussels (Williams et al. 1997, Amorim and Vasconcelos, 1999, Miller et al. 2010), farmed crustaceans (Vasconcelos et al. 2001, Zimba et al. 2006), corals (Richardson et al., 2007), fish (Malbrouk and Kestemont 2006), and crabs (Miller et al. 2010). Wood et al. (2012a) confirmed that benthic cyanotoxins can enter freshwater food webs by showing that nodularins had been incorporated into crayfish hepatopancreatic tissue after feeding on ^{13}C-labeled cyanobacterial mats in lake-based field experiments. The effects and propensity for bioaccumulation, of cyanotoxins in stream benthic macroinvertebrates have not been as well studied, and should be a focus of future research (Quiblier et al. 2013).

Potential for cyanotoxin loading to receiving waters

Coastal watersheds in many parts of California are mountainous, with a sizable proportion of waterways in the form of dense, low-order stream networks. The approximately 280,000 km of streams in California represents a potentially vast base for cyanotoxin production. Moreover, the general chemical stability of some cyanotoxins (Rapala et al. 1993, Jones et al. 1995, Tsuji et al. 1995, Twist and Codd 1997, Lahti 2001, Rapala et al. 2005) means that toxins produced in streams may not only have undesirable effects locally, but could also be exported, intact, to receiving waters, either in the form of pieces of cyanobacterial growths that detach from the benthos and are transported downstream, or in dissolved form released from lysed cells (Wood et al. 2011).

It will be important to understand the significance of cyanotoxin inputs from wadeable streams to drinking water reservoirs, recreational lakes, and coastal lagoons and estuaries that support wildlife. For example, are cyanotoxin concentrations in receiving waters meaningfully increased by contributions from tributaries? The answer to this question could influence whether it is deemed sufficient to rely on the appearance of *in situ* planktonic blooms for forecasting the likelihood of an impending toxic event, or whether additional monitoring of benthic cyanobacterial blooms in the contributing watershed are warranted. Future studies in streams should be directed toward determining 1) the typical concentrations (both cell-bound and dissolved) of toxins of benthic origin, 2) toxin fate and transport, 3) whether benthic blooms and toxin production have increased as a result of human activities, 4) the appropriate reference values to protect human and wildlife health, and 5) what concentrations may be of concern for listing purposes and other management actions (e.g., Wood and Williamson 2012).

Potential drivers of benthic cyanotoxin production in streams

Cyanotoxins were detected in California streams within both developed and undeveloped landscape settings, and concentrations were overall substantially higher in the latter. Thus it is difficult to ascertain, based on available data, whether any anthropogenic factors may promote toxic events in these systems, in contrast to the mounting evidence that human influences have exacerbated toxigenic planktonic blooms in lentic water bodies (Paerl and Huisman 2008, Paerl and Paul 2011, Paerl et al. 2011). It is possible that the patterns observed in the concentration of stream benthic microcystins could more be a function of what species are selected for by specific environments than site-specific drivers boosting cyanotoxin production *per se*. For example, *Nostoc*, a nitrogen fixer, is a likely candidate for producing microcystins in California streams, and it tends to flourish in oligotrophic, minimally disturbed systems (Stancheva et al. 2013, Fetscher et al. 2014). It may be that *Nostoc* is inherently a more prolific microcystin producer than other benthic cyanobacterial taxa (that inhabit other types of streams). This scenario could help explain the observation of greater microcystin concentrations in largely undeveloped catchments.

It is also worth noting that, if the high microalgal cover in certain sites is a symptom of low scour, the relationship between low scour and high microcystin production (Figure 2-6) suggests that some of the key taxa that produce microcystins may not be well adapted to high-flow conditions. Indeed, *Phormidium* is a benthic genus that has been observed to proliferate in lower-flow sites of rivers in New Zealand (Heath et al. 2011), presumably due to its weak physical connection with the streambed, thus reduced flows due to natural climate cycles, climate change, and/or hydromodification could potentially select for toxin-producing cyanobacteria in lotic water bodies. If there is a relationship with flow/scour, it should be explored, because climate change and future water management activities have the potential to alter streamflow patterns.

With respect to establishing a connection between human activities and cyanotoxin production in streams, an important consideration is that the toxin results presented here are based mostly on one-time grab samples during a restricted index period, which could miss toxic events due to the ephemeral and episodic nature of toxin production. This, in conjunction with the inherent patchiness of algae within stream benthos (Sheath et al., 1986), as well as the patchiness of toxin concentrations, even within individual cyanobacterial mats (Wood et al. 2010 2012b), suggests that the results presented may underestimate the true prevalence of cyanotoxins in California streams. Because of difficulties in drawing concrete conclusions about drivers of toxin production based on the opportunistically collected data available to date, further, more focused, efforts should be made to understand the potential for anthropogenic factors influencing toxin production in streams, as this has implications for what can be expected to be achievable via management actions. Issues that should be explored include: 1) temporal/seasonal

variability in toxin production (i.e., when to sample, and whether there is a need for multiple samples per year); 2) the necessary level of sampling effort (i.e., to account for spatial variability and patchiness of toxigenic benthic cyanobacteria); and 3) factors that may trigger or exacerbate toxin production.

Table 2-1. Toxigenic cyanobacterial genera, and (where applicable) species within those genera, that were recorded in California wadeable streams (N = 1,279 unique sites), along with toxins they can produce, according to the literature. Except where noted, only the results from literature for which chemical analyses were conducted on isolated cyanobacterial strains in culture conditions are included. Superscripts/bold-font indicate how species match with the toxins that they can produce and the literature source. Note: Morphological descriptions and photomicrographs for the cyanobacterial species from California streams are available at Stancheva, R., Fuller, C., Sheath, R.G. 2014: Soft-Bodied Stream Algae of California (http://dbmuseblade.colorado.edu/DiatomTwo/sbsac_site/major_groupCyanobacteria.html)

Genus	% of sites where genus recorded	Species	anatoxin-a	aplysiatoxin	β-methylamino alanine	cylindrospermopsin	debromoaplysiatoxin	lyngbyatoxin	microcystins	neosaxitoxins	nodularins	pahayokolide	saxitoxins	References
Anabaena	17		X			X			X	X			X	Vezie et al., 1998, Mohamed et al., 2006, Spoof et al., 2006
Anabaenopsis	< 1								X					Lanaras and Cook, 1994
Arthrospira	1								X					Ballot et al., 2005
Coelomoron	1	*C. pusillum*[a]							**X**[a]					**Dos S Vieira et al., 2005**[a‡]
Cylindrospermum	3	*C. stagnale*[a]	X						X				**X**[a]	**Sivonen et al., 1989**[a], Pandey and Tiwari, 2010, Borges et al., 2015
Dolichospermum	1	*D. flosaquae*[a], *D. planctonicum*[b]	**X**[a,b]						**X**[a]					**Sivonen et al., 1989**[a], Harada et al., 1991, **Bruno et al., 1994**[b]
Geitlerinema	6	*G. splendidum*[a], *G. amphibium*[b], *G. lemmermannii*[b]							**X**[a]				**X**[b]	**Aboal et al., 2005**[a], Myers et al., 2007, **Borges et al., 2015**[b]
Gloeotrichia	1								X					Carey et al., 2007
Hapalosiphon	1	*H. hibernicus*[a]							**X**[a]					**Prinsep et al., 1992**[a]
Leptolyngbya	78								X					Mohamed et al., 2006

Lyngbya	3	*L. wollei*[a,b]		X		**X**[b]	X	X			X	**X**[a]	Onodera et al., 1997, **Yin et al. 1997**[a], Berry et al., 2004, Dos S Vieira et al., 2005, **Seifert et al., 2007**[b], Harr et al., 2008
Microcystis	1								X				Botes et al., 1982
Nodularia	2	*N. spumigena*[a]								**X**[a]			**Sivonen et al., 1989**[a]
Nostoc	33	*N. carneum*[a]							**X**[a]				Sivonen et al., 1992, **Mohamed et al., 2006**[a]
Oscillatoria	4	*O. tenuis*[a]	X			X			**X**[a]				Sivonen et al., 1989, **Luukkainen et al., 1993**[a], Brittain et al., 2000, Mazmouz et al., 2010
Phormidium	38	*P. formosum*[a], *P. uncinatum*[b], *P. autumnale*[c]	**X**[a,c]		X	X			X			**X**[b]	**Skulberg et al., 1992**[a‡‡], Mez et al., 1997, Gugger et al., 2005, Mohamed et al., 2006, Izaguirre et al., 2007, **Harland et al., 2014**[b], **Harland et al., 2013**[c], **Borges et al., 2015**[b]
Rivularia	4	*R. biasolettiana*[a], *R. haematites*[a]							**X**[a]				**Aboal et al., 2005**[a]
Schizothrix	2			X									Sivonen and Jones, 1999
Scytonema	2	*S. crispum*[a]										**X**[a]	**Smith et al., 2011**[a]
Tolypothrix	12	*T. distorta*[a]							**X**[a]				**Aboal et al., 2005**[a]
Trichormus	1	*T. variabilis*[a]							**X**[a]				**Mohamed et al., 2006**[a]
Tychonema	10			X									Shams et al., 2015

‡ Extracts tested positive for toxicity, and toxin exhibited similarity to microcystin, but the exact chemical nature of the toxin was not conclusively determined.

‡‡ Specimen was not cultured.

Table 2-2. Frequency of occurrence and biovolumes of the microcystin-producing genera (based on Table 1) encountered in benthic samples from California wadeable streams. "Rank" refers to decreasing order of a "prevalence index", which is the product of the number of sites where the genus was observed and its mean biovolume across sites. Genera in bold exhibited the highest prevalence indices.

Genus	Rank (of 17 genera total)	# of sites where observed	Mean biovolume across sites ($\mu m^3\ cm^{-2}$)	Median biovolume across sites ($\mu m^3\ cm^{-2}$)	Maximum biovolume across sites ($\mu m^3\ cm^{-2}$)
Anabaena	**3**	**218**	**4.54E+09**	**2.89E+05**	**8.37E+11**
Anabaenopsis	16	1	4.06E+05	4.06E+05	4.06E+05
Arthrospira	15	10	1.17E+07	2.69E+03	1.17E+08
Coelomoron	17	11	3.05E+04	1.65E+04	9.01E+04
Cylindrospermum	8	38	4.25E+08	5.77E+06	3.24E+09
Dolichospermum	13	16	4.40E+07	5.87E+05	4.66E+08
Geitlerinema	10	73	6.38E+07	1.19E+05	1.87E+09
Gloeotrichia	9	10	1.06E+09	3.32E+08	3.84E+09
Hapalosiphon	12	16	1.22E+08	4.14E+06	1.42E+09
Leptolyngbya	**2**	**995**	**1.13E+09**	**7.36E+04**	**1.08E+12**
Microcystis	14	16	3.11E+07	2.68E+04	4.62E+08
Nostoc	**1**	**426**	**4.29E+09**	**2.89E+07**	**5.33E+11**
Oscillatoria	11	50	3.94E+07	8.02E+05	9.82E+08
Phormidium	**4**	**483**	**1.88E+08**	**4.58E+05**	**1.75E+10**
Rivularia	5	50	1.65E+09	1.25E+07	2.72E+10
Tolypothrix	6	152	2.96E+08	1.81E+07	7.62E+09
Trichormus	7	13	2.11E+09	1.48E+08	1.98E+10

Table 2-3. Results of indicator species analysis showing which genera were significantly associated with high-elevation sites (>700 m) supporting high microalgal cover (>70%) at the time of assessment. Indicator values can range from 0 to 100, with higher values corresponding to a stronger association between taxon and class of site. Shown are all genera with significant indicator values > 10. Cyanobacterial genera in the list (shown in bold) may be considered strong candidates for producing microcystins in California wadeable streams, based on their tendency to inhabit the high-elevation sites with high microalgal cover at the time of assessment (i.e., the type of site where microcystin concentrations were particularly high in the study data set, Figure 6). Genera are listed in order of decreasing indicator value.

Genus	Indicator Value	p
Chamaesiphon	42.3	0.0002
Nostoc	35.5	0.0002
Calothrix	28.9	0.0002
Homoeothrix	28.5	0.0002
Zygnema	26.7	0.0002
Phormidium	26.1	0.0020
Aphanocapsa	22.8	0.0354
"chantransia" stage (Rhodophyta)	22.1	0.0008
Tolypothrix	20.6	0.0002
Aphanothece	19.2	0.0216
Tribonema	17.5	0.0018
Ulothrix	12.6	0.0002
Microspora	11.2	0.0002
Closterium	11.0	0.0058

Figure 2-1. Benthic algae sampling sites where taxa capable of producing cyanotoxins (CYN) in general (panels on left), or microcystins (MCY) specifically (panels on right), were observed during spring-summer monitoring surveys from 2007-2013. Blue triangles correspond to sites where no toxigenic taxa, based on current knowledge (Table 2-1), were observed, yellow squares correspond to sites where toxigenic genera (but not species) were observed, and red diamonds correspond to sites where toxigenic species were observed. Top panels correspond to the state as a whole, and bottom panels are zoomed-in on Southern California, where the data density is highest.

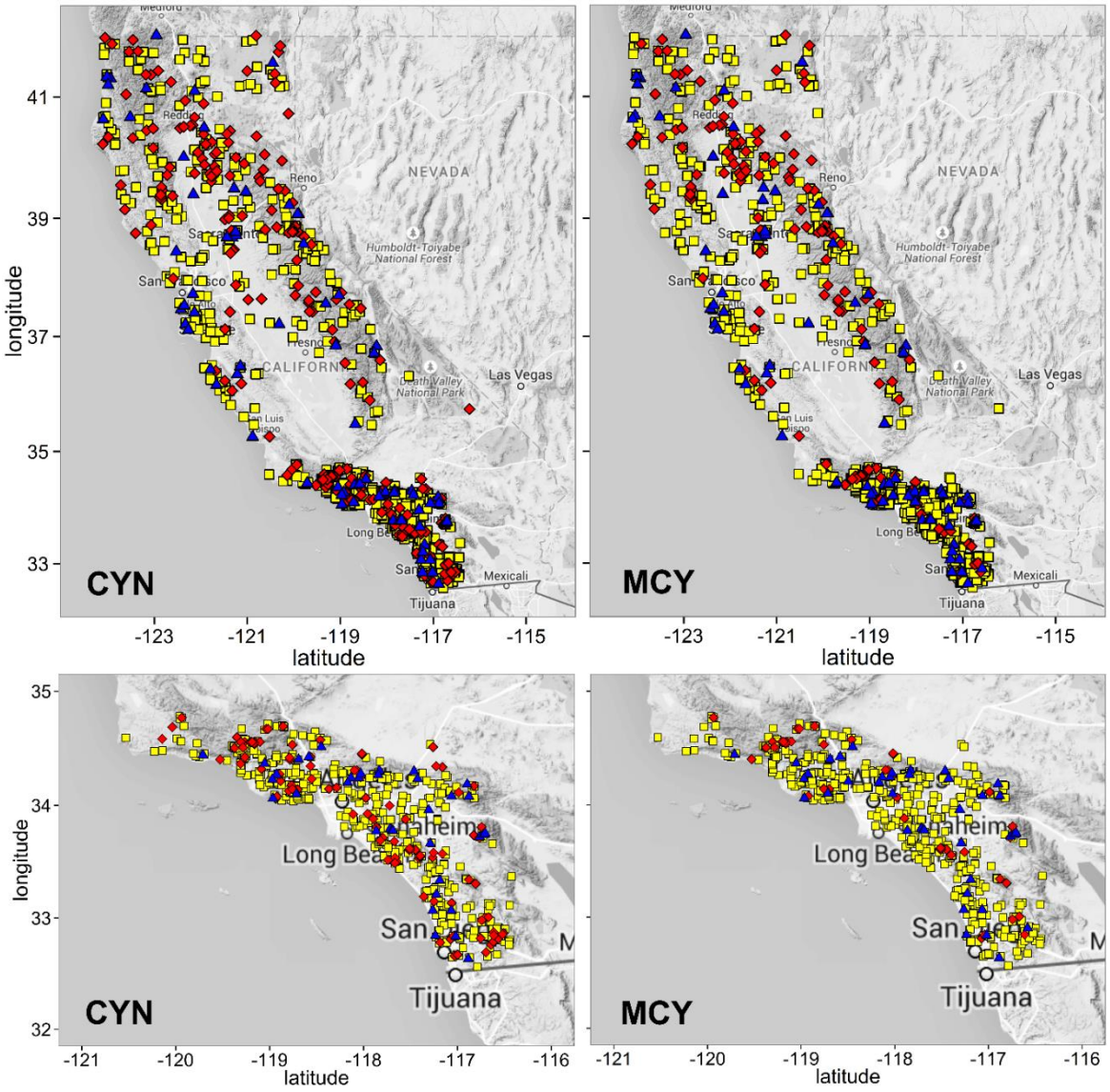


Figure 2-2. Statewide cumulative distribution function for benthic microcystin concentrations during the spring-summer time frame. The graph shows the estimated probability distribution of toxin concentrations relative to the cumulative proportion of length of California wadeable streams. Specifically, the y-axis refers to the percent of total stream kilometers, aggregated across the state, that are estimated to support the corresponding microcystin concentrations on the x-axis. The grey highlighted area delineates the 95% confidence interval for the estimate. Note: the x-axis is truncated at 400 μg m^{-2} to aid visualization.

