The Tide Turns: Episodic and Localized Cross-Contamination of a California Coastline with Cyanotoxins

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ABSTRACT

The contamination of coastal ecosystems from a variety of toxins of marine algal origin is a common and well-documented situation along the coasts of the United States and globally. The occurrence of toxins originating from cyanobacteria along marine coastlines is much less studied, and little information exists on whether toxins from marine and freshwater sources co-occur regularly. The current study focused on the discharge of cyanotoxins from a coastal lagoon (Santa Clara River Estuary) as a consequence of an extreme tide event (King Tides; December 3-5, 2017) resulting in a breach of the berm separating the lagoon from the ocean. Monthly monitoring in the lagoon throughout 2017 documented more than a dozen co-occurring cyanobacterial genera, as well as multiple algal and cyanobacterial toxins. Biotoxin monitoring before and following the King Tide event using Solid Phase Adsorption Toxin Tracking (SPATT) in the lagoon and along the coast revealed the co-occurrence of microcystins, anatoxin, domoic acid, and other toxins on multiple dates and locations. Domoic acid was ubiquitously present in SPATT deployed in the lagoon and along the coast. Microcystins were also commonly detected in both locations, although the beach berm retained the lagoonal water for much of the year. Mussels collected along the coast contained microcystins in approximately half the samples, particularly following the King Tide event. Anatoxin was observed in SPATT only in late December, following the breach of the berm. Our findings indicate both episodic and persistent occurrence of both cyanotoxins and marine toxins may commonly contaminate coastlines in proximity to cyanobacteria-laden creeks and lagoons.

INTRODUCTION

Harmful eukaryotic microalgae and cyanobacteria, and the toxins produced by many of these species, are increasing in frequency, intensity, and geographic range across the globe (Paerl and Paul 2012; Gobler et al., 2017). Collectively, these phenomena are being influenced by factors that may act in concert and have widespread effects throughout food webs (Sunda and Cai 2012; Tatters et al., 2018). The implicated drivers include environmental change and eutrophication due to increased emissions, land usage, agriculture, aquaculture, and erosion. The corresponding changes in environmental parameters such as a steady rise of annual mean sea surface temperature, elevated nutrient concentrations, and pH alteration may dramatically promote changes in phytoplankton species composition and linked predator/prey distributions (Sabine et al., 2004; Feely et al., 2008). The degree of this change is likely genus or even species-specific and involves several abiotic and biotic factors on both local and global scales (Tatters et al. 2018; Fu et al. 2012).

Marine algal toxins and the consequent human and animal health issues have been documented throughout waters along the coasts of the United States. The toxins that pose the greatest public health risk and are therefore the focus of most marine monitoring programs include brevetoxins, domoic acid, okadaic acid, and saxitoxins. The marine algal species that cause these harmful algal blooms (HABs), the conditions leading to these events and the illnesses attributed to them (neurologic, paralytic, amnesic, and diarrhetic shellfish poisoning, respectively) have been extensively studied (Gobler et al., 2017; Smith et al., 2018). Domoic acid and saxitoxins have a history of occurrence along the coast of southern California, although the most frequent recurring toxic events within the region have been attributed to domoic acid, produced by species within the diatom genus *Pseudo-nitzschia* (Smith et al. 2018).

Numerous harmful freshwater species, primarily cyanobacteria, are also well-known sources of toxins, including anatoxins, cylindrospermopsins, microcystins, nodularins, and saxitoxins. Their occurrence and effects on human health in various freshwater ecosystems have garnered considerable attention in recent years (e.g. Toledo) (Steffen et al., 2017) although our awareness regarding the diversity of species and toxins is still less complete than for marine taxa. In California, a growing body of studies have documented the presence of

toxins or potentially toxic species of cyanobacteria across a wide range of streams and lakes (Fetscher et al., 2015; Tatters et al., 2017; Tatters et al., 2019).

Marine and freshwater harmful algae and cyanobacteria, and the toxic conditions they produce, have generally been investigated separately despite the potential for overlapping occurrence in estuarine ecosystems. This dichotomy is largely because funding sources for research and monitoring have traditionally been divided along freshwater/marine lines, translating into comparatively little work on cyanotoxins in estuaries. Consequently, there has been little recognition that cyanobacterial toxins produced in freshwater ecosystems can also affect estuarine and coastal waters due to transport down streams and rivers. Cyanobacteria and cyanotoxins can be transported hundreds of miles downstream from the original bloom source (Bowling et al., 2013; Rosen et al., 2018; Graham et al., 2012) or present in coastal rivers (Miller et al., 2010; Otten et al., 2015; Bouma-Gregson et al., 2017; Kelly et al., 2019; Tatters et al., 2019). Indeed, cyanotoxin and cyanobacterial transport into estuarine or coastal marine ecosystems has been largely undocumented except for a small number of specific locations (c.f. Preece et al., 2017). Recent studies, however, have determined that these artificial demarcations between marine and freshwater HAB issues, and their health-related effects, are often contraindicated (Tatters et al., 2017, Tatters et al., 2019; Peacock et al., 2018).

As a consequence of these events, HAB studies have been implemented to determine the extent of cyanobacterial and cyanotoxin transport across the freshwater to marine continuum. We hypothesized that cyanotoxins produced in lakes, streams, wetlands, reservoirs, and estuaries accumulate at the bottom of the watersheds and enter marine waters episodically, particularly during storms or times of significant tidal exchange. Intermittent estuaries (created seasonally, separated from the ocean by berms) such as the Santa Clara River Estuary (SCRE), may generate or accumulate substantial amounts of biomass when not flowing and episodically deliver measurable amounts of cyanotoxins to marine waters once flow begins or berms are opened.

The SCRE is a seasonally connected river estuary system in the northern Southern California Bight (SCB). The estuary is stagnant, shallow, sunlit, and hypereutrophic (McLaughlin et al., 2014). These characteristics make it a natural cyanobacterial incubator. Since the fall of

2015, we have routinely observed this system and found substantial accumulation of cyanobacterial/algal biomass when river flows are relatively low and/or the system is closed to the sea. The SCRE is affected by a multitude of anthropogenic stressors (Tatters et al., 2017). This area is often cordoned off from the Pacific Ocean by a sand berm that is in place for most of the year. Natural breaches occur during high rainfall years (i.e. El Niño) and the berm has been sporadically opened in the past by the city of Oxnard/Ventura.

Exceptionally high tides, commonly referred to as "King Tides," are unique events that occur on an annual or biannual basis coinciding with a new or full lunar phase coupled with when the Earth's moon is at its perigee. These King Tides may encroach on areas that do not typically receive marine influence. The proximity of the SCRE to the Pacific Ocean lends itself to exchanges of ocean water into the low salinity estuary and vice versa. Although these episodes are minor erosion events and not considered a full breach of the lagoon and discharge of its contents, the tendency of the SCRE to accumulate biomass sets the stage for episodic delivery of cyanobacteria, potential cyanotoxins, and elevated nutrient concentrations to marine waters from connective events such as King Tides or other breaches of the sand berm from a variety of weather events.

We conducted a year-long campaign in the SCRE and along the adjacent coastline to expand our recent findings demonstrating the occurrence of cyanobacterial toxins in the river and estuary (Tatters et al., 2017). The overall objectives of this study were to perform monthly characterizations of the cyanobacterial composition and associated cyanotoxins in a compartmentalized estuary fed by a seasonal river from January to December 2017. During regular sampling, a King Tide provided an opportunity to observe a breach of the lagoon. In late November, we adapted our monthly sampling to a weekly schedule timed to impending King Tides. The event permitted us to document the relationship between river-ocean connectivity and the presence of biotoxins in the estuary and along the coast.

METHODS AND MATERIALS

Site Description

The Santa Clara River Estuary (SCRE) is a hypereutrophic lagoonal system located in Ventura County, California, in the northern region of the SCB (Figure 1). The estuary area is estimated to be 1.4 x 10^6 m^2 (McLaughlin et al., 2012) and the volume of the estuary fluctuates throughout the year. Volume estimates for the estuary ranged from 2.5 x 10^4 m^3 to 2.2 x 10^6 m^3 in a study conducted between 2009 and 2010 (Stillwater Sciences, 2011). The SCRE receives surface flows from the Santa Clara River (SCR), discharge from the Ventura Wastewater Reclamation Facility (VWRF), tidal influence from the Pacific Ocean, and to a lesser extent groundwater flow and subsurface flows with the ocean (Stillwater Sciences, 2011). The SCR is the largest river system in southern California and watershed area of the estuary is approximately 4.2 x 10^3 km^2 (McLaughlin et al., 2012). Riverine flow is influenced by a mix of urban and agricultural discharges and flows vary dramatically throughout the year, with long periods of low flow occasionally leading to episodic hydrologic disconnections and intermittent high flow periods driven by precipitation. Daily mean flow rates from the SCR have ranged from 0 to 90,000 cubic feet per second (cfs) between 1927 and 2007 (Stillwater Sciences, 2011). The SCR is the dominant flow into the estuary between the fall and spring during which time the estuary inlet is most likely to be open (Stillwater Sciences, 2011; McLaughlin et al., 2012). Treated effluent discharged by VWRF into the northern region of the estuary has historically ranged from 4 MGD to 10 MGD and is the primary flow into the estuary from approximately March to September (Stillwater Sciences, 2011). This system has been included on the 303(d) impaired waterbodies listing since 2010 for nutrients and bacteria.

Monthly Sample Collection

The SCRE lagoon was sampled monthly from January to December 2017 to assess the annual presence of cyanobacteria and cyanotoxins. A combination of sample types were collected in order to provide a holistic view of the toxin dynamics and transport. Discrete sub-surface water samples were collected for microscopic determination of cyanobacterial community composition, total and dissolved cyanotoxins, chlorophyll *a*, temperature, and salinity. Time-integrated toxin monitoring was conducted using passive sampling devices (Solid Phase Adsorption Toxin Tracking, or SPATT) which were deployed and recovered during each monthly site visit in 2017.

