Estuaries and Coasts
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Heterogeneity of Toxin-Producing Cyanobacteria and Cyanotoxins in Coastal Watersheds of Southern California

Avery O. Tatters[1] · Meredith D. A. Howard[2] · Carey Nagoda[3] · A. Elizabeth Fetscher[3] · Raphael M. Kudela[4] · David A. Caron[5]

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Abstract

Freshwater and marine harmful algal blooms are expanding on a global scale. Recent reports of toxic events have sparked a growing awareness of the importance of cyanobacteria and cyanotoxins at the land-sea interface and estuaries in general. A recent survey in the Southern California Bight documented a wealth of cyanobacteria-dominated communities at a variety of locations. To gain further insight into these assemblages, we repeatedly sampled several sites with different proximity and degrees of connectivity to the Pacific Ocean in four coastal watersheds along the coast of southern California. Our findings revealed temporal and spatial heterogeneity in the occurrence of potential toxin-producing cyanobacteria and associated toxins. Multiple toxins were measured in 45% of all samples (and 25% of shellfish examined), including samples testing positive for anatoxin-a, cylindrospermopsin, three congeners of microcystins, or the eukaryotic toxin domoic acid. The ecosystems are hydrologically connected to the Pacific Ocean and provide a source of cyanotoxins to marine and estuarine environments. Collectively, potential toxin-producing cyanobacteria were prevalent at all study sites and appeared to persist throughout the year in some locations. These findings indicate a need for implementation of coordinated monitoring programs across the land-sea interface.

Keywords Cyanobacteria · Cyanotoxins · Land-sea interface · SPATT

Introduction

Manifestations of harmful algal and cyanobacterial blooms may indirectly and directly impact human, animal, and environmental health. The detrimental effects of these increasingly common deleterious events are mostly attributed to chemicals biosynthesized by a relatively small number of species. These compounds include known and uncharacterized metabolites commonly referred to as "toxins" that elicit a spectrum of effects depending on concentration, duration and route of exposure, and biochemical activity (Carmichael 1992). Although naturally occurring phenomena, these events threaten the security of valuable resources including food and water (Carmichael 2001; Falconer and Humpage 2005).

A growing consensus among scientists is that freshwater and marine harmful algal and cyanobacterial blooms are increasing in frequency and severity worldwide (Hallegraeff 1993; Paerl and Huisman 2009; Anderson et al. 2012). Arguably, the most evident of these expansions, likely due to visibility, ubiquity, and human health concerns, are proliferations of cyanobacteria. Blooms of cyanobacteria have been primarily attributed to trends of eutrophication and warming of fresh waters (Paerl and Huisman 2008, 2009; Paerl and Paul 2012; O'Neil et al. 2012). Additional factors including hydrological modifications, drought(s) and altered rainfall patterns likely provide windows of opportunity for cyanobacterial growth due to differential and species-specific thermal and salinity tolerances that complement

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✉ Avery O. Tatters
tatters@g.ucla.edu

[1] California NanoSystems Institute, University of California Los Angeles, Los Angeles, CA, USA

[2] Central Valley Regional Water Quality Control Board, Rancho Cordova, CA, USA

[3] San Diego Regional Water Quality Control Board, San Diego, CA, USA

[4] Department of Ocean Sciences, University of California, Santa Cruz, Santa Cruz, CA, USA

[5] Department of Biological Sciences, University of Southern California, Los Angeles, CA, USA

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nutrient flexibility and allelopathy (Potts 1999; Moisander et al. 2002; Briand et al. 2004; Lehman et al. 2017; Chia et al. 2018). These natural- and human-mediated environmental transformations often co-occur, complicating our ability to tease apart and assess the contribution of individual variables to bloom events and the ecology of these organisms.

Our recognition of the problem and harm posed by cyanobacterial blooms has been heightened by recent findings that have documented potentially toxic algae and cyanobacteria and their associated toxins at the land-sea interface at various locations around the globe, including Argentina, Brazil, Estonia, Finland, Japan, Turkey, Uruguay, Portugal, and Washington State and Virginia, USA (Sathicq et al. 2014; Matthiensen et al. 1999; Magalhães et al. 2001; Tanner et al. 2005; Nikulina 2003; Karlsson et al. 2005; Takahashi et al. 2014; Taş et al. 2006; Vasconcelos et al. 1999; Wood et al. 2014; Preece et al. 2015; Buckaveckas et al. 2018). The movement of toxins from freshwater and marine ecosystems into estuaries has largely gone undetected because of a lack of monitoring and/or insufficient biomass to prompt concern but may constitute risks to human or animal health nonetheless. For instance, cyanotoxin transport from fresh to marine waters along the central California coastline was first discovered because of unexplained marine mammal mortality events (Miller et al. 2010). Specifically, microcystin contamination of multiple watersheds that flow into Monterey Bay, California, was documented and shown to be a persistent situation spanning years (Gibble and Kudela 2014). Documented contamination resulting from toxin transport is sporadic, but the phenomenon is probably more common than assumed. In addition to toxin transport from fresh water to the land-sea interface, cyanobacteria may also thrive and produce toxins within brackish ecosystems as has been demonstrated in Northern European seas and South American lagoons (Karlsson et al. 2005; Sivonen et al. 1989; Matthiensen et al. 1999; Dörr et al. 2010). The potential convergence of incoming chemicals from fresh waters, with those biosynthesized in place, and a marine contribution can result in a toxin cocktail with unknown and presently unstudied health and environmental consequences (Lopes and Vasconcelos 2011; Gibble and Kudela 2014; Gibble et al. 2016; Tatters et al. 2017; Peacock et al. 2018).

The frequency of occurrence and dynamics of cyanobacterial blooms at the coastal interface and potential toxin transport into estuarine and lagoonal ecosystems within the Southern California Bight (SCB) are poorly understood. Surprisingly few studies have focused on these topics in the SCB, despite the enormity of urbanization and heavy utilization of this coastal region (Dwight et al. 2007). Human activities have resulted in a tremendous amount of point and non-point source nutrient loading into fresh, brackish, and coastal waters that can stimulate algal/cyanobacterial growth (Kudela et al. 2008; Siegel et al. 2011). Nutrient sources include agricultural and storm runoff, wastewater treatment effluent, and other urbanization-related practices (Sengupta et al. 2013; Howard et al. 2014; McLaughlin et al. 2017). Additionally, climatologists predict a continuation of widespread temperature and precipitation anomalies for this region, conditions that appear to promote cyanobacterial dominance (Paerl and Huisman 2008; Swain et al. 2014; Mann and Gleick 2015). Taken together, the influence of these natural and anthropogenic factors on the ecology of cyanobacteria at the land-sea interface along the SCB warrants further study.

Cyanobacteria and co-occurring toxins were recently documented during a survey along the coastline of the SCB (Tatters et al. 2017) and as part of an assessment focused on San Diego estuaries and lagoons (Howard et al. 2017). These results prompted us to design higher resolution studies to better understand the spatial and temporal distribution and diversity of cyanobacteria in the region. The overall objectives of the current study were to characterize the cyanobacterial community composition and associated cyanotoxin dynamics at four sampling locations that represent different types of land-sea interfaces in the region (i.e., estuary/lagoon, river, seasonal river, and seasonal creek) that flow into the Pacific Ocean. Observational studies were conducted from one locality in Los Angeles County, CA, USA, where three sites extending into the watershed were evaluated monthly over a 1-year period in 2014–2015. We also examined three additional coastal tributaries in San Diego County, CA, USA, each consisting of multiple sampling sites visited on a weekly basis over a two-month period in 2016 during late summer, the season of peak cyanobacterial growth in California.

Methods

Sample Collection and Locality

Monthly samples from three locations in a seasonal creek located within the undeveloped Corral Canyon watershed in Malibu, CA, were obtained from October 15, 2014 to October 15, 2015 to assess the cyanobacterial community and associated toxins (Fig. 1a, b). Samples of sub-surface water and benthic mats were obtained and deposited in 250 mL amber glass bottles and whirl-pak™ bags respectively. The samples were immediately placed into a cooler and remained on ice until transported to the laboratory. Salinity was measured with a handheld refractometer.

