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Screening Assessment of Cyanobacteria and Cyanotoxins in Southern California Lentic Habitats

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Abstract

Harmful bloom-forming cyanobacteria (CyanoHABs) and associated toxins are increasingly prevalent world-wide. We conducted a screening-level study to determine if cyanobacteria and associated cyanotoxins were present in Southern California coastal lakes, ponds, and seasonally tidal lagoons. We evaluated waterbody nutrient status and physiochemical parameters, land use, waterbody type, and habitat type, to determine their utility as screening factors for risk of CyanoHAB blooms. One-time grab samples were collected from 30 sites during July–September 2009. Samples were analyzed for phytoplankton taxonomic composition, nutrients, other physiochemical parameters, and three cyanotoxins: microcystins (MCY), anatoxin-a, and cylindrospermopsin. Cyanobacteria was the predominant taxonomic group in most water bodies in this study, and *Microcystis* spp. was the predominant genus in 96% of the study sites. Cyanobacteria were equally prevalent among coastal lagoons, depressional wetlands, and lakes in this study. We detected MCY in high concentrations in 10% of our sites, but neither anatoxin-a nor cylindrospermopsin were detected. All of the MCY-positive sites exceeded California action levels for recreational use and World Health Organization (WHO) guidance for human health effects. The prevalence of *Microcystis* spp. from all study sites indicates a high potential for MCY in these water bodies, although the one-time toxin grab samples likely underestimated the overall toxicity of these sites. Landscape variables, such as developed land use and dominant habitat type, were not found to be predictive indicators of cyanobacterial dominance. However, because cyanobacteria become consistently dominant when chlorophyll-a levels exceed 15 μg L^{-1}, chlorophyll-a can serve as a significant predictor of MCY.

Keywords: Algae, Biodiversity, Cyanobacteria; Eutrophication, Microcystin, *Microcystis*.

1. Introduction

Cyanobacterial blooms are naturally occurring in freshwater, brackish, and marine ecosystems (Paerl 1988; Paerl and Fulton 2006). Harmful algal blooms (HABs) have increased in frequency and intensity worldwide in recent decades (Carmichael 2008; Heisler *et al.* 2008; Hudnell and Dortch 2008; Paerl and Huisman 2009; O'Neil *et al.* 2012; Paerl and Paul 2012). Cyanotoxins from harmful bloom-forming cyanobacteria (CyanoHABs) cause a number of negative impacts, including illness and mortality in humans, domestic pets, wildlife, and livestock; oxygen depletion; taste and odor problems; depressed growth of aquatic macrophytes; and loss of water quality (Paerl 1988; Carmichael 2001; Izaguirre and Taylor 2004; L évesque *et al.* 2004; Paerl and Fulton 2006; Backer *et al.* 2008; Backer *et al* 2010, Backer *et al.* 2013).

Microcystis and associated CyanoHAB genera and toxins were historically considered an issue in freshwater systems (such as freshwater lakes, reservoirs, larger rivers, public water supplies). However, recently in California, cyanotoxins have been shown to have far reaching effects downstream of their biological origin (Miller et al., 2010; Gibble and Kudela, 2014). The mortality of over 30 endangered marine sea otters in Monterey Bay, for example, were due to microcystin (MCY) intoxication (Miller *et al.* 2010). The origin of MCY was determined to be from a dense bloom in Pinto Lake that was carried downstream, bioaccumulated in marine

bivalves, and then ingested by marine sea otters (Miller *et al.* 2010; Kudela 2011). Cyanotoxins are potentially an issue not only in freshwater inland lakes and rivers, but also in estuarine and brackish coastal waterbodies. These phenomena have suggested risks to coastal waterbodies which are ecologically important, as they provide critical habitat for resident and migratory birds and fish, and are often sites of human recreational activities. One practical challenge for reducing such risks is the lack of sufficient data on CyanoHABs in these coastal lentic water bodies. While some documentation has been available for large scale toxic CyanoHABs along the U.S. west coast in Washington, Oregon, and California (Johnston and Jacoby 2003; Moisander *et al.* 2009a; Moisander *et al.* 2009b; Kudela 2011; Gibble and Kudela, 2014), there is a general data gap for the Southern California coast, a highly urbanized region of 20 million people (NRC 2008). Yet, toxin-producing cyanobacteria were detected in drinking water reservoirs in Southern California (Izaguirre and Taylor 2004) and have been responsible for canine deaths (Devries *et al.* 1993; Backer *et al.* 2013). Thus, it is important that additional data are collected on the prevalence of cyanobacteria and cyanotoxins in coastal Southern California lentic habitats.

A large number of environmental factors, including climate change, nutrient overenrichment, temperature, salinity, water residence time, vertical stratification, organic matter enrichment, and pH, have been linked to bloom increases (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). Historically, phosphorus was the primary nutrient attributed to controlling cyanobacterial blooms in freshwater systems. However, the specific nutrients controlling cyanobacterial blooms have been debated in recent years. Recent studies showed that nitrogen also controls cyanobacterial blooms, so that both nitrogen and phosphorous need to be considered in water quality management strategies (Conley *et al.* 2009; Scott and McCarthy 2010; Xu *et al.* 2010; Paerl *et al.* 2011; Wilhelm *et al.* 2011; Paerl and Otten 2013). In highly urbanized landscapes, significant point and non-point anthropogenic nutrient sources create eutrophic conditions that are ideal for cyanobacterial growth. An understanding of how landscape (such as land use and water body type), as well as site-specific factors (such as habitat type, water chemistry, and nutrient forms) influence the primary productivity, abundance of cyanobacteria, and presence/absence of cyanotoxins, can be useful information to screen sites for possible management action.

