Comparison of Novel Passive Sampling Methods to Identify Cyanotoxins and their Sources

Bowen Du
Jayme Smith
Keith Maruya
Charles S. Wong

Southern California Coastal Water Research Project

SCCWRP Technical Report #1123

Comparison of Novel Passive Sampling Methods to Identify Cyanotoxins and their Sources

Bowen Du, Jayme Smith, Keith Maruya, and Charles S. Wong

Southern California Coastal Water Research Project, Costa Mesa, CA

July 2020
SCCWRP Technical Report 1123

ACKNOWLEDGEMENTS

This project was funded by the Los Angeles Regional Water Quality Control Board, the California State Water Resources Control Board's Surface Water Ambient Monitoring Program (SWAMP) and the Southern California Coastal Water Research Project.

The authors thank the staff of Calleguas Municipal Water District, the City of Long Beach, California Department of Water Resources, Los Angeles Department of Water and Power, United Water Conservation District, Los Angeles Department of Recreation and Parks and the Big Valley Band of Pomo Indians and their Environmental Protection Office for sampling assistance. The authors also thank the following individuals for assistance: Jun Zhu (site selection and permission), Dario Diehl (sampling coordination), Lindsay Darjany (microcystin analyses), Ellie Wenger and Wenjian Lao (sampling and sample processing), Po Wang (passive sampling method development and sampling), and Dana Shultz (sample processing and map figures).

EXECUTIVE SUMMARY

Harmful cyanobacterial blooms (cyanoHABs) are an increasingly common water quality issue that poses a risk to human, wildlife, livestock, and pets due to the production of toxins by many cyanobacterial species. In California, lentic habitats are subject to cyanobacteria proliferation due to increased anthropogenic nutrient inputs from urban and agricultural point and nonpoint sources. Previously in Southern California, a recent study identified ubiquitous distributions of cyanobacteria in lakes, depressional wetlands and coastal lagoons, with *Microcystis* spp. dominating the community in 96% of the 39 sites surveyed. However, the occurrence of cyanotoxins, particularly microcystins, has been poorly characterized in waterbodies within the bounds of the Los Angeles Regional Water Quality Control Board (RWQCB; Region 4).

The goal of this project was to 1) document the occurrence of microcystins in water in selected Region 4 waterbodies; and 2) compare the utility of passive sampling methods for measuring the occurrence of microcystins in freshwater systems. Here, bench-scale tests on organic diffusive gradients in thin films samplers (o-DGT) were conducted with three microcystin (MC) congeners, MC-LR, MC-RR, and MC-YR, to evaluate the sorption and uptake capacities of the sampler. The o-DGT samplers were then piloted in the field alongside Solid Phase Adsorption Toxin Tracking device (SPATT) samplers and paired with traditional water grab sampling.

A total of 9 sites in the Los Angeles RWQCB's region were screened for the presence of microcystins during the summer-fall seasons of 2018 and 7 of these sites tested positive. No microcystins were detected at El Dorado Park lakes and Malibu Creek. At Pyramid Lake, microcystins were detected above California Cyanobacteria and Harmful Algal Bloom Network danger levels (Tier II) level (20 µg/L).

During the summer-fall of 2019, passive sampling devices (o-DGT and SPATT bags) were deployed concurrently at Pyramid Lake, Castaic Lake, Clear Lake (Northern California), and Huntington Harbor and microcystins were detected at all sites except Huntington Harbor. The result of this study indicated the potential advantages of using o-DGT over SPATT due to the more quantitative nature of o-DGT. The o-DGT results indicated a comparable prevalence as the grab sample results when microcystins were detected at low levels (under 100 ng/L).

Microcystins were detected almost ubiquitously in most waterbodies; however, the magnitude of microcystin concentrations detected varied by several orders of magnitude. Future research and monitoring efforts should focus on temporal/seasonal variability in toxin production and determine the sampling frequency needs to better understand the risks to human and animal health. Better ambient assessment of cyanotoxin prevalence and risk across waterbodies also will allow for more targeted monitoring of sites that are of higher risk. Our results demonstrate that reproducible sampling rates consistent with existing efforts on polar CEC uptake by o-DGT are possible, and that this device is feasible for monitoring of microcystins subject to successful field characterization. Additionally, more work is needed to understand the extent and magnitude of other cyanotoxin classes. The efficacy of o-DGT should be explored for anatoxin-a, cylindrospermopsin and saxitoxin.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Harmful cyanobacterial blooms (cyanoHABs) are an increasingly common water quality issue that poses a risk to humans, wildlife, livestock, and pets due to the production of toxins by many cyanobacterial species (Backer et al. 2008, Edwards et al. 1992, Mez et al. 1997, Pouria et al. 1998, Trevino-Garrison et al. 2015, Wood et al. 2010). Collectively, the toxins produced by cyanobacteria are referred to as cyanotoxins and represent multiple classes of toxins, including microcystins, cylindrospermopsin, anatoxin-a and saxitoxins. Cyanotoxin producing blooms have been occurring nationally at more locations with increasing frequency and severity and, as a result, several cyanotoxin classes have recently been integrated into monitoring programs by both USGS and EPA. Within the last decade, health advisory thresholds have also been developed by EPA for drinking and recreational waters for microcystins and cylindrospermopsin (USEPA, 2015a, 2015b, 2019). Similarly, the State of California has developed recreational trigger levels for microcystins, anatoxin-a, and cylindrospermopsin with the aim of safeguarding human and animal health (OEHHA, 2012; https://mywaterquality.ca.gov/monitoring_council/Cyanohab_network/docs/triggers.pdf)

Proliferation of planktonic cyanobacteria commonly occurs in lentic waterbodies, including lakes, reservoirs, depressional wetlands, and coastal lagoons, which are replete for nutrients, have calm and/or stratified water columns, and have plenty of irradiance and warm water temperatures, which may be exacerbated with climate change (O'Neil et al. 2012, Paerl and Huisman 2008, Paerl and Paul 2012, Xu et al. 2010). In California, lentic habitats are often subject to further risk of cyanobacteria proliferation due to increased anthropogenic nutrient inputs via point and nonpoint source runoff from urban and agricultural areas. Cyanotoxins can also be produced in benthic blooms of cyanobacteria, which can occur in lakes, rivers, and wadable streams. There are an increasing number of waterbodies in California that have recurrent toxic cyanobacteria blooms, including the Klamath River watershed, Clear Lake, Pinto Lake, lower Sacramento and San Joaquin Rivers and Delta, Lake Elsinore, and several East San Francisco Bay Area lakes. However, beyond these systems, the overall extent and magnitude of cyanobacteria and cyanotoxin prevalence is largely under-characterized in California, especially in Southern California.

Recent studies identified ubiquitous distributions of cyanobacteria in 30 lakes, depressional wetlands and coastal lagoons in Southern California. Of these sites, *Microcystis* spp. dominated the community in 96% of study sites (Magrann et al. 2015). Most species of *Microcystis* can produce one or more variants of microcystin, and some species and strains can produce additional classes of cyanotoxins. Microcystins are a group of over 90 hepatotoxins produced by cyanobacteria, of which microcystin-LR is the most common (Schmidt et al. 2014; Pearson et al. 2010). Additionally, microcystins were prevalent in all types of lentic waterbodies surveyed across the land-sea continuum in Southern California (Howard et al. 2017). To date, the occurrence of cyanotoxins, particularly microcystins, is poorly characterized in Region 4 waterbodies.

The occurrence of cyanotoxins in freshwater systems is challenging to characterize due to the ephemeral nature of toxin producing cyanobacterial blooms, creating challenges to capture toxin occurrence using conventional (grab) sampling techniques unless systems are monitored regularly. The large number of fresh and brackish waterbodies that can support cyanobacterial

growth makes routine monitoring of cyanoHABs in all potentially vulnerable systems challenging and resource intensive. To overcome these challenges, equilibrium passive sampling devices that utilize sorptive resins (e.g., the Solid Phase Adsorption Toxin Tracking device, or SPATT) have been developed as an assessment and monitoring tool to provide time integrated data on the presence of cyanotoxins. A key feature of SPATT is that the resin (HP20, polystyrene/divinylbenzene) used in the sampler both adsorbs and desorbs toxins depending on the ambient concentration in order to mimic bioaccumulating organisms. A key constraint of SPATT samplers is that they are not fully calibrated to allow for more quantitative estimations and comparisons of cyanotoxin concentrations across systems, and thus are difficult to put into the context of human health risks. The nature of the SPATT samplers also makes the devices sampling rates subject to environmental factors such as pH, ionic strength or flow rate (Kudela 2017).