Three systems in San Diego county were sampled (Fig. 1a, c): (1) Los Peñasquitos Creek flowing out to Los Peñasquitos Lagoon, (2) Sweetwater River flowing out to San Diego Bay, and (3) the Otay River flowing out to the southern tip of San Diego Bay. Each of these systems had 2–3 monitoring sites within the watershed: (1) in the tributary, just upstream from

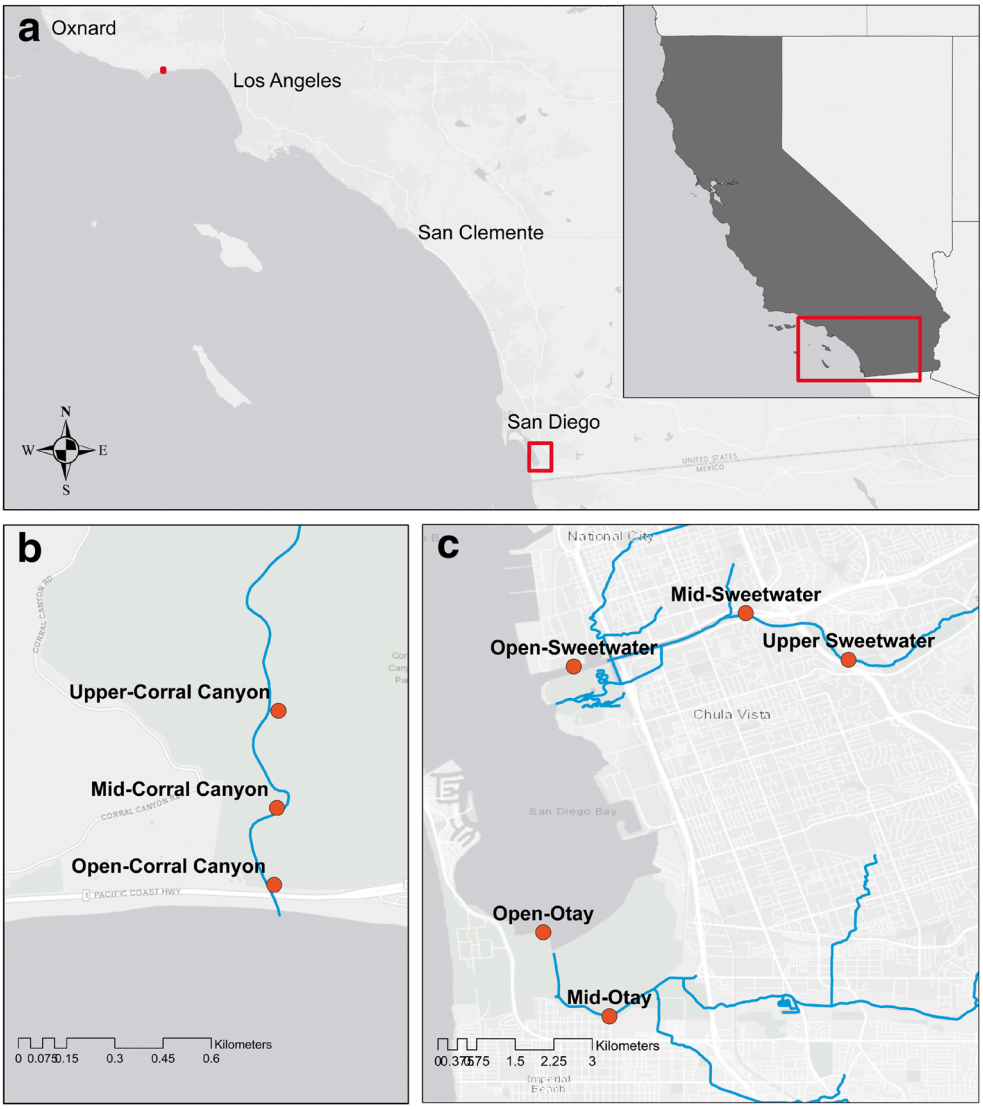

Fig. 1 Map depicting the sampling sites within the Corral Canyon watershed in Malibu, CA (**a**, **b**) and Los Peñasquitos lagoon, Sweetwater River, and Otay River (**a**, **c**)

the coastal discharge point (upper); (2) mid-slough (mid); and (3) coastal receiving water (lower/open). The field sampling period was July 27 through September 12, 2016, and samples were collected every 10 days for cyanotoxins, cyanobacterial taxonomy, salinity, and temperature.

Cyanotoxin samples were frozen in 250 mL plastic amber bottles which were thoroughly rinsed with sample water to prime/prevent significant adsorption of toxins while cyanobacterial taxonomy samples were placed into an incubator overnight and transported to the University of Southern California the day after collection for analysis. Salinity and temperature readings were collected using a Yellow Springs Instrument (YSI) MPS 556 handheld multiparameter meter.

Since oyster beds are present at the Sweetwater open site, oysters were collected concurrently with whole water samples to investigate potential relationships between cyanotoxin presence in tissue and water. Multiple medium-sized oysters of 9–13 cm were collected on the following dates: August 05, 2016 (16 oysters), August 15, 2016 (6 oysters), August 24, 2016 (8 oysters), September 12, 2016 (10 oysters).

Cyanobacterial Taxonomy

Water samples were gently homogenized, and aliquots poured into 20 mL tissue culture dishes and settled overnight at room temperature. Subsamples of benthic samples were placed into 5 mL tissue culture dishes charged with 2 mL of 0.22 μm filtered source water and also allowed to settle overnight. These samples were viewed live using an Olympus CKX41 inverted microscope at 40× to 200× magnification. Cyanobacteria were identified to the genus level according to Komárek (Anagnostidis and Komárek 1988; Komárek and Komárková 2002; Komárek and Komárková 2003; Komárek and Komárková 2004; Komárek and Zapomělová 2007; Komárek et al. 2010; Rajaniemi et al. 2005).

Cyanotoxin Detection

Malibu

The cyanotoxin assessment was aimed at determining the presence or absence of toxins; quantification was not a goal for the Malibu location because there are presently no human health thresholds established for benthic cyanobacteria, which were sampled in addition to the water column. Limiting the assessment to cyanotoxin presence/absence per site facilitated streamlining by combining water column and benthic samples prior to analysis. Sub-surface water samples of 50 to 250 mL were filtered in duplicate onto 47 mm GF/F filters and placed into 20 mL borosilicate scintillation vials charged with 10% aqueous methanol. Benthic samples were subsampled and representative material also placed into charged 20 mL scintillation vials. The vials were subjected to a series of three freeze/thaw cycles at −20 C and room temperature.

The volume was adjusted to ~1 mL with 50% aqueous methanol and agitated with a glass stirring rod under subdued light on ice. The slurry was filtered into 1.5mL amber glass Prominence vials using a glass Luer-Lok™ syringe fitted with 13 mm 0.22 μm PTFE filters. Extracts from the water column and benthic samples from a given site were combined prior to analysis. A replicate 25 mm GF/F filter was also taken for saxitoxin evaluation by enzyme-linked immunosorbent assay (ELISA).

Anatoxins and microcystins were analyzed with ultrahigh-performance liquid chromatography with ultraviolet detection (HPLC-UV) using an Agilent Infinity 1260 fitted with an Agilent Zorbax RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 μm column heated to 30 °C. The method, reported in Tatters et al. (2017), was adapted and modified from Harada et al. (1994), Spoof et al. (2010), and Pekar et al. (2016). Briefly, the mobile phase was a combination of A: H_20, 0.01%TFA and B: MeCN, 0.01% TFA. The method begins with 20% B over 4 min and increasing to 70% B by 4.2 min. 95% B is reached by 4.6 min and held until 5.4 min. There is a decrease to 20% B by 6 min and held until 7.5 min in preparation for the next sample. All anatoxin-like peaks were considered "anatoxin positive" because of the low resolution afforded by HPLC-UV analysis compared to mass spectrometry. Putative anatoxin positive extracts were followed with a fluorescent derivatization technique using n-BFD as reported in James et al. (1997, 1998) and only reported if separated from spike-ins of the interference compound phenylalanine. The presence of saxitoxins was assessed using a Biosense ELISA kit according to the manufacturer. Cyanotoxin detection methodology is summarized in Table 1.

Table 1 Toxin detection methodology employed for each site in the Corral Canyon-Malibu, Los Peñasquitos, Sweetwater, and Otay watersheds

Location, site	Sample type	Analysis method
Malibu-upper	Whole water	HPLC, ELISA
Malibu-mid	Whole water	HPLC, ELISA
Malibu-lower	Whole water	HPLC, ELISA
Los Peñasquitos-upper	Whole water	ELISA
	filtrate	ELISA
	SPATT	LC-MS
Los Peñasquitos-mid	Whole water	ELISA
	Filtrate	ELISA
	SPATT	LC-MS
Los Peñasquitos-open	Whole water	ELISA
	Filtrate	ELISA
	SPATT	LC-MS
Sweetwater-upper	Whole water	ELISA
	Filtrate	ELISA
	SPATT	LC-MS
Sweetwater-mid	Whole water	ELISA
	Filtrate	ELISA
	SPATT	LC-MS
Sweetwater-open	Whole water	ELISA
	Filtrate	ELISA
	SPATT	LC-MS
	Oysters	LC-MS
Otay-mid	Whole water	ELISA
	Filtrate	ELISA
	SPATT	LC-MS
Otay-open	Whole water	ELISA
	Filtrate	ELISA
	SPATT	LC-MS

San Diego

Cyanotoxin detection methodology employed on samples collected at each site is described in Table 1. Whole water samples were analyzed at the California Fish and Wildlife Water Pollution Control Lab (WPCL). Samples were analyzed for nine microcystin congeners (MCY-LA, MCY-LR, MCY-RR, MCY-YR, MCY-LW, MCY-LY, MC-desmethyl-LR, MC-desmethyl-RR, and MCY-LF) using LC-ESI-MS/MS as described in Mekebri et al. (2009) and following the EPA 3535 extraction method. Enzyme-linked immunosorbent assay (ELISA) plates from Abraxis were used for the analysis of anatoxin-a (PN 520060, Microtiter Plate) and cylindrospermopsin (PN 522011, Microtiter Plate) according to instructions provided by the manufacturer.