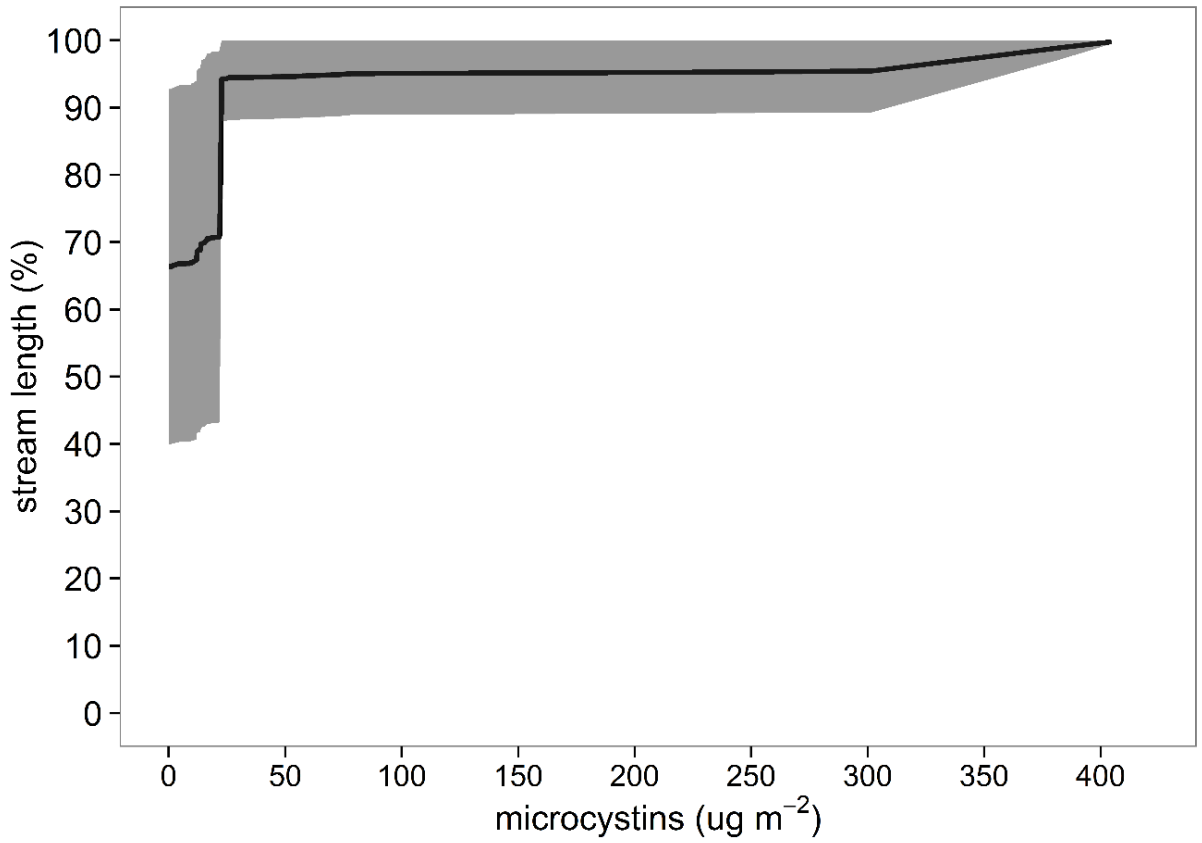


Figure 2-3. Benthic algae sampling sites where microcystins were assessed during spring-summer of 2011-2013 (N=368 sites sampled). Left insert panel corresponds to the state as a whole, and large panel is zoomed-in on Southern California, where the data density is highest. Colors correspond to concentration of total microcystins measured in benthic algal samples (µg m^{-2}) and open circles correspond to sites that were below the limit of detection. Appendix B provides zoomed in maps of the Bay Area and the Sierra Nevada).

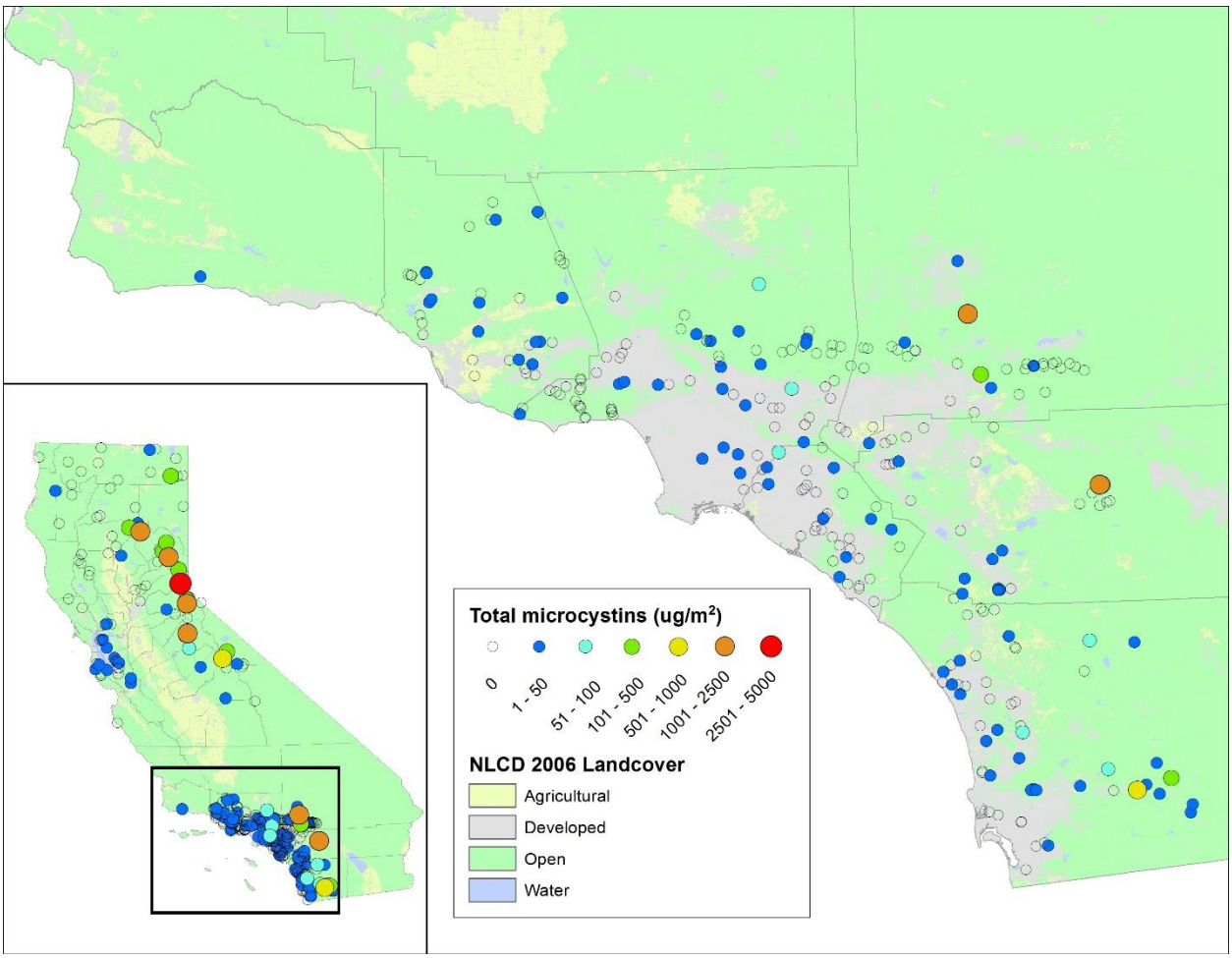


Figure 2-4. Relationship between land uses (proportion of agricultural land use, urban development, or undeveloped open space) surrounding the sampling site, and whether or not benthic microcystins were detected at that site. All nine panels show data from the same (full) dataset, but depicted in different ways. Proportions of land-use types are shown at three scales (i.e., within 10 km, 5 km, and 500 m radii of buffer around sampling sites) and add up to 100, for each site, within each scale. Boxplots within each land-use category are stratified by whether or not microcystins were detected at the site in question.

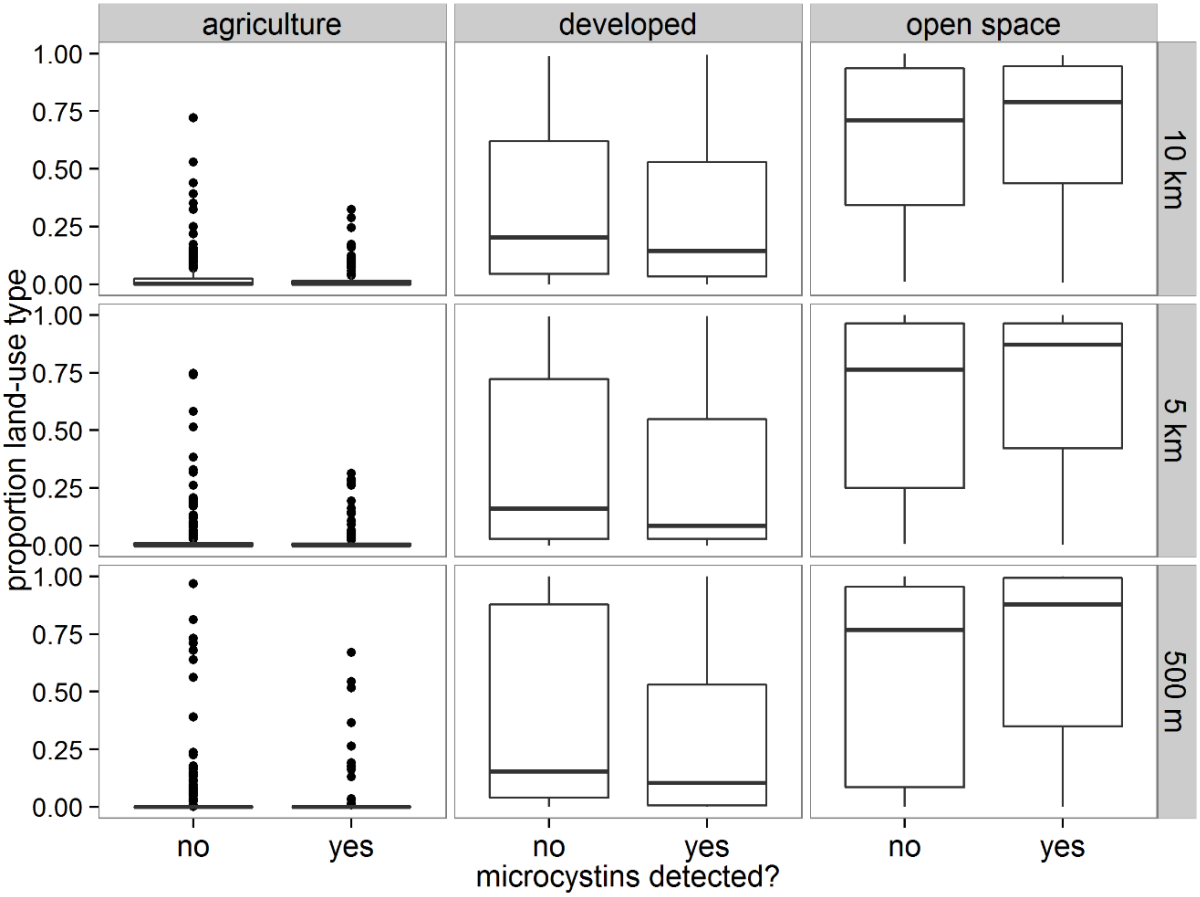


Figure 2-5. The relationship between the concentration of benthic microcystins and the proportion of land surrounding the sampling site that is open space (i.e., undeveloped). Data are shown for three spatial scales (buffer radii) around sampling sites: 10 km (dark grey squares), 5 km (light grey triangles), and 500 m (black diamonds). Non-detect samples are excluded.

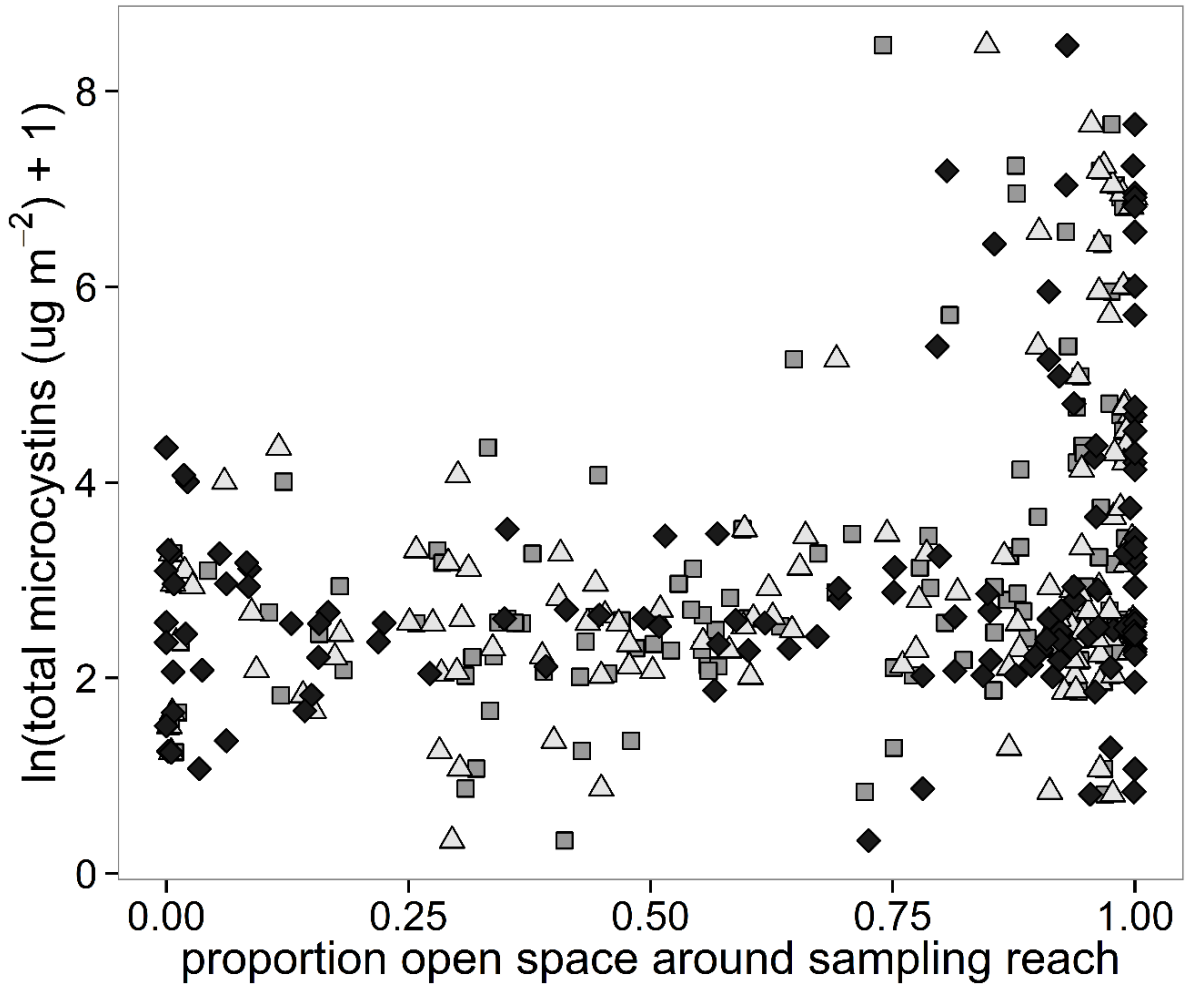
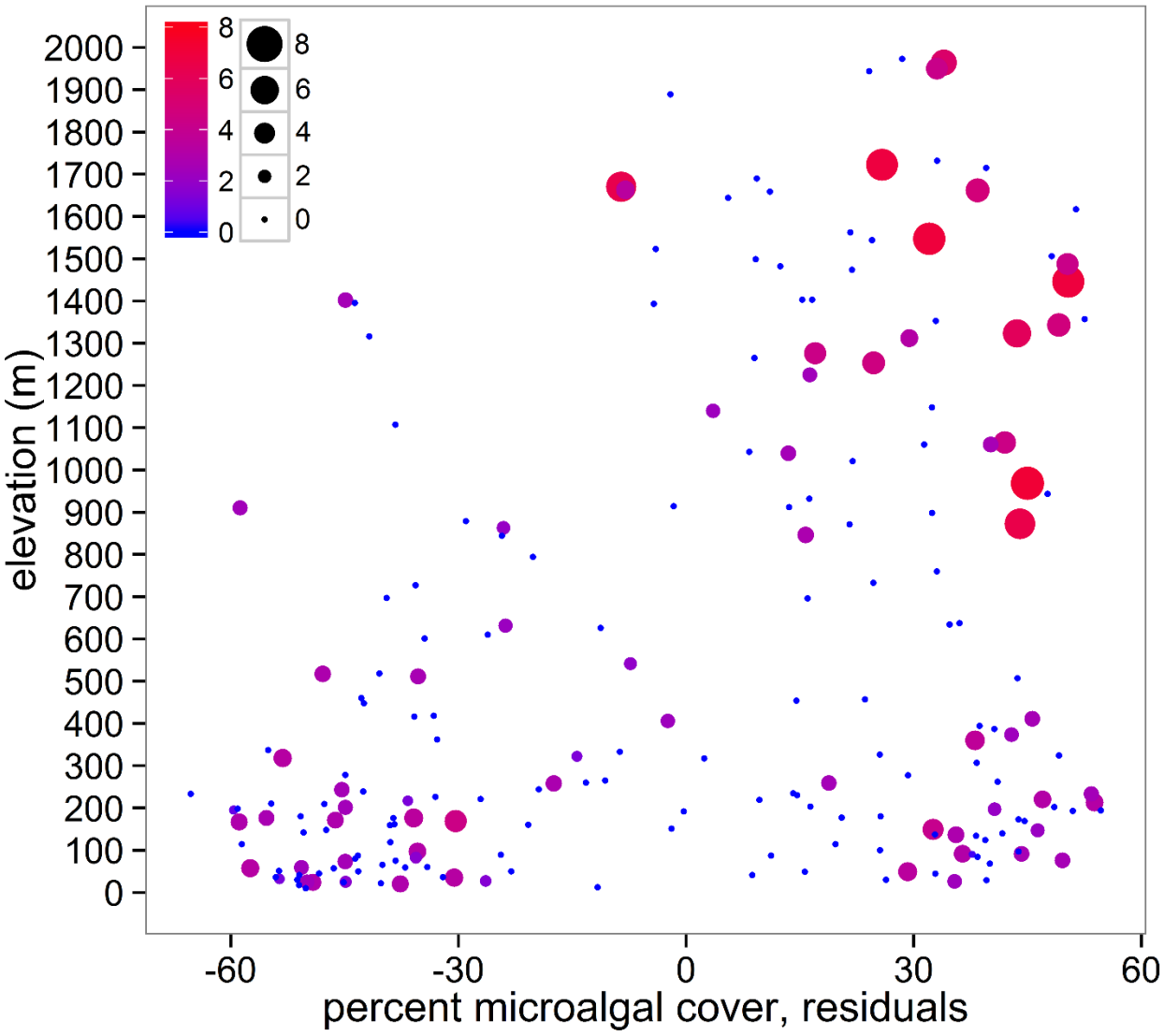


Figure 2-6. Microcystin concentration as a function of elevation and the percent cover of microalgae, presented as residuals after the effect of chlorophyll *a* concentration was removed. Icon size and shading indicate relative concentrations of microcystins, on a natural-log (ln) scale, with larger/pink corresponding to higher values (see legend).