In contrast, aquatic passive samplers such as the polar organic chemical integrative sampler (POCIS, (Alvarez et al. 2004)) and the organic diffusive gradients in thin films sampler (o-DGT, (Chen et al. 2012)), have been successfully calibrated for the continuous time-weighted-average quantitation of freely dissolved concentrations of polar organic contaminants in water and sediment. The o-DGT has advantages over other aquatic passive samplers for polar organic contaminants, including POCIS, in that sampling rates on o-DGT are dependent mainly on the thickness of the diffusive layer used to construct the sampler (Chen et al. 2012). As a result, sampling rates for o-DGT are controlled mainly by chemical diffusivity through the diffusive layer. This is a physical-chemical property that depends on chemical structure and on temperature, both of which are predictable (Challis et al. 2016). As a result, o-DGT chemical sampling rates, which are necessary for calculating time-weighted-average concentrations, are much less dependent on flow rate, ionic strength, pH, and other environmental factors than they are for kinetic samplers that are boundary-layer controlled, such as POCIS. Flow-rate dependence on such factors is often complex and difficult to predict, particularly in flowing environments, such as rivers, lake inlets/outlets and tidal lagoons. Additionally, o-DGT differs from SPATT in that the sorption of target analytes to the resins used in o-DGT samplers is typically irreversible in aqueous solution. However, the o-DGT, which has been validated for small polar organic contaminants (Challis et al. 2016, Chen et al. 2012, Stroski et al. 2018), has not been fully optimized for use with cyanotoxins in field monitoring projects and has been utilized far less than the SPATT samplers for monitoring cyanotoxins (Kudela 2017). Previous efforts to adapt o-DGT for cyanotoxins have been limited only to microcystin-LR (D'Angelo 2019, Yao et al. 2019).

In the current study, we focused on microcystins due to the previously reported dominance of *Microcystis* spp. in Southern California systems and because they are the most common cyanotoxin detected in the U.S. lakes in previous screening assessments (Fetscher et al. 2014, Magrann et al. 2015). Hence, this project aims to 1) document the occurrence of microcystins in water in selected Region 4 waterbodies; and 2) compare the utility of resin- and film-based passive sampling methods for measuring the occurrence of microcystins in freshwater systems.

METHODS

Study area

The field surveys were conducted at waterbodies within the Los Angeles Regional Water Quality Control Board (Region 4, LARWQCB). These waterbodies are in the most densely populated region in the state and typically receive nutrient loading through upstream runoff. The majority of these waterbodies serve as drinking water and recreational reservoirs, or as stormwater detention basins. Other sites of initial interest included ponds in city and state parks, recreational creeks, and gravel pits.

Site selection and sample collection for 2018 survey

The selection of study sites was based on the previous bloom events and on suggestions from the State Water Board. Fourteen waterbodies were initially prioritized for field surveys: Pyramid Lake, Lake Piru, Lake Casitas, Castaic Lake, Malibu Creek (near Century Dam and the Rock Pools), El Dorado East Regional Park Lake, Lincoln Park Lake, Bouquet Reservoir, Elderberry Forebay, Irwindale Gravel Pit, Lake Bard (Wood Ranch Reservoir), Morris Reservoir, Puddingstone Dam and Reservoir, and San Gabriel Reservoir. Due to logistical constraints and inability to receive site access permissions in a timely manner, Elderberry Forebay, Irwindale Gravel Pit, Morris Reservoir, Puddingstone Dam and Reservoir, and San Gabriel Reservoir were not able to be sampled in the preliminary survey.

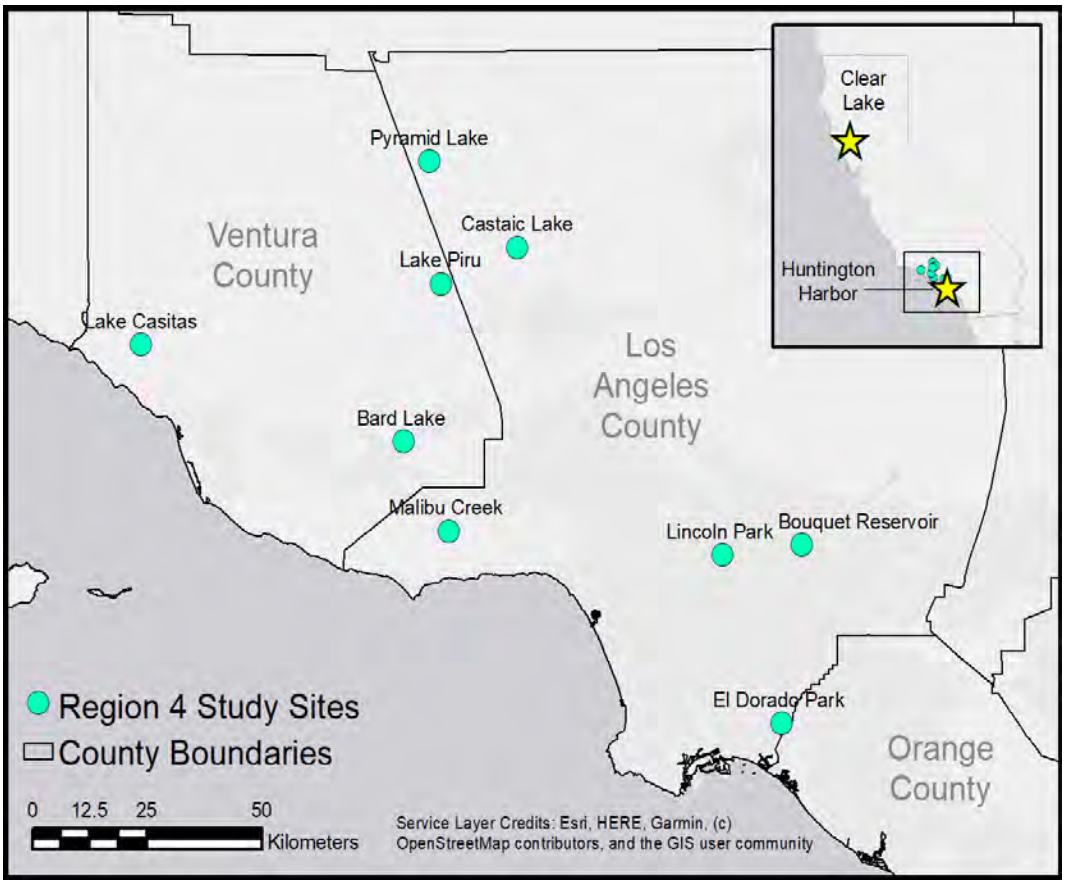


Figure 1. Map showing sampling locations in Region 4.

Grab water samples were collected during the summer-fall seasons of 2018 at each of the nine preliminary survey sites in Year 1. At each site, one sample was collected at a reference site at the center of the waterbody and another sample was collected along the windward shore. In cases where there was no access to the center of the waterbody, the second sample was collected at the opposite shore to serve as a reference site. In Malibu Creek, which is flowing system, samples were taken downstream and upstream of Century dam. Water samples were collected in 1-L pre-rinsed amber glass bottles without headspace and transported to the laboratory on ice. The sampling date, time, and coordinates of location are shown in the Table 1.

Table 1. List of study sites with sampling date, time and coordinates in 2018.

Date	Waterbody	Reference			Windward		
		Time	Latitude	Longitude	Time	Latitude	Longitude
9/18	Pyramid Lake	10:51	34.6546	-118.7754	11:40	34.6777	-118.7818
9/18	Castaic Lake	13:02	34.5263	-118.6041	13:14	34.5561	-118.6175
9/28	Lake Casitas	11:00	34.3813	-119.3399	11:53	34.4021	-119.3274
9/28	Malibu Creek	15:45	34.1027	-118.7381	16:05	34.0985	-118.7328
10/1	Lincoln Park	12:30	34.0670	-118.2017	12:10	34.0659	-118.2038
10/1	El Dorado Park	14:20	33.8149	-118.0857	14:40	33.8171	-118.0860
10/12	Bard Lake	9:49	34.2372	-118.8255	10:08	34.2338	-118.8198
11/2	Lake Piru	10:49	34.4726	-118.7525	11:23	34.4750	-118.7572
11/7	Bouquet Reservoir	11:42	34.0823	-118.0473	11:54	34.0861	-118.0389

Site selection and sample collection for 2019 survey

During the summer-fall of 2019, passive sampling devices (o-DGT and SPATT bags) were deployed at a single location in Pyramid Lake, Castaic Lake, Clear Lake (Northern California), and Huntington Harbor (Table 2). Sites were selected based on previous detection of toxins and feasibility of deployment. SPATT samplers have been a useful tool in monitoring the prevalence of microcystins and other algal toxins in multiple ambient monitoring and assessment programs. At present, SPATT samplers are only able to provide semi-quantitative estimates of toxin concentrations due to challenges in calibrating toxin concentrations in the environment. In the present study, SPATT samplers were co-deployed with o-DGT to compare and contrast the performance of both passive sampling approaches.

At each site, three o-DGT samplers and two SPATT bags were deployed for a period of 1-3 weeks. The duration of deployments was decided based on the suspected bloom intensity at the time of deployment, given that biofouling may reduce the adsorption capacity as well as complicate the evaluation of performance between o-DGT and SPATT. Due to the presence of a high biomass bloom of *Microcystis* spp. at Pyramid Lake in September 2019, we deployed again in October 2019 to assess the temporal differences during a bloom. Grab water samples were also collected at the time of deployment and retrieval.

Table 2. Waterbodies sampled by passive samplers in 2019.