Oysters were extracted and analyzed for microcystins and domoic acid at the University of California, Santa Cruz. The marine toxin domoic acid was included because

it demonstrates the potential for both marine and freshwater toxins (and prokaryotic and eukaryotic toxins) to be present at these land-sea interface sites. Tissue was extracted following the methods described in Peacock et al. (2018). Briefly, tissue was homogenized with 50% MeOH, centrifuged, and subsequently clarified using a 0.22-μm PTFE syringe filter, and cleaned using Biotage ISOLUTE SAX SPE columns. The SPE extract was subsequently analyzed by HPLC-ESI-MS with select ion monitoring (SIM) for domoic acid and epidomoic acid using an Agilent 1290 system coupled to an Agilent 6130 MS. Splits of the oyster material were processed and analyzed following Mekebri et al. (2009) after modification for MS with SIM rather than MS/MS, for MCY-LR, MCY-RR, MCY-YR, and MCY-LA.

Passive Sampling Devices

Passive sampling devices, called Solid Phase Adsorption Toxin Tracking, SPATT (MacKenzie et al. 2004; Lane et al. 2010; Kudela 2011), were deployed continuously between site visits and provided time-integrated dissolved cyanotoxin collection. SPATT samples were analyzed at the University of California, Santa Cruz, for four microcystin congeners (MCY-LA, MCY-LR, MCY-RR, MCY-YR) by LC-MS with electrospray ionization (ESI) with selected ion monitoring (SIM) on an Agilent 6130 with a Phenomenex Kinetix (100 × 2.10) C18 column. The method was adapted from Mekebri et al. (2009) with minor modifications to account for the choice of column and LC-MS/SIM instead of tandem mass spectrometry (Kudela 2011). The samples were prepared as described in Kudela (2011). Analysis included replicates and matrix-additions, with the quantification based on external standards.

Results

Multiple toxins were detected in at least 33% of samples at all locations across the SCB (Figs. 2, 3, 4, and 5). Overall, five classes of toxins and three additional congeners were identified (Figs. 2, 3, 4, and 5; Table 2).

Malibu Watershed

Six cyanobacterial genera were identified during the 13-month sampling campaign (Fig. 6). The cyanobacterial community was dominated by *Oscillatoria*, which was the only genus present at least once at all three sampling sites. Three classes of cyanotoxins—anatoxins, microcystins, and saxitoxins—were detected (Figs. 2 and 3). Microcystins were the most prevalent class and were detected using HPLC-UV in 19 of 39 (49%) collections across all sampling sites and dates. Anatoxins were detected with HPLC-UV/FL in 14 of 39 (36%) samples and one or more saxitoxin derivatives in 7 of 39 (18%) samples using ELISA (Fig. 2). Multiple toxin classes were detected in 12 of 39 (33%) collections (Figs. 2 and 3). The three toxin classes were identified within the watershed during sampling timepoints in May, June, October, and November, but never occurred together in a single, discrete sample (Figs. 2 and 3). Salinity was markedly different between the three sites. Values at the higher elevation site were consistently low and relatively unchanged throughout the study, ranging from 0 to ~0.5. Salinity measurements at the mid site ranged from 2 to 13, while the range at the lowest elevation was 20 to 32 (Table 3).

Five cyanobacterial taxa were documented at the upper sampling site, making it the most species rich location. The cyanobacteria recorded during the sampling included *Anabaena*, *Nostoc*, *Oscillatoria*, *Cylindrospermum*, and *Phormidium*. The genus *Anabaena* was the most commonly observed, followed by *Oscillatoria* (Fig. 6). Anatoxins, microcystins, and saxitoxin(s) were detected at the upper site and microcystins were the most commonly observed (7 of 13 samples; Fig. 2). Anatoxins and microcystins co-occurred in 5 of 13 collections (Fig. 2). Three potential toxin producers, *Anabaena*, *Oscillatoria*, and *Phormidium*, were documented at the mid site, and *Oscillatoria* was the most commonly observed (Fig. 6). Anatoxins and microcystins were detected in 8 of 13 (62%) and 7 of 13 (54%) samples, respectively (Fig. 2). Both toxin classes were observed in 5 of 13 (39%) samples at the mid site (Figs. 2 and 3). Two potential toxin-producing genera, *Oscillatoria* and *Geitlerinema*, were observed at the lower site (Fig. 6). Microcystins were identified in 5 of 13 (39%) samples and saxitoxin(s) were detected in 6 of 13 (54%) samples (Fig. 2). The presence of multiple cyanotoxins was confirmed in 2 of 13 (15%) collections at the lower site (Figs. 2 and 3).

Los Peñasquitos

Six potential toxin-producing genera were present at Los Peñasquitos (Fig. 7). Anatoxin-a and cylindrospermopsin were detected in 12 of 18 (66%) and 8 of 18 (44%) samples, respectively. Multiple toxins were confirmed in 6 of 18 (33%) samples (Figs. 4 and 5). Additional dissolved toxins, microcystin-RR and domoic acid, were low but detectable in SPATT using LC-MS (Table 2).

The water temperature measured during the sample collection at the upper site ranged from 19 to 22 °C and the salinity was approximately 2 during the study period (Table 4). Five cyanobacterial genera were documented, and the overall community was dominated by *Leptolyngbya*, which was also the most commonly observed genus. Anatoxin-a was present in 4 of 6 (66%) and cylindrospermopsin in 2 of 6 (33%) samples respectively (Fig. 4). These toxins co-occurred in 1 of 6 (17%) collections at the upper site (Figs. 4 and 5). The temperature at

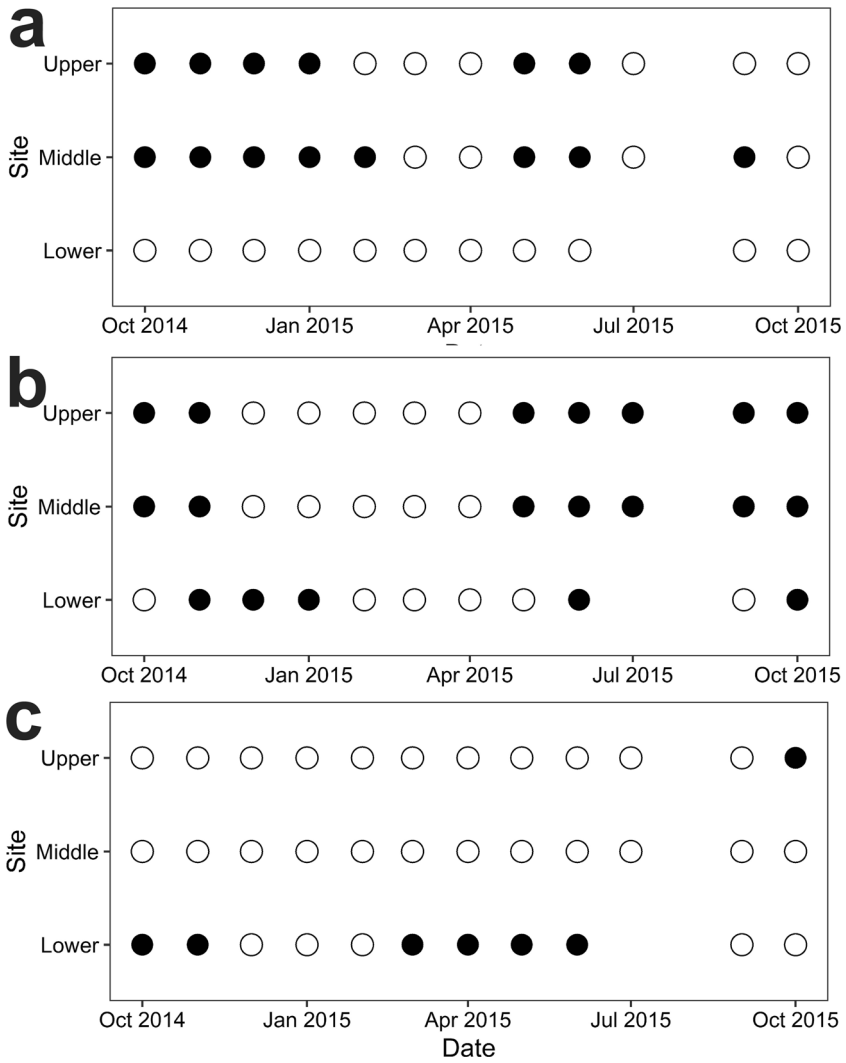

Fig. 2 The presence (indicated by filled circles) of anatoxins (**a**), microcystins (**b**), and saxitoxins (**c**) at each sampling site in Malibu, CA. Non-detects are shown as open circles

the mid site ranged from 25 to 31 °C and the salinity 3–9 (Table 4). Five potentially-harmful genera were documented, including *Leptolyngbya* and *Geitlerinema* (Fig. 7). Anatoxin-a was detected in 4 of 6 (66%), and cylindrospermopsin was confirmed in 2 of 6 collections (33%) (Figs. 4 and 5). These toxins were both present in 2 of 6 (33%) samples from mid Los Peñasquitos (Figs. 4 and 5). The water temperature at the open site varied from 20 to 30 °C and the salinity ranged from 6 to 13 (Table 4). There were six cyanobacterial genera present overall and *Leptolyngbya* was dominant (Fig. 7). Anatoxin-a was detected in 4 of 6 (66%) and cylindrospermopsin in 5 of 6