The objectives of this study were 1) to conduct a screening-level study to estimate the prevalence of cyanobacteria (with a focus on *Microcystis* spp.) and concentrations of common cyanotoxins (i.e. below or above action levels) in Southern California coastal lakes, ponds, and seasonally tidal lagoons, and 2) to examine the relationships between phytoplankton abundance, cyanobacterial abundance, and cyanotoxins with water body nutrient status and physiochemical parameters, habitat type, and surrounding land uses, to determine their utility as screening factors for the risk of CyanoHAB blooms.

2. Materials and Methods

2.1 Study Area

The Southern California Bight (SCB) comprises an open embayment of the coastline that stretches from Point Conception, California, to Cabo Colnette, Baja California. Southern California's climate exhibits a relatively dry summer and wet winter season. Over 90% of the precipitation generally occurs between late fall and early spring (October through March), with an average annual rainfall of 10–100 cm (Nezlin and Stein 2005). Approximately 100 watersheds drain into SCB estuaries and coastal waters, with storm water runoff contributing 95% of the total annual runoff volume (Akerman and Schiff 2003). In this Mediterranean ecosystem, aquatic habitats were historically dominated primarily by seasonal emergent wetlands with coastal lagoons at the terminus of the watersheds. These small coastal lagoons are closed seasonally to surface water due to the formation of sand-bars at their inlets during low freshwater inflow periods (Webb *et al.* 1991; Largier *et al.* 1996). Consequently, they are "lentic," with fresh to brackish water salinity regimes during closure, and increased susceptibility to eutrophication due to restricted flushing (Painting *et al.* 2007; Zaldivar *et al.* 2009). Urbanization of semi-arid Southern California coastal watersheds caused the import of water from Northern California and the Colorado River, dramatically changing regional water budgets and extending dry season river flow late into the season. Urbanization and associated irrigation runoff resulted in type conversion of seasonal freshwater emergent wetlands to perennial, open-water dominated ponds and small lakes that serve both as flood control and habitat.

2.2 Conceptual Approach and Site Selection

The approach for this study was to conduct a one-time assessment of phytoplankton biomass (measured as chlorophyll-a), phytoplankton community composition (measured as cell counts), and cyanotoxin concentrations, as well as explanatory variables in a sample of lakes, depressional wetlands, and coastal lagoons in the Southern California coastal watersheds of Santa Barbara, Ventura, Los Angeles, Orange and San Diego Counties. We chose to conduct the screening study during the mid-late summer to maximize the likelihood of finding CyanoHABs. At the time the survey was under development, a comprehensive inventory of these water bodies was not available. Therefore, we developed a master list of 60 candidate sites from previous studies and discussions with local water quality management agencies and non-profit organizations. Permission was granted by landowners to sample 14 coastal lagoons, 20 depressional wetlands, and five natural or man-made lakes. Thus, a total of 39 targeted water bodies were sampled in the five counties during June–September 2009 (Table 1).

Geographical information and total area for each site was collated using Geographic Information System (GIS) analysis. The sites were categorized into three main habitat types: open water, emergent marsh/flats, and scrub/other. The percentage of each habitat type was determined for each site (Table 1). The sites were also categorized by percent of land use category (open, agricultural, and urban) surrounding the site using a 1-km buffer from the center of the water body (Table 1).

Table 1. Southern California water bodies screened, with habitat type of open water (OW), emergent marsh or emergent flats (E), scrub or other (Sc), and land use of open (O), agricultural (A), and urban (U).

Site Name	Water Body	Total Area (m^2)	Habitat Type			Land Use		
			OW	E	Sc	O	A	U
Andree Clark Bird Refuge	Estuary	126,314	83	17	0	68	0	32
Ballona Marsh	Depressional	93,550	36	64	0	34	0	66
Barbara Lake	Lake	73,066	51	18	31	80	0	20
Big Canyon Post Marsh	Depressional	38,742	13	44	43	51	0	49
Big Canyon Pre-Marsh	Stream	50,828	0	4	96	48	0	52
Buena Vista Lagoon	Estuary	931,240	50	47	3	29	0	71
Calavera Lake	Lake	92,744	59	35	6	48	0	52
Devereux Slough Lagoon	Estuary	232,552	63	21	16	77	0	23
El Dorado Marsh	Depressional	159,037	11	9	80	38	0	62
El Dorado Pond	Depressional	10,348	83	17	0	41	0	59
Huntington Pond	Depressional	89,676	42	4	54	17	0	83
Irvine Regional Park Pond	Depressional	7,842	100	0	0	93	1	6
Irvine Ranch Water District Pond B	Depressional	9,682	100	0	0	58	0	42
Irvine Ranch Water District Pond C	Depressional	27,409	65	0	35	51	0	49
Lakewood Country Club Pond	Depressional	46,384	100	0	0	14	0	86
Machado Lake	Lake	322,283	36	17	47	18	0	82
Madrona Marsh	Lake	56,215	20	17	63	4	0	96
Malibu Lagoon	Estuary	130,679	40	60	0	79	0	21
Mason Lake	Depressional	37,472	100	0	0	37	1	62
McGrath Lagoon	Estuary	212,687	24	10	66	54	28	18
Newport Bay	Estuary	78,100	41	59	0	48	0	52
Nicholas Flat Pond	Depressional	8,869	75	25	0	100	0	0
Ormond Beach Wetlands	Estuary	168,512	34	66	0	52	11	37
Peters Canyon Lake	Lake	264,894	58	0	42	61	0	39
San Joaquin Marsh, Pond 4	Depressional	160,784	32	66	2	40	0	60
San Joaquin Marsh, Pond 7, 8, 9, 10, 11	Depressional	345,029	5	83	12	35	0	65
San Juan Creek	Estuary	100,181	0	100	0	31	0	69
San Marcos Lake	Depressional	224,987	100	0	0	42	5	53
San Mateo Lagoon	Estuary	156,033	11	13	76	59	12	29
Santa Ana Pond	Depressional	46,806	28	20	52	31	0	69
Santa Clara Lagoon	Estuary	1,264,302	0	65	35	59	31	10
Sepulveda Marsh	Depressional	1,450	100	0	0	45	0	55
Sims Pond	Depressional	22,313	58	4	38	21	0	79
Topanga Lagoon	Estuary	6,039	46	0	54	85	0	15
University of California, Santa Barbara Campus Lagoon	Estuary	124,229	96	4	0	67	0	33
Ventura POTW Pond	Depressional	22,417	100	0	0	64	15	21
Ventura River Mouth	Estuary	107,867	28	67	5	64	0	36
Zuma Lagoon	Estuary	26,480	41	22	37	76	0	24