174 **Weekly Sample Collection Pre- and Post-King Tides**

175 Weekly sampling was conducted during the last 5 weeks of 2017 to examine potential
176 toxin transport into the marine environment following breaching of the sand berm by King
177 Tides that occurred from December 3-5, 2017. Multiple sample types were collected that
178 included discrete water samples and integrative sampling, SPATT passive samplers, and mussel
179 (biotic) samples. SPATT samplers were deployed and recovered weekly for 5 weeks at coastal
180 locations northwest (n=4) and southeast (n=2) of the estuary and at two locations within the
181 lagoon (Figure 1). Marine mussels were collected concurrently at three marine locations with
182 SPATT deployment and recovery. Sample collection commenced while the SCRE remained
183 closed to the ocean and continued for 3 weeks after the breach occurred.

184 **Cyanobacterial Community Composition**

185 Water samples were obtained monthly for cell density enumeration and to
186 subsequently characterize the relative abundance of cyanobacterial taxa present. Two-hundred
187 mL of unpreserved water was collected in high-density polyethylene (HDPE) bottles and
188 transported back to the laboratory for analysis. Subsamples were aliquoted into 20-mL culture
189 dishes after gentle mixing and allowed to settle at room temperature overnight. The settled
190 samples were examined using an inverted microscope (Olympus CKX41, Centre Valley,
191 Pennsylvania, USA) at 40-200x magnification. Identified cyanobacterial genera were semi-
192 quantitatively categorized as the percent of the total phytoplankton community according to
193 the following categories: rare (<1%), present (1-10%), common (10-50%), and abundant (>50%).
194 Every 3 months, a fresh Lugol's fixed sample was also counted to assess the effectiveness of
195 using the live samples. After comparing the means of two independent counts of over 300 cells
196 on live and fixed preparations, there were no differences in the cell densities of the three most
197 abundant genera in each of the evaluation samples by Student's t-test ($p>0.05$).
198 Identifications were conducted as previously described (Anagnostidis and Komárek 1988;
199 Komárek 2002, Komárek and Komarkova 2004; Komárek and Zapomelova 2007).

200 **Chlorophyll *a*, Temperature and Salinity Measurements**

201 Water samples were filtered onto 25-mm Whatman GF/F filters (GE Whatman,
202 Marlborough, Massachusetts, USA) and frozen immediately after collection at -20^{o}C. Sample

volumes varied according to visual observations of biomass. Chlorophyll *a* samples were extracted by adding 4 mL of 100% acetone and stored in the dark at -20°C for 24 hours. Sample extracts were analyzed following the non-acidification method (Welschmeyer 1994) using a Trilogy Fluorometer (Turner Designs, Sunnyvale, California, USA). Temperature and salinity were measured with a handheld thermometer and refractometer, respectively.

Discrete Cyanotoxin Sample Collection and Analysis

Discrete water samples were collected monthly for the analysis of total (intracellular + dissolved phase) and dissolved toxins. Samples for the analysis of total toxin were collected in 250-mL amber glass jars that were rinsed three times with sample water and frozen at -20°C until analysis. Discrete dissolved toxin samples (data not included in this paper) were collected by filtering water through a combusted GF/F glass fiber filter (0.7-µm pore size). Filtrates were collected in 250-mL amber glass jars that were rinsed three times with the corresponding filtrate and also frozen at -20°C.

Water samples were prepared for analysis by conducting three sequential freeze/thaw cycles to lyse cells, filtration through 0.7-µm pore size filters, followed by an extraction of the filters using acidic methanol to improve recovery of hydrophobic microcystins and marine toxins. The filter extractions combined with the corresponding flow through represented total toxin or actual water concentrations. Dissolved toxin samples (data not included in this paper) were processed the same, but without three sequential freeze/thaw cycles. All samples were analyzed for the following cyanotoxins: anatoxin-a, cylindrospermopsin, nodularin, and 10 congeners of microcystin (MC); MC-HiLR, MC-HtYR, MC-LA, MC-LF, MC-LR, MC-LW, MC-LY, MC-RR, MC-WR, and MC-YR. Several marine toxins were also monitored in samples: domoic acid, gymnodimine, dinophysistoxin-1, dinophysistoxin-2, okadaic acid, 13-desmethyl spirolide c (SPX-1), and pectenotoxin (PTX). Sample extracts were stored frozen at -20°C prior to analysis at the Organic Geochemistry Research Laboratory at the U.S. Geological Survey Kansas Water Science Center by liquid chromatography with tandem mass spectrometry (LC/MS/MS). The analyses were conducted using an Agilent 1260 Bioinert LC coupled with an Agilent 6460 Triple Quadrupole Mass Spectrometer using a modified version of the method described in Loftin et al. (2016). Briefly, chromatographic separation was achieved using an Atlantis T3 analytical

column. Mobile phase A consisted of deionized water (18.2 MΩ/cm2, < 1 ppb total organic carbon), 0.1% formic acid, and 2mM ammonium formate. Mobile phase B consisted of 50/50 (v/v) methanol/acetonitrile, 0.1% tetrahydrofuran, and 2mM ammonium formate. Electrospray ionization (ESI) was used to ionize analytes, and multiple reaction monitoring (MRM) was used to detect precursor and fragment ions for each analyte. Calibration standards were sourced from the National Research Council of Canada or Enzo Life Sciences. Simetone was used as an internal standard and EDTA as a complexing agent in a stacked sample injection. Sample concentrations were quantitated using single-point standard addition for every sample with 1 µg L^{-1} of each analyte spiked into the sample.

SPATT Deployment and Analysis

Passive sampling devices (SPATT) (MacKenzie et al., 2004; Lane et al., 2010; Kudela 2011) were utilized as a monitoring tool to compliment traditional discrete water samples and to provide a time-integrated indicator of dissolved toxin presence. SPATT samplers were deployed monthly in two locations in the estuary from January through December 2017, and weekly at the same locations in the estuary, as well as at six additional coastal stations from November to December 2017. Given the long deployment times, the monthly SPATT samplers were likely behaving as equilibrium samplers while the weekly SPATT samplers were time-integrative (Kudela 2017). The samplers were constructed as described in Lane et al. (2010) and Kudela (2017). Briefly, 3 g (dry weight) DIAION HP20 resin was added to 100-µm Nytex mesh bags, activated in 100% methanol, rinsed in 18.2 MΩ/cm2, < 5 ppb total organic carbon water. Samplers were stored in ultrapure water prior to deployment. After collection, SPATT bags were stored at -20^{o}C until sample extraction and analysis. Extraction was performed as previously described by Kudela (2011).

SPATT extracts from the monthly samplings were analyzed for five congeners of microcystin (MC-RR, MC-YR, MC-LR, MC-LA, MC-LF), nodularin, anatoxin-a, cylindrospermopsin, domoic acid, okadaic acid, dinophysistoxin 1, and dinophysistoxin 2. SPATT extracts from the weekly samplings were analyzed for the same five microcystin congeners as the monthly samples with the addition of three derivatives (MC-LY, MC-WR, MC-dmLR). All extracts were analyzed at the University of California, Santa Cruz, via liquid chromatography/mass

spectrometry (LC-MS) with ESI and selected ion monitoring (SIM) on an Agilent 6130 with a Phenomenex Kinetix (100 x 2.1) C18 column. The method was adapted from Mekebri et al. (2009) with minor modifications to account for the choice of column and LC-MS/SIM instead of tandem mass spectrometry (Kudela 2011). Briefly, a mobile phase gradient was employed with solvent A consisting of water and solvent B consisting of acetonitrile acidified with 0.1% formic acid. Analysis included replicates and matrix additions, with quantification based on external standards. The detection limit for SPATT analyses was 0.05 ng g^{-1} HP20 resin for all congeners. The percent recovery was reported in Kudela (2011) and was ~58-100% for each derivative using a standardized recovery method. Data presented as ng g^{-1} HP20 resin.

Microcystin Analysis from Mussel Tissue

Non-commercial California mussels, *Mytilus californianus*, were collected opportunistically on a weekly basis from November to December 2017 at three coastal stations (Jetty 1, Ventura Harbor outside, and Channel Islands harbor) and frozen at -20^{o}C until extraction and analysis. Each week, three to six individual mussels were collected due to the limited number of organisms at each site. All collected mussels were homogenized and resulted in a total mass ranging between 3.4 grams and 13.2 grams. Two-gram aliquots of tissues were used for extraction with a protocol adapted from Amorim and Vasconcelos (1999), Vasconcelos (1995) and Eriksson et al. (1989). Briefly, 10 mL of 90:10 MeOH:H_2O with 0.1% trifluoroacetic acid was added to the homogenized tissue, vortexed for 30 seconds, and then sonicated for 10 minutes. After sonication, the samples were centrifuged for 10 minutes at 4000 rpm and the supernatant collected in a glass vial. The supernatant was prepared for analysis using the solid phase extraction protocol described by Mekebri et al. (2009). Mussel tissue extracts were analyzed for eight congeners of microcystin (MC-RR, MC-YR, MC-LR, MC-LA, MC-LF, MC-LY, MC-WR, MC-dmLR) and nodularin. All extracts were analyzed at the University of California, Santa Cruz, with LC-MS with ESI using the same method described above for SPATT extracts. Extraction efficiency as reported by Mekebri et al. (2009) ranged from 79.9-104% for mussels, 102% for oysters, and 106% for fish fillet. Mussels were not analyzed for anatoxins or domoic acid due to the small amount of tissue available and because the current extraction protocol for microcystins was incompatible with the analytical method for anatoxins or domoic acid.