CHAPTER 3: LENTIC WATERBODIES: DEPRESSIONAL WETLANDS, LAKES, RESERVOIRS, AND COASTAL WETLANDS

Across the continuum of aquatic habitats, lentic waterbodies, including lakes, reservoirs, depressional wetlands and coastal lagoons, represent conditions that readily support the proliferation of planktonic cyanoHABs. These conditions typically include ample supply of nutrients, calm water and stratification, plenty of irradiance and warm water temperatures due to the dominance of open water versus vegetated habitats (Carmichael 2008, Paerl and Huisman 2008, Hudnell 2008, 2010, Xu et al. 2010, O'Neill et al. 2012, Paerl and Paul 2012, Berg and Sutula 2015, Figure 3.1). In Southern California, these lentic habitats are often found within or downstream of urban and agricultural areas, and therefore subject to further risk of cyanoHAB proliferation due to increased anthropogenic nutrient inputs via point- and non-point source runoff. Magrann et al. (2015) found that cyanobacteria were ubiquitous in 30 lakes, depressional wetlands and coastal lagoons in Southern California, and *Microcystis spp.* dominated the community in 96% of study sites. Because this study had limited quantification of cyanotoxins and community composition was dominated by potentially toxic cyanobacteria, it generated interest in characterizing cyanotoxin risk across lentic waterbodies in Southern California.

The purpose of this chapter is to provide a snapshot of cyanotoxin risk in Southern California lentic waterbodies, summarized from three separate studies: 1) an ambient, probability-based assessment of depressional wetlands, 2) a targeted assessment of lakes, reservoirs and coastal lagoons in San Diego County, and 3) lakes in Riverside County.

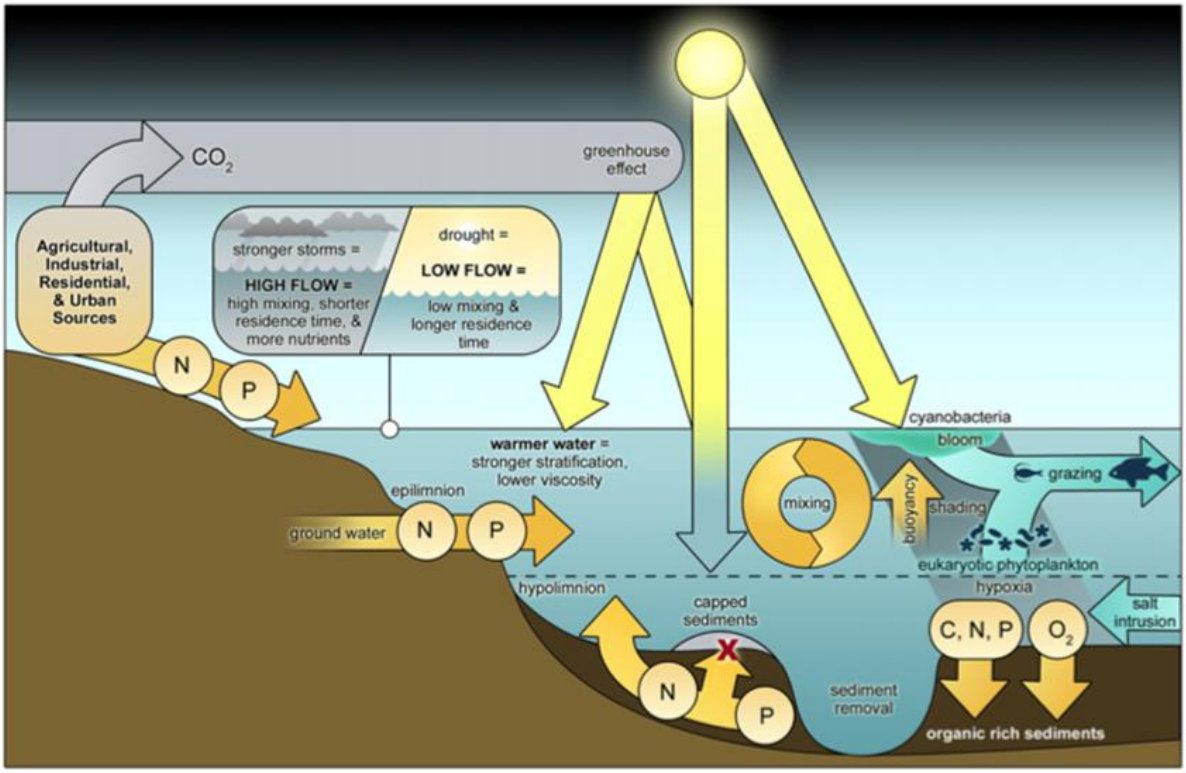


Figure 3.1. Conceptual model of factors affecting cyanobacteria blooms including warmer water, drought, decreased flow, decreased mixing, increased residence time, and increased N and P inputs from agricultural, industrial and urban sources. From Berg and Sutula (2015).

METHODS

Sampling Approach and Sample Collection

Ambient Survey of Depressional Wetlands

Depressional wetlands are topographic features positioned lower than the surrounding landscape thus allowing the accumulation of surface water. They can receive persistent surface or groundwater flows that connect them to other waterbodies or be isolated. These wetlands provide important seasonal refugia and breeding areas for a variety of fauna, particularly in dry habitats. As the most abundant wetland type in California, depressional wetlands comprise approximately 45% of the State's 3.6 million acres of wetland area (Sutula et al. 2008); nonetheless, there have been no regional assessment studies or systematic monitoring focused on depressional wetlands. Therefore, little is known about their extent or overall condition, or whether cyanotoxins are present in these waterbodies. The first ever systematic condition assessment of Southern California depressional wetlands was conducted as a new element of the cooperative regional monitoring and assessment program lead by California's Surface Water Ambient Monitoring Program (SWAMP). The goals of the assessment were to determine depressional wetland 1) extent and distribution 2) condition, and 3) major stressors to which they are exposed. Cyanotoxins were evaluated as a possible stressor to determine if they should be included in future depressional wetland monitoring programs.

SWAMP's probabilistic survey facilitated an evaluation of overall regional condition. Sites were chosen using the Generalized Random Tessellation Stratified (GRTS) (Stevens and Olsen 2004) technique to randomly select sites in a spatially balanced manner. Each site was sampled only once (following an initial reconnaissance survey). Cyanotoxins were not the focus of the regional condition assessment but were collected opportunistically through the field program associated with the assessment. As such, only the particulate fraction (i.e. plankton in the water column) was collected for cyanotoxin analysis, along with chlorophyll *a* samples, following the guidelines in the Standard Operating Procedures (SOP) (see Fetscher et al. 2014). Briefly, water was collected from 10 sampling nodes established around each wetland, and combined to form a composite sample in a 2L aluminum foil-covered bottle from which chlorophyll *a* and cyanotoxin samples were collected. Particulate samples of chlorophyll *a* and cyanotoxins were filtered onto GF/F filters and frozen immediately in the field. Cyanotoxin samples were not included in the original list of indicators for collection as part of the depressional wetlands assessment, therefore the approach to collect filtered particulate fraction samples in the same way as chlorophyll *a* samples were employed to ensure the samples would be collected with ease in a timely manner that allowed for completion of all of the indicator samples. However, this approach excluded collection of the dissolved fraction, and therefore, underestimates the total concentration of cyanotoxins.

Additional indicators collected as part of the depressional wetlands assessment and utilized in this analysis are nutrients (total ammonia, nitrate, nitrite, orthophosphate, total nitrogen or total Kjeldahl nitrogen (TKN) and total phosphorus), and alkalinity. Subsamples for dissolved nutrients were passed through a 0.45 µm polytetrafluoroethylene (PTFE) filter, while subsamples for total phosphorus and TKN were not. The total number of sites and the percentage of sites for each wetland function type are listed in Table 3-1. The study sites, year sampled, site name, wetland function and region are listed in Table 3-2. The samples collected in 2011 were part of the reconnaissance and method development component of the depressional wetlands condition assessment.

Table 3-1. Total number of sites and percentage of total sites for each wetland function type.

	Total number of sites	Percentage of total sites	Range of Microcystin (µg L^{-1})	Percentage of toxic sites
Golf Course	13	24.1	bd – 22.3	33.3
Habitat/Stormwater	23	42.6	bd – 2.5	13
Private Property	9	16.7	bd – 0.01	37.5
Stock pond	3	5.6	bd	0
recreation	2	3.7	bd – 0.45	50
flood control	4	7.4	bd – 0.09	50

Table 3-2. List of study sites from the depressional wetlands assessment survey including year sampled, site name, water regime, wetland function and region location. The site numbers correspond to the numbers listed on the maps (Figure 3-1) and the * indicates sites in San Diego that were revisited and more intensely assessed (see below Figure 3-2) in summer 2012.

Year	Site Name	Site number	Water Regime	Wetland Function	Region
2013	Admiral Baker Golf Course	1	Perennial	Golf course	San Diego
2011	Agua Hedionda	2	Perennial	Habitat/stormwater	San Diego
2011	Ballona freshwater marsh	3	Perennial	Habitat/stormwater	Los Angeles
2013	Big Canyon Golf Course	4	Perennial	Golf course	Riverside
2012, 2013	Buena Vista Park	5	Perennial	Habitat/stormwater	San Diego
2011, 2013	Calico Ranch Rd Julian	6	Seasonal	Habitat/stormwater	San Diego
2012	Calle Roxanne Fallbrook	7*	Perennial	Private property	San Diego
2011	Circle X	8	Seasonal	Stock pond	Los Angeles
2013	Costa del Sol Golf Course	9	Perennial	Golf course	San Diego
2012	Covina flood control basin	10	Perennial	Flood control	Los Angeles
2013	Creek Hollow Ranch	11	Seasonal	Habitat/stormwater	San Diego
2011	Dairy Mart Rd	12	Perennial	Habitat/stormwater	San Diego
2011	De La Garrigue Rd	13	Perennial	Stock pond	Los Angeles
2012	Dominguez gap west basin	14	Perennial	Flood control	Los Angeles
2012	Emerald Isle Golf Course	15*	Perennial	Golf course	San Diego
2012	Euclid Edison	16	Seasonal	Private property	Riverside
2011	Foss Lake Alkali Marsh	17	Seasonal	Habitat/stormwater	San Diego
2011	Guajome Lake	18	Perennial	Habitat/stormwater	San Diego
2012	Harbor Lake (Lake Machado)	19	Perennial	Habitat/stormwater	Los Angeles
2011	Harbor Lakes 2	20	Perennial	Habitat/stormwater	Los Angeles
2012	Hemet Golf Course	21	Perennial	Golf course	Riverside
2011	Irvine Turtle Ridge	22	Perennial	Flood control	Riverside
2013	IRWD San Joaquin Pond 5	23	Perennial	Habitat/stormwater	Riverside
2011	Laguna Lake	24	Perennial	Habitat/stormwater	Riverside

2012	Links at Summerly	25	Seasonal	Golf course	Riverside
2012	Lyons Valley Rd Jamul	26*	Seasonal	Private property	San Diego
2011	Madrona Marsh	27	Seasonal	Habitat/stormwater	Los Angeles
2012	Manchester Ave Encinitas	28	Seasonal	Private property	San Diego
2012	Michelson Marsh	29	Perennial	Habitat/stormwater	Riverside
2012	Mountain View Golf Course	30	Perennial	Golf course	Los Angeles
2011	Murrieta	31	Seasonal	Habitat/stormwater	Riverside
2011	Nicholas Flat pond	32	Perennial	Habitat/stormwater	Los Angeles
2011, 2012	Olive Hill Road Fallbrook	33*	Perennial	Private property	San Diego
2013	O'Neill Park pumping station	34	Perennial	Flood control	San Diego
2012	Pala Rey Ranch	35*	Perennial	Private property	San Diego
2012	Palm Lake Golf Course	36	Perennial	Golf course	Los Angeles
2011, 2012	Palomar Airport Rd	37	Seasonal	Habitat/stormwater	San Diego
2012	Pico Rivera Municipal Golf Course	38	Perennial	Golf course	Los Angeles
2012	Prado Recreation Inc.	39	Perennial	Habitat/stormwater	Riverside
2013	Robinson Ranch Golf Course	40	Perennial	Golf course	Los Angeles
2013	San Diego River Ponds P11BA Santee Recreation Lakes	41	Perennial	Recreation	San Diego
2011	San Diego River Santee	42	Perennial	Habitat/stormwater	San Diego
2012	San Dieguito River Calle Ambiente	43*	Perennial	Habitat/stormwater	San Diego
2012	Santee Lakes Recreation Preserve Lake #7	44*	Perennial	Recreation	San Diego
2013	Santo Rd San Diego	45	Perennial	Habitat/stormwater	San Diego
2013	Simi Hills Golf Couse	46	Perennial	Golf course	Los Angeles
2013	Sims Pond (Los Cerritos)	47	Perennial	Habitat/stormwater	Los Angeles
2012	Sunsol nursery	48	Perennial	Habitat/stormwater	San Diego
2012	Sweetwater Authority El Tae Rd	49*	Seasonal	Private property	San Diego
2012	Tumble Creek Lane	50	Perennial	Private property	San Diego
2013	Vista Valencia Golf Course	51	Perennial	Golf course	Los Angeles
2011	Zuniga Marsh	52	Seasonal	Stock pond	Los Angeles

A more intensive assessment was conducted in San Diego in 2012. Eight sites from the depressional wetlands condition assessment (sampled in May) that were revisited twice between July and September (indicated by an *next to the site number in Table 3-2). Passive sampling devices (Solid Phase Adsorption Toxin Tracking, SPATT bags) were deployed continuously between site visits and were used as a screening-level assessment tool that provided a time-integrated sample for detecting microcystin presence in the waterbodies. Grab samples have been shown to underestimate or miss toxin presence in a waterbody due to spatial and temporal variability in toxin production by cyanobacteria (Kudela 2011). Therefore, the SPATT bags were used as a supplement in order to provide insight into the overall toxin prevalence during the summer season in these depressional wetland waterbodies. SPATT bags are sampling devices constructed of resins that adsorb specific toxins, and are deployed in a waterbody for a fixed amount of time (Kudela 2011, Gibble and Kudela 2014). SPATT were deployed for approximately 1-month intervals between July and September 2012. Grab samples were collected during each SPATT deployment and retrieval, for a total of three sampling events per site. Grab samples consisted of chlorophyll *a*, cyanotoxins and dissolved nutrients (ortho-phosphate, nitrate, nitrite, ammonium), total phosphorus and nitrogen, TKN, and particulate nitrogen.