2.3 Field and Laboratory Methods

We collected grab samples within 3 m of the shoreline for discrete samples of phytoplankton community composition, phytoplankton biomass (measured as chlorophyll-a), cyanotoxins, and water chemistry. Using formalin-preserved samples that were settled for 24 hours in Utermöhl settling chambers, we determined the algal assemblage for each site sampled. After 50 mL settled for each sample, the fields were counted and identified for five algae divisions: the Bacillariophyta, Euglenophyta, Chlorophyta, Cyanophyta, and Pyrrophyta. Microcystis spp. was the only genus that was counted due to the ease of identification, prevalence of this genus worldwide as well as in other parts of California, and because it is a known producer of MCY. Relative abundance was calculated by dividing cells mL^{-1} of each division by the total cells mL^{-1} at the site. At each sample site, measurements of temperature, pH, electrical conductivity (EC), and total dissolved solids (TDS) were acquired with a Hanna HI98129 Digital Tester Meter (Hach Company, CO, USA). Dissolved oxygen (DO) was recorded with a Milwaukee SM600 DO Meter (Hach Company, CO, USA). A Secchi disc was also lowered into the water until the black and white lines were no longer visible, and depth at that point (i.e., visibility) was recorded in centimeters. Between sites, equipment was bathed in 2M HCl for 5 minutes, then rinsed with Milli-Q water and allowed to air dry. Samples were obtained at approximately three sites per day, three days per week, and were transported in cool, dark bins, and processed within 6 hours. Alkalinity was measured with a Hach Digital Titrator Model 16900 test kit (Hach Company, CO, USA) titrated with sulfuric acid to a colorimetric end point. Total alkalinity reflected all carbonate, bicarbonate, and hydroxide present.

We collected chlorophyll-a samples by filtering whole water samples onto Whatman GF/F filters, then samples were immediately frozen and stored in the dark in aluminum foil until analysis. Samples were analyzed for chlorophyll-a using a model 10AU fluorometer (Turner Designs, CA, USA), with acidification analyzed by the EPA Method 445.0 (Rigler 1956). Samples were analyzed for dissolved organic carbon (DOC), total dissolved nitrogen (TDN), total dissolved phosphate (TDP), total phosphorus (TP), particulate phosphorus (PP), particulate nitrogen (PN), and total nitrogen (TN). Total dissolved nitrogen and TDP were analyzed using persulfate to digest unfiltered and filtered water samples to convert nitrogen (N) from all N components into nitrate, and phosphorus (P) from all P components into orthophosphate for simultaneous determination of TN and TP. The resulting digests were analyzed by automated colorimetry for nitrate-N and orthophosphate using a Colorimeter (Alpkem, TX, USA) by APHA standard methods (APHA 1999). Soluble Reactive Phosphorus (SRP) was analyzed via the automated ascorbic acid reduction method, using a QuikChem 8000 Flow Injection Analyzer (Lachat, CO, USA). Nitrate + nitrite (NO_3 + NO_2) was analyzed using cadmium reduction, nitrite (NO_2) by using colorimetry, ammonium (NH_4) using gas diffusion, and phosphate (PO_4) using the molybdate method (Piper and Lovell 1981). From those results, values were calculated for dissolved organic phosphorus (DOP), particulate phosphorus (PP), nitrate (NO_3), dissolved inorganic nitrogen (DIN), particulate nitrogen (PN), total dissolved solids (TDS), and dissolved organic nitrogen (DON).

Cyanotoxin samples were analyzed for Microcystins (MCY), Anatoxin-a, and Cylindrospermopsin by the Monitoring and Event Response for Harmful Algal Blooms –

Lower Great Lakes Laboratory (MERHAB-LGL), at the State University of New York, College of Environmental Science and Forestry. Samples were extracted with 10 ml of acidified 50% methanol, and sonicated according to Boyer (2007). Functional assays were performed for microcystins and nodularins using Protein Phosphatase Inhibition Assays (PPIA) according to the method of Carmichael and An (1999). Samples for which greater than 0.5 μg L^{-1} was detected with PPIA for microcystins were further analyzed via High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) (Boyer, 2007). All of the sample assays were analyzed via HPLC-MS for anatoxin-a and cylindrospermopsin (as described in Boyer, 2007).