Waterbody	Latitude	Longitude	Deployment	Retrieval	Temperature (ºC, ±SD)
Pyramid Lake-Sep	34.6777	-118.7829	9/13	9/23	22.9 ± 1.1
Pyramid Lake-Oct	34.6777	-118.7829	10/14	10/21	20.4 ± 0.5
Castaic Lake	34.5192	-118.6008	10/21	10/29	18.5 ± 0.5
Clear Lake	39.0662	-122.8431	10/8	10/31	16.7 ± 1.6
Huntington Harbor	33.7271	-118.0763	10/2	10/23	20.9 ± 0.8

Construction of passive samplers

o-DGT sampler construction

Binding and diffusive gels for o-DGT were created following the procedures of Stroski et al. (2018). In brief, agarose binding gels were prepared by casting a 1.5% agarose mixture with 0.35 g Oasis HLB resin into sheets using a Bio-Rad Mini-Protean® casting system. Once set, the gels were cut into 1" diameter disks, rinsed with pure water (Milli-Q, 18 MΩ-cm), then stored in 5 mM KNO_3 solution until use. Polyacrylamide diffusive gels were prepared using 12.5 mL of 30% acrylamide monomer, 2.5 mL of 1% *N,N'*-methylenebisacrylamide cross-linker, 9.79 mL Milli-Q water, 200 µL of 10% ammonium persulfate initiator, and 10 µL of *N,N,N',N'*-tetramethylethylenediamine catalyst. After casting to a thickness of 0.75 mm, the diffusive gel sheets were hydrated with Milli-Q for 24 h to swell them to stable dimensions (0.90 mm final width). This procedure results in gels with 0.1% cross-linker and 15% polyacrylamide monomer, a well-studied amount in the DGT literature (Scally et al. 2006). Both binding and diffusive gels were placed into standard-sized plastic DGT holders (DGT Research, Lancaster, UK) with the binding gel against the base, sorbent side up, and the diffusive gel on top and sealed with a standard cap. Polyethersulfone membranes were not used, given potential issues with sorption of some analytes to these membranes that would confound sampling rates (Challis et al. 2016).

SPATT sampler construction

SPATT bags were constructed with Diaion HP20 resin (Sorbtech; Norcross, GA) and 100 µm mesh (Wildco; Yulee, FL) and supported by an embroidery hoop ring (Figure 2B). After construction, the bags were activated in 100% MeOH at 4°C for 24 hrs, then rinsed and stored in ultrapure water at 4°C until use as described in Lane et al. (2010).

Development of o-DGT

The o-DGT passive sampling device consists of a diffusive gel and a binding gel (Figure 2A). Based on Fick's First Law of Diffusion (Davison and Zhang 1994), dissolved-phase chemicals diffuse through the diffusive gel and attach to the resin in the binding gel. The time-averaged aqueous concentration (C_w) can then be calculated:

$$M_{DGT} = (DA/\Delta g)C_w t = R_s C_w t$$

where M_{DGT} is the measured mass of the chemical associated with the binding gel, D is the chemical's diffusivity through the diffusive gel, A is the cross-sectional area of the sampler, Δg is the measured thickness of the diffusive gel, t is the amount of time that the sampler has been deployed. The sampling rate $R_s = DA/\Delta g$ (units cm^3/d) is a measure of how much water is effectively sampled per day by the device.

Calibration of o-DGT passive sampler for Microcystins

The Diffusive Gradients in Thin-Films (DGT) sampling technique was originally developed to determine dissolved metal concentrations (Davison and Zhang 1994), but has since been used to monitor dissolved inorganic and organic pollutant (o-DGT) concentrations in different matrices (Challis et al. 2016, D'Angelo 2019, Kim et al. 2016, Mason et al. 2010). Unlike the solid phase adsorption toxin tracking (SPATT) sampler, the DGT includes a diffusive layer that allows chemicals that are sampled by the device to accumulate in the resin at a controlled diffusion rate. Bench-scale tests were conducted with three microcystins (MC), including MC-LR, MC-RR, and MC-YR, to evaluate the sorption and uptake capacities of the sampler.

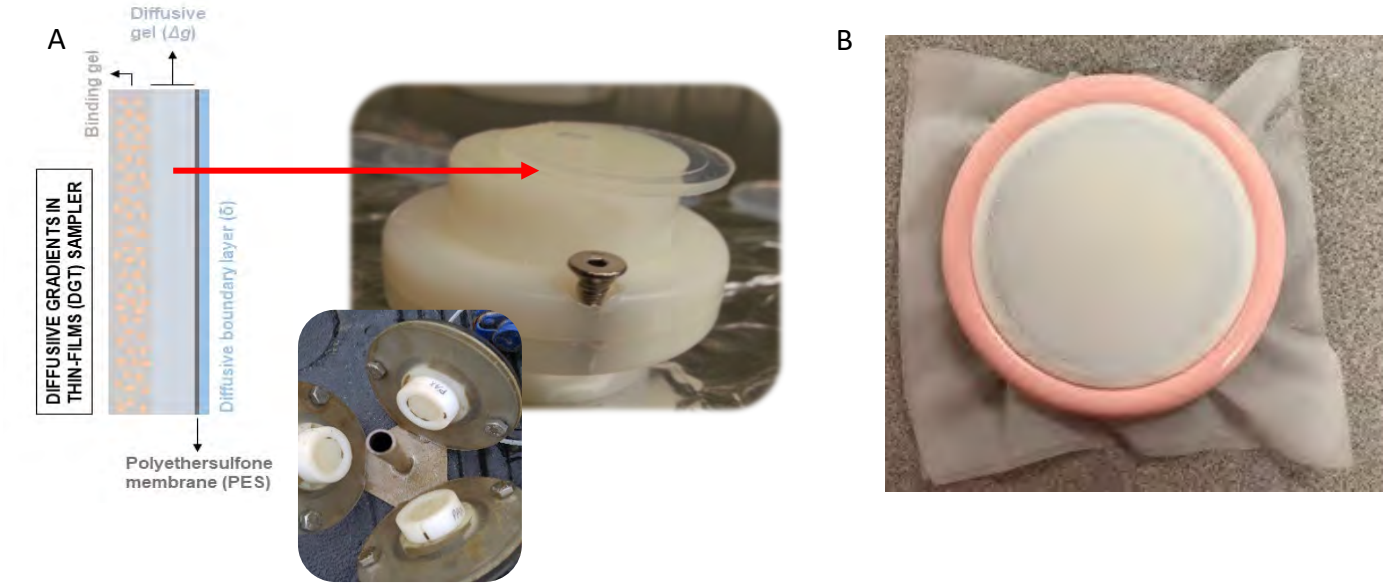


Figure 2. Schematic of an o-DGT passive sampling device (A) and a SPATT bag (B).

Sorption experiment

The polyacrylamide diffusive (PA) gels and hydrophilic-lipophilic balance (HLB) binding gels were separately immersed in glass tubes in triplicate both with microcystins at the concentration of 5 µg/L and blank control. Each treatment was agitated for 20 h at 80 rpm. Triplicate aliquots of water samples were collected directly from the tubes prior to and post the experiment to capture the change of exposure concentration in response to sorption to either PA gels or HLB binding gels.

Table 3. Summary of methods used to quantify microcystins.

Quantification Approach	Sample Type(s)	Congeners/Metabolites Quantified	Sample Set
LC-MS/MS	Whole water grabs*	MC-LA, MC-LR, MC-RR, MC-YR, MC-LY, MC-LW, MC-LF, MC-HilR, and MC-WR,desmethyl-MC-LR, desmethyl-MC-RR	2018 Survey
LC-MS/MS	Whole water grabs*, o-DGT, SPATT	MC-LR, MC-RR and MC-YR	2019 Survey

Total MC ADDA ELISA	Whole water grabs*, o-DGT, SPATT	Quantifies total of all MC congeners	2018 Survey, 2019 Survey

*Whole water grabs refer to samples collected and extracted for the particulate + dissolved fractions of microcystins.

Calibration experiment

Uptake kinetics for o-DGT were determined by deploying PA-HLB assembled o-DGT in a mixture with KNO_3 of 5 mM an microcystin -LR, -RR, -YR each at a concentration of 1 µg/L at 20 ± 0.5°C. Over the 10-day duration, two o-DGTs were collected each on days 1, 2, 4, 7, and 10. To ensure constant aqueous concentration throughout the experiment, water renewals were conducted at day 5. Aliquots of grab water samples were collected from all the containers prior to and post water renewal and at the end of the experiment to verify the constant exposure levels.

Laboratory analyses of microcystins via LC-MS/MS

The whole water samples collected in 2018 were analyzed for 9 microcystin congeners (MC-LA, MC-LR, MC-RR, MC-YR, MC-LY, MC-LW, MC-LF, MC-HilR, and MC-WR) and 2 microcystin metabolites (desmethyl-MC-LR and desmethyl-MC-RR) by liquid chromatography tandem mass spectrometry (LC-MS/MS) or (for the eleven in aggregate) by enzyme-linked immunosorbent assay (ELISA). The whole water samples and passive sampler samples collected in the field in 2019 were analyzed with the same fashion. In addition, those samples from bench-scale o-DGT experiments were analyzed for the three major microcystin congeners (MC-LR, MC-RR, and MC-YR) by LC-MS/MS. Note that, hereafter, "microcystins" refers to the combined values for all amenable microcystin congeners detectable by the quantification method (Table 3).

Table 4. Instrumental settings of LC-MS/MS.