Lakes, Reservoirs, and Coastal Wetlands

San Diego Screening Assessment

A targeted screening assessment study was conducted in San Diego during the summer and fall of 2013. There were 19 sites sampled three times between July and October including 10 lakes and reservoirs and 9 coastal waterbodies, with a variety of designated beneficial uses (Tables 3-3 and 3-4). The sampling design was similar to the intensive assessment of depressional wetlands in San Diego in 2012. SPATT bags were deployed for two 1-month intervals, typically from July to August and from August to September. Grab samples were collected during each SPATT deployment and retrieval, for a total of three (3) sampling events per site. Grab samples consisted of chlorophyll *a* (collected as described above), particulate microcystins (collected as described above) and phycoerythrin and phycocyanin pigments (collected on 1 µm Whatman nucleophore polycarbonate filters, covered in aluminum foil and frozen immediately). *In situ* readings were also recorded at each site, which included temperature, pH, DO, conductivity, alkalinity and salinity.

Table 3-3. List of study sites and beneficial uses for San Diego lakes and reservoirs (beneficial use data from San Diego Region – The Basin Plan). The site numbers correspond to Figure3-5.

Reservoirs and Lakes	Site Number On Map	Beneficial Use												
		MUN	AGR	IND	PROC	GWR	FRSH	REC1	REC2	WARM	COLD	WILD	RARE	POW
Lake Henshaw	3	•	•	•	•		•	•[1]	•	•		•	•	•
Cuyamaca Reservoir	1	•	•	•	•			•[1]	•	•	•	•	•	
Lower Otay Reservoir	8	•	•	•	•			•[1]	•	•	•	•		
Lake Murray	6	•		•				•[1]	•	•	•	•		•
Morena Reservoir	9	•	•	•	•		•	•[1]	•	•	•	•	•	
Vail Lake	10	•	•	•	•	•		•[1]	•	•		•		
Lake Hodges	4	•	•	•	•			•[1]	•	•	•	•	•	
Lake Sutherland	7	•	•	•	•			•[1]	•	•	•	•	•	
El Capitan Lake	2	•	•	•	•			•[1]	•	•	•	•	•	
Lake Miramar	5	•		•				•[1]	•	•		•		•

MUN = municipal and domestic supply, AGR = agricultural supply, IND = industrial service supply, PROC = industrial process supply, GWR = ground water recharge, FRSH = freshwater replenishment, REC1 = contact water recreation, REC2 = non-contact water recreation, WARM = warm freshwater habitat, COLD = cold freshwater habitat, WILD = wildlife habitat, RARE = rare, threatened or endangered species, POW = hydropower generation.

[1] Fishing from shore or boat permitted, but other water contact recreational (REC-1) uses are prohibited.

Table 3-4. List of study sites and beneficial uses from San Diego coastal waters.

Coastal Waters	Site Number on Map	Beneficial Use														
		IND	NAV	REC1	REC2	COMM	BIOL	EST	WILD	RARE	MAR	AQUA	MIRG	SPWN	WARM	SHELL
San Elijo Lagoon	17			•	•		•	•	•	•	•		•	•		
San Elijo Pond*	18															
Los Penasquitos Lagoon[1]	11			•	•		•	•	•	•	•		•	•		•
San Diego Bay near Silver Strand Bikeway[2, 3, 4]	14	•	•	•	•	•	•	•	•	•	•		•	•		•
San Diego Bay near Sweetwater[2, 3, 4]	15	•	•	•	•	•	•	•	•	•	•		•	•		•
San Diego Bay near Naval Training Center[2, 3, 4]	13	•	•	•	•	•	•	•	•	•	•		•	•		•
San Diego River Estuary*	16															
Mission Bay	12	•		•	•	•		•	•	•	•		•	•		•
Tijuana River Estuary	19			•	•	•	•	•	•	•	•		•	•		•

*Beneficial uses not described for these sites in the San Diego Region Basin Plan. IND = industrial service supply, NAV = navigation, REC1 = contact water recreation, REC2 = non-contact water recreation, COMM = commercial and sport fishing, BIOL = preservation of biological habitats of special significance, EST = estuarine habitat, WILD = wildlife habitat, RARE = rare, threatened or endangered species, MAR = marine habitat, AQUA = aquaculture, MIRG = migration of aquatic organisms, SPWN = spawning, reproduction, and/or early development, WARM = warm freshwater habitat, SHELL = shellfish harvesting.

[1]Fishing from shore or boat permitted, but other water contact recreational (REC-1) uses are prohibited.

[2] Includes the tidal prisms of the Otay and Sweetwater Rivers.

[3] The Shelter Island Yacht Basin portion of San Diego Bay is designated as an impaired water body for dissolved copper pursuant to Clean Water Act section 303(d). A Total Maximum Daily Load (TMDL) has been adopted to address this impairment.

[4] The shoreline segment along Shelter Island Shoreline Park within San Diego Bay is designated as a water quality limited segment for indicator bacteria pursuant to Clean Water Act section 303(d). Total Maximum Daily Loads have been adopted to address these impairments.

Due to an influx of visible bloom notifications to the San Diego Regional Water Control Board, an *ad hoc* event response survey was conducted in 2014. Samples were collected at 13 lakes between June and August (Table 3-5) for HAB species identification and particulate toxin analysis. The potentially toxic HAB genera and species were identified for all samples, and this information was used to determine the samples that were analyzed for cyanotoxins.

Table 3-5. Sites sampled during the 2014 ad hoc screening assessment in San Diego. Site numbers are listed for sites where cyanotoxin samples were analyzed (not all samples collected were analyzed).

Name	Site Number on map	Month Site Was Sampled
Barrett Lake	20	August
Chollas Reservoir		August
Discovery Lake		June
Guajome Lake		August
Harveston Lake	21	June, August
Lake Barbara	22	June
Lake Hodges	4	June
Lake Henshaw	3	June
Lake Morena		August
Lake Poway		August
Lake Sutherland	7	June
Lindo Lake	23	June, August
Santee Lake #5	24	July

Riverside Lake Sampling

There were four lakes sampled on May 21, 2014 in Riverside County that included Lake Elsinore, Canyon Lake, Lake Menifee and Lake Skinner. These highly frequented recreational lakes were visibly surveyed for high biomass (i.e. “bloom”) areas and one grab sample was collected in a glass bottle from these areas in each lake for toxin analysis and HAB species identification. Both the particulate and dissolved fractions were collected for toxin samples by filtration onto GF/F filters (particulate), collection of filtrate into small glass bottles (dissolved) and frozen immediately in the field. The HAB species identification samples of whole water were collected with plastic 1L bottles, stored in an incubator overnight and analyzed live the following day.

One sample was also collected from San Joaquin Marsh in Irvine from Pond C after a citizen reported a green visible bloom. The San Joaquin Marsh and Wildlife Sanctuary encompasses over 300 acres of coastal freshwater wetlands and has 12 miles of nature trails open to the public, as well as a chapter office for The Audubon Society and The Duck Club, a facility for non-profit organizations. Pond C is the long-term emergency storage for the Michelson Water Reclamation Plant. Under normal operations the water in the pond is circulated into the plant; however, due to the drought, all recycled water was used to meet demands of the recycled water systems and Pond C became stagnant (which likely contributed to the dense bloom). The sample was collected in a plastic container and brought to SCCWRP, where a total toxin sample (whole water) was collected in a glass bottle and frozen immediately.

Laboratory Analysis

Depressional Wetlands

Laboratory analysis of cyanotoxin samples

Particulate grab samples were analyzed by enzyme-linked immunosorbent assay (ELISA) for both total microcystins and saxitoxins. Microcystins were analyzed by ELISA using the Envirologix QuantiPlate™ kit (Envirologix, Portland, ME, Cat. No. EP 022, as described in Kudela 2011). The BIOO Scientific MaxSignalTM Saxitoxin (PSP) test kit (BIOO Scientific Corp., Austin, TX, Cat. No. 1034) was used for saxitoxin analysis. Prior to analysis, the sample-containing filters were extracted in 3 mL of Milli-QTM water, sonicated for 30 seconds to ensure cell disruption, and centrifuged for 10 min at 2147 g (as described in Seubert et al. 2014). The extract was then analyzed according to the manufacturer's instructions for both toxins.

SPATT samples were analyzed at the University of California, Santa Cruz for four microcystin congeners (MCY-LA, MCY-LR, MCY-RR, MCY-YR) by liquid chromatography/mass spectrometry (LCMS) with electrospray ionization (ESI) with selected ion monitoring (SIM) on an Agilent 6130 with a Phenomenex Kinetix (100x2.10) C18 column. The method was adapted from Mekebri et al. (2009) with minor modifications to account for the choice of column and LCMS/SIM instead of tandem mass spectrometry (Kudela 2011).

Laboratory analysis of discrete samples

Chlorophyll *a* and all nutrient samples were collected as part of the depressional wetlands assessment and the laboratory analysis is summarized in the corresponding report (Brown et al., in prep). In short, the following analysis methods were used for each analyte: Chlorophyll *a* (EPA 445.0), Total phosphorus (SM 4500-P E), nitrate and nitrite (EPA 300.0), TKN (EPA 351.2), orthophosphate (EPA 300.0) and alkalinity (SM 2320B). Phycoerythrin and phycocyanin pigment samples were sent to the laboratory of Dr. Gregory Boyer at the State University of New York and analyzed using a Milton-Roy MR3000 UV-VIS Spectrophotometer.

Lakes, Reservoirs, and Coastal Wetlands

Laboratory analysis of cyanotoxin samples

The particulate microcystin samples collected in San Diego in 2013 and 2014 were analyzed for 9 microcystin congeners (MCY-LA, MCY-LR, MCY-RR, MCY-YR, MCY-LW, MCY-LY, MC-desmethyl-LR, MC-desmethyl-RR, and MCY-LF) and anatoxin-a at the California Fish and Wildlife Water Pollution Control Lab (WPCL) using LC-ESI-MS/MS as described in Mekebri et al. (2009). SPATT samples were analyzed at University of California, Santa Cruz as described previously.

Both particulate and dissolved cyanotoxin samples collected from the four lakes in Riverside in 2014 and the San Joaquin Marsh sample were analyzed at University of California, Santa Cruz by LC-MS, electrospray ionization (ESI) with selected ion monitoring (SIM) on an Agilent 6130 with a Phenomenex Kinetix TM C18 column. This 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). Samples were analyzed for four microcystin congeners (MCY-LA, MCY-LR, MCY-RR, MCY-YR), anatoxin-a and cylindrospermopsin (San Joaquin Marsh sample was only analyzed for microcystins based on microscopy results; see below).

HAB species identification samples

HAB taxonomic identification samples were collected in 2014 at the Riverside lake sites and from the event-response survey in San Diego and analyzed at the University of Southern California. Samples were examined live within one day of collection. Briefly, homogenized water was aliquoted into shallow plastic tissue culture dishes (Falcon) and allowed to settle for approximately 30 minutes. The subsamples (5 mL) were viewed with an Accu-Scope 3032 inverted microscope and cyanobacteria were identified to the genus level and, when possible, the species was identified. The San Joaquin Marsh sample was analyzed on the CellScope Aquatic at SCCWRP.

Statistical Analysis

The relationships between environmental variables, toxin presence and chlorophyll *a* were determined using statistical packages of R, version 2.15.0 (2012-03-30) and the α level was set at 0.05 for all statistical analysis. Logistic regression was used to determine if chlorophyll *a* (log transformed, log10), was a significant predictor of microcystin from grab samples and additional environmental variables were included when data was available (alkalinity, total nitrogen, total phosphorus, cyanobacteria pigments, phycocyanin and phycoerythrin).

Results

Depressional Wetlands

Southern California Spring 2011-2013

Over three years, microcystins were detected at 25% of sites, though the percentage of sites varied widely from year to year (Table 3-6, Figure 3-2), as did the detectable concentrations (Table 3-7). Microcystins were detected in all three years, ranging from 12.5% of all sites in 2011 and 2013 to 47% in 2012. The range of microcystin concentrations found varied from below detection to 2.5 µg L^{-1} in 2011, up to 0.45 µg L^{-1} in 2012, and up to 22 µg L^{-1} in 2013. Interestingly, though the highest percentage of positive sites was in 2012, in no sites did the concentrations of microcystins exceed the California recreational action thresholds. In contrast, there were only 2 sites in the 2013 results where microcystins were detected, however, the concentrations at both sites exceeded the California recreation action thresholds (Table 1-1). Microcystins were detected at every wetland function type except stock ponds (Table 3-1). The percentage of toxic sites was highest for recreation and flood control wetland types, however, there were very few sites of that function included in the assessment. Golf course wetland types were also relatively toxic a third of the time and had the highest concentrations of microcystin detected for all depressional wetlands sites. Private property wetlands had very low concentrations of microcystins in 37% of the region. Appendix C shows pictures of blooms at several sites.

In contrast to microcystin, saxitoxin was detected at only one site during 2012-2013 (Santee Lakes Recreation Preserve Lake, site number 44 on Table 3-2 and Figure 1) and at very low concentrations (<0.4 µg L^{-1}).

Table 3-6. Summary of depressional wetlands toxin results collected during the spring assessments in 2011, 2012 and 2013 throughout Southern California.

Year sampled	% sites Microcystin Detected	% sites Saxitoxin detected	Range of MCY concentrations (µg L^{-1})
2011	12.5	NA	bd – 2.5
2012	47	5	bd – 0.45
2013	12.5	0	bd - 22
All years combined	25.4	2	

bd = below the method detection limit, NA = not analyzed

Table 3-7. Summary of MCY concentrations detected at depressional wetlands sites collected in the spring for all years (2011-2013).

Site Name	Region	MCY concentration (µg L^{-1})
Guajome Lake	9	2.5*
Santee	9	0.007
Covina Flood Control Basin	4	0.09
Dominguez Gap West Basin	4	0.03
Palm Lake Golf Course	4	0.07
Calle Roxanne Fallbrook	9	0.01
Emerald Isle Golf Course	9	0.01
Manchester Ave	9	0.07
San Dieguito River	9	0.02
Santee Lake	9	0.45
Lyons Valley Road	9	0.01
Simi Hills Golf Couse	4	22*
Vista Valencia Golf Course	4	1.5*

*Exceeded California Action Thresholds for recreational water (OEHHA 2012)

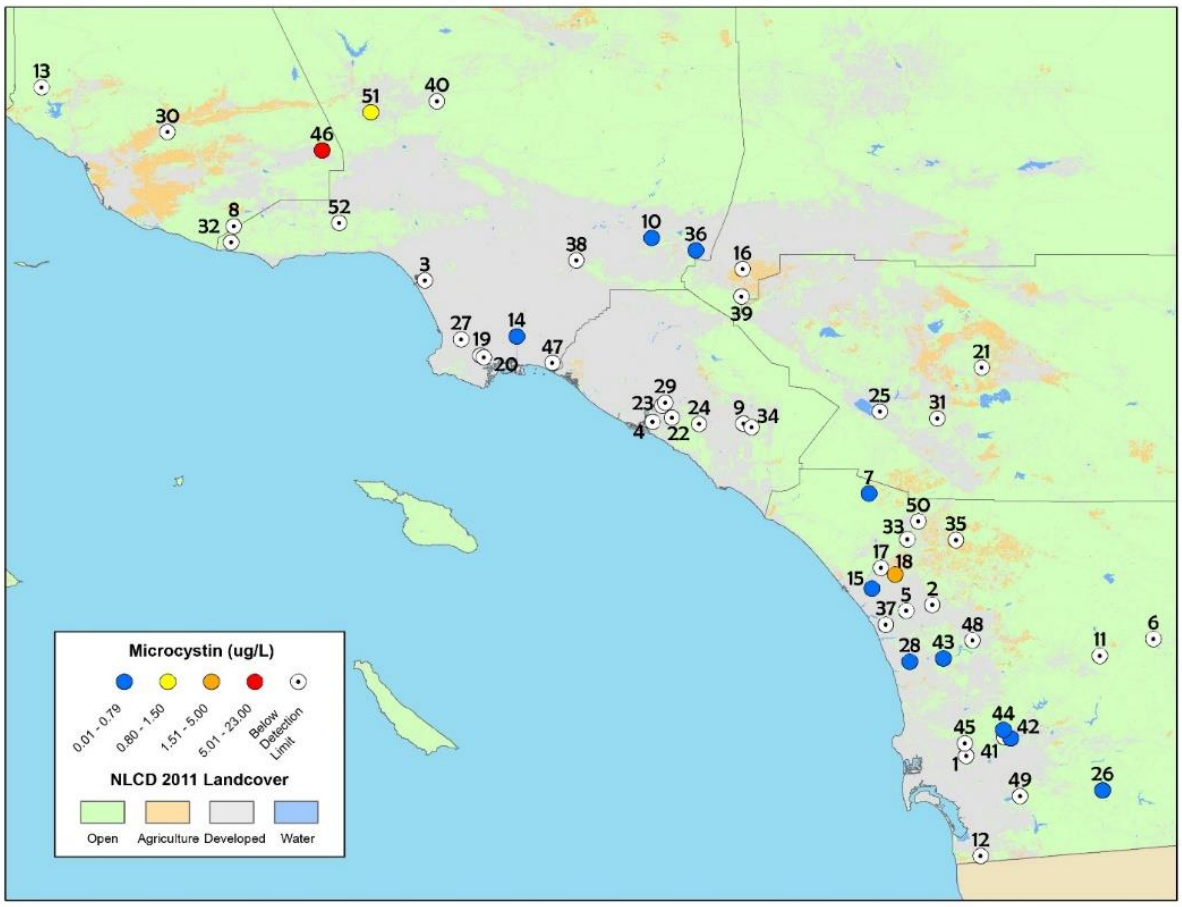


Figure 3-2. Map of the microcystin concentration results from grab samples collected in the spring depressional wetlands survey. The site numbers on the map correspond to Table 3-1. The white circles with a black dot in the center indicate sites that were below detection while blue circles indicate sites that had microcystin concentrations ranging from 0.01-0.79 µg L^{-1}. The yellow, orange and red circles indicate microcystin concentrations that exceeded the California recreational action threshold for microcystins (0.8 µg L^{-1}).