Results of cyanotoxins analyses were evaluated relative to existing statewide or international cyanotoxins action levels. The action levels for MYC concentrations are 0.8 μg L^{-1} for human recreational uses, and 2.0 μg L^{-1} for subchronic water intake for dogs. The human recreational use action level for anatoxin-a is 90 μg L^{-1}, and cylindrospermopsin is 4 μg L^{-1}. The World Health Organization (WHO) has also established guidance values for recreational exposure to cyanobacteria and MCY with low probability of acute health effects at <10 μg L^{-1} MCY, < 10 μg L^{-1} chlorophyll-a, or < 20,000 cells mL^{-1} of cyanobacteria; moderate probability at 10–20 μg L^{-1} MCY, 10-50 μg L^{-1} chlorophyll-a, or 20,000–100,000 cells mL^{-1} cyanobacteria; and high probability of acute health effects at 20–2,000 μg L^{-1} MCY, 50–5,000 μg L^{-1} chlorophyll-a, or 100,000–10,000,000 cells mL^{-1} cyanobacteria (Chorus and Bartram 1999).

2.4 Statistical analysis

The relationships between response variables and explanatory variables were investigated using SAS (Version X) and RStudio (version 0.96.122) statistical packages. Response variables included chlorophyll-a, cyanobacterial abundance, percent of total cells present as cyanobacteria, and toxin presence/absence of cyanotoxins, whereas explanatory variables included water body type (estuaries, lakes, and depressional wetlands), nutrients (PN + DON, PP + DOP, PO_4, and NH4, as well as TN:TP and DIN:DIP ratios), surrounding land use (% developed), and habitat type (% open water). Analysis of variance (ANOVA) with Tukey's pairwise comparisons were used to determine if there were significant differences in the chlorophyll-a, cyanobacteria cell count, and percent of total cells as cyanobacteria by water body type. Stepwise regression was used to assess the relative influence of continuous explanatory variables (land use, habitat type, nutrient concentrations and ratios, and physio-chemical variables). All data were log-transformed (log_{10}) and the significance level was set at 0.05 for all statistical analyses, unless otherwise specified. Logistic regression was used to determine if chlorophyll-a, cyanobacteria abundance, N:P ratios, and Microcystis spp. abundance were significant predictors of toxin presence.

3. Results

3.1 Phytoplankton Assemblage and Presence of Cyanotoxins

Overall, chlorophyll-a concentrations were moderately high (median of 12, $\bar{x} = 49 \pm 17$ μg L^{-1} SE). The mean distributions of phyla throughout the study sites were Cyanophyta (92%),

Chlorophyta (3%), Bacillariophyta (3%), Euglenophyta (2%), Pyrrhophyta (0.03%), and Cryptophyta (0.02%; Figure 1). In 96% of the study sites, the cyanobacterium *Microcystis* spp. was the predominant genus. In 3% of the study sites, *Euglena* was predominant, and the diatom, *Attheya* was predominant at one site. The five genera with the highest cell counts throughout all the study sites combined were the Cyanophyta, *Microcystis* ($\bar{x}$ = 1.9 x 10^5 cells mL^{-1} ± 1.2 x 10^6 SE, range 0–7.9 x 10^5 cells mL^{-1}), the Euglenophyta, *Euglena* (3.3 x 10^3 cells mL^{-1} ± 2.2 x 10^5, range 0–4.0 x 10^4), the Cyanophyta, *Merismopedia* (2.6 x 10^3 cells mL^{-1} ± 1.8 x 10^4, range 0–7.5 x 10^3–1.3 x 10^5), the Chlorophyta, *Chlamydomonas* (2.5 x 10^3 cells mL^{-1} ± 1.6 x 10^5, range 0–8.6 x 10^4), and the Bacillariophyta, *Nitzschia* (1.8 x 10^3 cells mL^{-1} ± 1.2 x 10^5, range 0–3.5 x 10^4). Genera with the lowest cell counts were the Chlorophyta, *Spirogyra*, and the Bacillariophyta, *Melosira*, each averaging 125 cells mL^{-1} ± 1.2 x 10^2. Cyanobacterial dominance was not significantly different by water body type (Figure 2).