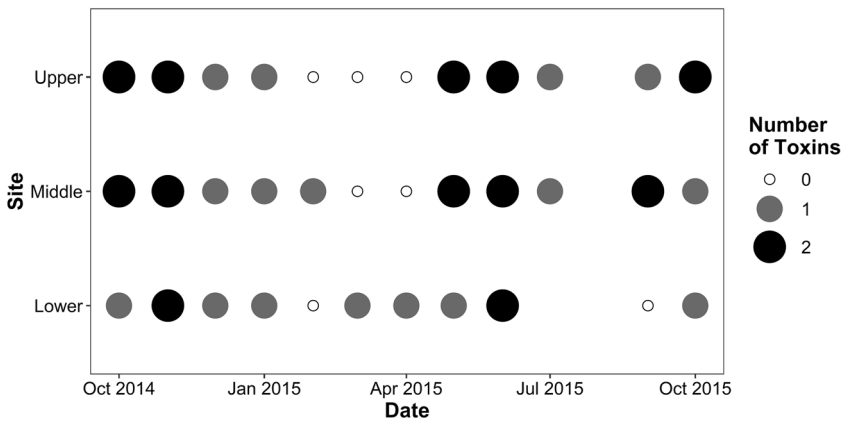

Fig. 3 Bubble plot portraying the number of toxins detected per sampling event and site in Malibu, CA

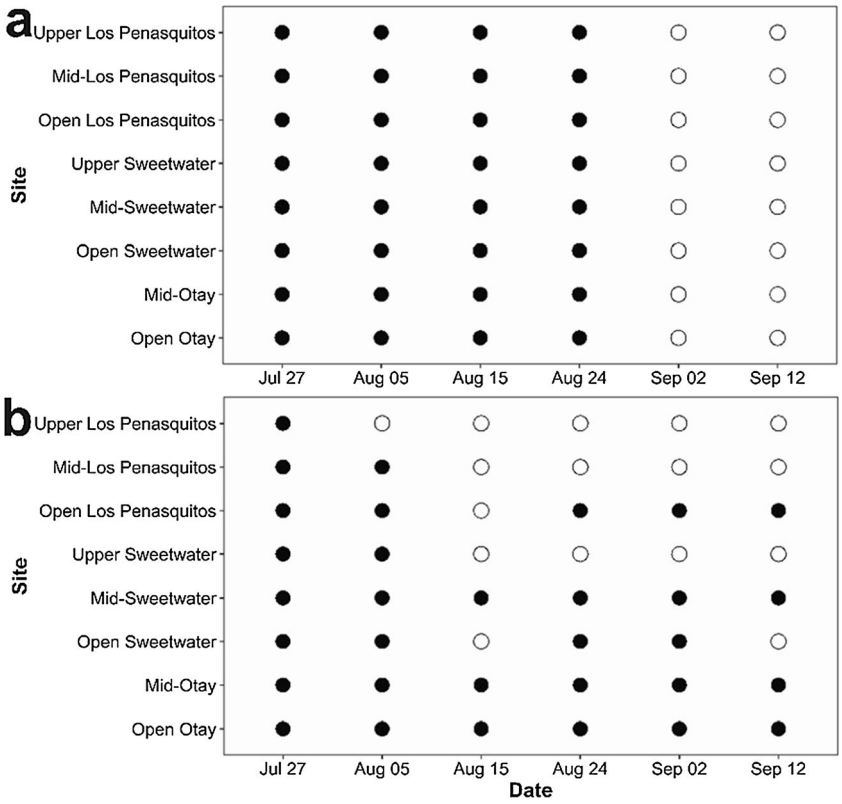

Fig. 4 The presence (indicated by filled circles) of anatoxin-a (**a**) and cylindrospermopsin (**b**) from each sampling site in San Diego watersheds. Non-detects are shown as open circles

(83%) samples (Fig. 4). Multiple toxins were present in 3 of 6 (50%) samples from open Los Peñasquitos (Figs. 4 and 5).

Sweetwater River

Nine potential toxin-producing cyanobacteria were documented in the system (Fig. 8).

Anatoxin-a and cylindrospermopsin were each detected in 12 of 18 (66%) samples using ELISA (Fig. 4). These compounds co-occurred in 9 of 18 (50%) collections (Figs. 4 and 5). Additionally, the dissolved microcystin congeners RR and LA were detected by SPATT using LC-MS (Table 2).

The water temperature measured during sample collection at the upper site ranged from 19 to 22 °C and the salinity was approximately 3. Six cyanobacterial genera were present, and *Leptolyngbya* was the most abundant and frequently observed overall (Fig. 8). Anatoxin-a and cylindrospermopsin were identified in 4 of 6 (66%) and 2 of 6 (33%) samples respectively (Fig. 4). Multiple toxins were detected in 2 of 6 (33%) collections at the upper site (Figs. 4 and 5). The mid site temperature ranged from 25 to 30 °C and the salinity variance was 6–12. There were seven potential toxin-producing cyanobacteria observed and the most abundant and commonly observed genus was *Phormidium* (Fig. 8). Anatoxin-a was detected in 4 of 6 (66%) and cylindrospermopsin in 6 of 6 (100%)

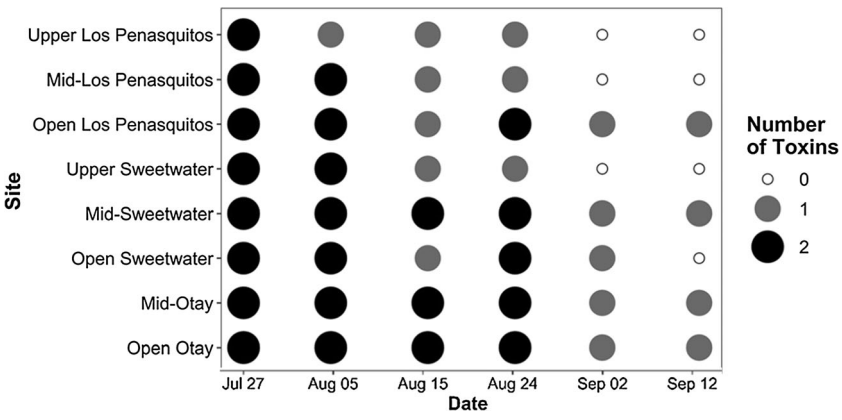

Fig. 5 Bubble plot portraying the number of toxins present in whole water per sampling event and site in San Diego watersheds

Table 2 Results of toxin prevalence at each sampling site in the Los Peñasquitos, Sweetwater, and Otay watersheds based on analysis of SPATT deployments

Date Retrieved	8.5			8.24			9.2			9.12		
	MC-RR	MC-LA	DA	MC-RR	MC-LA	DA	MC-RR	MC-LA	DA	MC-RR	MC-LA	DA
Los Peñasquitos												
U				0.11								
M				0.49					5.81			33.25
O				.28						1.30		
Sweetwater												
U	0.71	0.64					0.31			6.28		
M	1.18											
O							1.70					
Otay												
M	0.93						1.01			1.36		
O							1.20		6.67			

Values are reported as ng (g resin)$^{-1}$

U upper site, *M* mid site, *O* open site, *MC-RR* microcystin-RR, *MC-LA* microcystin-LA, *DA* domoic acid

samples (Fig. 8). These toxins co-occurred in 4 of 6 (66%) collections at the mid site (Figs. 8 and 9). The temperature at the open Sweetwater spanned 25–28 °C and the salinity range was 6–12. The most abundant cyanobacterial genus was *Leptolyngbya* (Fig. 7). Anatoxin-a and cylindrospermopsin were each found in 4 of 6 (66%) samples, while both were detected in 3 of 6 (50%) collections from the open site (Figs. 8 and 9).