Statistical analysis revealed that chlorophyll *a* was not a statistically significant predictor of microcystin concentration (p-value, 0.3), neither across years nor in any individual year. Additionally, no statistically significant relationships were identified between microcystin concentration and environmental variables, such as alkalinity (p-value = 0.8), total nitrogen (p-value = 0.3), and total phosphorus (p-value = 0.7).

San Diego Surveys of Depressional Wetlands, Lakes, Reservoirs and Estuaries

A summary map of all 47 lentic sites (depressional wetlands, coastal wetlands and lakes and reservoirs) tested for microcystins in San Diego from 2011 to2014 is presented in Figure 3-3, with detailed analyses by year and habitat given in subsequent sections below. In the case of sites visited multiple times, the highest microcystin concentrations detected are presented. Overall, 31 sites had measureable microcystin concentrations while 16 sites had no measureable toxin at the time of sampling.

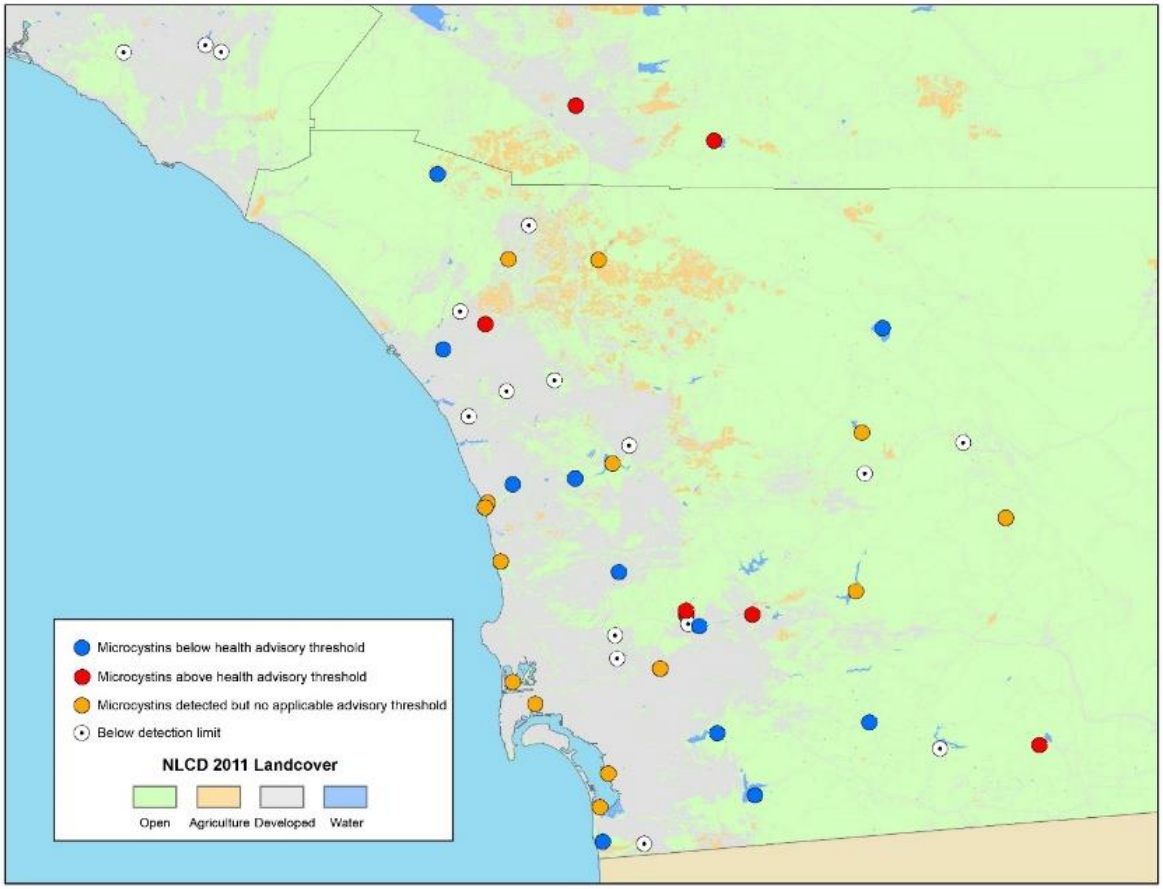


Figure 3-3. Map of all microcystin sample results from lentic waterbodies in San Diego (2011-2014). The colors correspond to the concentration of microcystins detected in relation to the California health advisory threshold. Red circles exceed the threshold, blue circles are within the threshold, yellow circles are SPATT data for which there is no applicable threshold, and white circles with a dot in the center were below the method detection limit.

San Diego 2012

The depressional wetlands sites sampled in the spring of 2012 in San Diego were revisited in the summer and early fall (Figure 3-4). During the spring of 2012, microcystins were detected at 60% of sites based on grab sample results. However, in the summer and fall time points, microcystins were detected at only 29% of sites based on grab sample results and 83% of sites based on SPATT results, a substantial difference (Figure 3-3, Table 3-8). The comparison of results between SPATT samplers and grab samples from all seasons in 2012 is illustrated in Table 3-8. The most common microcystin congener detected from the SPATT samples was MCY-LR, followed by MCY-RR and MCY-LA. MCY-YR was not detected in the samples (Table 3-9).

Table 3-8. The percentage of sites where microcystins were detected based on grab samples compared with SPATT samples in San Diego sites, sampled in 2012.

Season	% of toxic sites based on grab samples	% of toxic sites based on SPATT samples
Spring	60	Not collected
Summer	29	83
Fall	29	

Table 3-9. Results from SPATT samplers from San Diego sites sampled in 2012 for all microcystin congeners (in ng g^{-1}).

Site Name	Site Number on Map	Total MCY	MCY-LR	MCY-RR	MCY-YR	MCY-LA
Calle Roxanne Fallbrook	7	0.05	bd	0.05	bd	bd
Emerald Isle Golf Course	15	0.37	bd	0.37	bd	bd
Lyons Valley Rd Jamul	26	7.36	7.36	bd	bd	bd
Olive Hill Road Fallbrook	33	0.17	bd	0.17	bd	bd
Pala Rey Ranch	35	2.18	2.18	bd	bd	bd
San Dieguito River Calle Ambiente	43	0.06	bd	0.06	bd	bd
Santee Lakes Recreation Preserve Lake #7	44	97.02	81.18	bd	bd	15.84

bd = below the method detection limit.

Based on grab samples collected throughout all three seasons, saxitoxin was detected at one site (equivalent to 10% of sites), the Santee Lakes Recreation Preserve lake (site number 44 on the map in Figure 3-2), and in that one site the concentrations were extremely low (<0.02 µg L^{-1}).

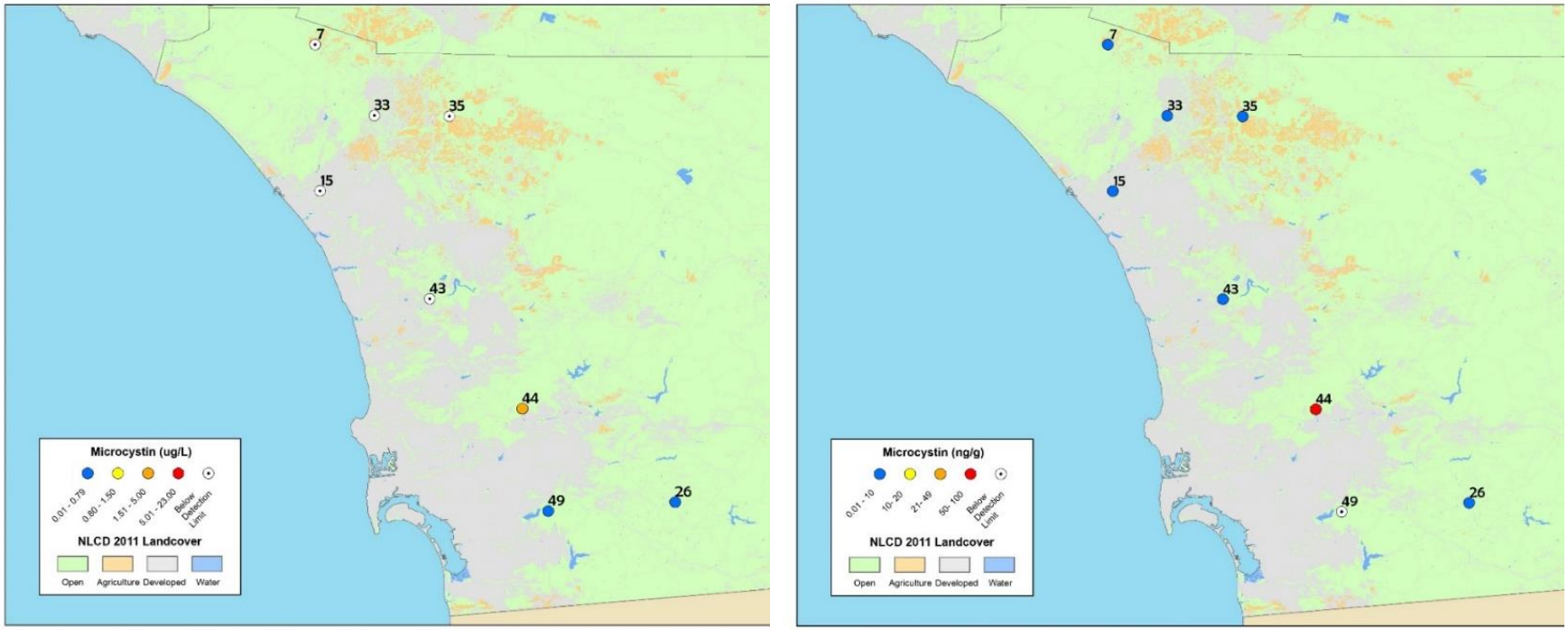


Figure 3-4. Maps of the microcystin concentrations detected from San Diego depressional wetlands samples collected in the summer and fall 2012 from particulate grab samples (left map) and SPATT sample results (right map). The site numbers on the map correspond to Table 3-1, and the highest microcystin concentrations detected are shown (sites were sampled multiple times for grab samples).

Lakes, Reservoirs, and Coastal Wetlands

San Diego 2013

The San Diego targeted survey of lakes, reservoirs, and coastal lagoons had similar results to the depressional wetlands such that SPATT sample results revealed microcystin to be more prevalent than grab sample results (Figure 3-5, with site numbers corresponding to Tables 3-3 and 3-4). The results from SPATT samplers show all sites had measurable microcystins during at least one-time point of the field survey and 7 sites were toxic throughout the entire study period (Table 3-10).

Table 3-10. Sites for which microcystins were detected throughout the entire study period in 2013 and the range of microcystin concentrations detected by both grab and SPATT samples.

Site Name	Range of microcystin concentrations determined from grab samples (µg L^{-1})	Range of microcystin concentrations determined from SPATT samples (ng g^{-1})
Lake Henshaw	Bd – 0.08	1.3 – 2.1
San Elijo Lagoon	bd	1.2 – 1.5
San Elijo Pond	bd	2.3 – 4.5
Lake Hodges	bd	0.5 – 2.7
Lake Miramar	bd – 0.1	5.6 – 7.0
San Diego Bay (near Naval Training Center)	bd	3.2 – 6.0
Tijuana River Estuary	bd – 0.09	2.7 – 100.8

bd = below the method detection limit.

The toxin concentrations ranged from below the method detection limit (bd) to 23.6 µg L^{-1} from grab sample results (Tables 3-10 and 3-12) and below the method detection limit (bd) to 100.8 ng g^{-1} from SPATT sample results (Table 3-11). Among SPATT samples, MCY-RR is the most commonly detected of the four analyzed microcystin congeners, detected at every site and in 64% of the samples overall (Table 3-11). MCY-LR was only detected at 6 sites and MCY- LA at 5 sites (out of the total 18 for which there are results). MCY-YR was not detected at any of the sites. There are no corresponding recreational toxin thresholds that are applicable to the SPATT results; therefore, health implications cannot be made from these results. SPATT samplers were deployed but never recovered at the lower Otay Reservoir site; therefore, no SPATT data are available for this site.

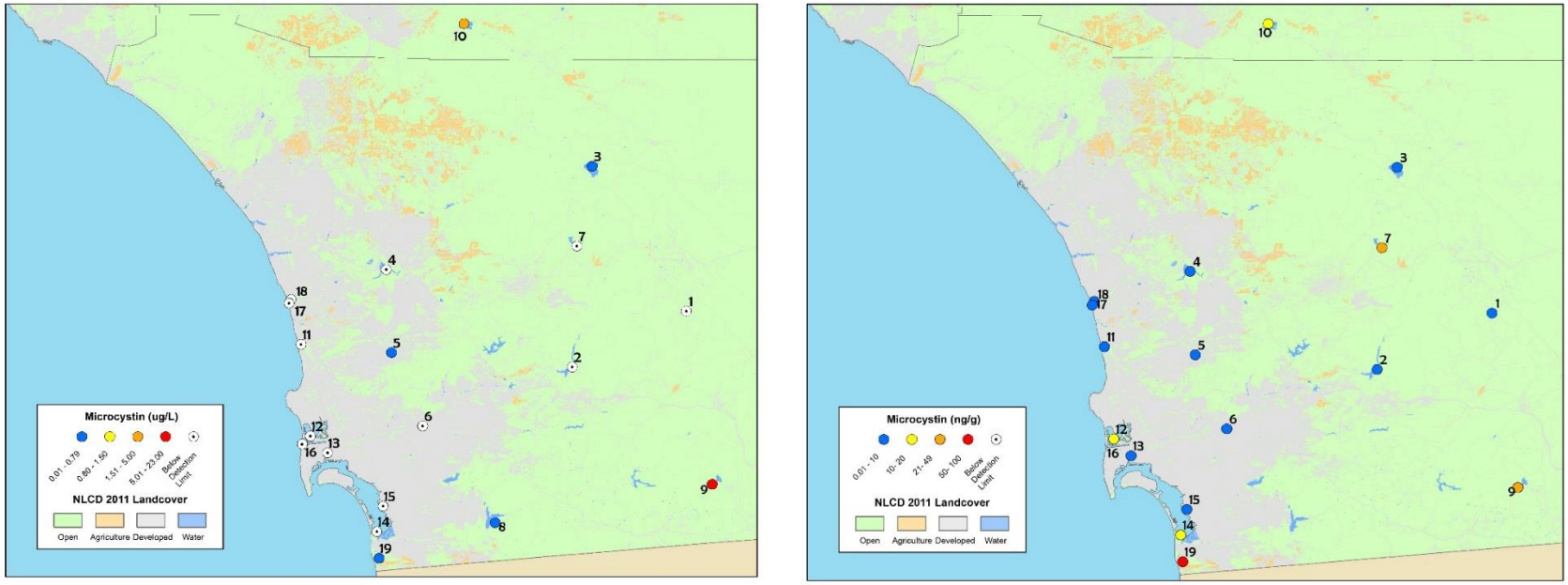


Figure 3-5. Maps of microcystin concentration detected from grab samples (left) and SPATT samples (right) collected in the summer of 2013. The highest concentration of microcystin is reported (sites were visited multiple times).

Table 3-11. Results from SPATT samplers from San Diego sites sampled in 2013 for all microcystin congeners (in ng g^{-1}). Concentrations on the top correspond to the summer samples from August and the bottom concentrations represent the early fall samples in late September/early October.

Site Name	Site Number on Map	Total MCY	MCY-LR	MCY-RR	MCY-YR	MCY-LA
Lakes and Reservoirs						
Lake Henshaw	3	2.1 1.3	bd bd	2.1 1.3	bd bd	bd bd
Cuyamaca Reservoir	1	0.9 NA	bd NA	0.9 NA	bd NA	bd NA
Lower Otay Reservoir	8	NA NA	NA NA	NA NA	NA NA	NA NA
Lake Murray	6	8.5 bd	bd bd	8.5 bd	bd bd	bd bd
Morena Reservoir	9	NA 44.7	bd 30.8	bd 13.9	bd bd	bd bd
Vail Lake	10	bd 13.3	bd 12.6	bd 0.7	bd bd	bd bd
Lake Hodges	4	2.7 0.5	bd bd	2.7 0.5	bd bd	bd bd
Lake Sutherland	7	44.3 bd	bd bd	16.3 bd	bd bd	28 bd
El Capitan Lake	2	1.6 bd	bd bd	1.6 bd	bd bd	bd bd
Lake Miramar	5	7.0 5.6	bd 4.9	6.0 0.6	bd bd	1.0 bd
Estuaries						
San Elijo Lagoon	17	1.5 1.2	bd bd	bd 1.2	bd bd	1.5 bd
San Elijo Pond	18	4.5 2.3	bd bd	4.5 2.3	bd bd	bd bd
Los Penasquitos Lagoon[1]	11	bd 2.3	bd bd	bd 2.3	bd bd	bd bd
San Diego Bay near Silver Strand Bikeway[2, 3, 4]	14	12.2 bd	bd bd	7.7 bd	bd bd	4.5 bd
San Diego Bay near Sweetwater[2, 3, 4]	15	bd 0.2	bd bd	bd 0.2	bd bd	bd bd
San Diego Bay near Naval Training Center[2, 3, 4]	13	3.2 6.0	bd 1.6	3.2 4.4	bd bd	bd bd
San Diego River Estuary	16	bd 2.4	bd bd	bd 2.4	bd bd	bd bd
Mission Bay	12	bd 15.1	bd 12.9	bd 2.2	bd bd	bd bd
Tijuana River Estuary	19	100.8 2.7	81.6 bd	bd 2.7	bd bd	19.2 bd

bd = below the method detection limit, NA = not analyzed.