LC conditions	
Column	Agilent ZORBAX Eclipse Plus C18 2.1*50mm, 1.8µm
Column temperature	35°C
Injection volume	20 µL
Mobile phase	A) Water, 0.1% formic acid B) Acetonitrile, 0.1% formic acid
Linear gradient	Time (min) %B 0 6 1 6 4 50 5 50 8 95 9 95 9.01 6 12 6 Post time 2 min
Flow rate	0.4 mL/min
MS/MS conditions	
Ionization Mode	ESI positive, dMRM
Drying gas temperature	350°C
Drying gas flow	11 L/min
Nebulizer gas	40 psi
Capillary voltage	4000 V

For the samples analyzed by LC-MS/MS (Table 4), electrospray ionization (ESI) with dynamic multiple reaction monitoring (dMRM) was employed using an Agilent 1260 liquid chromatograph and Agilent 6470 triple quadrupole mass spectrometry system (Santa Clara, CA). An Agilent Zorbax Eclipse Plus C18 column (2.1×50 mm, 1.8 µm) was employed. This method was adapted from EPA method 544 with minor modifications, to account for the choice of supplies and instrument parameters. Aqueous samples were spiked with internal standards (MC-LR d_7) then filtered through Whatman GF/F filters. The filtrate was extracted with Waters Oasis HLB solid phase extraction (SPE) cartridges 6cc (200 mg). The lowest concentration of calibration curves was adopted as the reporting limit.

The o-DGT samplers were extracted with 100% methanol. The HLB binding gels were collected and used to verify the extraction efficiency. HLB binding gel from the sorption experiment was extracted for 3 times with 3 mL of methanol (×3) with sonication for 2 min. The extracts were combined and spiked with the internal standard prior to LC-MS/MS analysis. An additional methanol rinse following the extraction was analyzed separately to determine the remaining mass left on the binding gel, which help characterize the extraction recovery.

Toxin extractions from SPATT samplers was conducted as described in Lane et al. (2010), resulting in 3 extracts from each individual SPATT bag. Extract 1 and Extract 2 was eluted with 10 mL of 50% MeOH (v/v) and Extract 3 was eluted with 20 mL of 50% MeOH. The final concentration extracted from the bag was calculated by summing the concentrations of all 3 extracts.

Laboratory analyses of microcystins via ELISA

Microcystins were analyzed in grab water samples, SPATT and o-DGT extracts by ELISA using the ADDA ELISA test kits (Abraxis, Part No. 520011OH, Warminster, PA). This assay detects all microcystin and nodularin variants with the ADDA side group in bulk and does not provide data about concentrations of specific variants.

Water samples collected in 2018 and 2019 were analyzed for microcystins in the dissolved and particulate pools following EPA method 546. Prior to analysis, the samples were lysed by freeze-thaw three times to ensure cell disruption. The extract was then analyzed according to the manufacturer's instructions. Samples with concentrations higher that the standard curve were serially diluted with kit provided dilution buffer until sample concentration was within the working range of the kit.

Extractions of o-DGT samplers followed the same protocol as described for analysis via LC-MS/MS with slight modifications. O-DGT extracts used for analysis via ELISA were not spiked with an internal standard. Extracts were blown to dryness under nitrogen, reconstituted with MeOH:H_2O 1:1, and then filtered. Finally, after extracts were combined, the sample was blown to half the volume to volatize the MeOH. Sample extracts were loaded directly on to kit without dilution following manufacturer's instructions.

Extractions of SPATT samplers followed the same protocol as described for analysis via LC-MS/MS Extracts were analyzed following the manufacturer's guidelines and extracts were diluted a minimum of 1:10 in the manufacturer supplied dilution buffers to reduce the MeOH concentration prior to analysis.

RESULTS AND DISCUSSION

Occurrence of microcystins in water grab samples from Region 4 waterbodies in 2018

Of the 9 sites sampled for microcystins analysis using ELISA, 7 sites tested positive during the preliminary survey in 2018. No microcystins were detected in the samples collected from El Dorado Park Lake and Malibu Creek. At 5 of 7 sites where microcystins were detected, total-microcystin concentrations were below the caution trigger level (0.8 µg/L) for human health based on California recreational health trigger levels (OEHHA, 2012; https://mywaterquality.ca.gov/monitoring_council/Cyanohab_network/docs/triggers.pdf). At Lincoln Park Lake and Pyramid Lake, microcystins were detected above the warning (Tier I) level (6 µg/L). At the windward locations, where higher concentrations were typically observed than the reference locations, very high concentrations of microcystins (78 µg/L) were detected at Pyramid Lake. This concentration is well above the danger level (Tier 2) of 20 µg/L. The occurrence of microcystins at all sites is shown in Table 5.

Given the lack of specificity from the ELISA analysis, the initial results from ELISA screening were further confirmed by LC-MS/MS to quantify the individual microcystin congeners. The whole water samples from 9 study sites were tested for 9 individual microcystin congeners and 2 microcystin metabolites. The most commonly detected congeners were microcystin-LR and microcystin-LA, both of which were detected at 4 sites and exhibited a maximum concentration of 137 µg/L and 0.33 µg/L, respectively. At the Pyramid Lake shore location, all targeted microcystins were detected and the concentrations were substantially higher than those at the rest of sampling locations. The total concentration of all 11 microcystin compounds was 184 µg/L at the shoreline location at Pyramid Lake, confirming the highest detection level among study sites from the ELISA analyses. Although the microcystin congeners varied at each of the other 8 study sites, the summed concentrations of total microcystins detected at these sites were all below the caution trigger level (Table 5).

Table 5. Occurrence of microcystins detected by LC-MS/MS and ELISA in Region 4 waterbodies (µg/L) in 2018.

	des-MC-RR[a]	MC-RR	MC-YR	des-MC-LR[a]	MC-LR	MC-HilR	MC-WR	MC-LA	MC-LY	MC-LW	MC-LF	LC-MS/MS Total	ELISA
Pyramid Windward	0.45	13.3	1.77	8.80	137	2.14	5.63[e]	0.868	7.03	5.70[e]	1.54	184	78
Pyramid Reference	ND[b]	0.007	0.001	0.002	0.73	0.002	ND	0.001	ND	ND	ND	0.743	2.10
Castaic Windward	ND	0.004	ND	0.011	0.6	0.005	ND	0.004	ND	ND	ND	0.623	<0.15
Castaic Reference	ND	ND	ND	ND	0.002	ND	ND	0.005	ND	ND	ND	0.007	<0.15
Casitas Windward	ND	ND	ND	ND	<0.0006	ND	ND	ND	ND	ND	ND	ND	<0.15
Casitas Reference	ND	ND	ND	ND	<0.0006	ND	ND	ND	ND	ND	ND	ND	<0.15
Malibu downstream[g]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Malibu upstream	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lincoln N. shore[f]	ND	ND	ND	0.013	0.18	0.022	<0.03[c]	<0.0006	ND	ND	ND	0.215	12.0
Lincoln S. shore[f]	ND	ND	ND	ND	0.006	ND	ND	ND	ND	ND	ND	0.006	0.50
El Dorado N. shore[f]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
El Dorado S. shore[f]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bard lake Windward	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bard lake Reference	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<0.15
Piru Windward Dup[d]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<0.15
Piru Windward	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<0.15
Piru Reference	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<0.15
Bouquet Windward	ND	ND	ND	ND	ND	ND	ND	0.112	ND	ND	ND	0.112	ND
Bouquet Reference	ND	ND	ND	ND	ND	ND	ND	0.332	ND	ND	ND	0.332	<0.15

a. Metabolites of MC-RR and MC-LR, desmethyl-microcystin-RR and desmethyl-microcystin-LR
b. ND = not detected.
c. less than a value means the microcystin was detected, but not quantifiable.
d. Dup = duplicate
e. Field estimate
f. If access of geographic centers are not logistically possible, another sample was collected from the opposite shore.
g. Flowing system

Reference vs. windward

There are a few study sites where sampling at the geographic center of the waterbody was not logistically possible. Therefore, we sampled these waterbodies at a site that was opposite to the windward shore. Not surprisingly, a higher concentration of microcystins were detected at windward locations, sometimes up to more than two orders of magnitude compared to those at reference locations. For example, ELISA results showed that microcystins at the windward shore of Pyramid Lake were 37 times greater than those at the center. The difference was also confirmed by LC-MS/MS, but the difference appeared to be even higher (249 times greater at the windward shore). At sites with low concentrations detected via ELISA, no obvious differences were observed between concentrations at the reference and windward locations. Unlike the other survey sites, microcystin-LA was the sole microcystin detected at Bouquet Reservoir at a greater concentration at the center of the waterbody than at the windward sampling location.