Oysters collected at the mouth of the Sweetwater River were extracted and analyzed for microcystins and domoic acid at the University of California, Santa Cruz, as described above (Table 5). A representative oyster obtained in August 05 yielded only trace amounts of domoic acid, both microcystin-LR and domoic acid in August 15, trace amounts of domoic acid in August 24, and the highest amount of microcystin-LR in September 12. Microcystin-RR, YR, and LA were not detected (Table 5).

Otay River

There was a distinctive community from all other ecosystems examined in the Otay River with six cyanobacterial taxa present. The genus *Spirulina* was dominant overall followed by *Leptolyngbya* and *Phormidium* (Fig. 9). Anatoxin-a was found in 8 of 12 (66%) and cylindrospermopsin in 12 of 12 (100%) samples using ELISA (Fig. 4) and both toxins co-occurred in 8 of 12 (66%) collections (Figs. 4 and 5). Two additional dissolved toxins, microcystin-RR and domoic acid, were detectable on SPATT using LC-MS (Table 3).

The water temperature at the mid site ranged from 23 to 28 °C and the salinity 5–16. The most abundant genus was *Spirulina*. Two potentially toxigenic taxa, *Leptolyngbya* and *Phormidium*, were also common (Fig. 4). Anatoxin-a was detected in 4 of 6 (66%) samples and cylindrospermopsin in 6 of 6 (100%) (Fig. 4) Multiple toxins were present in 4 of 6 (66%) samples at mid Otay (Figs. 4 and 5). The temperature at the open site ranged from 24 to 27 °C and the salinity 5–16. There were four cyanobacterial genera documented with *Spirulina* and *Heteroleiblenia* being most abundant (Fig. 9). Identical to the mid site, anatoxin-a and cylindrospermopsin were detected in 4 of 6 (66%) and 6 of 6 (100%) samples respectively (Fig. 4). Multiple toxins co-occurred in 4 of 6 (66%) collections at open Otay (Figs. 4 and 5).

Discussion

The specific aim of this study was to contribute to the sparse body of knowledge regarding the dynamics of cyanobacteria and cyanotoxins across the land-sea interface in the SCB. Here, we provide information on their occurrence at multiple sites within four watersheds with hydrologic connectivity to the ocean. Regular sampling carried out over the course of months revealed substantial temporal and spatial heterogeneity with respect to potential toxin-producing cyanobacteria and associated cyanotoxins. Taxa and toxins were more prevalent and widely distributed than anticipated, including commonplace detections of anatoxins and cylindrospermopsin. The presence of one or more cyanotoxins in nearly every sample across the different collection sites and sampling dates indicated this condition may persist throughout the year in some areas of the SCB. To our knowledge, this is the first

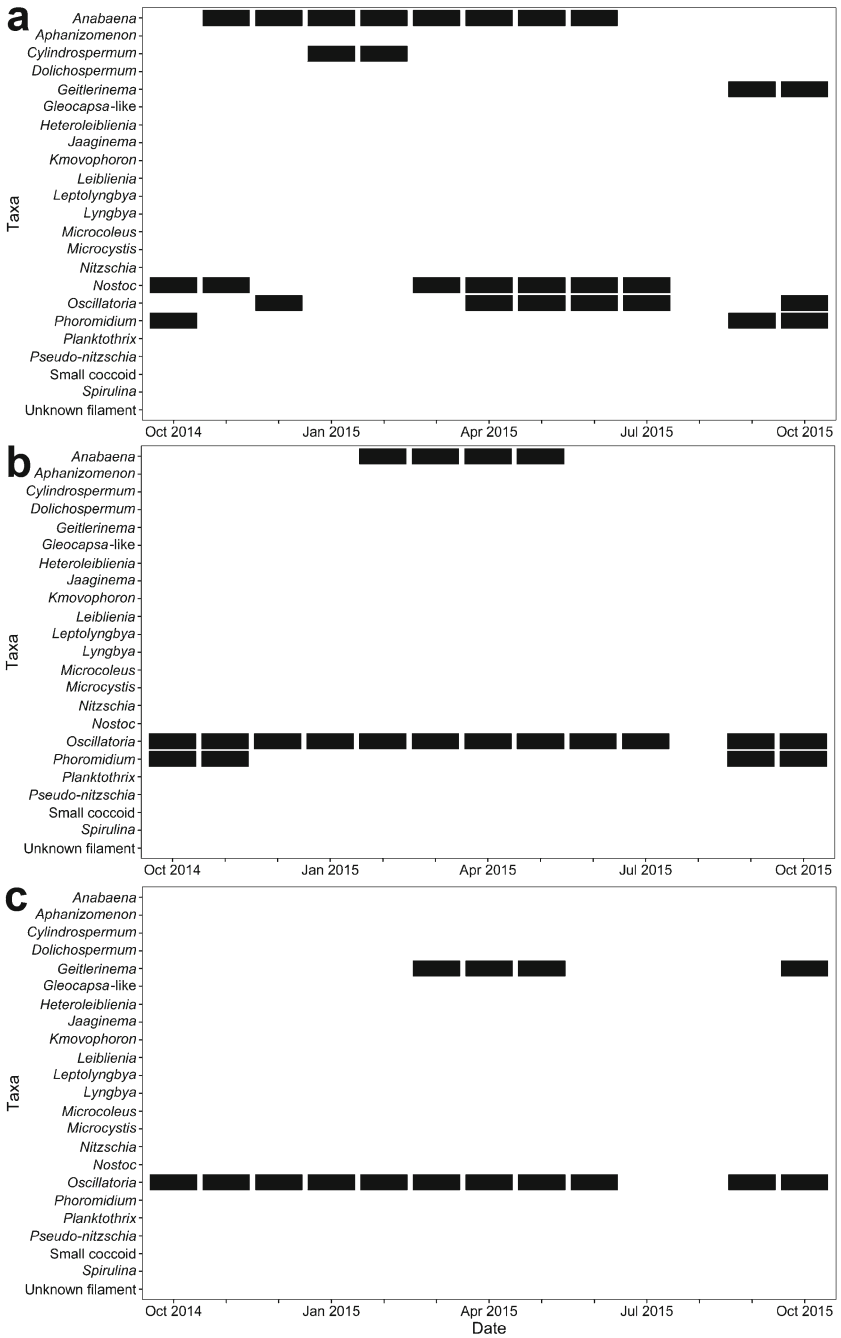

Fig. 6 Multiple panels depicting the cyanobacterial composition from the upper (**a**), mid (**b**), and lower (**c**) sites in Malibu, CA

study to comprehensively examine cyanobacterial taxa in conjunction with cyanotoxin presence at the land-sea interface of a semi-arid region.

The land-sea interface is poorly characterized with respect to algal/cyanobacterial toxins compared to coastal marine ecosystems. Locally, this is due to the lack of

Table 3 Salinity measurements of water samples collected at each site in the Corral Canyon-Malibu watershed

Site	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct
Malibu-U	0	0	0	0	0	0	0	~0.5	~0.5	~0.5	–	0	0
Malibu-M	8	5	6	4	4	6	3	8	13	10	–	3	2
Malibu-L	30	25	22	20	20	20	23	32	32	–	–	25	22

U upper site, *M* mid site, *L* lower site.