RESULTS

The SCRE lagoon exhibited high levels of cyanobacterial and microalgal biomass during much of the sampling period in 2017 prior to breaching of the barrier beach and exchange with ocean water in December (Figure 2). Visible discoloration of the water (Figure 2D), and noticeable accumulations (Figure 2A, B, C, E) were present on most visits. Evidence of the exchange of lagoonal and coastal ocean water was evident at the time of the King Tides in December (Figure 2F).

Monthly occurrence of cyanobacteria in the Santa Clara River Estuary

Eleven cyanobacterial genera were identified during the year-long study at the SCRE (Figure 3). All of these taxa are potential toxin-producers. There were monthly fluctuations in community composition, but the temporal dynamics were most pronounced seasonally. The most prevalent cyanobacterial taxa present in the lagoon during winter months (January-March) were *Geitlerinema* and *Microcystis*. During the spring (April-June), *Geitlerinema*, *Oscillatoria,* and *Microcystis* were the most abundant. The estuary was again dominated by *Microcystis* throughout the summer months (July-September) with genus richness increasing during the fall (October-December) as *Cylindrospermopsis* and *Planktothrix* shared dominance with *Microcystis* as the most common cyanobacteria. During the King Tide event there were six genera present in the lagoon—*Microcystis*, *Planktothrix*, *Phormidium*, *Cylindrospermopsis*, *Geitlerinema*, and *Leptolyngbya* (Figure 3).

Toxins in discrete, monthly water samples

Whole water (total) samples collected on a monthly basis represented a combination of intracellular and dissolved phase toxins (i.e. total toxin concentrations). Three toxin classes were detected in these samples - microcystins, domoic acid, and cylindrospermopsin (Figure 4A). Cylindrospermopsin was detected in January and November, and domoic acid was detected only during the spring (April-June) (Figures 4A, B). Two toxin classes, microcystin and domoic acid, were observed concurrently in May and June (Figures 4A, C, D). Microcystins were observed at the highest overall concentrations during summer and fall (August-December) and were common throughout the year (Figures, 4A, D). No toxins were detected in March and

July's sample was compromised in the freezer (Figure 4A). In addition, whole water samples were analyzed for 10 microcystin congeners, nine of which were detected in the lagoon- MC-HiLR, MC-HtYR, MC-LA, MC-LF, MC-LR, MC-LY, MC-RR, MC-WR, and -YR (Figure 5). The five most abundant microcystin congeners were MC-LR, MC-RR, MC-WR, MC-YR, and MC-HiLR. No nodularin or anatoxins were detected in whole water samples.

Toxins in monthly SPATT samples

One or more toxins were detected each month using SPATT samplers, with the exception of March when the SPATT sampler was lost (Figure 6A). Four classes of toxins were revealed during the year (Figure 6A) with one or more compounds detected in every deployment. Similar to the observations in whole water samples, a dominance of total microcystins was observed during the summer and fall. Both nodularin and anatoxin were detected occasionally at low relative concentrations with SPATT samplers although neither toxin was detected in whole water samples. These compounds were present as minor constituents of total toxin concentrations in six and three deployments, respectively (Figures 6A, B, C). Domoic acid was detected in 10 of 11 monthly samples in SPATT (Figures 6A, D). Microcystin concentrations were highest during summer and fall (July-December) and were present in all monthly deployments that were recovered (Figures 6A, E). Okadaic acid, dinophysistoxin 1, and dinophysistoxin 2 were not detected in SPATT (or whole water) samples. Nodularin co-occurred with MC-RR on six occasions, but never with MC-LA, MC-LR, MC-YR, MC-LA, or anatoxin (data not shown). Three toxin classes were present in eight of the 11 monthly SPATT samples analyzed (Figure 6A). The most abundant microcystin congeners detected using SPATT were MC-LR, MC-RR, and MC-YR (Figure 7).

SPATT- King Tide

The high temporal resolution sampling using SPATT samplers that was conducted from November 25 through December 22 revealed the presence of microcystins, domoic acid, and anatoxin (Figure 8A, B, C). Prior to the King Tide event both microcystins and domoic acid were observed within and outside the lagoon (Figure 8A, B). Although microcystins were detected with SPATT samplers outside the estuary before the King Tides, concentrations were low relative to values obtained from

SPATT samplers deployed inside the lagoon (sites Estuary north and Estuary mid, Figure 8A). Microcystin concentrations detected in SPATT samples were higher at all coastal sites immediately following the King Tides that occurred on December 3-5 (shaded area in Figure 8A). The highest microcystin concentrations were measured inside the lagoon before (6236 ng g^{-1}) and after breach (2312 ng g^{-1}) of the sand berm. Domoic acid was detected in nearly every SPATT sample (39 of 40 samples) along the coastline and within the estuary prior to and following the breach event, with the highest concentration detected at Jetty south on Dec. 22 (Figure 8B). Domoic acid and microcystins occurred in the same sample in 36 of 40 SPATT deployments during the high-resolution sampling period. Anatoxins were detected using SPATT samplers only during the last sampling event in December (7 of 40 samples), approximately 3 weeks following the King Tides (Figure 8C). The highest concentrations were observed within the estuary, but five of the six coastal sites also had detectable levels of anatoxin.

Mussels

Mussels were collected opportunistically on a weekly basis twice before (November 25 and December 2) and three times after the King Tide (December 9, 16, and 22) from three of the coastal stations (Jetty 1, Ventura Harbor outside, and Channel Islands harbor, Figure 1). Tissue extracts were analyzed for microcystins and nodularin (Figure 9). Microcystins were detected in mussels collected on November 25 from all three locations within a range of 2 to 108 ng g^{-1} of wet mussel tissue, but in none of the samples on December 2 just prior to the lagoon breach. A total microcystin concentration of 292 ng g^{-1} mussel tissue was measured south of the estuary on December 9 at Channel Islands harbor and at all three stations (ranging from 14-232 ng g^{-1} mussel tissue) on December 16 (Figure 9). Microcystins were still detectable in mussels collected at the Ventura Harbor outside station approximately 3 weeks after the estuary was breached. Eight congeners of microcystin were analyzed (MC-RR, MC-YR, MC-LR, MC-LA, MC-LF, MC-LY, MC-WR, MC-dmLR). MC-RR and predominately MC-dmLR were detected in mussels sporadically at the three sampling sites prior to and following the breach of the estuary, while MC-YR, MC-LR, and MC-LA were only

detected after the King Tides (data not shown). Microcystin concentrations in mussel tissue were not significantly different between locations or in concentrations or by date using a Kruskal-Wallis test ($p>0.05$). Nodularin, while present in water samples, was undetectable in mussel tissue.

DISCUSSION

The appearance of traditional 'freshwater' or freshwater-sourced toxins in coastal ecosystems has been sporadically reported from various parts of the world. Microcystin contamination has been observed in coastal areas in California (Miller et al., 2010; Gibble et al., 2016; Tatters et al., 2017; Peacock et al., 2018;), Washington State (Preece et al., 2015), the Adriatic Sea (Rita et al., 2014), Isahaya Bay, Japan (Umehara et al., 2015), and France (Bormans et al., 2019). However, recent observations indicate that the situation may be more prevalent spatially and temporally than realized, at least in some regions (Peacock et al., 2018).

Previous sampling at our study site in the SCRE ecosystem along the eastern North Pacific Ocean demonstrated the presence of particulate-associated cylindrospermopsin and microcystins in the river entering the estuary along with anatoxins and saxitoxins inside the lagoon (Tatters et al., 2017). The SCRE is a well-documented hypereutrophic system with cyanobacterial blooms making it an ideal location to test our hypothesis that intermittent estuaries can accumulate biomass and cyanotoxins that are released into the marine environmental during connectivity events. In addition, these estuaries may also provide habitat for growth. The objective of this study was to add to the sparse body of knowledge regarding the dynamics of cyanobacteria and biotoxins across the land-sea interface in the SCRE system. Monthly sampling over the course of a 1-year period revealed temporal fluctuations in the dominant cyanobacterial taxa and cyanotoxins. Greater temporal resolution of sampling (weekly) during the final month of the study at the time of King Tides revealed contamination of the adjacent coastal ocean with cyanotoxins originating in the SCRE and potentially elsewhere in estuaries along the coast. Overall, the prevalence of both toxin-producers and toxins in the SCRE lagoonal system illustrated a persistence of cyanotoxins in SCB and highlighted the shortcomings of current monitoring programs that do not routinely measure cyanotoxins.

Toxigenic Algal Genera

Recognized toxin-producers dominated the cyanobacterial assemblage in the SCRE during the present study. There were pronounced differences in monthly, but most notably in seasonal, cyanobacterial composition and *Microcystis* was among the most abundant genera when present (Figure 3). The next most prevalent genera in the lagoon were *Planktothrix* during the winter, *Oscillatoria* during the spring, *Geitlerinema* during the summer, and *Planktothrix* again during the fall. Although *Geitlerinema* was never dominant in terms of relative abundance in any monthly sample, this genus was previously shown to produce several different toxin classes (Gantar et al., 2009, Borges et al., 2015, Tatters et al., 2019). Cultured isolates of this genus obtained from the SCRE during a prior study produced anatoxin-a (Tatters et al., 2017; Tatters et al., 2019), likely linking the genus with the presence of anatoxins in the lagoon.