Sorption

Microcystin (-LR, -RR, and -YR) sorption by diffusive gel (PA) and binding gel (HLB) was assessed by monitoring changes in aqueous concentration before and after the experiment. Sampler components other than the binding gel should not bind target microcystins. The aqueous concentration of the PA diffusive gel remained at the initial level, indicating that any sorption of microcystins by the PA diffusive gel was minimal (≤ 5%) and could therefore be usable as the diffusive layer for o-DGT. In contrast, the binding gel should have high binding capacity for microcystins, and should also have good microcystin extraction recovery from the binding gel. During the 20-h binding test, microcystins in the exposure pool were reduced by 69% to 80% of the original concentrations (Figure 3), which indicated sufficient binding capacity of the HLB binding gel for microcystins. Greater than 98% of the sorbed microcystins were extracted from the HLB binding gel via the 100% MeOH solvent extraction described extraction procedure in the methods section above. These results are consistent with the use of HLB for effective solid-phase extraction of microcystins in grab samples of water (Kaloudis et al. 2013, Zervou et al. 2017). As a result of these tests, both the agarose-HLB binding gel and polyacrylamide diffusive gel were suitable for further evaluation. This configuration is identical to that of Stroski et al. (Stroski et al. 2018) for suite of 31 polar organic contaminants (e.g., pharmaceuticals and personal care products), suggesting that this configuration may be usable not only for anthropogenic polar CECs, but also simultaneous monitoring of natural-origin cyanotoxins as well.

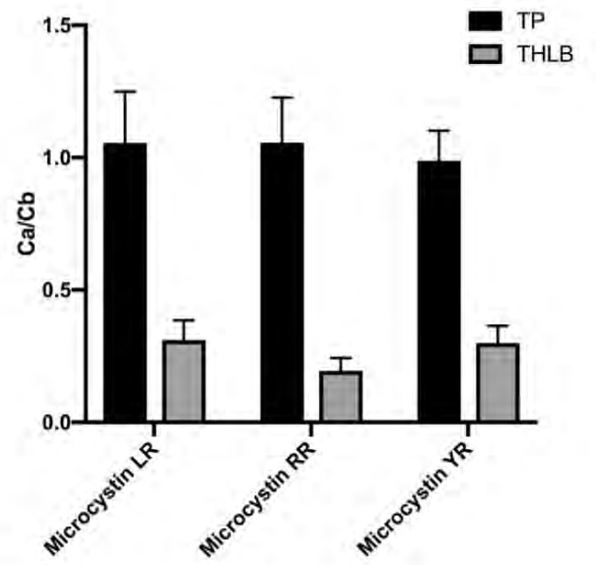


Figure 3. Relative change in concentrations of microcystin-LR, microcystin-RR, and microcystin-YR at the start (Cb) and end (Ca), respectively, for TP (PA diffusive gel) and THLB (HLB binding gel).

Uptake kinetics

Water concentrations remained constant and near the nominal 1 μg/L target value (Figure 4), including renewal of the aqueous solution half-way through the 10-day evaluation period. This result indicated that the concentration gradient driving uptake of microcystins to the binding gel remained constant over the course of the experiment. We renewed the water in the uptake experiment halfway through the evaluation period, and measurements showed that concentrations were stable throughout the entire period (Figure 4).

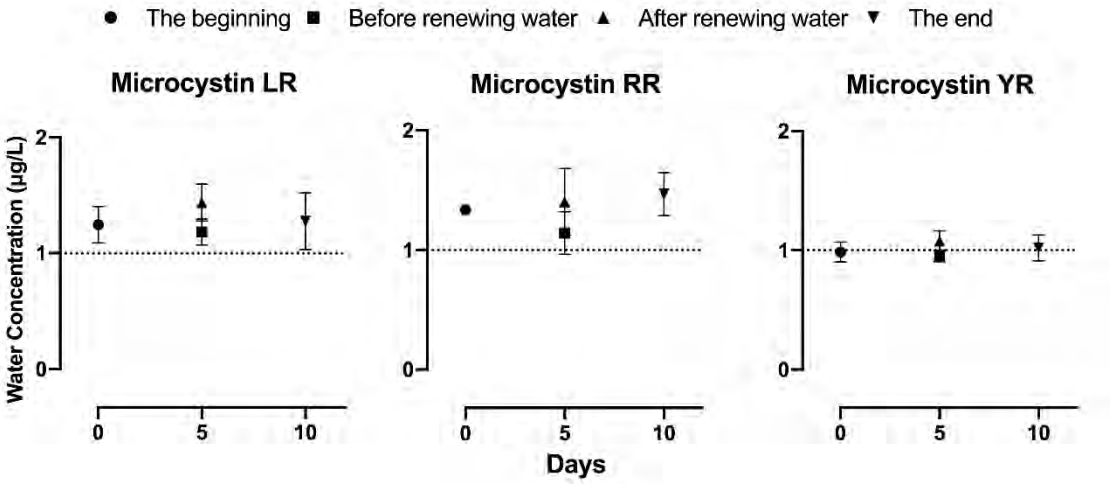


Figure 4. Aqueous concentrations of microcystin-LR, microcystin-RR, and microcystin-YR over the uptake test. Error bars were calculated from the standard deviation (SD) of three replicates.

As shown in Figure 5, uptake of all three microcystins appears to be similar and linear over the 10-day test. This is expected, given that these microcystin congeners are of comparable molecular weight. Accordingly, they would have similar diffusivities through the diffusive gel and by extension through water given the high porosity of diffusive gels (Challis et al. 2016, Stroski et al. 2018). The linearity observed in this uptake test is consistent with that observed for dozens of polar organic contaminants, ranging from pharmaceuticals to personal care products to pesticides (Challis et al. 2016) over at least 21 days. These observations suggest that the linear uptake phase of the o-DGT configuration that we have used is at least 10 days and is likely to be up to 21 days (Challis et al. 2016). Linearity is important for kinetic passive samplers. Calculation of sampling rates for samplers in the linear phase of chemical uptake is straightforward, and is unencumbered by corrections for a myriad of factors such as accounting for changes in concentration gradients that can occur if the resin is nearing the point of saturation (Booij et al. 2007). Our results are in contrast with the fast uptake of MC-LR reported by Yao et al. 2019, in which MC-LR reached a plateau within an hour by using a different diffusive layer. In our case, the uptake of the three major microcystin congeners in the current study never reached saturation, a point supporting the feasibility of our configuration of o-DGT for passive sampling of microcystins.

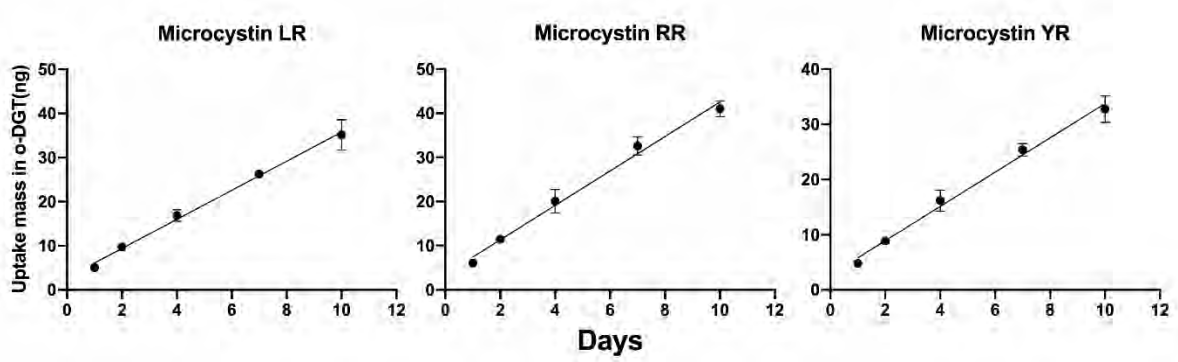


Figure 5. Mass uptake of microcystin-LR, microcystin-RR, and microcystin-YR using PA-HLB o-DGT over 10 days.

The sampling rates and diffusion coefficients are consistent with known parameters for polar organic molecules. Because Rs is inversely proportional to diffusion through the diffusive gel in the sampler, larger molecules will diffuse more slowly than smaller molecules and hence have a smaller sampling rate. The three microcystins are considerably larger in molecular weight than the small polar molecules for which sampling rates for o-DGT have typically been measured (Belles et al. 2017, Challis et al. 2016, Chen et al. 2012, Guibal et al. 2017, Stroski et al. 2018). Measured room temperature sampling rates and gel diffusivities for polar pesticides, pharmaceuticals, personal care products and other small polar CECs with molecular weight ranges in the 100-400 Da range on o-DGT are typically 10-15 cm^3/d and $3\text{-}5\times10^{-6}$ cm^2/s, respectively, while those for larger analytes (e.g., macrolide antibiotics of 700-800 Da) are approximately 5 cm^3/d and $2\text{-}3\times10^{-6}$ cm^2/s, respectively. Our measured values for each microcystin (Table 6) are consistent with this trend of decreasing diffusivity with increasing molecular weight.

Table 6. Molecular weights (Da), and measured sampling rates (Rs, $cm^3\ d^{-1}$) and 15% polyacrylamide diffusion coefficients (D, $\times 10^{-6}\ cm^2 s^{-1}$) of microcystin-LR, microcystin-RR, and microcystin-YR.