In grab samples, only 4 sites (out of 19 surveyed) were positive for any of the nine microcystin congeners analyzed (Table 3-12). Within those 4 sites, MCY-LR and MCY-YR were the most common congeners, detected at 3 of the sites. Two sites exceeded the California recreational action thresholds for microcystins, Vail Lake (2.1 µg L^{-1}) and Morena Reservoir, (two time points, August and September, 6.1 µg L^{-1} and 23.6 µg L^{-1}, respectively). Anatoxin-a was not detected in any of the grab sample results. Bloom pictures from the 2013 survey are in Appendix D (coastal wetlands) and E (lakes and reservoirs).

Table 3-12. Microcystin concentration results for all microcystin congeners (in µg L^{-1}) from grab samples collected in 2013 from the San Diego field survey.

Site Name		Total MCY	LA	LR	RR	YR	LW	LY	des-LR	des-RR	LF*
Vail Lake	June*	2.1	bd	1.3	0.2	0.5	NA	NA	NA	NA	NA
	July	bd									
	Sept	bd									
Lake Henshaw	June*	0.1	bd	0.1	0.02	bd	NA	NA		NA	
	July	bd									
	Aug	0.08	bd	0.08	bd	bd	bd	bd	bd	bd	bd
	Sept	bd									
Morena Reservoir	July	0.02	bd	0.02	bd	bd	bd	bd	bd	bd	bd
	Aug	6.1	0.1	3.1	0.3	1.9	0.1	<0.1	bd	0.04	0.3
	Sept	23.6	10.1	9.9	1.2	1.3	0.2	bd	0.6	0.1	bd
Tijuana River Estuary	July	0.09	bd	bd	bd	0.09	bd	bd	bd	bd	bd
	Aug	0.05	bd	bd	bd	0.05	bd	bd	bd	bd	bd
	Sept	bd									

Abbreviations above are as follows: MCY-LA = LA, MYC-LR = LR, MCY-RR = RR, MCY-YR = YR, MCY-LW = LW, MCY-LY = LY, MC-desmethyl-LR = des-LR, MC-desmethyl-RR = des-RR, MCY-LF. bd = below the method detection limit. *These samples were collected during the site reconnaissance and analyzed at UCSC as described above for the Riverside samples. Therefore, only 4 MCY congeners were analyzed. NA = not analyzed.

Phycocyanin pigment (PC), an indicator of freshwater cyanobacteria, was detected in most samples analyzed throughout the study period except for a couple of samples from San Diego Bay (both Sweetwater and Naval Training Center sites). The concentrations ranged from 0.03 to over 5,000 µg L^{-1}. Phycoerythrin pigment (PE), an indicator of marine cyanobacteria, red algae and cryptophytes, were much less prevalent and only detected throughout the study period at three sites, Vail Lake, Lake Hodges and Lake Sutherland at low concentrations (<2 µg L^{-1}).

The statistical analysis revealed that chlorophyll *a* was a statistically significant predictor of microcystin concentration (p-value, 0.004). There was no statistically significant environmental predictor (such as alkalinity or nutrients) of microcystins (p-values > 0.05). The pigment results were similar in that neither PC nor PE concentrations were statistically significant predictors of microcystins.

San Diego 2014 Ad Hoc Survey in response to bloom reports

The potentially toxic HAB taxa were identified to genus level and where possible to species level and the results are summarized in Table 3-13. *Microcystis sp*., *Cylindrospermopsis spp*., and *Anabaena spp*. were the three most common genera identified in 40-50% of the sites. The species that were identified included *Cylindrospermopsis raciborskii, Anabaena variabilis, A. spiroides*. These results indicated the potential

for many types of cyanotoxins including microcystins, saxitoxin, cylindrospermopsin, anatoxin-a and β-Methylamino-L-alanine (BMAA).

The grab sample microcystin concentration results are shown in Table 3-14. MCY-LR was detected at every site and MCY-RR was detected at 3 of the 4 sites. All sites where microcystin was detected were well above the recreational action thresholds for California (0.8 µg L^{-1}) (OEHHA 2012) and ranged from 2.4-11.7 µg L^{-1}. These results are shown in Figure 3-6. There were 4 sites where potentially toxin producing genera were observed but microcystins were not detected. Anatoxin-a was not detected in any of the samples. Bloom pictures from the 2014 survey are in Appendix D (coastal wetlands) and Appendix E (lakes and reservoirs).

Table 3-13. Summary of potentially toxic species identified in San Diego Sites sampled in summer of 2014. The sample collection month in 2014 is listed next to the name of the waterbody. The genera and species identification of HAB organisms in the sample are listed, as well as total microcystins detected in µg L^{-1}. All samples analyzed for microcystins were also analyzed for nodularin and anatoxin-a, and all were below the limit of detection for those two toxins.

Month	Name	HAB Genera and Species Identification	Total MCY
August	Barrett Lake	*Cylindrospermopsis raciborskii,* *Cylindrospermopsis spp.,* *Anabaena spp.*	bd
August	Chollas Reservoir	Low abundance of non-nitrogen fixing filaments	NA
June	Discovery Lake	*Planktothrix sp., Anabaena variabilis, Anabaena spiroides,* *Cylindrospermopsis sp.* (minor component)	NA
August	Guajome Lake	*Cylindrospermopsis sp., Planktothrix sp.*	NA
June	Harveston Lake	NA	10.0
August	Harveston Lake	Sparce *Microcystis sp.*	NA
June	Lake Barbara	no cyanobacteria observed	bd
June	Lake Hodges	*Anabaena sp.*	bd
June	Lake Henshaw, downstream	*Microcystis sp.*	bd
August	Lake Morena	Mainly eukaryotes, shoreline dominated by *Microcystis spp.*	NA
August	Lake Poway	Sparse *Microcystis sp.* colonies	NA
June	Lake Sutherland	*Microcystis sp.*	bd
June	Lindo Lake	*Planktothrix spp., Anabaena variabilis, Anabaena sp.* *Cylindrospermopsis sp.* (minor component)	2.5
August	Lindo Lake	*Planktothrix sp.* and *Cylindrospermopsis spp.* dominant *Microcystis sp.,* and *Anabaena spp.* observed	2.4
July	Santee Lake #5	*Microcystis sp*. floating on surface, *Cylindrospermopsis sp.* dominated water column, *Cylindrospermopsis raciborskii* and *Anabaena spiroides*	11.7

NA = not analyzed; bd = below the method detection limit.

Table 3-14. Grab sample results of microcystin analysis for all 2014 samples analyzed.

Name	Total MCY	MCY-LR	MCY -RR	MCY -YR	MCY -LA	MCY -desmethyl -RR	MCY-desmethyl -LR	MCY -LF	MCY -LW	MCY -LY
Harveston Lake	10.0	2.23	4.49	2.99	bd	0.7	0.31	bd	bd	bd
Lindo Lake (June)	2.56	1.26	0.844	b.d.	bd	bd	bd	bd	bd	0.46
Lindo Lake (August)	2.44	1.11	0.55	0.06	bd	0.08	0.08	bd	bd	0.56
Santee Lake	11.71	7.69	b.d.	b.d.	3.3	b.d.	0.5	0.06	0.09	0.09

bd = below the method detection limit.

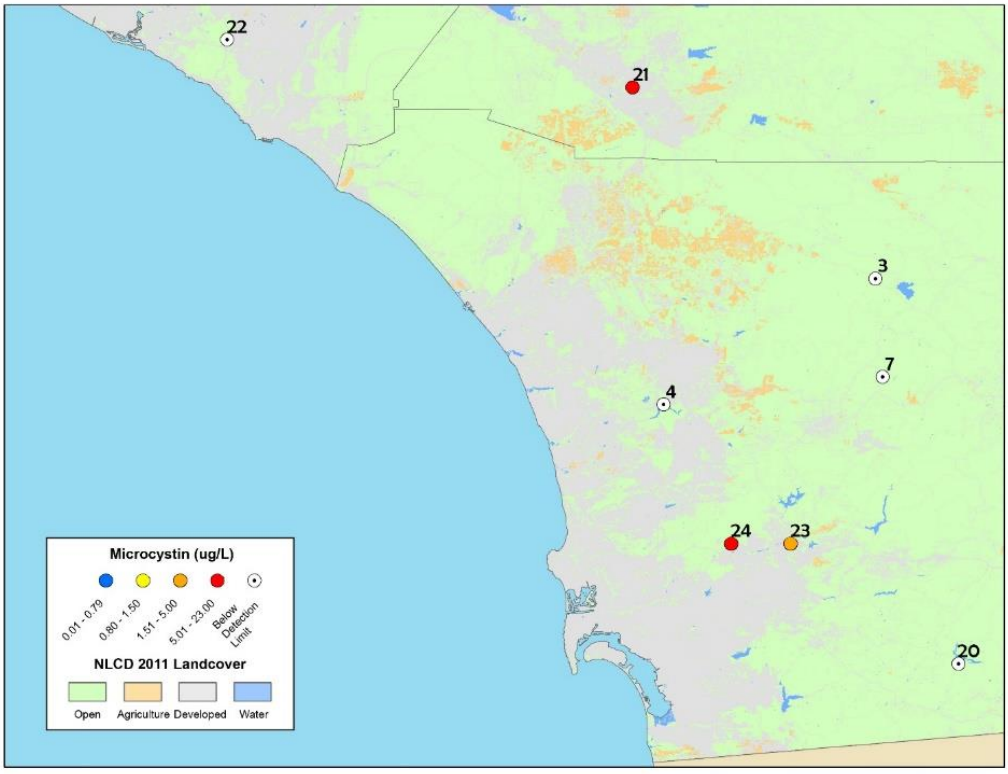


Figure 3-6. Map of microcystin concentration results from grab samples collected in the summer, 2014 in the San Diego event response survey. The highest concentration of microcystin is reported for sites that were visited multiple times.

Riverside 2014

The results from the lake samples collected in Riverside County in 2014 are summarized in Table 3-15. Lake Elsinore had 2 measurable cyanotoxins present, microcystins and cylindrospermopsin, but anatoxin-a was not detected. The clindrospermopsin exceeded the California recreational health advisory level (4 µg L^{-1}) (OEHHA 2012). The potential toxin producing genera identified were *Anabaena sp*. and *Cylindrospermopsis spp*. Canyon Lake was toxic for all three cyanotoxins tested, but did not exceed the California recreational health advisory levels for any toxin. The potential toxin producing species identified were *Anabaena sp*. and *Raphidopsis sp*. Lake Menifee had a fish kill in April and the lake had been treated with copper sulfate at that time due to a bloom of the golden alga, *Prymnesium* (D. Caron, pers. comm.). Two cyanotoxins were detected at Lake Menifee, cylindrospermopsin and anatoxin-a and potential toxin producing genera identified in the sample was *Cylindrospermopsis spp.* and *Raphidopsis sp.* Pictures taken at Lake Elsinore and Canyon Lake are in Appendix E.

The results from the San Joaquin Marsh sample were the highest detected within California to date, total microcystins were 36,549 µg L^{-1} and the concentration of each congener analyzed were the following: MCY-LR 32,540 µg L^{-1}, MCY-RR 2,487 µg L^{-1}, MCY-YR 721 µg L^{-1}, MCY-LA 801 µg L^{-1}. *Microcystis sp.* was identified through microscopy and dominated the sample, which resembled a monoculture.

Table 3-15. Summary of potentially toxic genera and species identified in Riverside Lakes sampled on May 21, 2014 and the San Joaquin Marsh sample collected on May 20, 2015. The total microcystins (MCY), cylindrospermopsin (CYN) and anatoxin-a (ANA) detected in µg L^{-1}.

Name	Site Number on Map	HAB Genera and Species Identification	Total MCY	Total CYN	Total ANA
Lake Elsinore	26	*Anabaena sp., Cylindrospermopsis spp.*	0.01	4.1	bd
Canyon Lake	25	*Anabaena sp., Raphidopsis sp.*	0.01	2.7	4.6
Lake Menifee	27	*Cylindrospermopsis sp. Raphidopsis sp.*	bd	2.9	3.6
San Joaquin Marsh	28	*Microcystis sp.*	36,549	NA	NA

bd = below the method detection limit; NA = not analyzed.

Discussion

Microcystin Prevalence

Depressional Wetlands Assessment and San Diego Regional Survey

Microcystins were detectable and prevalent in all types of lentic waterbodies surveyed across the land-sea continuum in Southern California. Microcystin concentrations were generally low across all habitats, exceeding California recreational health thresholds for acute toxicity at only a small number of sites, similar to findings in the California Sacramento-San Joaquin Delta (Lehman et al. 2005, Berg and Sutula 2015). However, the persistence of detectable microcystins in repeat samplings over years and seasons underscores the likelihood of a low-level, but chronic risk through direct exposure as well as via bioaccumulation and the potential transfer of toxin to higher trophic levels. This chronic threat is relevant for both human health and wildlife, from depressional wetlands to lakes, reservoirs and coastal lagoons. The sites in this study were chosen due to the number beneficial uses and the high frequency of use (Tables 3-3, 3-4). All of the lake and reservoir sites are used for municipal and domestic water supply (including drinking water), agricultural water supply for farming, irrigation etc. (except Lake Miramar) and both contact and non-contact water recreation. These particular beneficial uses pose the most concern due to the toxin exposure pathways for humans, such as ingestion (through drinking water or contaminated shellfish or vegetables), inhalation of aerosolized toxins and dermal contact (Mohamed et al. 2009, Backer et al. 2008, 2009, Levesque et al. 2014). Aquatic-life related uses (i.e. preservation or enhancement of aquatic and terrestrial habitats, wildlife, vegetation and invertebrates) are also of concern due to the bioaccumulation and transfer of toxins to higher trophic levels, such as warm and cold freshwater habitats and wildlife habitats. Among the highest concentrations found were in highly frequented recreational sites such as golf course ponds and the recreational campground, Guajome Regional Park. The park is a highly used recreational site (activities include camping, hiking, fishing, playgrounds), and it is a refugium for a wide variety of migratory birds.

The regional assessment of depressional wetlands was conducted in the spring, however, previous studies (Lehman et al. 2008, Moisander et al. 2009, Gibble and Kudela 2014) have observed seasonal patterns that indicate summer and fall seasons are the most conducive to cyanobacteria growth, bloom formation and toxin production, suggestive that the current study results may underestimate the prevalence of microcystin in depressional wetlands.

Traditional grab samples have been shown to miss toxic events or underestimate toxin prevalence, and passive samplers such as SPATT may be a better indicator of toxin presence within a waterbody (Lane et al. 2009, Kudela 2011). In this study, SPATT was successfully used as a monitoring and assessment tool to determine the prevalence and persistence of microcystins. SPATT results indicated a much higher prevalence throughout the region than the grab sample results, again, pointing towards the probability of a low-level but chronic exposure via direct as well as indirect pathways. There are several explanations for the differences in results from grab and SPATT samples. First, grab samples measure toxin presence on the day and at the time of sample collection while SPATT samplers are deployed for weeks to a month, are time-integrative, and are, therefore, more likely to capture ephemeral toxin events. Second, grab samples measure particulate (intracellular) toxin whereas the SPATT samplers measure dissolved (extracellular) toxin. While there could be some difference based on these fractions, comparative studies have shown that when the grab samples collected at deployment and recovery of SPATT samplers are time-averaged, there is good correlation with SPATT results (Kudela 2011). The range of SPATT-derived concentrations for microcystins were mostly less than 50 ng g^{-1}, and based on SPATT analyses from other studies (Kudela 2011), correlate to particulate samples in the range of <1 µg L^{-1}, which is close to

regulatory action levels for acute toxicity. These results support the conclusion of low level, chronic risk and exposure to microcystins in these waterbodies.

San Diego Ad Hoc Bloom Response Survey

The San Diego bloom response survey results indicated almost half of the reported blooms had very high concentrations of microcystins, and all toxin concentrations were above the recreational action thresholds (0.8 µg L-1; OEHHA 2012). These lakes and reservoirs had acute toxin concentrations that have implications for human, wildlife and domestic pet health and in contrast to the 2013 survey, did not indicate a low level risk but rather a high level chronic risk. The HAB genera and species identification indicated that microcystin and cylindrospermopsin producing cyanobacteria were prevalent in the region, and suggests that both cylindrospermopsin (not analyzed) and saxitoxins (not analyzed) could have been present in those waterbodies.