Co-occurrence of freshwater and marine toxins

Co-occurrences of 'freshwater' cyanotoxins with those of marine origin are a relatively new finding, having been reported only in recent years (Peacock et al., 2018; Tatters et al., 2019). During this study, cyanotoxins were present in combination with domoic acid during nine of 12 months in the SCRE. These toxins were also detected in the coastal ocean in late November and December around the timing of the King Tide event. Along the central California coastline, Peacock et al. (2018) reported the presence of dinophysis toxins, domoic acid, microcystins, and saxitoxins in 37% of mussel samples from San Francisco Bay. During that study, all SPATT deployments were positive for domoic acid or microcystins and 73% for both toxins. These authors were the first to report traditional 'freshwater' and marine toxins co-occurring in environmental mussel samples (Peacock et al., 2018). Similarly, domoic acid and microcystins were present in 25% of oysters examined at the mouth of the Sweetwater River, Chula Vista, California, marking the first report of this mixture in oysters (Tatters et al., 2019). The same study also revealed the presence of domoic acid and MC-RR at the mouth of the Otay River, San Diego, California, using SPATT samplers.

Microcystins in marine shellfish

There is a growing body of studies documenting the bioaccumulation of microcystins in marine shellfish including California (Miller et al., 2010; Gibble et al., 2016; Peacock et al., 2018;

435 Tatters et al., 2019), Washington (Preece et al., 2015), Virginia (Buckaveckas et al., 2018) and
436 Louisiana (Garcia et al., 2010). There are currently no regulatory guidelines or health thresholds
437 addressing microcystin ingestion in the United states for shellfish; however, California's Office
438 of Environmental Health and Hazard Assessment (OEHHA) set a guidance level for human
439 consumption at 10 μg kg^{-1} of wet fish tissue. Gibble et al. (2016) conducted experiments to
440 examine the update and depuration of particulate and dissolved microcystins in California
441 marine mussels and oysters. The results from mussels indicated microcystins were detectable
442 for 8 weeks post-exposure of particulate toxins but dissolved microcystins were depurated
443 more rapidly (Gibble et al. 2016). Therefore, in addition to microcystins introduced by the King
444 Tide, it is possible that the mussels had been exposed to microcystins from an alternate source
445 in the 2 months prior to the adapted sampling portion of the current study. The presence of
446 domoic acid in these waters also renders the circumstances probable that mussels likely
447 contained domoic acid, though insufficient mussel tissue was available to confirm uptake. This
448 study adds to the growing body of literature that highlights the potential for cyanotoxin
449 exposure in addition to potential co-occurrence with marine algal toxins in estuarine
450 environments.

451 **Multiple toxins**

452 Multiple toxins (two or more toxins) co-occurred in whole water or SPATT samples
453 collected from the SCRE in most months (Figures 4, 6) and in samples obtained at higher
454 temporal resolution from November through December (Figure 8). Five classes of toxins were
455 detected in the lagoon, including domoic acid, anatoxins, cylindrospermopsins, microcystins,
456 and nodularins. These included a collection of dissolved and particle- or cell-associated toxins
457 present throughout the year. In the sample obtained in October, four toxin classes were
458 documented including nine microcystin derivatives and 13 different compounds overall. Nine
459 cyanobacterial genera were observed at that time (*Anabaena, Cylindrospermopsis*,
460 *Dolichospermum*, *Geitlerinema*, *Jaaginema*, *Leptolyngbya*, *Microcystis*, *Phormidium*, and
461 *Planktothrix*). Such complex mixtures of taxa and toxins are consistent with other reports of
462 genus/toxin co-occurrence at the land-sea interface along the southern California coastline
463 (Tatters et al., 2017; Tatters et al., 2019) and central California (Peacock et al., 2018). As in the

current study, five classes of toxins (domoic acid, microcystins, cylindrospermopsins, anatoxins, and saxitoxins) were reported from watersheds in the same region (Tatters et al., 2017). In the latter study, the incidence of multiple toxins (i.e., two of more toxins) was collectively 45% in all samples from the Otay River, Sweetwater River, Los Penasquitos Lagoon, and Malibu creek (Tatters et al., 2019). Similarly, a survey of 52 locations along the SCB revealed the presence of multiple toxins at 23% of sites (Tatters et al., 2017). Three classes of toxins were detected in Buena Vista Lagoon and Santa Margarita River. As previously noted, that study also documented co-occurring cyanotoxins in the SCR and SCRE during 2015. Due to the retentive nature of the hypereutrophic SCRE, the potential for coastal transport events is virtually ever-present. Anytime the estuary is breached by rain and increased river flow, mechanical disruption, high energy wave events, or King Tides as in the present study, sequestered biomass and associated toxins may flow into the Pacific Ocean.

Dissolved toxins and SPATT Samplers

The potential importance of dissolved toxins is becoming increasingly recognized. Routine analysis of dissolved toxins in natural systems have lagged that of particulate or cell-based measurements, due to the assumption that the highest toxin concentrations are intracellular. The ability of passive (adsorptive) sampling devices such as SPATT samplers to concentrate low levels or episodically present toxins have alleviated some of the difficulties of obtaining sufficient material to measure these substances, permitting a better understanding of toxin classes or derivatives present at low levels or from ephemeral events that may be missed by whole water sampling. Compared to particulate- and cell-associated toxins that may be ingested or sink out of suspension and deposited in the benthos, we now recognize that dissolved compounds can travel long distances, have a relatively ubiquitous distribution in the water column, and exhibit impressive stability (Schnetzer et al., 2017; Peacock et al., 2018; Tatters et al., 2019). These attributes allow soluble toxins to readily move through the environment and infiltrate food webs (Gibble et al., 2016).

Our previous studies have highlighted particulate and dissolved cyanotoxins in the SCB (Tatters et al., 2017; Tatters et al., 2019). Here we report differences between toxins detected from two sample types, discrete whole water samples and SPATT deployments (Figures 5, 7).

These differences are expected because discrete samples provide a measurement of toxin in a water parcel at a single location on a specific day and time. On the other hand, passive SPATT samplers represent the adsorption of toxins onto the resin from the surrounding water that has flowed past the sampler. Depending on a variety of factors including sampler design and flow (turbulence), SPATT samplers can act as a time-integrative or equilibrium sampler and therefore can provide a good measurement to indicate toxin patterns and toxin prevalence within a system. Therefore, discrete water samples and SPATT samplers used together for monitoring provide increased insight into the toxin dynamics and transport.

Discrepancies between toxins observed in whole water and SPATT samples may be a consequence of cell-associated toxins not being secreted in measurable quantities that would then be available for adsorption onto SPATT devices or could be representative of temporal differences reflected by the different sample types. Cylindrospermopsin was only detected in two whole water samples. Conversely, anatoxins and nodularin were only found via SPATT analysis. These differences may indicate the ability of SPATT to concentrate these toxins, spatial heterogeneity in the production, or the contribution of a benthic cyanobacterium such as *Geitlerinema*. The presence of *Geitlerinema* could explain the anatoxins (Tatters et al., 2017), but not nodularin, as no known producers of the latter toxin were identified in the estuary. Nodularin therefore may have been produced by an unknown organism, in the river, or upstream in cyanobacterial hot spots such as Sespe or Piru Creek. Finally, dissolved domoic acid was more consistently detected by SPATT deployments compared to whole water samples.

Microcystins (Environmental regulation of toxin composition)

Mixtures of microcystin congeners are not atypical in environmental or culture samples. The toxicity of individual congeners is variable and structure-dependent, with differences due to the extent of protein phosphatase inhibition and interaction with entry-level transporters that dictate downstream potency (Chen et al., 2006a; Niedermeyer et al., 2014). It is likely that multiple microcystin producers were present in the SCRE lagoon. It is also not uncommon for culture isolates of *Microcystis aeruginosa* or other genera to produce several different toxin congeners (J.L.C. Wright, UNC-W/MARBIONC, oral communication, 2017). For instance, an

isolate of *Planktothrix agardhii* collected from Loma Alta Creek during fall of 2015, produced three distinct microcystin congeners (Tatters, oral and written communication, 2017).

Additionally, nitrogen availability has been shown to affect microcystin congener composition in laboratory studies (Puddick et al., 2016). Nitrogen-depleted cultures have been shown to contain less arginine containing derivatives, reduced total microcystin quotas, and lower corresponding toxicity (Puddick et al., 2016). In this study, the predominant forms found in whole water and/or SPATT samples were MC-RR, MC-LR, MC-YR, MC-WR, and MC-Hi-LR. Each of these derivatives contain arginine, which is in line with the high nitrogen concentrations (up to 280 µM during December of 2017, data not shown) in this eutrophic system. Here we report the presence of nine microcystin congeners detected and quantified in the SCRE and adjacent coastal waters (Figures 5, 7). Interestingly, there were two prominent spikes in total microcystin concentrations during the year (Figure 5), yet *Microcystis* was notably absent from the initial occurrence. The genus *Oscillatoria,* present in a nearby creek with a strong salinity gradient, was implicated as the primary microcystin producer in that system (Tatters et al., 2019).

Unexpected findings

The presence of the 'marine' toxin domoic acid in the SCRE lagoon, and 'freshwater' microcystins in the coastal ocean near the SCRE prior to breaching of the beach by the King Tide event was particularly unexpected in the present study. These occurrences may be a function of unrecognized producers, or undocumented mechanisms of transport between the two environments. The genus *Pseudo-nitzschia* was absent from the SCRE, yet domoic acid was detected throughout the year inside the estuary, albeit at considerably lower levels compared to the coastal zone merely steps away. Many diatom species inhabit the lagoon, occasionally rivaling cyanobacteria in dominance. In fact, changes in the relative abundances of diatoms and cyanobacteria is seasonal in the SCRE (Tatters et al., 2017). Not surprisingly, the highest particulate domoic acid concentration in whole water samples was observed during the spring when diatoms were most abundant. One of the diatom genera commonly present in the lagoon throughout the year was *Amphora*. This genus consists of marine, brackish, and freshwater species and has been putatively implicated in domoic acid production (Dhar et al., 2015). The

potential for other diatoms as well as Rhodophytes including *Chondria* spp. to produce domoic acid is also possible.