		PA HLB o-DGT	
	Molecular weight	Rs	D
Microcystin-LR	995.189	2.68 ± 0.23	0.90 ± 0.08
Microcystin-RR	1038.20	3.06 ± 0.33	1.03 ± 0.11
Microcystin-YR	1045.19	3.22 ± 0.18	1.08 ± 0.06

The only other measured diffusion coefficient in diffusive gels was 2.5×10^{-6} cm^2/s for agarose at 25°C, for microcystin-RR (Yao et al. 2019). Estimations of aqueous diffusivity for this congener are 3×10^{-6} cm^2/s at 23°C (D'Angelo 2019). These values are larger than our measured values (Table 6) due to several factors. First, while estimations of aqueous diffusivity are typically quite accurate for small molecules (Schwarzenbach et al. 2017), the empirical expressions typically used for such estimations are generally based only on measurements of small molecules (Wilke and Chang 1955) and may not take into account changes in molecular conformation and shape that may affect diffusivity and are more likely for large molecules. Second, polyacrylamide pore sizes are smaller than those of agarose (Stroski et al. 2018) used in these other studies (D'Angelo 2019, Yao et al. 2019). Also, diffusivity increases with temperature. Aqueous diffusion coefficients at a given temperature (D_T) can be corrected for temperature (T) and the diffusivity at 298 K (D_{298K}):

$$\log D_T = 1.37023(T - 25) + 0.000836(T - 25)^2/(100 + T) + \log[D_{298K}(273 + T)/298]$$

Such temperature corrections can be used in field deployments if temperature is also co-monitored, as they were in this study. Finally, aqueous diffusivity is greater than diffusivity through gels given that the structure of the gel can impede molecular diffusion. This effect has been shown to be minor for small molecules, particularly in agarose gel for which the porosity is large (98%, (Chen et al. 2012)). However, larger molecules such as microcystins may be more impeded in diffusion through gel pores that are less of a hindrance for smaller molecules.

While we did not evaluate the effects of pH or ionic strength on uptake of microcystins by o-DGT, these have been shown to be negligible in previous experiments on microcystin-RR (Yao et al. 2019). Our results demonstrate that reproducible sampling rates consistent with existing efforts on polar CEC uptake by o-DGT are possible, and that this device may be feasible for monitoring of microcystins subject to successful field characterization.

Field characterization using passive sampling devices in the 2019 survey

In the current study, the performance of o-DGT samplers was evaluated in natural waters over the summer-fall of 2019. We selected Pyramid Lake and Castaic Lake, where microcystins were detected in the preliminary survey in 2018 and deployed both o-DGT and SPATT passive samplers for between 7 to 23 days to determine the prevalence of microcystins. Opportunistically, we included Huntington Harbor (Orange County) and Clear Lake (Northern California) in the current study for comparison purposes. In the 2019 survey, we only focused on

MC-LR, MC-RR, and MC-YR in the evaluation of passive samplers and comparison among samplers and grab events.

Of the 4 distinct sites sampled for microcystins, 3 sites had detectable microcystins in the water grab samples during at least one of the site visits (Table 7). Microcystins exceeded the caution trigger level (0.8 µg/L) during the deployment sampling of Pyramid Lake on September 13th based on LC-MS/MS results. However, the ELISA results were 2.6-fold higher than the LC-MS/MS results and, microcystins exceeded the warning trigger level (6 µg/L). Ten days later, the microcystins in the water grab samples decreased, but were still above the caution trigger level (0.8 µg/L) during the retrieval sampling of Pyramid Lake on September 23 according to both the LC-MS/MS and ELISA results. No grab samples from the other sites exceeded any of the California trigger levels.

Table 7. Occurrence of microcystins in water grab samples in surveyed waterbodies (µg/L) in 2019 via LC-MS/MS and ELISA.

	MC-LR	MC-RR	MC-YR	LC-MS/MS Total	ELISA
Pyramid Lake Sept Deployment	2.7	1.5	0.037	4.2	10.9
Pyramid Lake Sept Retrieval	0.68	0.26	0.015	0.954	3.36
Pyramid Lake Oct Deployment	0.009	0.005	ND	0.015	0.21
Pyramid Lake Oct Retrieval	0.004	0.001	ND	0.005	ND
Castaic Lake Deployment	0.019	0.002	ND	0.021	0.16
Castaic Lake Retrieval	0.04	0.003	0.007	0.050	0.44
Clear Lake Deployment	0.01	0.003	ND	0.015	ND
Clear Lake Retrieval	0.003	0.002	ND	0.005	ND
Huntington Harbor Deployment	ND	ND	ND	ND	ND
Huntington Harbor Retrieval	ND	ND	ND	ND	ND

In this study, we compared grab sample results, o-DGT results, and SPATT results by LC-MS/MS analysis to assess the relative performance of these approaches. The o-DGT results were comparable in prevalence and concentrations to the grab sample results when microcystins were detected at low levels (under 0.1 µg/L) (Figure 6). Specifically, a good correspondence between MC-LR concentrations in the water grab samples and the o-DGT samplers was observed in the Pyramid Lake deployment in October, at Castaic Lake and at Clear Lake (Figure 6). Interestingly, there was a weak correspondence between the o-DGT and water grabs concentrations of MC-LR during the Pyramid Lake deployment in September when water concentrations of MC-LR were 1 to 2 orders of magnitude higher than the other sites surveyed. MC-RR and MC-YR were rarely detected by o-DGT throughout the study sites while they were more frequently detected in the grab samples. The correspondence between the calculated MC-RR and MC-YR concentrations in o-DGT and the grab samples was weaker than that of MC-LR.

Figure 6. Comparison of microcystin-LR, -RR, -YR concentrations measured by o-DGT and grab sampling. The asterisk (*) shows the average of the deployment and retrieval grab water samples concentrations.

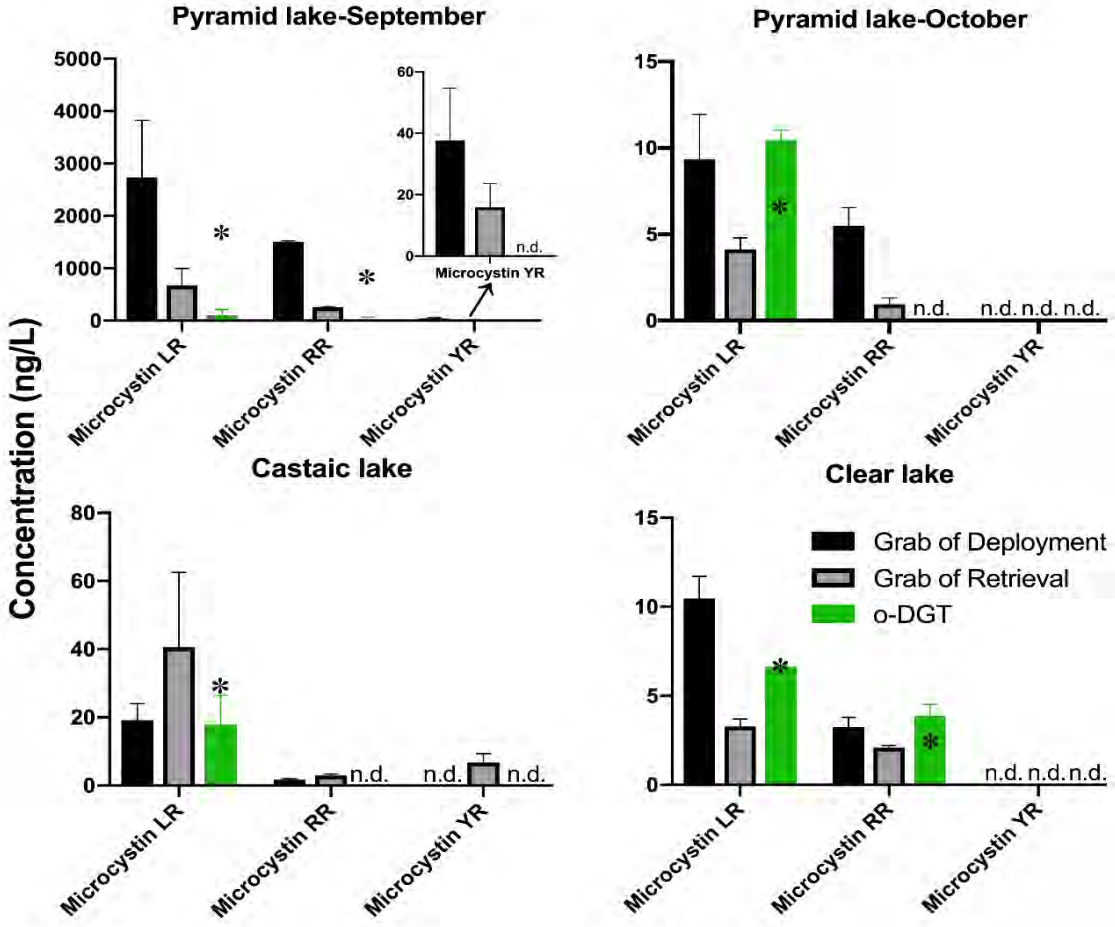


Table 8. LC-MS/MS results for grab samples and o-DGT samples in µg/L and results for SPATT as presence (+) or absence (-).