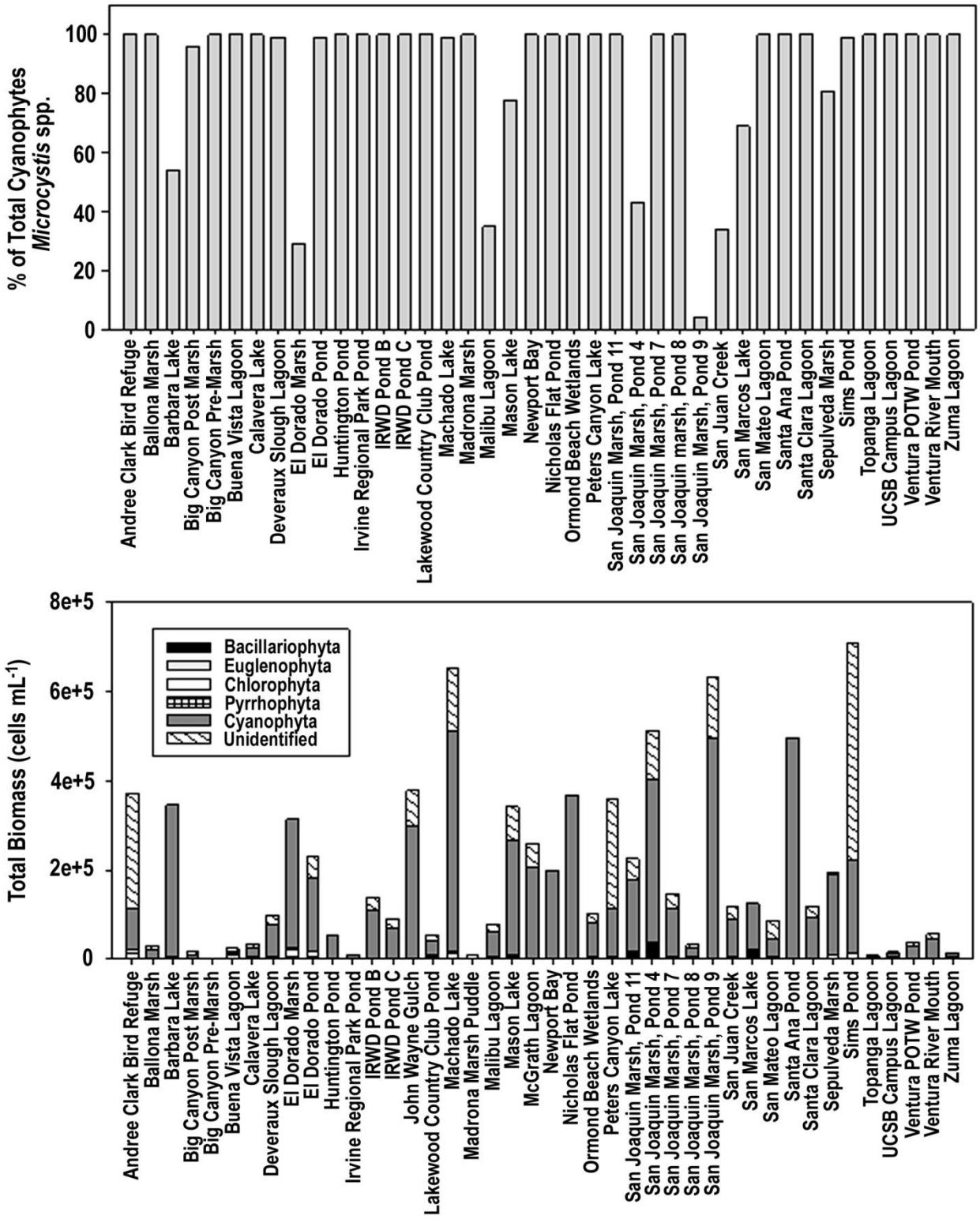

Fig. 1. Phytoplankton community composition by site: percentage of cyanobacteria comprised of *Microcystis* spp. (top panel) and composition (bottom panel).

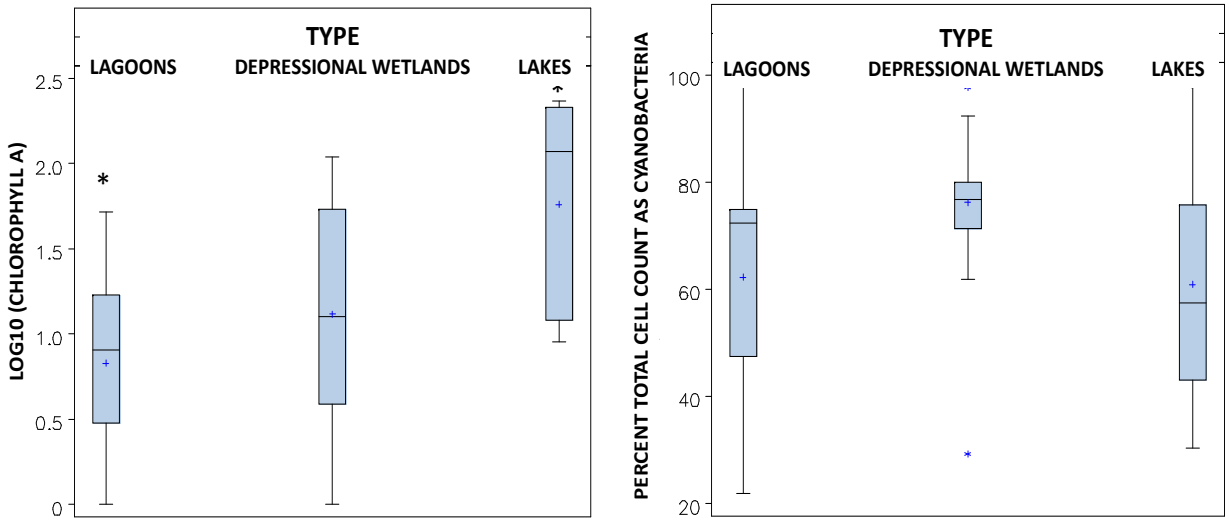

Fig. 2. Comparison of $\log_{10}$ [chlorophyll-a] (left panel) and % Cyanobacteria (right panel) by water body type.

Four sites (10% of study sites) were positive for MCY, and no sites were positive for anatoxin-a or cylindrospermopsin (Table 2; Figure 3). All sites that were positive exceeded both the California action levels for recreational use for MCY (0.8 μg L^{-1}) by 3–24 fold, and WHO levels (1.0 μg L^{-1}) by 3–19 fold. The most common MCY variant was MCY-LR, as it comprised 100% of the MCY in both Mason Lake and San Joaquin Marsh. Interestingly, MCY-FR was the dominant variant at the Andree Clark Bird Refuge site, a coastal lagoon. There was insufficient sample material collected at the Irvine Regional Water District Pond C to quantify MCY below the sample limit of detection (1.2 μg L^{-1}), which was above the CA and WHO action levels. Therefore, MCY could also have been present at this site.