Extremely high concentrations of microcystins were detected in discrete water samples from the lagoon through the latter part of the year that exceeded California recreational health thresholds and U.S. Environmental Protection Agency recreational water quality criteria (EPA 2016). These compounds were also observed at low levels in the coastal ocean and in mussels just prior to the King Tide event. The potential sources of these microcystins could be a result of benthic cyanobacteria in adjacent harbors with slightly lower salinities (enabling growth of the cyanobacteria and transport to the coastal ocean), discharge from other conduits such as Calleguas Creek to the south of our study area, or even groundwater transport (Chen et al., 2006b; Mohamed et al., 2009; Yang et al. 2016). Our study site, located within the Oxnard Plain Sub-basin, has a high proportion of sand that allows for percolation into the Pacific Ocean, a scenario that might allow groundwater to influence the coastal ocean (Santa Clara River Valley Groundwater Basin 2006). Microcystins along the coast could also have originated from minor, undocumented breaches of the beach berm at the SCRE river mouth as a consequence of early winter storms, from beach erosion by such storms if they expose buried, cyanobacteria-laden sand, dissolved-phase transport through the berm, or an unknown producer.

Implications for monitoring the land-sea interface

Ultimately, the source(s) of the pre-King Tide microcystins in the coastal zone have not yet been elucidated, but their presence presents a worrisome and potentially complex scenario for assessing health risks attributable to these freshwater toxins in the coastal ocean. Recent studies, including this one, have heightened recognition of the connections between freshwater and marine ecosystems with respect to toxin occurrence and transport. Over the last decade, there have been a series of cases documenting cyanotoxin contamination of marine resources in coastal California waters (Peacock et al., 2018; Buckaveckas et al., 2018; Tatters et al., 2019). In this study we documented a natural King Tide-mediated erosion/transport event that resulted in massive amounts of cyanobacteria and cyanotoxins sequestered in the SCRE lagoon being delivered directly into the coastal ocean. Low salinities 0-10 ppt were shown to promote the growth and permit toxin production by *Microcystis aeruginosa* (Orr et al., 2004; Tonk et al.,

2007). However, it is likely that any toxin-containing cells present at low salinities (i.e., 4 ppt inside the estuary) that are rapidly subjected to full strength seawater would eventually lyse, releasing intracellular toxins in the coastal ocean (Orr et al., 2004; Ross et al., 2006; Miller et al., 2010; Preece et al., 2017). These mechanisms for cyanotoxin transport across the land-sea interface are likely not unique to our study area.

Local transport mechanisms

Seasonal flushing primarily due to episodic winter rains delivers vast amounts of stormwater runoff into the SCB. The extent of these flushing events depends on several factors including the timing and magnitude of storms. This mechanism of transporting terrestrial and inland waterway contaminants is a well-documented health threat along the coast. These events deliver a spectrum of bacteria and viruses, including human pathogens, stemming from a multitude of sources into the coastal ocean that can even be transported offshore (Ahn et al., 2005). In addition, various pollutants such as pesticides, fertilizers, sediment(s), and heavy metals are routinely introduced into coastal waters during these runoff events (DiGiacomo et al., 2004; Ahn et al., 2005; Rogowski et al., 2015; Vikas et al., 2015). Our results demonstrating elevated levels of cyanotoxin contamination in lagoonal waters add an additional issue to consider along the SCB coastline during rain and erosion events.

Summary

This study examining the intra-annual variability in toxin presence provides quantitative data on multiple toxins and highlights an unrecognized toxin transport mechanism in the SCRE. Nearly every sample over the course of 1 year yielded the presence of co-occurring 'freshwater' and 'marine' toxins. The potential human and environmental health consequences of these mixtures are poorly understood. In particular, the implications of dissolved toxins, which were commonly detected, may constitute a larger public and environmental health concern than is presently perceived. Toxin producers may or may not co-occur with dissolved toxins, toxin concentrations based on analyses of particulate material rarely reflect total toxins present, and phytoplankton surveillance is not an adequate proxy for proper ecosystem assessment and monitoring. To thoroughly characterize toxin presence in a dynamic system such as the land-sea interface, multiple samples and sampling approaches are required to provide a holistic and

608 comprehensive determination of toxin presence and prevalence in these systems. The
609 combination of monitoring tools using both discrete whole water samples and time-integrative
610 SPATT samples and/or mussel samples provided robust insight into toxin dynamics. Research
611 studies focused across hydrologically connected waterbodies that span freshwater to estuarine
612 and marine waters are needed to conclusively determine the origin of cyanotoxins that enter
613 estuarine and marine waters. Alternative approaches and mechanisms may warrant
614 consideration in future studies. Our results reinforce the importance of routine monitoring of
615 microcystins, anatoxins, nodularins, and potentially cylindrospermopsins at the land-sea
616 interface, and especially in estuarine and marine waters in the SCB.
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618
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Figure Legends

Figure 1: A map of the study region along the coast of southern California. Panel A shows the sampling locations for the monthly and weekly sampling conducted during 2017 within the Santa Clara River Estuary and along the coast to the north and south. The yellow square indicates the site of monthly revisits within the lagoon from January - December 2017. Weekly samples were also collected at that location from Nov-Dec 2017. Mussels and SPATT samples were collected weekly from Nov-Dec 2017 at stations indicated by red circles, while SPATT samples were collected weekly from Nov-Dec 2017 at stations indicated by yellow circles. Panel B shows a more detailed depiction of the region outlined

by a red box in panel A. Panel C shows the southern California coast with a filled red box indicating the location of the study area within the Southern California Bight.

Figure 2: Collage depicting common sampling observations at the Santa Clara River Estuary. Accumulations of cyanobacterial at the surface of the water were common within the lagoon (A, C, E) and in hand-collected samples (B, D) prior to exchange with coastal ocean water. Evidence of recent breaches of the beach berm were apparent at the time of the King Tides (F).

Figure 3. Diversity of cyanobacterial genera and other dominant phytoplankton taxa in monthly discrete water samples collected in the Santa Clara River Estuary during 2017.

Figure 4. Toxin classes detected in monthly discrete whole water samples collected in the Santa Clara River Estuary during 2017. The composition of toxin classes per month is depicted as pie charts (A) and individual concentrations of cylindrospermopsin (B), domoic acid (C) and total microcystin (D) as line graphs.

Figure 5. Microcystin congeners detected in monthly discrete whole water samples collected in the Santa Clara River Estuary during 2017.

Figure 6. Toxin classes detected in SPATT samplers deployed in the Santa Clara River Estuary during 2017. The composition of toxin classes per month is depicted as pie charts (A) and individual concentrations of anatoxin (B), nodularin (C), domoic acid (D), and total microcystin (E) as line graphs.

Figure 7. Microcystin congeners detected in SPATT samplers deployed in the Santa Clara River Estuary during 2017.

Figure 8. Total microcystin (A), domoic acid (B) and anatoxin (C) concentrations in SPATT samplers deployed in the Santa Clara River Estuary area during December 2017.

Figure 9. Total microcystin concentrations in mussels collected weekly in the Santa Clara River Estuary area during December 2017.