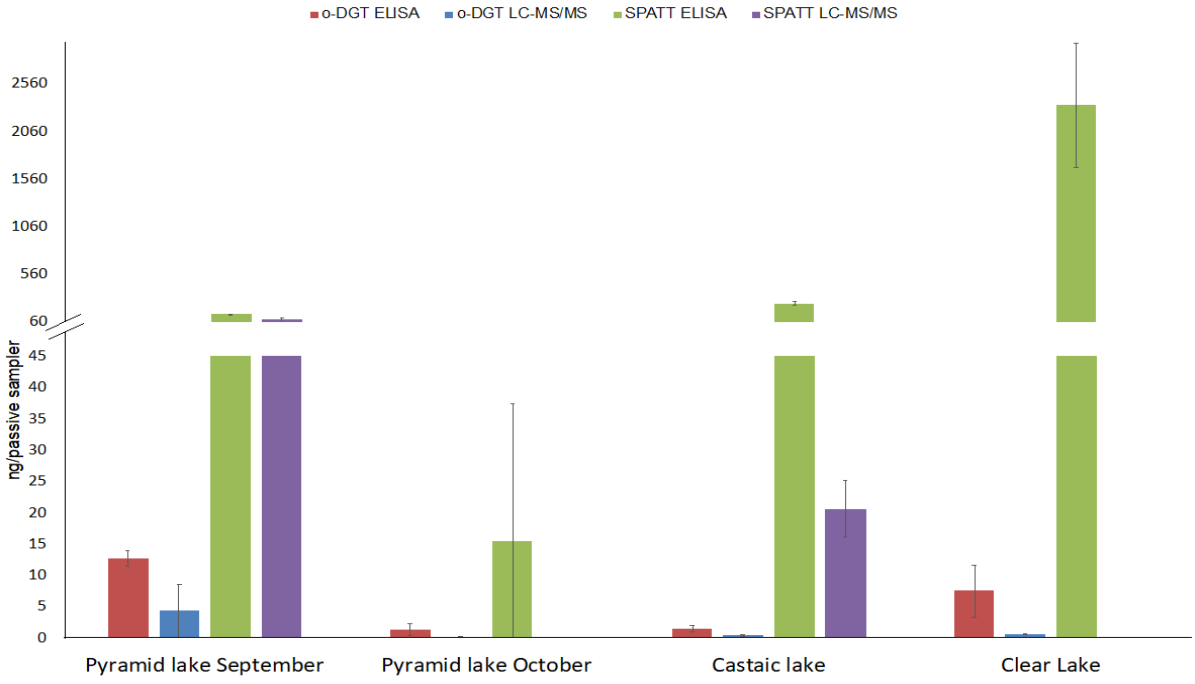
	MC-LR				MC-RR				MC-YR			
	Grab		o-DGT	SPATT	Grab		o-DGT	SPATT	Grab		o-DGT	SPATT
	Deploy	Retrieval			Deploy	Retrieval			Deploy	Retrieval		
Pyramid Lake - Sept	2.7	0.68	0.11	+	1.5	0.26	0.035	+	0.037	0.015	ND	-
Pyramid Lake - Oct	0.009	0.004	0.011	-	0.005	0.001	ND	-	ND	ND	ND	-
Castaic Lake	0.019	0.04	0.017	+	0.002	0.003	ND	-	ND	0.007	ND	-
Clear Lake	0.01	0.003	0.007	-	0.003	0.002	0.004	-	ND	ND	ND	-

Figure 7. Visible colonies of *Microcystis* spp. present in the water column at Pyramid Lake on September 13, 2019.

To date, SPATT samplers have not been calibrated and toxin concentrations detected in SPATT bags are most typically reported semi-quantitatively as mass of toxin per gram of resin (e.g., ng of microcystins/g of resin). The most appropriate comparisons between SPATT, grab samples, and o-DGT at this time is to compare the presence or absence of the toxins between the sampling approaches (Table 8). Overall, SPATT detections of MC-LR and MC-RR correspond well to grab samples and o-DGT when one of the grab sample concentrations was >0.01 µg/L. Below this concentration, the microcystins were not detected in the SPATT sample extracts via the LC-MS/MS, whereas o-DGT samplers appeared to be slightly more sensitive. SPATT samplers from all sampling locations did not have detectable concentrations of MC-YR, similar to the o-DGT results.

There are several rationales for the differences in results from grab samples and passive samplers. Passive samplers were deployed for 7 or more days while grab samples only measured the occurrence at a discrete point in time during passive sampler deployment and retrieval, therefore an exact comparison is not expected (Kudela 2011).

Figure 8. Comparison of passive sampler extracts run on LC-MS/MS and ELISA



However, in this work, we observed a good correspondence between MC-LR concentrations from o-DGT samplers and time-averaged grab sample concentrations from deployment and retrieval at lower toxin concentrations (< 0.1 µg/L) (asterisks in Figure 6). However, the o-DGT concentrations were substantially lower than grab sample results in Pyramid Lake in September when grab sample concentrations were higher (> 0.1 µg/L). Grab samples were processed to measure both intracellular and extracellular toxins whereas passive samplers only adsorb extracellular toxins. A high biomass bloom was observed with visible flocks of *Microcystis* spp. colonies present in the water column (Figure 7), which suggests much of the toxin was intracellular, and therefore was unavailable to bind to the passive sampler resins and reflect bioavailable microcystins. This dynamic could explain the observed disconnect in concentrations when cells were at a higher biomass. However, in October, the overall cyanobacterial biomass in the lake was greatly reduced with fewer visible algal cells observed. As the bloom senesced between September and October, it is likely that more toxin was present in the extracellular pool improving the relationship between grab sample concentrations and o-DGT samplers. This dynamic will need to be further explored to assess the performance and comparability of o-DGT sampler results to California trigger levels, which are based on both intracellular and extracellular toxins, in high biomass bloom scenarios.

Differences in passive sampler performance across analysis platforms

The concentrations of microcystins in o-DGT and SPATT extracts from analysis via LC-MS/MS and ELISA were compared as an initial assessment of the performance of these analysis approaches with passive sampler extracts. Qualitive comparisons between o-DGT and SPATT were made on the basis of nanograms of microcystin per device, as the most conservative approach for comparison. When comparing the passive samplers on a presence absence basis, overall agreement in detections was observed at Pyramid Lake in September and in Castaic Lake. Regardless of quantification approaches, SPATT samplers from these locations seemed to accumulate a relatively larger mass of microcystins per device compared to o-DGT, which is possibly due to the larger mass of sorbent material in the SPATT bag compared to the o-DGT. Similarly, toxin mass estimations from SPATT bags analyzed via ELISA were higher than the LC-MS estimations. This trend is particularly strong in SPATT bags deployed in Pyramid Lake in October and in Clear Lake. In these locations, microcystins were below the detection limit for the LC-MS/MS method but were detected when analyzed via ELISA. The ELISA method estimates the concentration of all microcystin variants by targeting the ADDA side chain, whereas the LC-MS/MS method employed for these samples only quantifies 3 variants, which could have resulted in the underestimation via LC-MS/MS.

Temporal patterns in toxin occurrence

While the full extent of temporal variability has not been resolved for Pyramid Lake, our observations points to the necessity to understand these trends. Pyramid Lake had 2 deployment events in September (Summer) and October (Fall) of 2019, respectively. The current study suggests that seasonal patterns in warm water temperature are a contributing factor to cyanobacteria growth and toxin production (Table 2), similar to global observations of cyanobacterial blooms (Paerl and Paul 2012). Lake turnover from either decreasing temperatures in the autumn or from changes in wind mixing could also affect concentration of microcystins in a manner beyond the scope of this study. During the deployment period in September, water

temperatures at Pyramid Lake were 22.9 ± 1.1 °C and by the end of the survey period in October, water temperatures decreased by ~2°C. This decrease in temperature corresponded in a decrease in visible cyanobacterial biomass and a concurrent decrease in microcystins. The grab sample results indicated the bloom had high concentrations of microcystins, and summed concentrations of the three microcystins congeners (4.2 µg/L) were above the caution trigger level (0.8 µg/L) in September 2019 while the toxin concentrations were well below the all trigger levels in October (Table 7; Table 8). Notably, the total concentration of microcystins detected in Pyramid Lake in September 2018 was above the danger trigger level (20 µg/L). In the three sampling events at Pyramid Lake between 2018 and 2019, concentrations of microcystins ranged from well below California trigger levels to above the danger trigger level. More comprehensive monitoring at Pyramid Lake and other sites with detections of microcystins in Region 4 is needed to gain a better understanding of variability in toxin concentrations and environmental drivers of that variability.

RECOMMENDATIONS

1) Ambient monitoring for microcystins and other cyanotoxins is needed in Region 4 waterbodies.

Currently, ambient monitoring of cyanobacteria and cyanotoxins has been identified as a need by the California Freshwater HAB Assessment and Support Strategy (SWAMP 2016). The results of this work highlight that microcystins were commonly detected in some waterbodies, however, the magnitude of microcystins detected varied by several orders of magnitude. The results in Pyramid Lake are particularly illustrative of this point, with grab samples ranging from well above the danger trigger level (20 µg/L) to well below across sampling events during the study. Future research and monitoring efforts should also heavily focus on temporal/seasonal variability in toxin production and determine the sampling frequency needs to adequately characterize risk. Ambient monitoring approaches need to be developed to effectively protect the designated beneficial uses of the waterbody. For example, protection of recreational uses (e.g. protection of human and domestic animal health) will require a different approach than assessment on how cyanotoxins impact aquatic life beneficial uses. Better ambient assessment of cyanotoxin prevalence and risk across waterbodies also will allow for more targeted monitoring of sites that are of higher risk. Ambient monitoring for toxins can be paired with a characterization of water quality parameters, such as temperature or nutrients, to better resolve specific bloom drivers and to gain a better understanding of which sites are at risk of developing cyanoHAB blooms. Additionally, more work is needed to understand the extent and magnitude of other cyanotoxin classes.