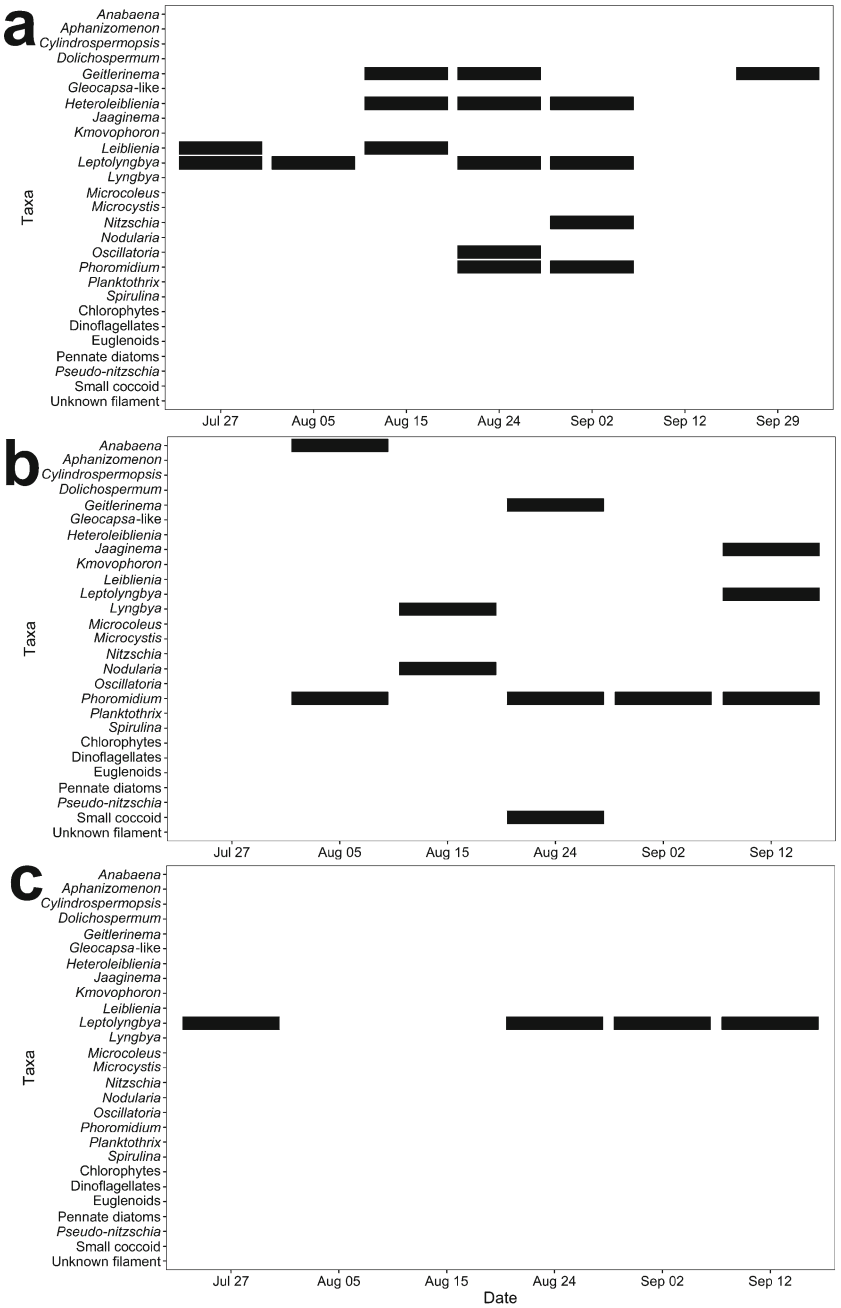

Fig. 7 Multiple panels depicting cyanobacterial communities from the **a** upper, **b** mid, and **c** open sites at Los Peñasquitos

cohesive monitoring in the SCB, particularly in freshwater environments. Nonetheless, a recent survey of > 1200 wadeable streams across California reported a high frequency of occurrence (> 90%) of potentially toxigenic benthic cyanobacteria, encompassing several taxa. Benthic material from one third of the streams harbored microcystins, and several other cyanotoxins were detected at lower frequencies (Fetscher et al. 2015). Additionally, one recent survey documented the presence of several potential toxin-producing cyanobacteria along the coastline in the SCB. The prevalence of multiple toxins in estuarine sites with constant or intermittent connectivity to the ocean was 23% in that study (12 of 53 sites) (Tatters et al. 2017). These pioneering studies in the region document several previously unrecognized occurrences of potentially toxic cyanobacteria and their toxins across a wide array of freshwater and estuarine ecosystems.

Predicting the spatial and temporal nature of occurrences of cyanobacteria and associated toxins at the land-sea interface is difficult and involves high levels of uncertainty, thus complicating management efforts. The commonplace occurrence of multiple potential producers in a community, coupled with non-constitutive production, presents a challenge to characterizing these systems. Examples of heterogeneity of both

Table 4 Salinity measurements of water samples collected at each site in the Los Peñasquitos, Sweetwater, and Otay watersheds

Site	Salinity						
	Jul 27	Aug 5	Aug 15	Aug 24	Sept 2	Sept 12	Sept 29
Los Peñasquitos							
U	1.85	1.84	1.76	1.89	2.50	2.06	
M	8.27	9.03	7.77	6.87	2.62	3.35	
O	8.64	9.93	7.82	6.74	6.07	7.00	12.7
Sweetwater							
U	2.76	2.81	2.64	2.73	3.33	2.82	3.10
M	9.14	11.72	11.69	5.47	9.85	6.07	
O	11.97	9.88	9.49	5.75	7.72	7.74	
Otay							
M	9.24	10.77	8.89	8.56	15.92	5.02	
O	9.31	10.46	9.23	8.04	16.05	4.79	

U upper site, *M* mid site, *L* lower site.

cyanobacteria and associated toxins in estuarine systems have been described (Laamanen et al. 2002; Malazarte et al. 2017) and was particularly evident across a salinity gradient in the adjacent Buena Vista Lagoon, San Diego County, USA (Tatters et al. 2017). The overall complexity of the ecological mosaic is not often captured with limited sampling coverage as differences can exist on a scale of meters or less (Carmichael and Gorham 1981; Wood et al. 2012).

A general lack of understanding concerning cyanobacterial dynamics is also evident when considering production and transport of toxins, which may include several compounds including anatoxins, cylindrospermopsins, microcystins, nodularins, and saxitoxins. In addition to transport of these chemicals, cyanobacterial biomass can be dispersed from a freshwater source, colonize along the route, grow, and even proliferate across salinity gradients that extend to nearly full-strength sea water (Nubel et al. 2000; Laamanen et al. 2002; Rejmankova et al. 2004; Whitton and Potts 2011; Wood and Young 2011; Graham et al. 2012). Physical transport can occur from a variety of mechanisms including upstream scouring during unfavorable conditions or turbulence, advection of isolated populations in "hot spots," on particles, sediment, and via runoff and natural flows (Wormer et al. 2011; Wood and Young 2011; Graham et al. 2012). Depending on community composition, environmental conditions, and potential for degradation, a portion of these cells and toxins can remain intact for extended periods of time (i.e., days to months) and travel large distances (i.e., kilometers) (Imanishi et al. 2005; Lemes et al. 2008; Graham et al. 2012; Preece et al. 2017). The species responsible for toxin production can be difficult to discern because of a spectrum of potential producers, toxic and non-toxic strains, and toxigenic organisms not constitutively producing a specific chemical (Kardinaal et al. 2007; Tatters et al. 2017). The current study expands our knowledge by examining cyanobacterial taxonomy and associated toxins in four representative coastal systems over time and space.

Cyanobacteria Prevalence and Frequent Cyanotoxin Detections

Pronounced spatial and temporal differences in cyanobacterial composition and detectable toxins were documented at each study location across the SCB. In Malibu, *Oscillatoria* was dominant in the system, yet was found in abundances that varied through time at each site. The genera *Anabaena*, *Cylindrospermum*, and *Nostoc* were likely influenced by salinity as they proliferated only at locations with values less than 4. This is in line with previous findings that indicate a presence of these genera only at salinities under 4 in the region (Tatters et al. 2017; Tatters unpublished). Multiple toxins were detected during the year-long campaign at the Malibu creek indicating possible linkages with causal cyanobacteria as well as temperature and salinity. The seasonally fluctuations in taxa identified during the year imply multiple producers, not a single taxon, of both anatoxins and microcystins. Anatoxins were identified only at mid and upper Malibu, sites that were characterized by more species rich cyanobacterial communities compared to the lower area where only one or two genera were usually present. Recent work in France found anatoxin concentrations to be positively correlated with community diversity when the primary producer, *Anabaena* sp., was not dominating the assemblage (Sabart et al. 2015). Microcystins were found simultaneously at all sites on three separate occasions (9 samples; Fig. 4b). In 8 of these samples, *Oscillatoria* was abundant. Particulate microcystins were less abundant during cooler months, when the toxins were either non-detectable at low levels or absent in some instances (Fig. 4b). These results corroborate reports from other locations, where microcystin levels were positively correlated with temperature (Davis et al. 2009; Bui et al. 2018; Lurling et al. 2017; Buckaveckas et al. 2018). The genus *Oscillatoria* was always present when microcystins were detected at the lower and mid sites, possibly implicating this genus as a producer. Saxitoxins were always detectable when the genus *Geitlerinema* was observed. This genus has been previously demonstrated to produce anatoxins, microcystins, and saxitoxins (Gantar et al. 2009; Borges et al. 2015; Tatters, unpublished).

At the Los Peñasquitos sites, the cyanobacterial communities changed on a weekly basis. Cylindrospermopsin detections were sporadic while anatoxin-a displayed a repeatable pattern, i.e., always positive or negative for all sites. Multiple toxins were detected from Los Peñasquitos lagoon and putative linkages with associated cyanobacteria were identified.