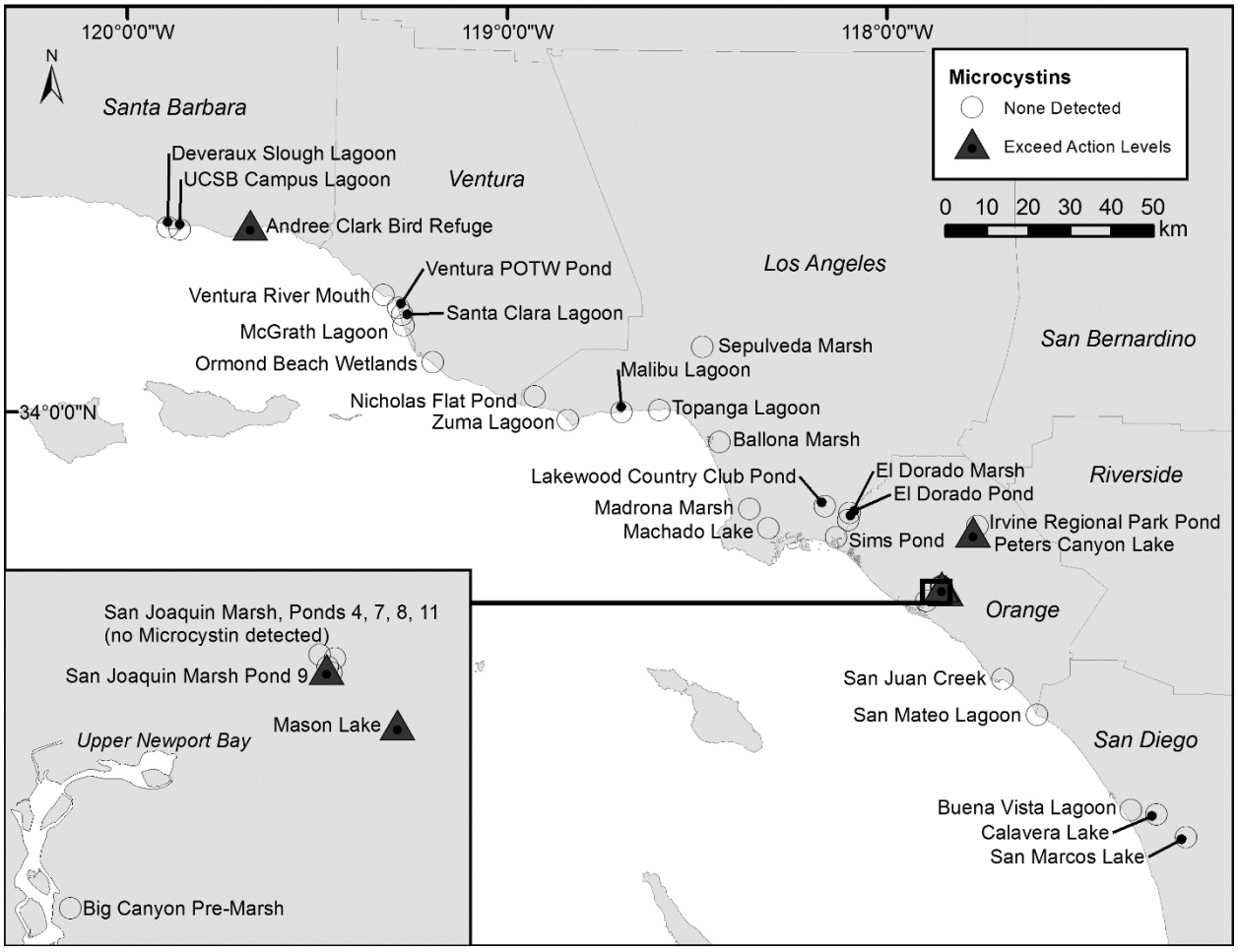

Fig. 3. Map of Southern California with all study sites labelled. Open circles indicate sites where no microcystins were detected and black triangles indicate sites where microcystin concentrations exceeded California action levels.

Table 2. Sites where microcystins were detected and quantified by either the Protein Phosphatase Inhibition Assay (PPIA) or High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) methods. The microcystin variant composition is shown for microcystin-LR (MCY-LR) and microcystin-FR (MCY-FR) by percent of total microcystins. NQ = not quantified.

Site Name	Sample Collection Date	Microcystin Concentration ($\mu g\ L^{-1}$)		Microcystin Variant Composition (%)	
		PPIA	HPLC-MS	MCY-LR	MCY-FR
Andree Clark Bird Refuge	8/18/2009	18.642	7.499	32	68
Mason Lake	8/24/2009	19.6	5.883	100	0
San Joaquin Marsh, Pond 9	8/24/2009	4.705	3.336	100	0
Peters Canyon Lake	7/7/2009	3.113	NQ	NQ	NQ

3.2 Nutrient Concentrations and Ratios

Nutrient concentrations in Southern California lentic water bodies were skewed towards an enriched status, with 70% of sites having concentrations exceeding 3.5 µM TP and 100 µM TN (Table 3). On average, greater than 85% of TN was PN + DON, and roughly half of TP was PP + DOP. Approximately 70% of sites had dissolved inorganic N:P ratios below the Redfield ratio of C:N:P = 106:16:1 (Redfield 1934), indicating that these sites exhibited N limitation, whereas only 46% had total N:P ratios less than 16:1, indicating a roughly equal split between N- and P-limitation with respect to total nutrient availability.

Table 3. Mean, minimum, and maximum values for phytoplankton, nutrient, and physiochemical variables.