A
Jetty 1
Jetty 2
Ventura Harbor inside
Ventura Harbor outside
Estuary North
Estuary Mid
B
Ventura Harbor inside
Ventura Harbor outside
Estuary North
Estuary Mid
Oxnard Airport
N
0 0.350.7 1.4 2.1 2.8
Kilometers
Port Hueneme Naval CBC
Channel Islands Harbor
Channel Islands Beach
Jetty South
C
Santa Maria
Lompoc
Santa Barbara
Lancaster
Palmdale
Victorville
Santa Clarita
Los Angeles
Riverside
Anaheim
Santa Ana
Murrieta
Oceanside
San Dieg
Tijua
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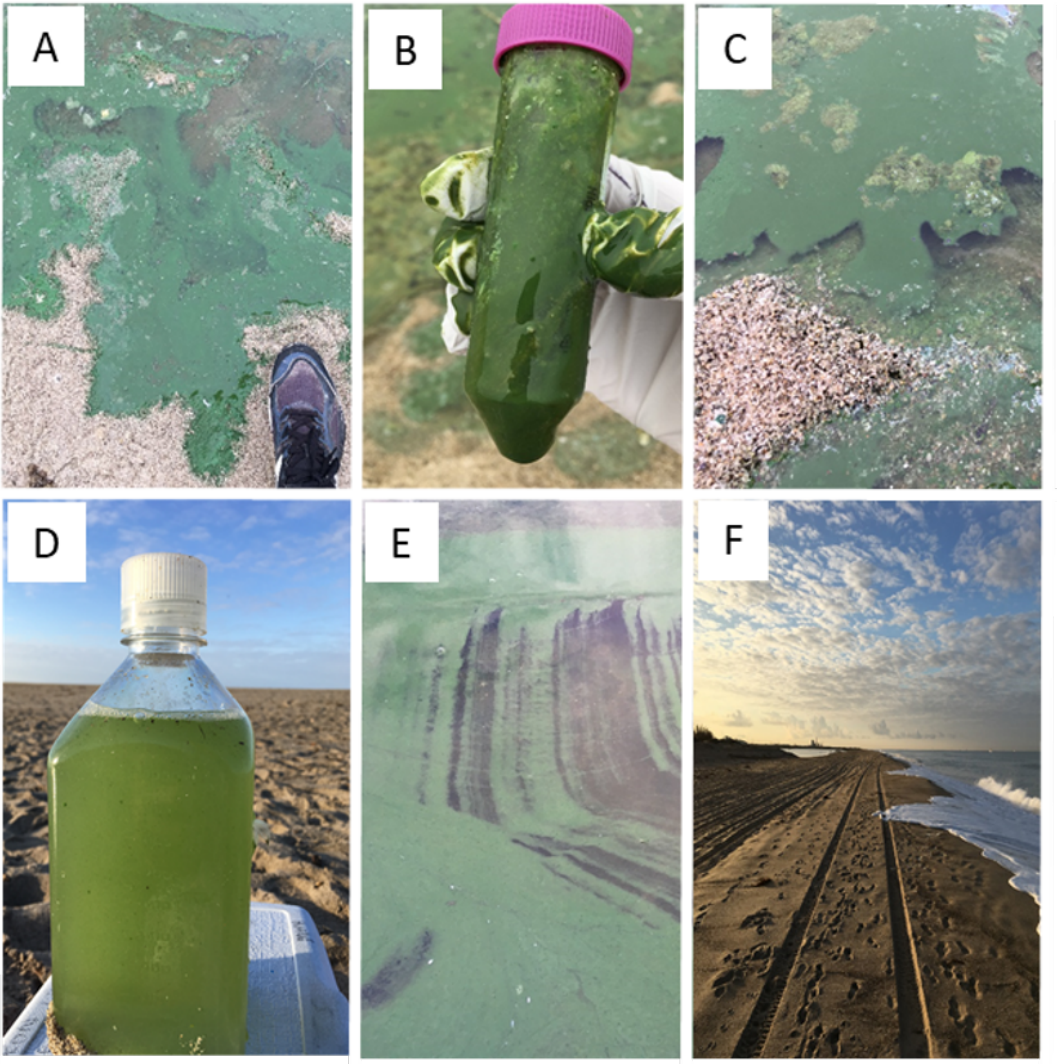
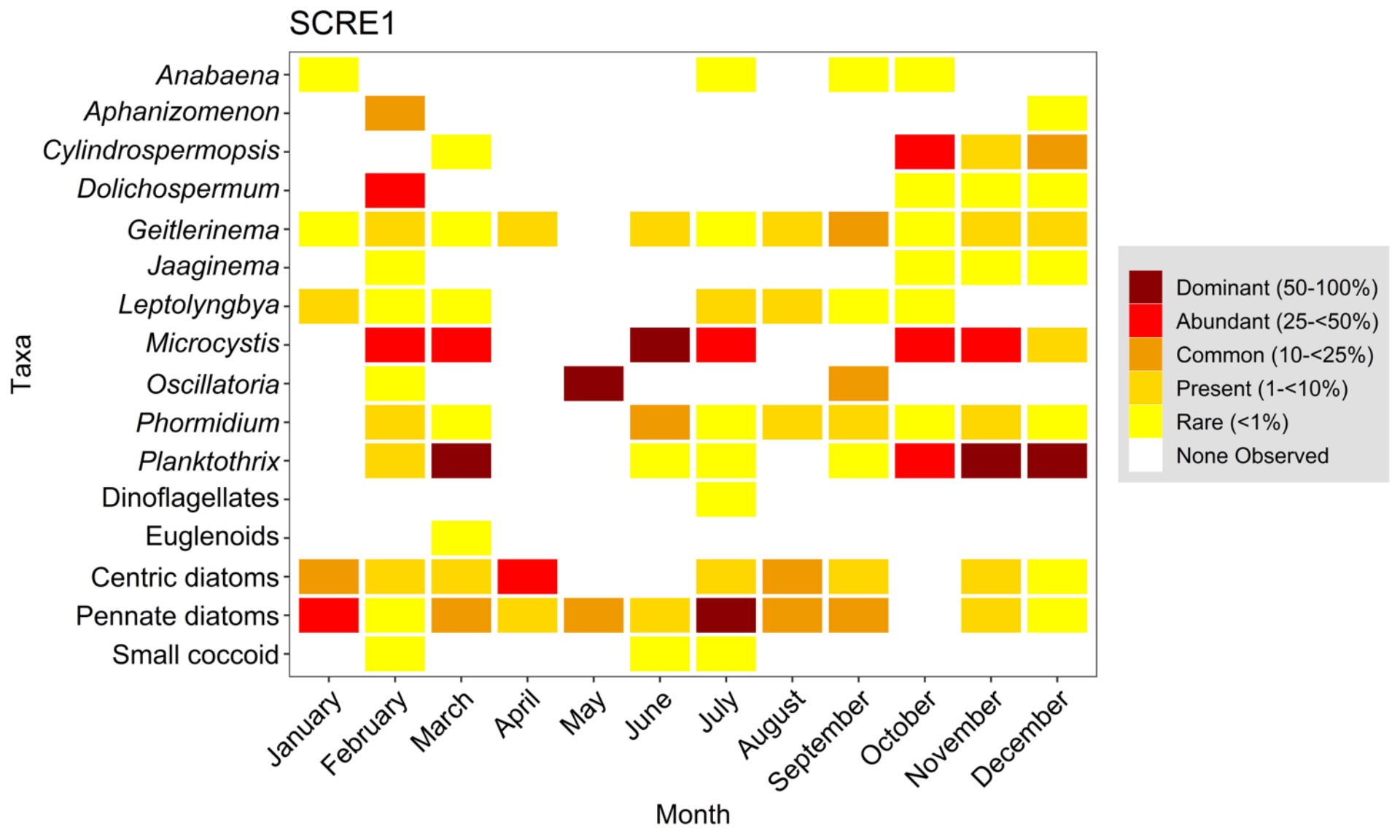
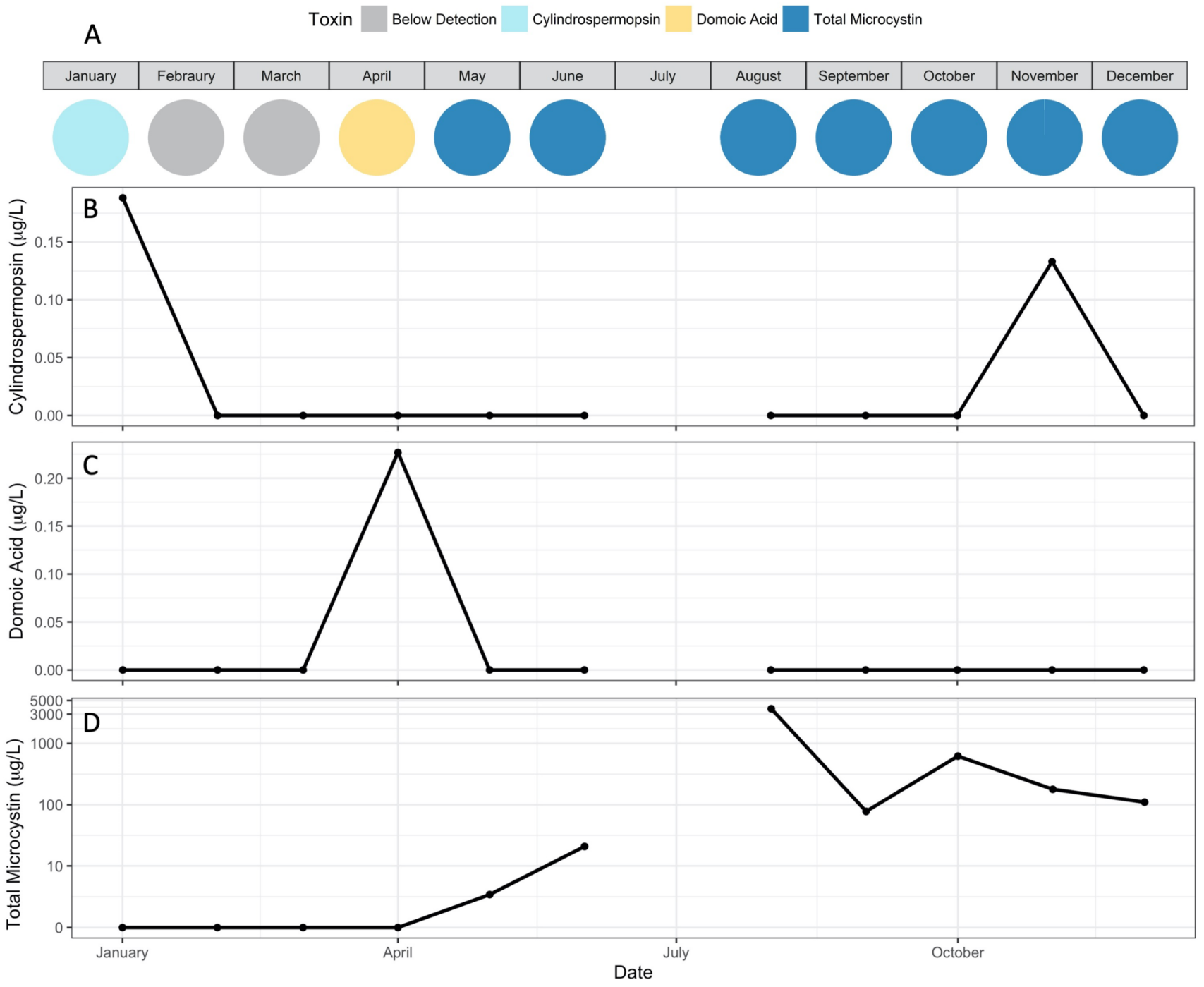
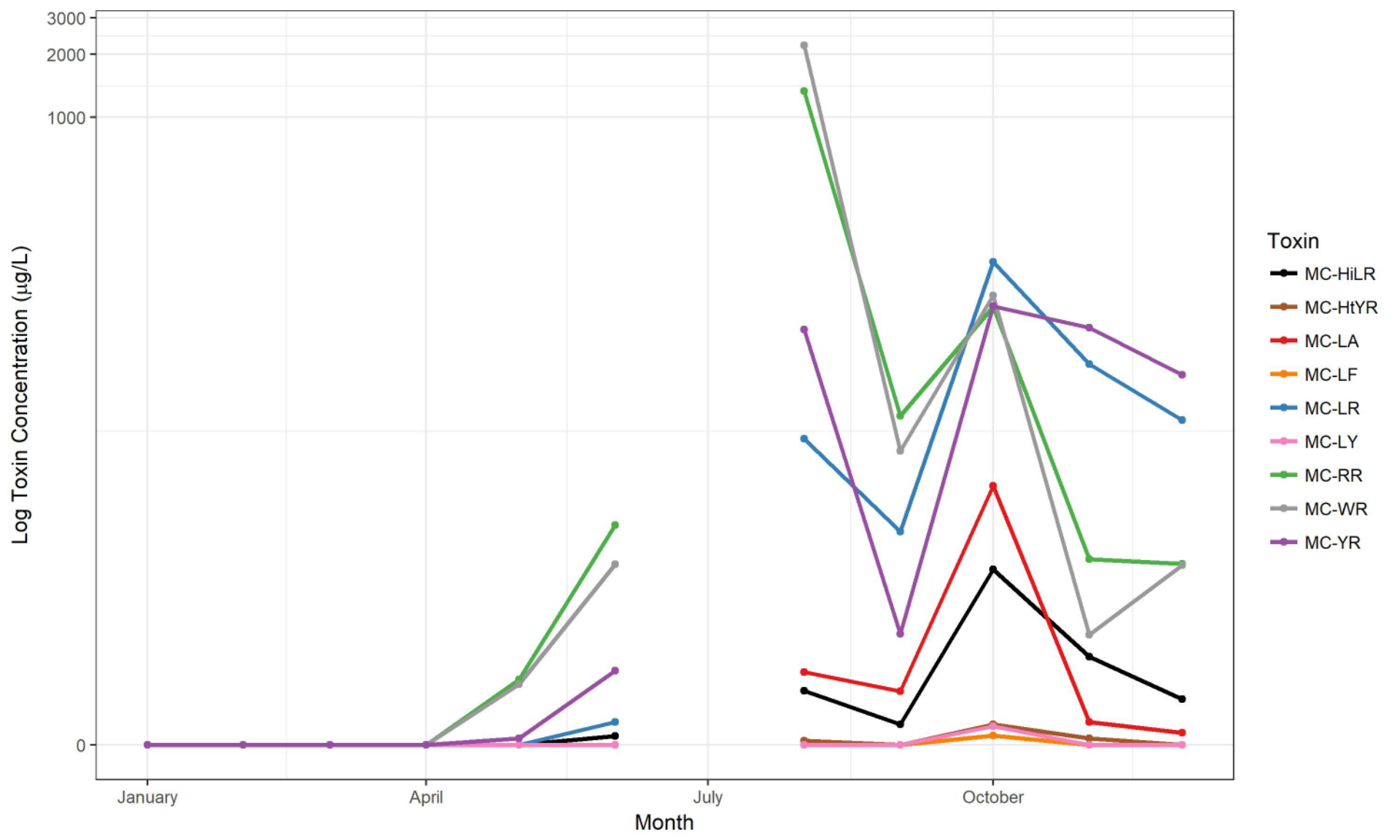
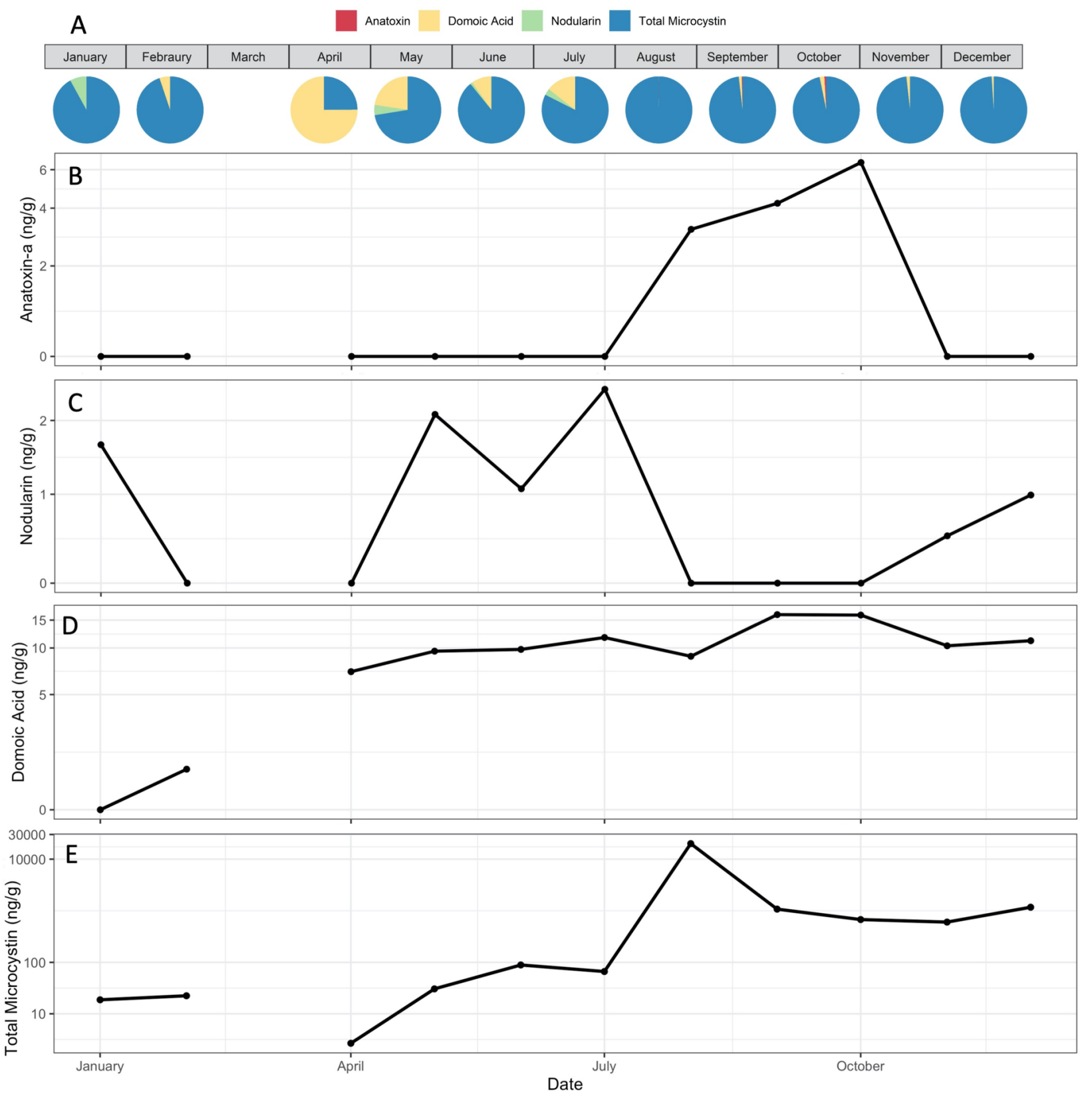
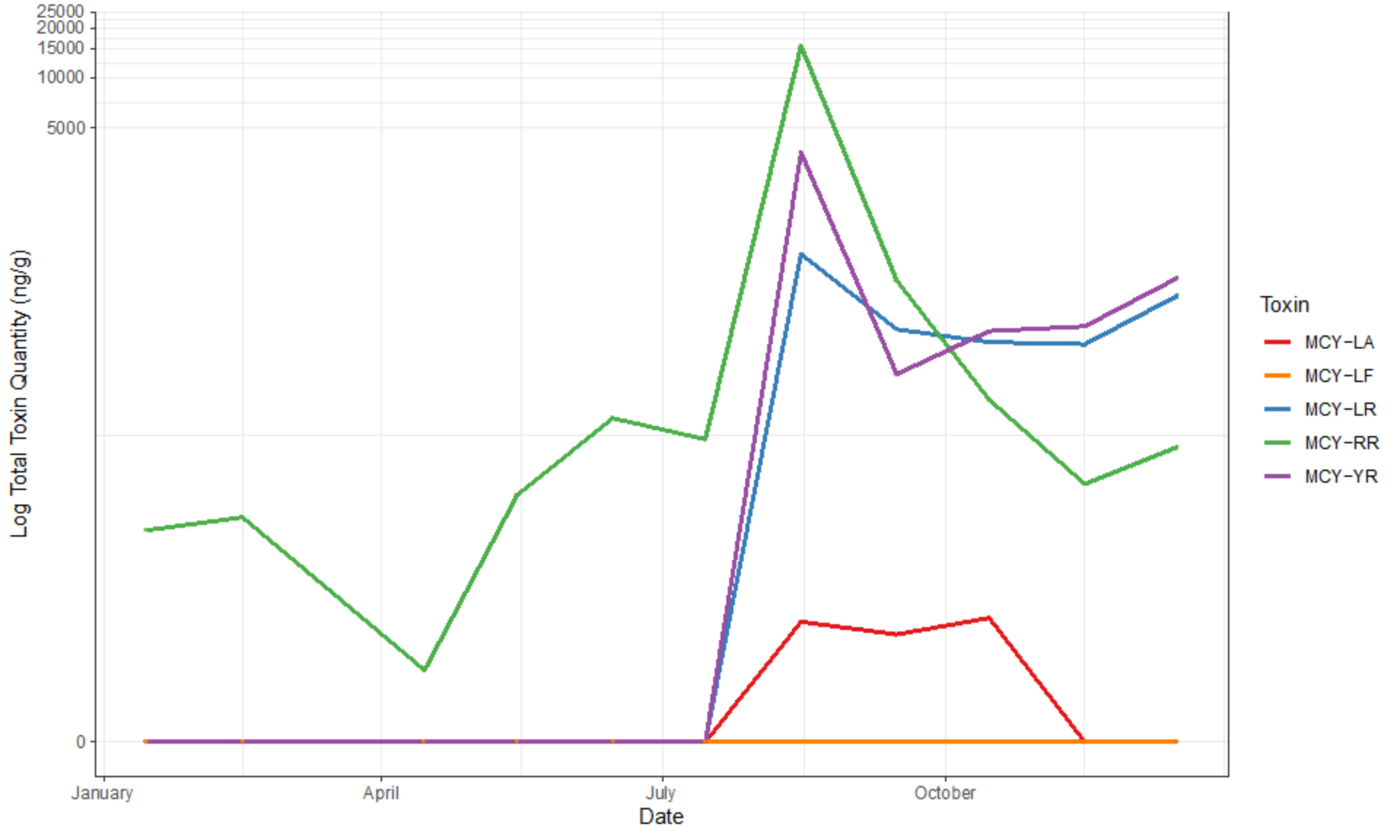