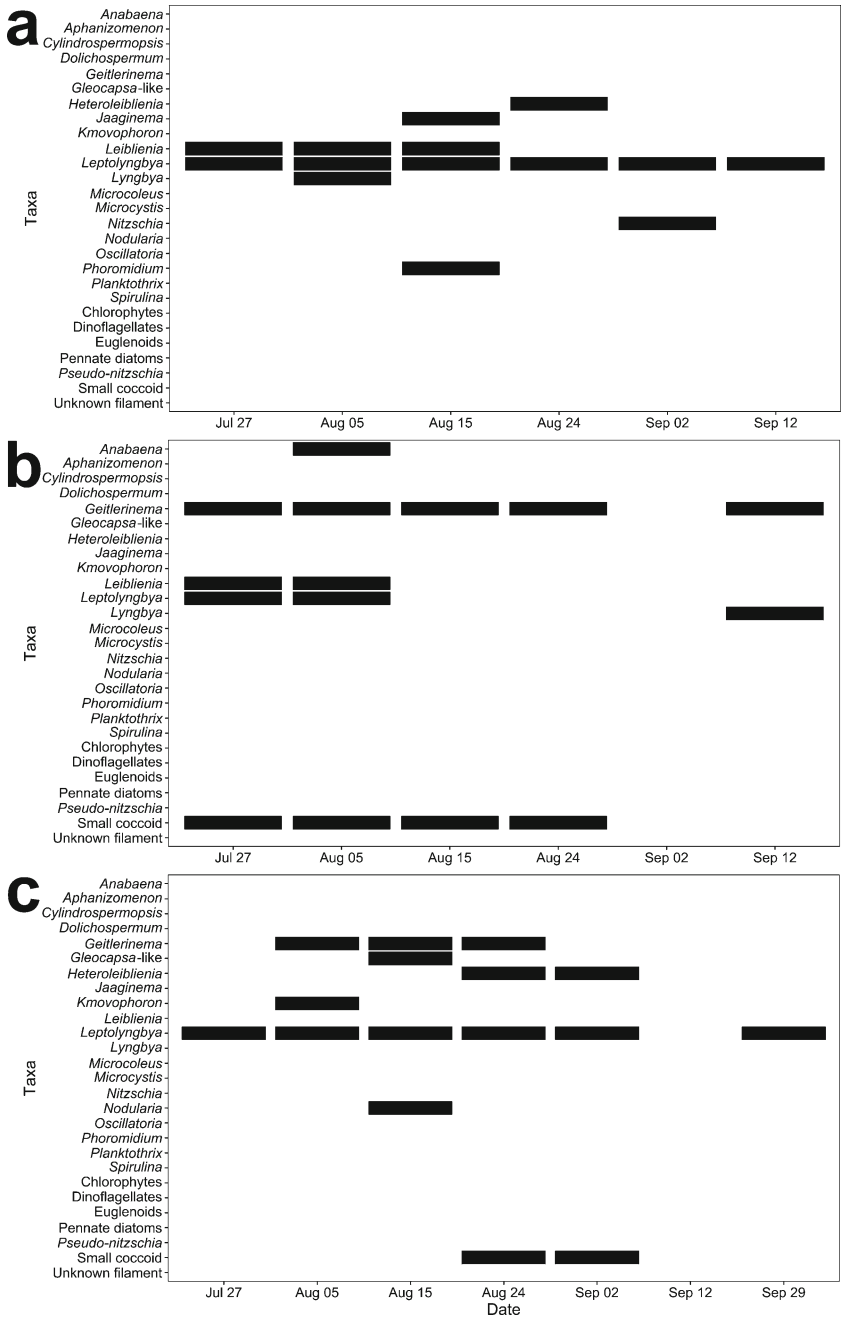

Fig. 8 Multiple panels depicting cyanobacterial communities from the **a** upper, **b** mid, and **c** open sites at the Sweetwater River

Anatoxin-a was present at all sites during the initial four collection dates. An absence of anatoxin-a detection for the final two collecting dates followed a 3–5 °C decrease in water temperature and salinity fluctuations at Los Peñasquitos, along with other San Diego sites. A general decline in cyanobacterial species richness was also observed at each site following the aforementioned temperature and salinity changes, likely indicating a shift away from environmental optima for some taxa. The presence of anatoxins was correlated with temperature in an Italian lake (Cerasino and Salmaso 2012). Elevated toxin incidence in this study at the open site compared to mid and upper sites hinted to possible transport but these findings were not conclusive. It is possible that a benthic component of the system was overlooked and not sampled, i.e., *Phormidium*, and the samples were providing just enough biomass for toxin detection. In fact, a considerable portion of the filament biomass was in dispersal form. Los Peñasquitos was included in the 2015 survey where benthic *Lyngbya* and *Calothrix* were found to be the dominant cyanobacterial genera. No toxins were detected in sub-surface water samples at that time (Tatters et al. 2017). The results contrast with those of the current study in terms of cyanobacteria genera and toxins present.

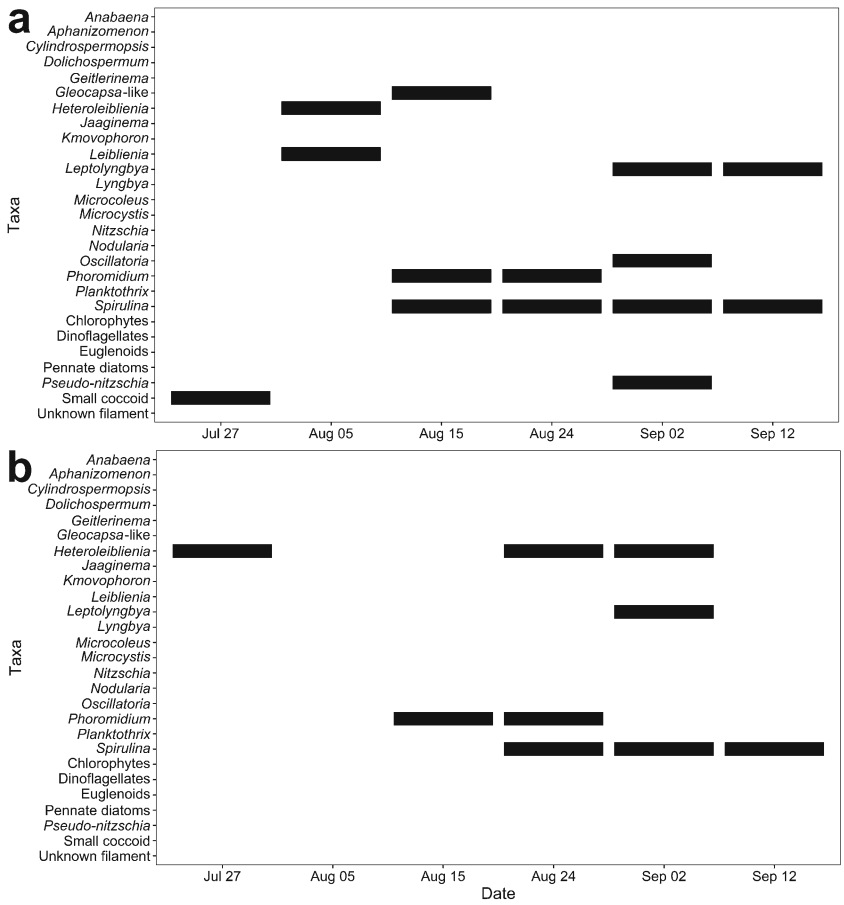

Fig. 9 Multiple panels depicting cyanobacterial communities from the **a** mid and **b** open sites at the Otay River

The Sweetwater River had a dynamic cyanobacterial community composition, yet it exhibited relatively uniform occurrence of anatoxin-a. After detection of cylindrospermopsin at all sites during the initial two sampling days, the occurrence was inconsistent across the upper and open areas, but the mid site was always positive. In addition to anatoxin-a and cylindrospermopsin, analysis of SPATT also revealed the occasional presence of microcystin-RR, microcystin-LA, and domoic acid. Microcystin-LA was detected only once and was absent at the other San Diego sites. Oysters obtained from the open/mouth of the Sweetwater River always yielded the presence of microcystin-LR or domoic acid. One of four oysters examined contained detectable and quantifiable amounts of both toxins (Table 5). We previously found *Anabaena*, *Merismopedia*, *Nodularia*, *Oscillatoria*, and *Phormidium* in the cyanobacterial community at this site (Tatters et al. 2017). In addition, multiple toxins including microcystins and at least one saxitoxin derivative were detected in samples from that survey. There were both similarities and differences compared to the current study. The genus *Leptolyngbya* was not documented in the fall of 2015 but was identified on subsequent collection dates. We did, however, find many of the same taxa including *Anabaena*, *Nodularia*, *Oscillatoria*, and *Phormidium* as well as detectable levels of microcystins.