Variable	Min	Max	Median	Mean	Standard Deviation
Phytoplankton					
Chlorophyll-a (µg L^{-1})	1	587	12	49	17
Cyanobacteria (cells Ml^{-1})	5,303	494,142	83,961	138,618	23,175
% Total Cells as Cyanobacteria	22	99	74	69	3
Nutrients					
DIN:DIP molar ratio	0.3	1878	7.1	92.0	50.7
TN:TP molar ratio	2.3	939	18.6	52.2	23.8
TN (µM)	24.1	1264.6	150.2	298.2	49.0
PN + DON (µM)	0.01	936.6	125.1	250.1	43.1
NH_4 (µM)s	0.5	105.0	2.45	8.7	2.9
NOX (µM)	0.2	823.2	0.8	39.7	22.3
TP (µM)	0.2	91.4	7.6	20.2	3.8
PP + DOP (µM)	0.01	75.2	4.4	12.3	43.1
PO_4 (µM)	0.08	66.8	0.7	7.9	2.7
Physiochemical Parameters					
Alkalinity (MG L^{-1} $CACO_3$)	38	359	160	174	13
TOTAL DISSOLVED SOLIDS (MG L^{-1})	171	2000	1523	1,360	106
Conductivity (MS CM^{-1})	315	61300	3045	10,682	2443

3.3 Factors Associated with Chlorophyll-a, Cyanobacterial Abundance and Microcystin Concentration

Among landscape-scale explanatory variables (surrounding land use, water body, and habitat type), only water body type was an important predictor. Statistically significant differences were found by water body type for chlorophyll-a ($p = 0.002$, $R^2 = 0.19$), with lagoons showing significantly lower concentrations (6 µg L^{-1}) than lakes (57 µg L^{-1}). Waterbody type approached significance for cyanobacteria cell count ($p = 0.08$), and was not significant for percent of total cells as cyanobacteria ($p = 0.14$).

Among nutrient forms, ratios (TN:TP and DIN:DIP), and physio-chemical variables, only TN was retained as a significant variable at an $\alpha = 0.1$ significance level in stepwise regression models for both chlorophyll-a and cyanobacterial cell counts. No variables were retained for percent cyanobacteria. Linear regression models of $\log_{10}$ chlorophyll-a and $\log_{10}$ cyanobacterial counts yielded significance values of 0.005 and 0.02, respectively, although model fits were somewhat low ($R^2 = 0.18$ and 0.10, respectively). Further analyses of nutrient forms illustrated that the strengths of these regressions were driven by the PN + DON, which was the most significantly correlated fraction ($p = 0.008$ and 0.009, respectively). The similarity of input variables for both chlorophyll-a and for the cyanobacterial cell count models was not surprising, given that the cyanobacterial cells counts generally represent dominant contributions to the chlorophyll-a concentrations. The three sites with the highest microcystin toxin concentrations (Andree Clark Bird Refuge, Mason Lake and San Joaquin Marsh) all had low N:P ratios (8.8, 0.2, and 2.4, respectively).

No significant water chemistry predictors were found for percent of total cell counts as cyanobacteria in linear regression models. However, plots of percent cyanobacteria as a function of chlorophyll-a (Figure 4) and, to a lesser extent, TN and TP showed clear break points above which cyanobacteria dominated phytoplankton abundance. Visually, these cut points occurred at approximately 15 µg L^{-1} chlorophyll-a, 325 µM TN, and 25 µM TP. Logistic regression results showed chlorophyll-a to be a significant predictor of MCY ($p = 0.02$; coefficient = 3.7). The other three variables which failed to predict MCY included abundance of cyanobacteria ($p = 0.2$), abundance of Microcystis spp. ($p = 0.8$), and N:P ratios ($p = 0.8$).

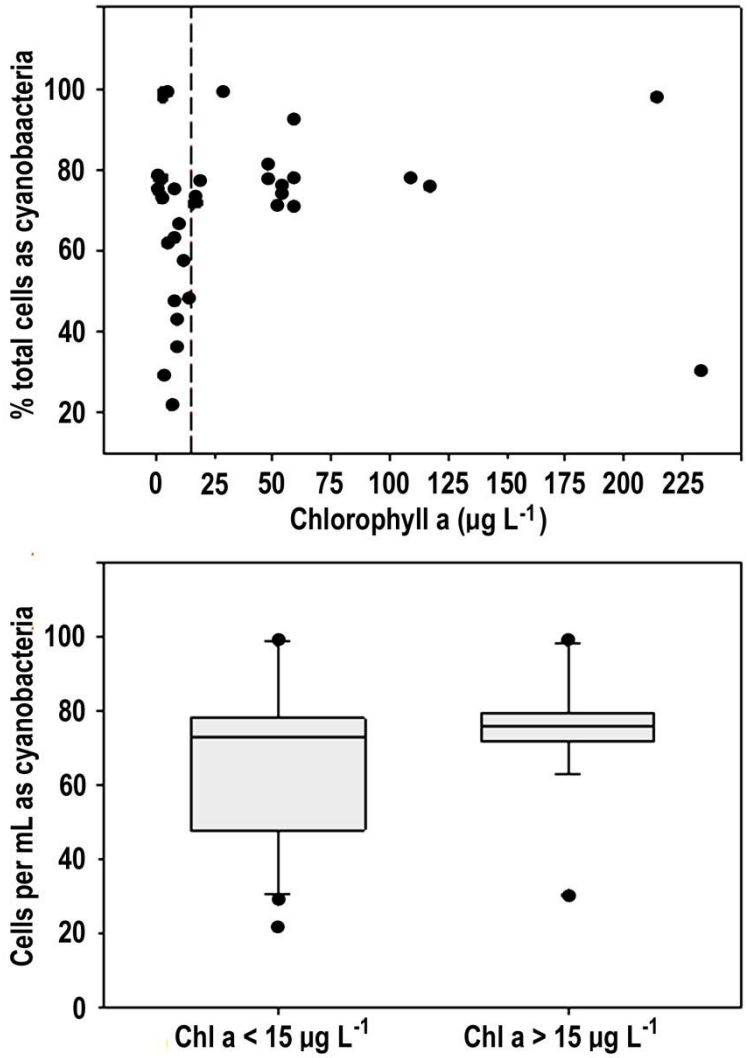

Fig. 4. Percentage of total cells as cyanobacteria as a function of chlorophyll-a (top panel) showing change points at approximately 15 µg L^{-1} where cyanobacteria become consistently dominant. Bottom panel illustrates box plots showing mean and interquartile range of percentage of total cell count as a function of chlorophyll-a above and below a change point of 15 µg L^{-1}.