The San Joaquin Marsh sample had the highest microcystin concentration detected to date in California. Other known toxin hotspots, such as Pinto Lake have 1,000 µg L^{-1} microcystins annually with maximum values reaching 3,500 µg L^{-1} and Lake Chabot had a maximum concentration of 11,000 µg L^{-1} (Kudela 2011, R. Kudela, pers. comm.). This site is open to the public, not for direct recreational contact, but rather as indirect contact with trails alongside the site and a nature center used by the public.

Chlorophyll a as a toxin indicator

Chlorophyll *a* has been shown to be a meaningful screening variable in lentic waterbodies for the risk of cyanotoxins, as it is cheaper and easier to collect and analyze (or remotely sense) than toxin concentration (Kudela 2011, Otten et al. 2012, Magrann et al. 2015). In this study, we found a significant correlation between chlorophyll *a* and microcystin concentrations in lakes, reservoirs, and coastal wetlands, however, no such relationship was identified in the depressional wetlands waterbody type. There are two possible reasons for the lack of correlation. First, the depressional wetlands study design was based on condition assessments used to evaluate chemical contaminants that rely on a one-time grab sample to determine the condition of a waterbody and the stressors present. This type of design is not appropriate for HAB toxins as they are biologically produced, and thus toxin production can change on the order of days. Second, as discussed above, the grab sample results underestimated microcystin prevalence in depressional wetlands and were not a good indicator of toxin presence in these systems.

Other cyanotoxins and multiple stressors

The single day of sampling in the Riverside area lakes (Table 3-15) documented the first detection of cylindrospermopsin and anatoxin-a in Southern California. Cylindrospermopsin was detected at every site tested, and was above the recreational action threshold at Lake Elsinore. *Cylindrospermopsis spp*., *Anabaena spp*., and *Raphidopsis sp.* were the three potential toxin producing genera identified in these lakes (Table 3-15). The co-occurrence of multiple cyanotoxins detected simultaneously indicated multiple toxin stressors present in these waterbodies. Previous studies of cyanobacteria species identification in 2003 and 2010 in Lake Elsinore indicate the historical potential for multiple cyanotoxins (Oza 2003, Tobin 2011). Cyanobacteria were the dominant phytoplankton group found during the summer and fall seasons in 2010, and *Cylindrospermopsis raciborskii* was detected throughout all seasons (a potential producer of cylindrospermopsin and saxitoxins) (Tobin 2011).

The genera and species identifications from the 2014 San Diego survey indicate a high potential for multiple cyanotoxins to be routinely produced and co-occurring HAB species to be present in these systems. There were a wide variety of potential toxin producing cyanobacteria identified, but the most

common genera were *Microcystis* sp. and *Cylindrospermopsis spp*. The combination of multiple cyanotoxin producing genera/species identified, and the detection of multiple cyanotoxins simultaneously, suggests a poor understanding of the commonly occurring toxins, and the toxin-producing organisms. As with the interplay between acute and chronic risk, the interaction of multiple stressors on humans and other wildlife make the risk more uncertain, because most regulatory action levels are typically based on exposures to single organisms, and are based on single toxins.

Because of the dominance of cyanobacteria in these lentic waterbodies and the ubiquitous and persistent detection of microcystins in these heavily utilized aquatic habitats, more frequent monitoring should be considered in order to better quantify this risk to the beneficial uses of these waterbodies. Future studies, assessment and monitoring programs need to characterize the cyanobacteria species that are producing toxins and determine which toxins and concentration ranges are routinely present in these systems. The potential for bioaccumulation of multiple cyanotoxins into the food web suggest the influence of toxic cyanobacteria blooms are a much more complex stressor than presently recognized and should be considered a high priority measurement to be included in condition assessments and water quality monitoring programs. Both high acute concentrations and low chronic concentrations of cyanotoxins should be considered predominant stressors within lentic waterbodies. Management and restoration decisions should include considerations of cyanobacteria growth and toxin production.

Recommendations

1) **Cyanotoxins should be included in ambient water quality monitoring and assessment programs for all lentic waterbodies**, a challenge in that State Water Board-sponsored surface water ambient monitoring programs only regularly sample the water quality of wadeable streams. Both USGS and EPA have recommended cyanotoxins to be prioritized in ambient water quality monitoring programs. Fifteen cyanotoxins were prioritized as part of the National Target Analyte Strategy (NTAS) workgroup effort due to human-health concerns, and 12 of these have been listed as NTAS Tier 1 constituents, categorizing them as the highest priority for ambient monitoring (Olsen et al. 2013). These 12 include anatoxin-a, cylindrospermopsin, microcystin variants (LA, LF, LR, LW, LY, RR and YR), saxitoxins, nodularin, and deoxyclindrospermopsin. Lyngbyatoxin was listed in Tier 2, intermediate priority for ambient monitoring (Olsen et al. 2013). Anatoxin-a, cylindrospermopsin and microcystin-LR are also listed as part of the U.S. Environmental Protection Agency Contaminant Candidate List 3 (CCL3) for drinking water. The sampling approach for cyanotoxins should be carefully considered as the results from this study show that traditional chemical contaminant assessment approaches consisting of one-time grab samples will underestimate toxin prevalence and will not protect human health or provide insight on the persistence of cyanotoxins. Care should be taken to distinguish whether the objective is to characterize human versus ecosystem health risk to cyanotoxins, as the method of sampling differs depending on the focus. Monitoring to assess human health risk should be more frequent than traditional chemical contaminant sampling because cyanotoxins are a biological contaminant, and therefore, the detection and concentrations can fluctuate on the order of days. A tiered sampling approach should be formally developed in order to ensure efficient use of resources for long-term routine monitoring. An example of such a program is the Marine Biotoxin Monitoring Program which monitors marine HABs. This program uses a tiered system. The first tier is visual microscopic analysis in the field to determine the relative abundance of potential toxin producing HABs. If HABs are present, toxin samples are analyzed (second tier); however, if there are no HABs observed in the water sample, toxin samples are not analyzed. Additionally, toxin field strip tests are also utilized to determine a course estimation of toxin

concentration. This type of system should be developed for cyanotoxins in order to make efficient use of resources.

Future research and monitoring efforts should focus on several issues including: 1) temporal/seasonal variability in toxin production (i.e. when to sample, and determination of how frequently sampling needs to occur to minimize risk); 2) the necessary level of sampling effort (i.e., how many samples are needed to account for spatial variability, patchiness and extent of the bloom); and 3) factors that may trigger or exacerbate toxin production. Management and restoration decisions should include considerations of cyanobacteria growth and toxin production.

2) **Develop capacity to analyze, interpret and use passive sampling technologies in cyanotoxin monitoring.** Monitoring and assessment tools such as passive samplers (i.e. SPATT) were successfully used to determine prevalence of microcystins and should be incorporated to future ambient monitoring and assessment programs. These sampling devices are particularly useful at capturing ephemeral events that traditional grab samples do not capture, and provide a more comprehensive view of cyanotoxins in a waterbody or region. Currently, most analytical laboratories in California do not provide analytical services for SPATT samplers, despite the published methodology and similar instrumentation used for grab sample analysis (Lane et al. 2010, Kudela 2011). A technology training for State, County and Local laboratories is needed in order to accommodate the growing use of SPATT sampling throughout California. Additionally, the use of SPATT data for waterbody advisories should be evaluated. At the time of this writing, the California Blue-Green Algae Voluntary Guidance Document (outlines process for posting warnings in notifying the public of waterbodies experiencing a bloom) is being updated, and SPATT results could be used as part of the decision tree to determine the signage for posting and de-posting a waterbody.

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APPENDICES

Appendix A: Frequency of occurrence and biovolumes of potential microcystin-producing species in benthic samples from California wadeable streams

Frequency of occurrence and biovolumes of potential microcystin-producing species (based on membership in a toxigenic genus; Table 2-1) encountered in benthic samples from California wadeable streams. "Rank" refers to decreasing order of a "prevalence index", which is the product of the number of reaches where the species was observed and its mean biovolume across reaches. Species in bold exhibited the highest prevalence indices. "NA" indicates that biovolume data are not available because the species in question was recorded only in the epiphyte fraction of composite samples and/or in the qualitative samples, thus biovolume was non-quantifiable.

Species	Rank (of 98 species total)	# of reaches where observed	Mean biovolume across reaches ($\mu m^3\ cm^{-2}$)	Median biovolume across reaches ($\mu m^3\ cm^{-2}$)	Maximum biovolume across reaches ($\mu m^3\ cm^{-2}$)
Anabaena aequalis Borge	28	6	3.61E+08	4.60E+08	5.23E+08
Anabaena californica Borge	32	1	1.64E+09	1.64E+09	1.64E+09
Anabaena cylindrica Lemmerm.	20	5	1.29E+09	1.32E+09	2.54E+09
Anabaena inaequalis (Kütz.) Bornet et Flahault	34	11	1.09E+08	5.10E+06	5.82E+08
***Anabaena iyengarii* Bharadwaja**	**3**	**5**	**2.09E+11**	**3.84E+07**	**8.37E+11**
Anabaena oscillarioides Bory ex Bornet et Flahault	8	44	8.97E+08	1.88E+07	1.40E+10
Anabaena sp. 1	21	156	3.27E+07	1.69E+05	1.04E+09
Anabaena sp. 2	38	28	3.01E+07	5.92E+05	2.12E+08
Anabaena sp. 5	52	2	1.16E+08	1.16E+08	2.31E+08
Anabaena sp. 8	83	3	2.64E+05	4.88E+04	7.23E+05
Anabaenopsis elenkinii V. V. Mill.	87	1	4.06E+05	4.06E+05	4.06E+05
Arthrospira sp. 1	56	10	1.17E+07	2.69E+03	1.17E+08
Coelomoron pusillum (Goor) Komárek	89	10	3.31E+04	2.28E+04	9.01E+04
Coelomoron sp. 1	96	1	4.86E+03	4.86E+03	4.86E+03
Cylindrospermum sp. 1	27	14	1.78E+08	4.16E+05	1.41E+09
Cylindrospermum stagnale (Kütz.) Bornet et Flahault	15	25	5.59E+08	3.20E+07	3.24E+09
Dolichospermum flosaquae (Bréb. ex Bornet et Flahault) P. Wacklin, L. Hoffm. et Komárek	81	2	5.53E+05	5.53E+05	8.24E+05
Dolichospermum planctonicum (Brunnth.) P. Wacklin, L.Hoffm. et Komárek	46	10	4.72E+07	5.87E+05	4.66E+08
Dolichospermum solitarium (Kleb.) P. Wacklin, L. Hoffm. et Komárek	77	3	5.30E+05	2.28E+05	1.30E+06
Dolichospermum sp. 1	95	1	3.44E+04	3.44E+04	3.44E+04
Dolichospermum sp. 2	53	1	2.29E+08	2.29E+08	2.29E+08
Geitlerinema acuiforme (Skuja) Anagn.	92	3	5.29E+04	6.13E+03	1.49E+05

Species	Rank (of 98 species total)	# of reaches where observed	Mean biovolume across reaches ($\mu m^3\ cm^{-2}$)	Median biovolume across reaches ($\mu m^3\ cm^{-2}$)	Maximum biovolume across reaches ($\mu m^3\ cm^{-2}$)
Geitlerinema amphibium (C.Agardh ex Gomont) Anagn.	24	45	7.92E+07	2.37E+05	1.87E+09
Geitlerinema ionicum (Skuja) Anagn.	67	12	1.12E+06	2.06E+04	1.15E+07
Geitlerinema lemmermannii (Wołosz.) Anagn.	94	1	1.33E+05	1.33E+05	1.33E+05
Geitlerinema sp. 1	86	7	8.15E+04	3.49E+04	2.79E+05
Geitlerinema sp. 2	88	2	1.82E+05	1.82E+05	3.36E+05
Geitlerinema splendidum (Grev. ex Gomont) Anagn.	36	15	6.82E+07	9.62E+04	8.08E+08
Gloeotrichia natans Rabenh. ex Bornet et Flahault	16	10	1.06E+09	3.32E+08	3.84E+09
Hapalosiphon hibernicus W. West et G. S. West	30	15	1.30E+08	7.02E+06	1.42E+09
Hapalosiphon sp. 1	80	1	1.16E+06	1.16E+06	1.16E+06
***Leptolyngbya foveolarum* (Mont. ex Gomont) Anagn. et Komárek**	**2**	**786**	**1.43E+09**	**5.89E+04**	**1.08E+12**
Leptolyngbya granulifera (Copel.) Anagn.	37	26	3.72E+07	6.45E+04	4.86E+08
Leptolyngbya laminosa (Gomont) Anagn. et Komárek	45	9	5.26E+07	1.28E+05	3.96E+08
Leptolyngbya nostocorum (Bornet ex Gomont) Anagn. et Komárek	85	18	3.91E+04	5.80E+03	2.76E+05
Leptolyngbya notata (Schmidle) Anagn. et Komárek	61	133	4.56E+05	4.33E+04	2.89E+07
Leptolyngbya sp. 1	72	26	9.63E+04	4.80E+04	6.07E+05
Leptolyngbya tenuis (Gomont) Anagn. et Komárek	29	362	5.85E+06	2.50E+04	5.20E+08
Leptolyngbya valderiana (Gomont) Anagn. et Komárek	23	10	3.80E+08	1.32E+06	2.49E+09
Microcystisfirma (Kütz.) Schmidle	43	16	3.11E+07	2.68E+04	4.62E+08
Nostoc caeruleum var. *planctonicum* (V.S.Poretsky & V.K.Tschernow) B.A.Whitton (Syn. *Nostoc kihlmanii* Lemmer.)	41	1	6.20E+08	6.20E+08	6.20E+08
Nostoc carneum C. Agardh ex Bornet et Flahault (Syn. *Nostoc spongiaeforme* C. Agardh ex Bornet et Flahault)	18	11	7.87E+08	4.98E+08	3.12E+09
Nostoc commune Vaucher ex Bornet et Flahault	17	6	1.56E+09	1.65E+09	2.88E+09
Nostoc linckia (Roth) Bornet ex Bornet et Flahault	84	1	7.89E+05	7.89E+05	7.89E+05
Nostoc microscopicum Carmich. ex Bornet et Flahault	31	5	3.28E+08	3.94E+05	1.62E+09
Nostoc muscorum C. Agardh ex Bornet et Flahault	79	2	7.42E+05	7.42E+05	7.42E+05
Nostoc paludosum Kütz. ex Bornet et Flahault	64	1	2.82E+07	2.82E+07	2.82E+07

Species	Rank (of 98 species total)	# of reaches where observed	Mean biovolume across reaches ($\mu m^3\ cm^{-2}$)	Median biovolume across reaches ($\mu m^3\ cm^{-2}$)	Maximum biovolume across reaches ($\mu m^3\ cm^{-2}$)
***Nostoc parmelioides* Kütz. ex Bornet et Flahault**	**4**	**60**	**1.66E+09**	**4.91E+08**	**1.75E+10**
Nostoc pruniforme C.Agardh ex Bornet et Flahault	60	5	1.41E+07	1.41E+07	2.82E+07
Nostoc punctiforme (Kütz.) Har.	12	23	1.16E+09	2.44E+07	1.24E+10
Nostoc sp. 1	22	124	3.70E+07	1.11E+05	8.53E+08
Nostoc sp. 2	97	1	NA	NA	NA
***Nostoc sphaericum* Vaucher ex Bornet et Flahault**	**5**	**3**	**2.30E+10**	**2.86E+09**	**6.58E+10**
***Nostoc verrucosum* Vaucher ex Bornet et Flahault**	**1**	**265**	**6.40E+09**	**2.43E+08**	**5.33E+11**
Oscillatoria anguina Bory ex Gomont	63	2	1.61E+07	1.61E+07	3.22E+07
Oscillatoria jenensis G.Schmid	78	1	1.50E+06	1.50E+06	1.50E+06
Oscillatoria limosa C. Agardh ex Gomont	26	24	1.13E+08	1.15E+07	9.82E+08
Oscillatoria princeps Vaucher ex Gomont	98	1	NA	NA	NA
Oscillatoria sp. 1	57	13	7.95E+06	1.26E+05	9.15E+07
Oscillatoria sp. 2	59	2	3.90E+07	3.90E+07	7.60E+07
Oscillatoria tenuis C. Agardh ex Gomont	73	3	7.37E+05	7.37E+05	1.42E+06
Oscillatoria ucrainica Vladimirova	68	10	8.78E+05	1.63E+05	6.92E+06
Phormidium ambiguum Gomont	19	30	2.42E+08	3.25E+05	2.32E+09
Phormidium autumnale Gomont	14	43	4.85E+08	2.95E+06	1.09E+10
Phormidium bulgaricum (Komárek) Anagn. et Komárek	50	1	3.52E+08	3.52E+08	3.52E+08
Phormidium chalybeum (Mert. ex Gomont) Anagn. et Komárek	42	25	2.20E+07	8.08E+05	3.51E+08
Phormidium formosum (Bory ex Gomont) Anagn. et Komárek	48	8	4.57E+07	3.45E+05	2.27E+08
Phormidium incrustatum (Nägeli) Gomont ex Gomont	47	12	3.15E+07	1.75E+06	1.58E+08
Phormidium inundatum Kütz. ex Gomont	49	14	2.52E+07	2.93E+05	3.29E+08
Phormidium irriguum (Kütz. ex Gomont) Anagn. et Komárek	44	7	6.87E+07	2.89E+04	3.43E+08
Phormidium lucidum Kütz. ex Gomont	70	5	1.56E+06	3.43E+05	6.61E+06
Phormidium nigroviride (Thwaites ex Gomont) Anagn. et Komárek	65	2	1.24E+07	1.24E+07	2.23E+07
Phormidium retzii (C. Agardh) Kütz. ex Gomont	25	46	6.89E+07	4.40E+05	8.66E+08
Phormidium sp. 1	69	60	1.34E+05	7.31E+04	1.67E+06
Phormidium sp. 2	74	14	1.41E+05	4.01E+04	1.29E+06
Phormidium sp. 3	62	16	2.40E+06	1.50E+05	2.34E+07
Phormidium sp. 4	82	4	2.13E+05	1.18E+05	6.18E+05
Phormidium sp. 5	75	5	3.63E+05	1.70E+05	9.80E+05
Phormidium sp. 6	93	1	1.39E+05	1.39E+05	1.39E+05
***Phormidium subfuscum* Kütz. ex Gomont**	**6**	**250**	**2.29E+08**	**9.40E+05**	**1.75E+10**