A
B
C
D
E
F

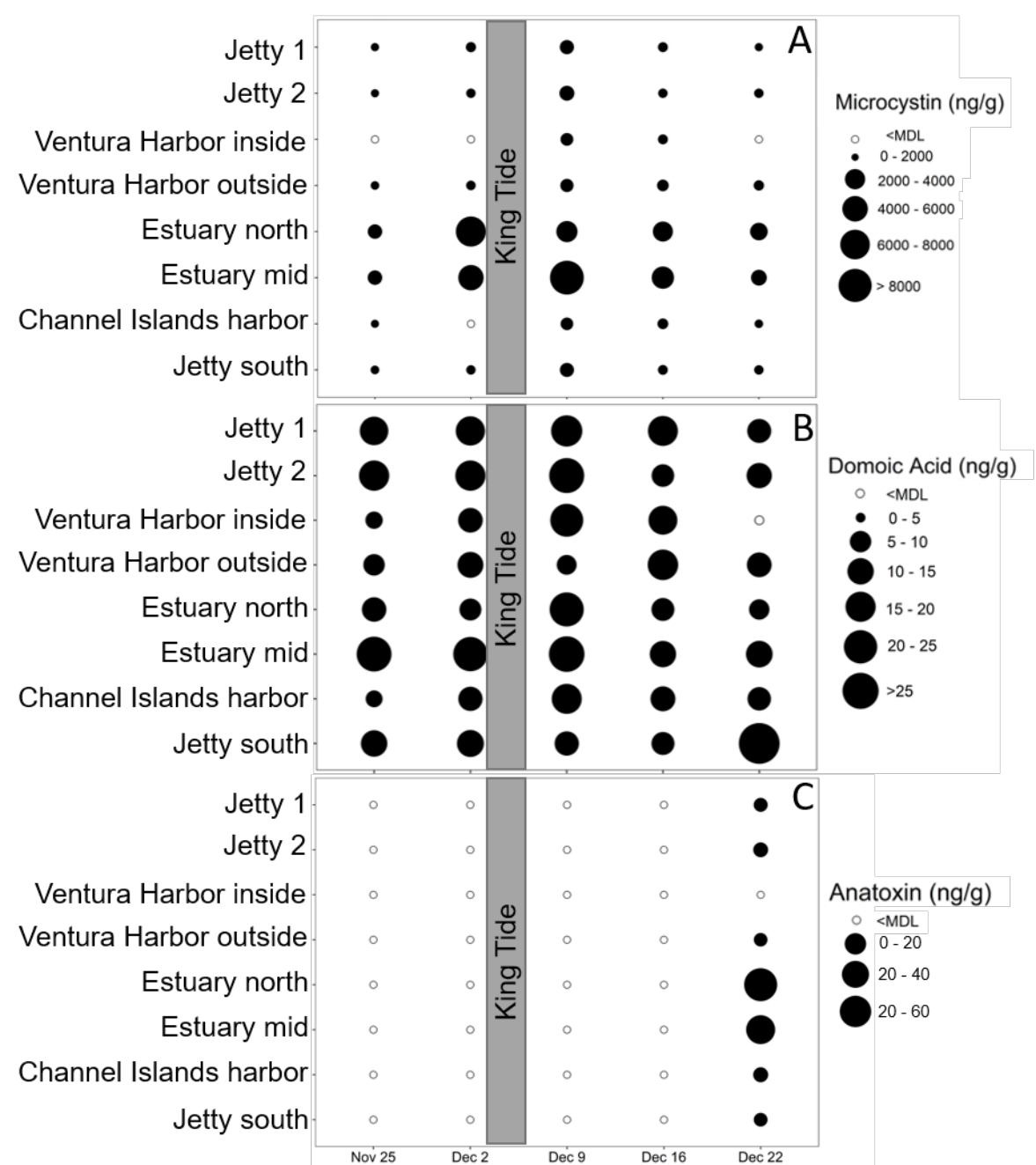

SCRE1
Anabaena
Aphanizomenon
Cylindrospermopsis
Dolichospermum
Geitlerinema
Jaaginema
Leptolyngbya
Microcystis
Oscillatoria
Phormidium
Planktothrix
Dinoflagellates
Euglenoids
Centric diatoms
Pennate diatoms
Small coccoid
Taxa
January
February
March
April
May
June
July
August
September
October
November
December
Month
Dominant (50-100%)
Abundant (25-<50%)
Common (10-<25%)
Present (1-<10%)
Rare (<1%)
None Observed

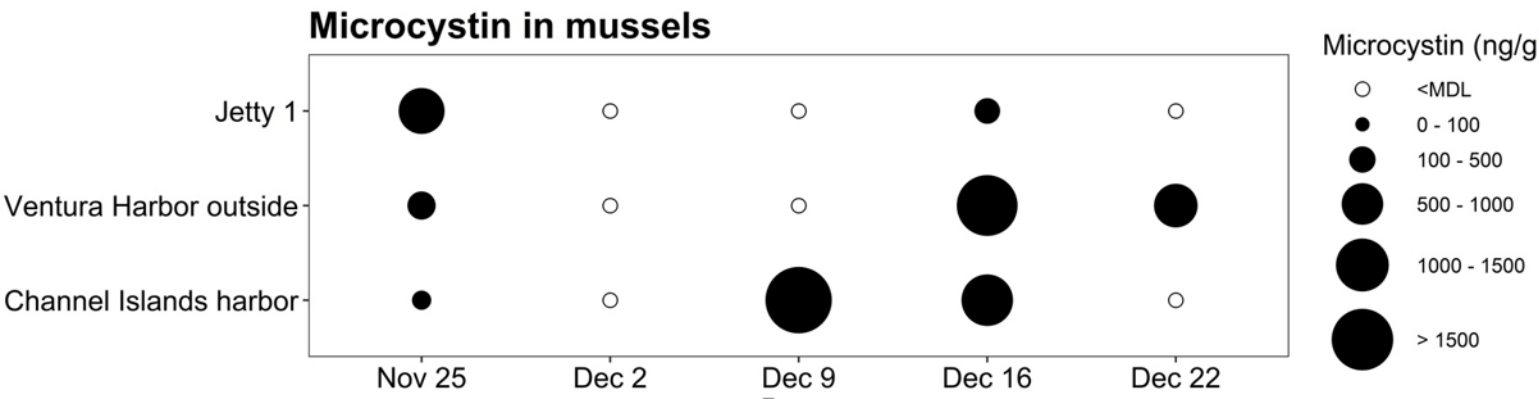

Toxin
Below Detection
Cylindrospermopsin
Domoic Acid
Total Microcystin
A
January
Febraury
March
April
May
June
July
August
September
October
November
December
B
Cylindrospermopsin (µg/L)
0.15
0.10
0.05
0.00
C
Domoic Acid (µg/L)
0.20
0.15
0.10
0.05
0.00
D
Total Microcystin (µg/L)
5000
3000
1000
100
10
0
January
April
July
October
Date


3000
2000
1000
0
Log Toxin Concentration (µg/L)
January
April
July
October
Month
Toxin
MC-HiLR
MC-HtYR
MC-LA
MC-LF
MC-LR
MC-LY
MC-RR
MC-WR
MC-YR


A
Anatoxin
Domoic Acid
Nodularin
Total Microcystin
January
Febraury
March
April
May
June
July
August
September
October
November
December
B
Anatoxin-a (ng/g)
0
2
4
6
C
Nodularin (ng/g)
0
1
2
D
Domoic Acid (ng/g)
0
5
10
15
E
Total Microcystin (ng/g)
10
100
10000
30000
January
April
July
October
Date


25000
20000
15000
10000
5000
0
Log Total Toxin Quantity (ng/g)
January
April
July
October
Date
Toxin
MCY-LA
MCY-LF
MCY-LR
MCY-RR
MCY-YR


A
B
C
Jetty 1
Jetty 2
Ventura Harbor inside
Ventura Harbor outside
Estuary north
Estuary mid
Channel Islands harbor
Jetty south
King Tide
Nov 25
Dec 2
Dec 9
Dec 16
Dec 22
Microcystin (ng/g)
<MDL
0 - 2000
2000 - 4000
4000 - 6000
6000 - 8000
> 8000
Domoic Acid (ng/g)
<MDL
0 - 5
5 - 10
10 - 15
15 - 20
20 - 25
>25
Anatoxin (ng/g)
<MDL
0 - 20
20 - 40
20 - 60


Microcystin in mussels
Jetty 1
Ventura Harbor outside
Channel Islands harbor
Nov 25
Dec 2
Dec 9
Dec 16
Dec 22
Microcystin (ng/g)
<MDL
0 - 100
100 - 500
500 - 1000
1000 - 1500
> 1500