4. Discussion

Microcystis spp dominated the overall phytoplankton assemblage in 92% of the Southern California lentic water bodies sampled in this study. MCY was detected in only 10% of all sites tested; however, the concentrations were extremely high, and all positive sites exceeded the California action levels for human and dog recreational use for MCY (0.8 and 2.0 µg L^{-1}). In applying WHO guidelines to these toxic sites, there are 3 metrics that can be used to evaluate the risk; (1) chlorophyll-a concentration, (2) MCY concentrations and (3) cyanobacterial cell counts. We found several sites in our study that qualified as high risk for adverse human health effects based on chlorophyll-a concentrations (50–5,000 µg L^{-1}). When based on MCY concentrations, two of our sites were considered moderate risk (10–20 µg L^{-1}) and another two sites were considered low risk (<10 µg L^{-1}). When considering cyanobacterial cell counts, we

found three sites to be high risk (100,000–10,000,000 cells mL^{-1}) and one to be moderate risk (20,000–100,000 cells mL^{-1}). The two sites with the highest MCY concentration are ecologically important areas that also have a human recreational aspect. Andree Clark Bird Refuge (Santa Barbara, CA) is a wildlife refuge for migrating birds, but is also encircled by a public walking path that is frequented by pets and humans. Mason Lake is within the heavily used recreational area of the William R. Mason Regional Park, and is located very close to the Newport Back Bay Estuary, which is a habitat for a number of rare and endangered birds. The high concentrations of MCY at these sites suggest serious health implications for both wildlife and humans since there are multiple routes of recreational exposure (Lévesque *et al.* 2004; Backer *et al.* 2008; Backer *et al.* 2010). The ubiquity of cyanobacteria is of concern in these coastal water bodies, which provide critical habitat for resident and migratory birds and fish, and are often sites of human recreational activities in this urbanized landscape (McLaughlin *et al.* 2013).

While the number of toxic sites was low, the toxic events in these systems are likely underestimated by the current study results for at least two reasons. First, toxin tests were only performed on grab samples obtained during the late summer period and are not indicative of the overall toxicity of a waterbody. If samples had been obtained throughout the year, more episodes of toxicity may have been noted. Second, grab samples have been shown to miss toxic events (Kudela 2011) due to the ephemeral nature of blooms. Therefore, the results from this study are not likely indicative of toxic events, but rather indicate that a more comprehensive and routine study is warranted. Interestingly, the sites with the highest MCY also had the lowest N:P ratios, and so were nitrogen limited (Andree Clark Bird Refuge, Mason Lake and San Joaquin Marsh, Pond 9). These results agree with other studies (Liu *et al.* 2011; Paerl and Otten 2013) that have documented high MCY concentrations and cyanobacterial blooms coinciding with low N:P ratios, and are consistent with the growing body of literature (Conley *et al.* 2009; Moisander *et al.* 2009a; Scott and McCarthy 2010; Xu *et al.* 2010; Wilhelm *et al.* 2011; Paerl and Paul 2012; Paerl and Otten 2013) showing that nitrogen limitation, as well as phosphate limitation, drive cyanobacterial blooms.

The concept of the use of landscape variables as a screening measure to prioritize types of water bodies could not easily be applied in this region. No significant statistical relationship was found between variables, such as percent urban or agricultural land use or habitat type (% of water body as open water), and cyanobacterial cell count or cyanobacterial dominance. However, chlorophyll-a was a significant predictor of MCY presence. These results are consistent with other recent studies (Rinta-Kanto *et al.* 2009; Lehman *et al.* 2010; Kudela 2011; Otten *et al.* 2012; McLaughlin *et al.* 2013) that have shown a strong positive correlation between MCY and chlorophyll-a for lakes and other freshwater habitats. Our results also demonstrated a cut point of approximately 15 $\mu g\ L^{-1}$ chlorophyll-a, above which cyanobacteria consistently dominated the phytoplankton assemblage. Thus, our results reinforce the concept that chlorophyll-a can be a meaningful screening variable for cyanoHABs, either through direct field measures or via remote sensing (Kahru *et al.* 1993; Kahru 1997), and can be prioritized for more intensive field monitoring.

TN had the strongest linear relationship with chlorophyll-a and cyanobacterial cell counts, with the particulate and dissolved organic fraction driving the strength of the relationship, indicating that nutrients have been incorporated into cyanobacterial growth or were recycling as dissolved organic forms within the water column. Other studies have found significant relationships between CyanoHABs and organic nitrogen sources, such as DON and urea, that are utilized by phytoplankton, and stimulate cyanobacterial growth (Kudela *et al.* 2008; Wawrik *et al.* 2009; Altman and Paerl 2012). The significant correlations between cyanobacterial cell counts, TN, and chlorophyll-a suggest that a primary target for management and mitigation strategies should be reduction in nutrient loading.

5. Conclusions

In summary, cyanobacteria were dominant during the summer study period in most (92%) of Southern California lentic waterbodies and were mostly comprised of the genus *Microcystis* spp. Therefore, there is a high probability of persistent MCY prevalence in these waterbodies. Routine monitoring is needed to determine the concentrations of cyanotoxins that are present in these water bodies seasonally, particularly for recreational areas that have high human contact. MCY intoxication should be considered for any wildlife illnesses or mortalities in regions surrounding these sites, given the high MCY concentrations detected and the high potential for cyanotoxins in these water bodies (Miller *et al.* 2010). In contrast to the expensive and laborious taxonomic composition measurements, future studies in this area should focus on chlorophyll-a as a general indicator of the likelihood of MCY.

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