Species	Rank (of 98 species total)	# of reaches where observed	Mean biovolume across reaches ($\mu m^3 cm^{-2}$)	Median biovolume across reaches ($\mu m^3 cm^{-2}$)	Maximum biovolume across reaches ($\mu m^3 cm^{-2}$)
Phormidium terebriforme (C. Agardh ex Gomont) Anagn. et Komárek	91	2	8.26E+04	8.26E+04	8.26E+04
Phormidium uncinatum (C.Agardh) Gomont ex Gomont	54	7	3.15E+07	3.28E+05	1.73E+08
Phormidium versicolor Wartm. ex Gomont	39	8	9.74E+07	4.40E+06	6.40E+08
Rivularia atra Roth ex Bornet et Flahault	58	6	1.47E+07	3.82E+06	4.74E+07
Rivularia biasolettiana Menegh. ex Bornet et Flahault	66	1	1.83E+07	1.83E+07	1.83E+07
Rivularia globiceps G. S. West	40	2	3.85E+08	3.85E+08	7.22E+08
Rivularia haematites (DC.) Bornet et Flahault	10	5	5.58E+09	1.21E+07	2.72E+10
Rivularia manginii Frémy	51	1	2.90E+08	2.90E+08	2.90E+08
Rivularia minutula (Kütz.) Bornet et Flahault	13	35	7.34E+08	9.61E+06	1.32E+10
Rivularia polyotis Roth ex Bornet et Flahault	11	2	1.39E+10	1.39E+10	1.60E+10
Rivularia sp. 1	90	1	2.48E+05	2.48E+05	2.48E+05
Tolypothrix distorta Kütz. ex Bornet et Flahault	7	140	3.16E+08	1.91E+07	7.62E+09
Tolypothrix lanata Wartm. ex Bornet et Flahault	71	2	3.40E+06	3.40E+06	3.40E+06
Tolypothrix penicillata (C. Agardh) Thuret ex Bornet et Flahault	55	3	6.31E+07	1.93E+07	1.69E+08
Tolypothrix sp. 1	76	2	9.02E+05	9.02E+05	1.75E+06
Tolypothrix tenuis Kütz.	35	9	1.18E+08	1.52E+07	4.91E+08
Trichormus fertilissimus (C. B. Rao) Komárek et Anagn.	33	5	2.92E+08	3.81E+08	4.75E+08
Trichormus variabilis (Kütz. ex Bornet & Flahault) Komárek et Anagn.	9	9	3.10E+09	1.19E+07	1.98E+10

A note on cyanobacteria taxonomy

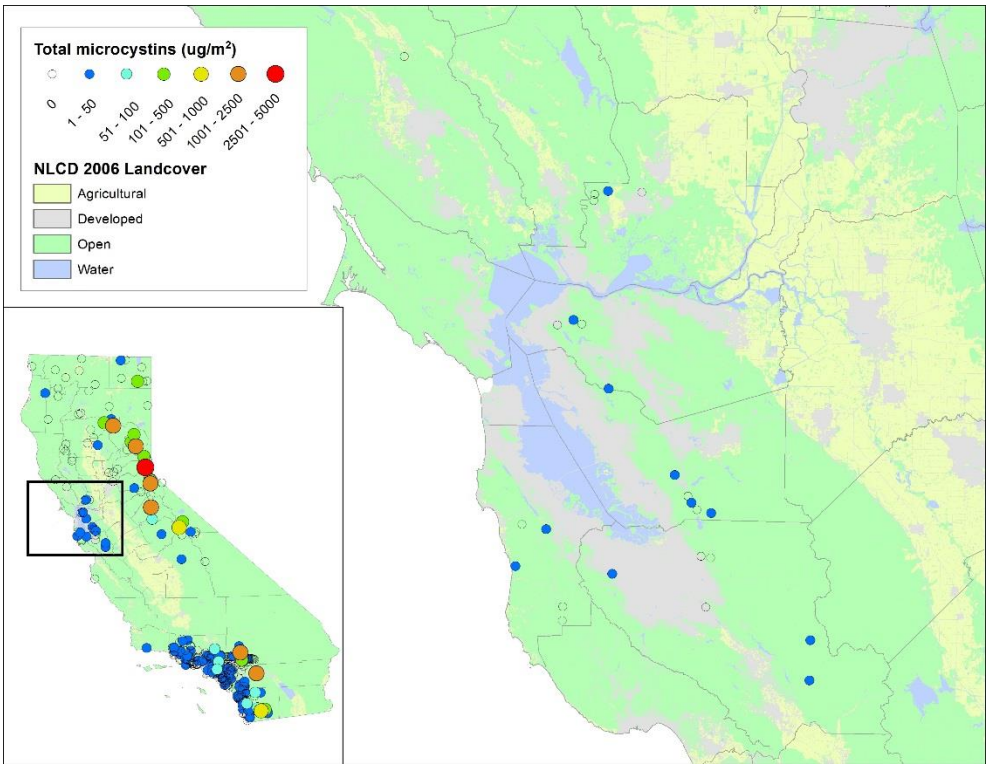
In the California wadeable stream reaches studied, a total of 118 species of cyanobacteria were recorded, which belonged to 22 genera that contain infrageneric taxa capable of cyanotoxin production, according to the literature (Table 1). These potentially toxigenic cyanobacterial species comprised 39% of the total number of cyanobacterial taxa in the study's data set and are included in an online identification tool (Stancheva et al. 2014). However, due to the recent rapidly changing taxonomy and phylogeny of the cyanobacteria (Komárek 2010), their high morphologic plasticity, and the need of molecular analysis, the

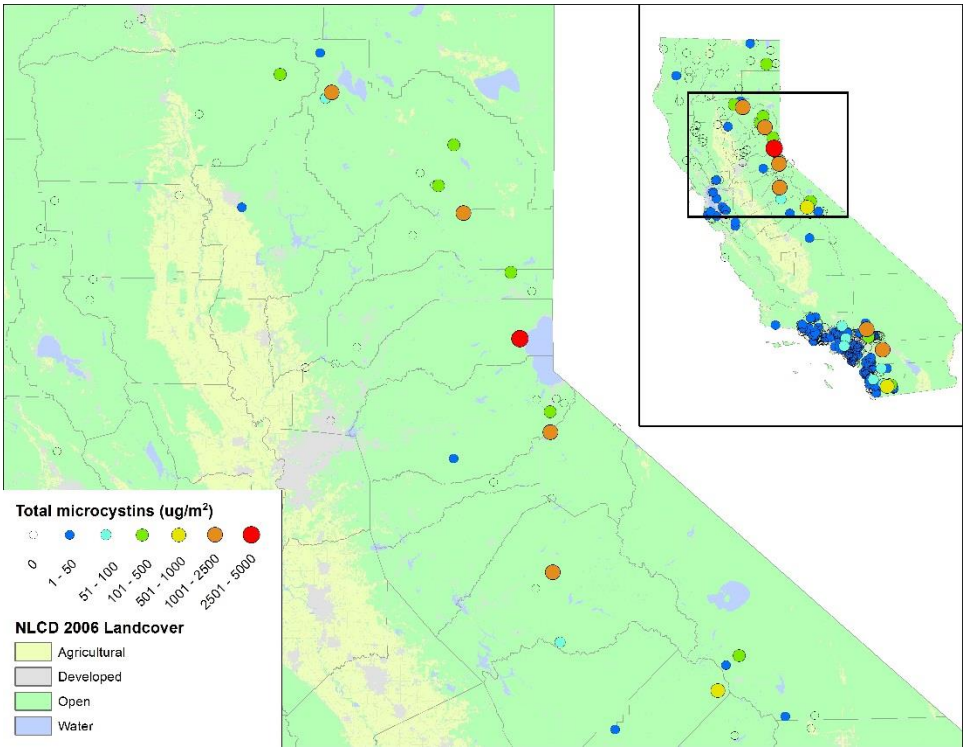
transfer of knowledge among studies can be challenging. For instance, closely related species of *Phormidium* with overlapping and variable morphology (such as *P. autumnale*, *P. uncinatum* and *P. subfuscum*) have been differently treated by researchers (Whitton, 2011 (and references therein); Smith 2011). Whitton (2011) suggested that nutrient limitation experiments could help to evaluate the taxonomic importance of morphological features in this *Phormidium* group. In this work, the cyanobacteria taxonomic concept and nomenclature of Komárek and Anagnostidis (1999, 2005) and Komárek (2013) were followed.

Furthermore, the diversity of stream benthic cyanobacteria in California has not been completely explored. In this study, several species of *Anabaena* and *Trichormus* were recorded that need additional taxonomic and molecular analysis in order to be properly identified to species level, and might be new to science. Collection of fresh qualitative samples from each stream reach (Fetscher et al. 2009) provides an opportunity to isolate and culture cyanobacteria of interest.

Appendix B: Maps of the microcystin concentrations detected from benthic algae during the spring-summer assessments from 2011-2013.

The top map is a zoomed in to the Bay Area and the bottom map is zoomed in to the Sierras.





Appendix C: Bloom pictures from depressional wetlands

Olive Hill Road Fallbrook site, with *Lemna* covering the surface. SPATT deployed 7/6/2012 – 9/18/2012, MCY-RR detected. All grab samples were below the method detection limit. Photo date: 7/6/2012. Photo credit: Carey Nagoda

Emerald Isle Golf Course. Cyanobacteria bloom obvious on 8/31/2012 (top picture), but microcystins were below method detection limit in grab sample. Bottom picture taken on 10/12/2012, and grab sample detected MCY-RR = 0.03 μg/L. SPATT deployed 8/31-10/12 detected MCY-RR. Photo credit: Carey Nagoda

Appendix D: Bloom pictures from coastal wetlands

Mission Bay, Rose Creek (Site #90606MISS). SPATT deployed July-August 2013. SPATT deployed 8/2013 – 9/2013, MCY-LR and MCY-RR detected. All grab samples below method detection limit. Photo date: 9/4/2013. Photo credit: Carey Nagoda.

Tijuana River Estuary. Microcystins detected in July and August from grab samples and in July through September from SPATT samples. Photo date: 5/21/2013. Photo credit: Carey Nagoda.

San Diego River Estuary. No microcystins detected in grab samples. SPATT deployed August-September, MCY-RR detected. Photo date: 9/4/2013. Photo credit: Carey Nagoda.

San Elijo Lagoon, upstream of railroad crossing and San Elijo Pond in foreground of photo. Microcystins detected in both locations during both SPATT deployments (MCY-RR and MCY-LA in lagoon, MCY-RR in pond). No toxins detected in grab samples. Photo date: 5/23/2013. Photo credit: Carey Nagoda.

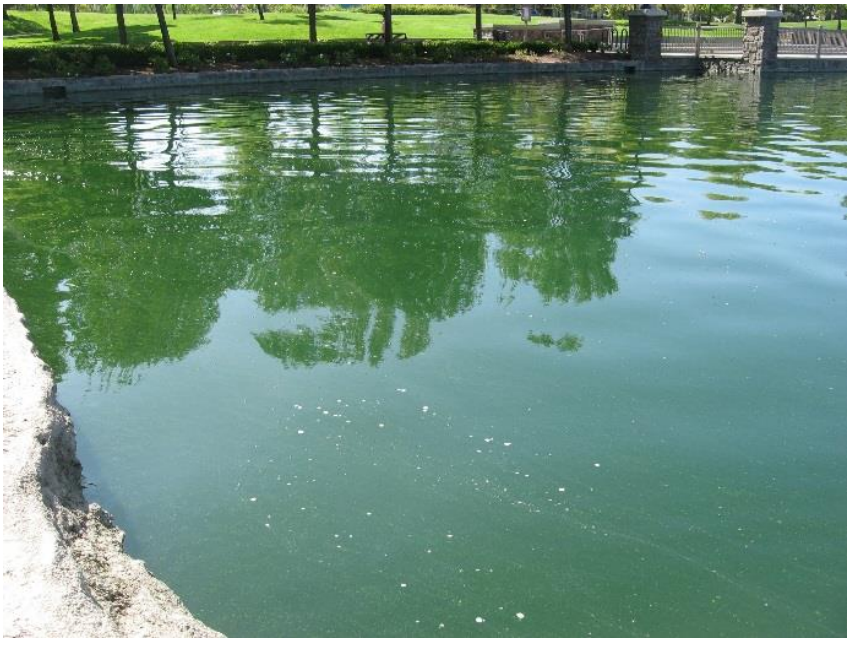
Appendix E: Bloom pictures from lakes and reservoirs

Lake Henshaw. Microcystins detected in both 2013 SPATT deployments (July-August and August-September, MCY-RR detected) and from grab samples in August and September. Bottom right photo is the outflow from the lake. Photo date: 7/8/2013. Photo credit: Carey Nagoda.

Morena Reservoir. Microcystins detected from July to September 2013 and exceeded the California recreational thresholds in August and September. Photo credit: Carey Nagoda.

Lake Hodges. Microcystins detected from SPATT samplers deployed from July-August and August-September in 2013. Grab sample results were below the method detection limit. Photo date: 7/22/2013. Photo Credit: Carey Nagoda

Harveston Lake. Sampled 6/23/2014. Particulate Microcystins = 10.7 µg/L. Photo credit: Carey Nagoda.

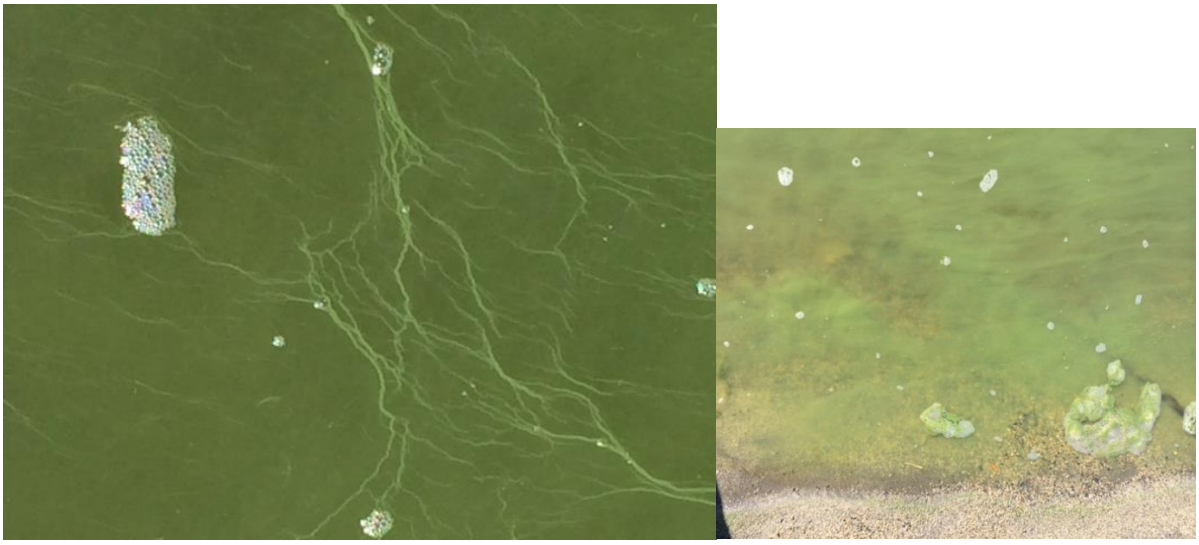


Santee Lake #5. Sampled 7/22/2014. Particulate Microcystins = 11.7 μg/L. Photo credit: Carey Nagoda.

Lindo Lake. Sampled 6/20/2014. Particulate microcystins = 2.5 µg/L. Photo credit: Carey Nagoda

Vail Lake. Sampled June 22, 2013. Photo credit: Carey Nagoda and Chad Loflen.

Lake Elsinore. Microcystins and cylindrospermopsin detected from grab samples collected on May 21, 2014. Photo credit: Meredith Howard.



Canyon Lake. Microcystins, cylindrospermopsin and anatoxin-a detected from grab samples collected on May 21, 2014. Photo credit: Meredith Howard.

San Joaquin Marsh. Microcystins detected from grab sample collected on May 21, 2014 and CellScope photo of *Microcystis sp*. Photo credit: Meredith Howard.