Table 5 Results from toxin analysis of oysters collected at the mouth of the Sweetwater River

	MC-LR	MC-RR	MC-YR	MC-LA	Domoic Acid
Oyster 8.5	–	–	–	–	0.004
Oyster 8.15	4.378	–	–	–	0.082
Oyster 8.24	–	–	–	–	0.003
Oyster 9.12	6.629	–	–	–	–

All units are expressed in ng (g tissue)$^{-1}$

MC-LR microcystin-LR, *MC-RR* microcystin-RR, *MC-YR* microcystin-YR, *MC-LA* microcystin LA and domoic acid

Although the cyanobacteria community was distinctively different across sampling at the Otay River, the presence of anatoxin-a mirrored the other San Diego locations and cylindrospermopsin was always detectable. Sampling was reduced from three to two sites due to a drought-induced dry upper area throughout the study. During the period between August 24 and September 2 there were four toxins detected in the Otay watershed (two dissolved, two particulate). In addition to anatoxin-a and cylindrospermopsin, analysis of SPATT deployments revealed microcystin-RR in the mid and open sites as well as the diatom-associated toxin, domoic acid, at the open site (Table 2). The Otay River was not part of the 2015 survey.

Anatoxins

Anatoxins were analyzed from whole water samples in Malibu and both whole water and SPATT from each San Diego location. Anatoxins were detected in particulate samples across the entire SCB, while SPATT was negative or below detection. In fact, water samples from San Diego sites were often positive for anatoxin-a (or congeners) by ELISA, but SPATT deployments at the same sites were always negative (LC-MS). This discrepancy has multiple possible explanations, including transformation by environmental conditions or heterotrophic bacteria, low dissolved concentrations, adsorption to particles, presence as an anatoxin-a congener or derivative, desorption, and degradation from ultraviolet light. If anatoxin derivatives were present, they were not identified by LC-MS due to a lack of commercially available standards and thus were potentially overlooked. Additionally, the extraction methodology for anatoxin recovery and analysis on HP20 resin has been significantly improved, but not employed in this study (R.M. Kudela, personal communication). Anatoxin-a is notorious for its ephemeral nature as it is readily degraded by a variety of environmental factors. Outside the cell, anatoxin-a is not considered a persistent chemical, unlike microcystins and domoic acid.

Cylindrospermopsin

Cylindrospermopsin was always present, in particulate and dissolved forms, at both sites in the Otay River and mid Sweetwater. Collectively, cylindrospermopsins are a class of small bioactive alkaloids that have exhausted a variety of effects ranging from hepato- to neurotoxicity (Hudnell 2008). The principal producer is *Cylindrospermopsis raciborskii*, but other Nostocalean and even Oscillatorialean genera may produce the toxin. Cylindrospermopsin was also detected samples from other San Diego sites, but it was more sporadic in occurrence. Nonetheless, the toxin was identified at all San Diego sites at least once during the study period. Sites in the Malibu creek never had measurable cylindrospermopsin at any sampling timepoint. In addition to being present in dissolved and cell-associated form, the toxin may also be particle-associated. This compound is not considered to be common in the USA and even North America (Loftin et al. 2016). The frequent presence of cylindrospermopsin at these sites was unexpected and represents a unique finding that warrants attention.

Difficulties in Tracing the Sources of Cyanotoxins (Microcystins)

A prime example of the vagaries of predicting the presence of toxins are microcystins. Salinity tolerance of these compounds contributes to the inherent difficultly of identifying their source in estuaries. Studies have reported a salinity of 0–10 will support the growth of *Microcystis* (Orr et al. 2004; Tonk et al. 2007). Microcystin production has also been documented in saline waters (Orr et al. 2004; Lehman et al. 2005; Tonk et al. 2007, reviewed in Preece et al. 2017). In another report, sudden exposure to salinity greater than 10 dramatically increased extracellular toxin concentrations (Ross et al. 2006). Microcystins were detected at each sampling site from subsurface samples or SPATT deployments (Fig. 4b; Table 3). These chemicals are a major concern in nearly all waterbody-types including rivers, creeks, inland lakes, reservoirs, and lagoons. Known microcystin producers include *Anabaena*, *Aphanizomenon*, *Dolichospermum*, *Cylindrospermopsis*, *Microcystis*, *Planktothrix*, *Oscillatoria, Phormidium*, and *Nostoc* (Hawkins et al. 1985; Sivonen and Jones 1999), and many possible sources of these chemicals were found during this study. The list consists of benthic and planktonic producers/genera as well as nitrogen-fixing and non-nitrogen-fixing forms. Unfortunately, food products including seafood are not routinely screened for microcystins (Mulvenna et al. 2012; Vareli et al. 2013; Peacock et al. 2018). The salinity tolerance of producers coupled with transport potential afforded by stability and persistence of microcystins warrants further attention and consideration in the SCB.

SPATT

The utilization of SPATT to complement whole water samples by capturing the integrated dissolved phase provides a useful platform to characterize toxins in these dynamic environments and an alternative to discrete water or benthic samples whose effectiveness are affected by the heterogeneous nature of cyanobacterial distributions. Importantly, SPATT permits the ability to detect overlooked dissolved compounds, often due to concentration-related issues, and alleviates the limitations of temporal resolution associated with traditional sampling. Overall, the contribution of SPATT may provide a more holistic picture of the system by integrating dispersal potential, sources, pulses, and pools of important toxins. At present however, toxin detections by SPATT cannot yet be directly related to particulate or dissolved toxins at the time of sampling because SPATT theoretically provides a time-average measurement throughout the period of deployment.

There were marked differences between toxins identified in whole water and SPATT. Microcystins were commonly found in particulate samples from Malibu but not detected in whole water from the San Diego sites. SPATT however was positive for microcystins on a regular basis in San Diego. These compounds were likely being produced away from the sampling sites, in non-target benthic mats, or were present at low ambient concentrations. Cylindrospermopsin was not detected in particulate samples from Malibu or San Diego SPATT but

were routinely identified in whole water from San Diego. In addition, particulate domoic acid was not detected in Malibu or San Diego, but dissolved toxin was detected from SPATT at each San Diego site.

Multiple Cyanotoxins Frequently Detected

Many unknowns surround the presence, occurrence, and significance of toxin mixtures. The consequences of acute and chronic exposure of animals and humans to multiple chemicals have not been extensively assessed (Metcalf et al. 2008; Fire et al. 2011; Ferriss et al. 2017; Peacock et al. 2018). In this study, the same general trends were evident across geographical locations where mixtures of cyanotoxins were commonly detected at each watershed, i.e., 66% and 50% at the Otay and Sweetwater rivers respectively. Three toxins were identified during 1 week in the Sweetwater River and the Malibu creek, albeit using different detection methods. Four toxins were found in the lagoon at Los Peñasquitos and in the Otay River. These results corroborate with recent reports from the San Francisco Bay Estuary where four different classes of toxins were present simultaneously (Peacock et al. 2018). Not surprisingly, communities of multiple potential toxin producers were usually observed at each site. Their presence likely contributes to the regularity of particulate and/or dissolved toxins in these watersheds. The co-occurrence of toxins in a given water sample, once considered a rare phenomenon, seems to be more common than originally assumed.

Conclusions

This study furthers our understanding of cyanobacterial heterogeneity within watersheds and connectivity between freshwater, brackish, and marine ecosystems. It sheds light on another mechanism of how inland waterways may directly and indirectly influence estuaries and coastal waters in the SCB. From our results and study design, however, it is not possible to definitively differentiate toxin transport to, or production at, the sampling sites. We observed a high frequency of cyanotoxin detection at multiple sites in the SCB, and an unanticipated overlap of potential toxin-producing organisms and toxins adjacent to the Pacific Ocean. The observed diversity and heterogeneity is likely a function of having a continuum of microhabitats likely promoted by differential flows, salinity, temperature, light, nutrients, and community composition in these dynamic ecosystems.

Only a few studies have investigated cyanobacterial dynamics at the land-sea interface along the SCB and in general (Currin et al. 2011; Howard et al. 2017; Tatters et al. 2017; Peacock et al. 2018). This work improves our understanding of this complex system by documenting diverse cyanobacterial communities and a spectrum of toxins from multiple sites in different watersheds. Some of these communities and toxins changed on a near-weekly basis. The presence of co-occurring cyanotoxins (45% of total samples) coupled with their potential to co-occur with toxins of marine origin represent a significant public and environmental health concern (i.e., in the co-occurrence in 25% of analyzed shellfish samples from the Sweetwater River mouth). Although studies have examined impacts in other systems (Lehman et al. 2010; Ferrão-Filho and Kozlowsky-Suzuki 2011), there is a relatively poor understanding of the consequences stemming from the presence of toxin mixtures on the environment and marine resources in the SCB. In the case of Los Peñasquitos and the Sweetwater River, the strong temporal and spatial heterogeneity in component cyanobacteria and toxins between sampling years make a case for improved characterization of these systems, as a baseline has only been loosely established. These results strengthen the need for expanded monitoring and observational strategies to examine seasonal and longer-term patterns. The dynamics and heterogeneity of these systems suggest that one collection method is likely insufficient for characterizing the presence and location of toxins in the environment and in marine resources. This reporting on the prevalence and incidence of the co-occurring toxins may eventually assist in influencing a re-evaluation of cyanotoxin thresholds and guidelines. Efforts to characterize chemical transport in similar systems are now underway.

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