2) Robust passive sampling techniques should be developed to support cyanotoxin monitoring efforts.

Integration of passive samplers into HAB monitoring is a valuable approach to better understanding the extent and magnitude of microcystins. Both passive samplers utilized in this performed well for detecting microcystins, showing utility of this tool as an assessment tool. Additional work is needed to improve and fully validate if passive sampler results can be compared to currently actionable thresholds. Increasing confidence and interpretability of these comparisons will improve the utility of passive sampling tools. Passive samplers may never replace grab sampling approaches, but a better understanding of the relationship between these two approaches could result in management action being taken based on passive sampler results (e.g., initiate more intensive ambient monitoring, posting preliminary caution signage, etc.). The results of this study show promise in calibrating these techniques to be actionable in this way. Passive samplers providing quantitative results (e.g., o-DGT) need to be applied to additional cyanotoxin classes. The efficacy of o-DGT should be explored for anatoxin-a, cylindrospermopsin and saxitoxin.

3) Sediment-based sampling approaches for cyanotoxins need to be developed.

Microcystins can deposit into the surficial sediments by binding to particulates and settle to the lake bottom. As well, cyanotoxins that may be associated with dead cyanobacteria could also settle to the bottom. Live cyanobacteria may also be present in the sediment. Both could conceivably affect benthic organisms directly, or could be resuspended back into the water

column. Currently the impacs and human health risks of cyanotoxins in lake sediments in not fully understood. All of these possibilities warrant the integration of sediment characterization into future studies for cyanotoxins. It was not possible to collect fine sediments at a number of sites in this study, as the bottoms were rocky; therefore, analysis of microcystins in the sediment was not carried in this study. Future investigation will explore suitable approaches in characterizing microcystins and other cyanotoxins in the sediment. This can pose a challenge, given that bioavailable cyanotoxins are those in the dissolved phase i.e., sediment porewater, which is difficult and time-consuming to separate from bulk sediments through techniques such as centrifugation or through the deployment of sediment peepers to sample porewater. A potential passive sampling approach for analyzing cyanotoxins and other polar CECs in sediment porewaters is through DGT sheets physically larger than the water samplers described in this report. Such sheets have been successfully used for chemical imaging of metals (Kreuzeder et al. 2013) and can be adapted to sequester and map organic chemicals such as cyanotoxins.

REFERENCES

Alvarez, D.A., Petty, J.D., Huckins, J.N., Jones-Lepp, T.L., Getting, D.T., Goddard, J.P., and Manahan, S.E. 2004. Development of a passive, in situ, integrative sampler for hydrophilic organic contaminants in aquatic environments. Environmental Toxicology and Chemistry 23(7), 1640-1648.

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

Belles, A., Alary, C., Aminot, Y., Readman, J.W., and Franke, C. 2017. Calibration and response of an agarose gel based passive sampler to record short pulses of aquatic organic pollutants. Talanta 165, 1-9.

Booij, K., Vrana, B. and Huckins, J.N. 2007. Theory, modelling and calibration of passive samplers used in water monitoring. *Comprehensive analytical chemistry*, *48*, pp.141-169.

Challis, J.K., Hanson, M.L., and Wong, C.S. 2016. Development and Calibration of an Organic-Diffusive Gradients in Thin Films Aquatic Passive Sampler for a Diverse Suite of Polar Organic Contaminants. Analytical Chemistry 88(21), 10583-10591.

Chen, C.-E., Zhang, H., and Jones, K.C. 2012. A novel passive water sampler for in situ sampling of antibiotics. Journal of Environmental Monitoring 14(6), 1523-1530.

D'Angelo, E. 2019. Development and evaluation of a sensitive, Diffusive Gradients in Thin-Films (DGT) method for determining microcystin-LR concentrations in freshwater and seawater. Harmful Algae 89, 101668.

Davison, W. and Zhang, H. 1994. In situ speciation measurements of trace components in natural waters using thin-film gels. Nature 367(6463), 546-548.

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

Fetscher, A.E., Stancheva, R., Kociolek, J.P., Sheath, R.G., Stein, E.D., Mazor, R.D., Ode, P.R., and Busse, L.B. 2014. Development and comparison of stream indices of biotic integrity using diatoms vs. non-diatom algae vs. a combination. Journal of Applied Phycology 26(1), 433-450.

Guibal, R., Buzier, R., Charriau, A., Lissalde, S., and Guibaud, G. 2017. Passive sampling of anionic pesticides using the Diffusive Gradients in Thin films technique (DGT). Analytica Chimica Acta 966, 1-10.

Howard, D.A.M., Nagoda, C., Kudela, M.R., Hayashi, K., Tatters, A., Caron, A.D., Busse, L., Brown, J., Sutula, M. and Stein, D.E. 2017. Microcystin Prevalence throughout Lentic Waterbodies in Coastal Southern California. Toxins 9(7).

Kaloudis, T., Zervou, S.-K., Tsimeli, K., Triantis, T.M., Fotiou, T., and Hiskia, A. 2013. Determination of microcystins and nodularin (cyanobacterial toxins) in water by LC–MS/MS. Monitoring of Lake Marathonas, a water reservoir of Athens, Greece. Journal of Hazardous Materials 263, 105-115.

Kim, M.S., Choi, M.S., and Kim, C.-K. 2016. Monitoring trace metals in seawater using a diffusive gradient in thin film probe in Ulsan Bay, East Sea, Korea: Comparison with transplanted mussels. Ocean Science Journal 51(2), 169-182.

Kreuzeder, A., Santner, J., Prohaska, T., and Wenzel, W.W. 2013. Gel for simultaneous chemical imaging of anionic and cationic solutes using diffusive gradients in thin films. Analytical Chemistry 85, 12028-12036.

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

Kudela, R.M. 2017. Passive sampling for freshwater and marine algal toxins. Compr Anal Chem, Book 78. pp.379-409. Elsevier.

Lane, J.Q., Roddam, C.M., Langlois, G.W., and Kudela, R.M. 2010. Application of Solid Phase Adsorption Toxin Tracking (SPATT) for field detection of the hydrophilic phycotoxins domoic acid and saxitoxin in coastal California. Limnology and Oceanography: Methods 8(11), 645-660.

Magrann, T., Meredith D. A, H., Martha, S., Danilo S, B., William K, H., and Stephen G, D. 2015. Screening Assessment of Cyanobacteria and Cyanotoxins in Southern California Lentic Habitats. Environmental Management and Sustainable Development; Vol 4, No 2 (2015)DO - 10.5296/emsd.v4i2.8036.

Mason, S., McNeill, A., McLaughlin, M.J., and Zhang, H. 2010. Prediction of wheat response to an application of phosphorus under field conditions using diffusive gradients in thin-films (DGT) and extraction methods. Plant and Soil 337(1), 243-258.

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

Miller, T.R., Bartlett, S.L., Weirich, C.A., and Hernandez, J. 2019. Automated Subdaily Sampling of Cyanobacterial Toxins on a Buoy Reveals New Temporal Patterns in Toxin Dynamics. Environ Sci Technol 53:5661-5670

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

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

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

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

Scally, S., Davison, W., and Zhang, H. 2006. Diffusion coefficients of metals and metal complexes in hydrogels used in diffusive gradients in thin films. Analytica Chimica Acta 558(1), 222-229.

Schwarzenbach, R.P., Gschwend, P.M., and Imboden, D.M. 2017. Environmental Organic Chemistry, Wiley.

Stroski, K.M., Challis, J.K., and Wong, C.S. 2018. The influence of pH on sampler uptake for an improved configuration of the organic-diffusive gradients in thin films passive sampler. Analytica Chimica Acta 1018, 45-53.

SWAMP. 2016 California Freshwater Harmful Algal Blooms Assessment and Support Strategy. Surface Water Ambient Monitoring Program of the California State Water Resources Control Board; Sacramento, CA, USA. SWAMP-SP-SB-2016-0001

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

USEPA. 2015a. Drinking Water Health Advisory for the Cyanobacterial Toxin Cylindrospermopsin; United States Environmental Protection Agency: Washington, DC, USA. EPA- 820R15101

USEPA. 2015b. Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins; United States Environmental Protection Agency: Washington, DC, USA. EPA-820R15100

USEPA. 2019. Recommended Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin; USEPA Office of Water: Washington, DC, USA. EPA 822-R-19-001.

Wilke, C.R. and Chang, P. 1955. Correlation of diffusion coefficients in dilute solutions. AIChE Journal 1(2), 264-270.

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

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

Yao, L., Steinman, A.D., Wan, X., Shu, X., and Xie, L. 2019. A new method based on diffusive gradients in thin films for in situ monitoring microcystin-LR in waters. Scientific Reports 9(1), 17528.

Zervou, S.-K., Christophoridis, C., Kaloudis, T., Triantis, T.M., and Hiskia, A. 2017. New SPE-LC-MS/MS method for simultaneous determination of multi-class cyanobacterial and algal toxins. Journal of Hazardous Materials 323, 56-66.