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Detection of persistent microcystin toxins at the land–sea interface in Monterey Bay, California

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ABSTRACT

Blooms of toxin-producing *Microcystis aeruginosa* occur regularly in freshwater systems throughout California, but until recently potential impacts in the coastal ocean have been largely ignored. Twenty-one sites in and around Monterey Bay were surveyed for evidence of microcystin toxin (2010–2011) at the land–sea interface. Following this initial survey four major watersheds in the Monterey Bay area were surveyed (2011–2013) for microcystin concentration, nutrients, alkalinity and water temperature to identify potential environmental factors correlated with the abundance of microcystin at the land–sea interface. During the first year microcystin was detected in 15 of 21 sites. Data from years two and three were analyzed by principal components analysis and mixed effects model. Results indicated that coastal nutrient loading (nitrate, phosphate silicate, ammonium, urea), were statistically significant predictors of the microcystin concentrations in the watersheds with clear evidence for seasonality at some sites. Microcystin was frequently at highest concentration in the autumn; however, at some locations high levels of toxin were measured during spring. Because this toxin has the ability to biomagnify and persist within food webs, elevated levels within the watershed may decrease potential for health and survival of wildlife and humans exposed to freshwater and marine waters. The widespread occurrence of microcystin at low to moderate levels throughout the year and throughout the sampled watersheds demonstrates the potential difficulty for management.

1. Introduction

Harmful algal blooms (HABs) are a global problem in both freshwater and marine ecosystems. The prevalence of HABs and subsequent toxic events may be intensified by a warming climate in tandem with increases in environmental degradation and eutrophication (Zehnder and Gorham, 1960; Welker and Steinburg, 2000; Guo, 2007; Paerl and Huisman, 2008; Davis et al., 2009; Kudela, 2011). Production of the toxin microcystin by the cyanobacterium *Microcystis aeruginosa*, was originally recognized by Ashworth and Mason (1946) in American waters in the 1940s. *M. aeruginosa* blooms are now common in lakes and rivers throughout North America, including California (Chen et al., 1993; Lehman et al., 2005). *M. aeruginosa* bloom formation and consequent toxin generation increases with environmental variables such as: high nutrient supply, elevated light levels, and warm temperatures (Zehnder and Gorham, 1960; Tsuji et al., 1994; Jacoby et al., 2000; Welker and Steinburg, 2000; Paerl and Huisman, 2008; Davis et al., 2009; Paerl and Otten, 2013a, 2013b).

Recently toxins associated with the ostensibly freshwater cyanobacterium *Microcystis aeruginosa* have been detected in the near-shore marine ecosystem of central California, and have been confirmed as a danger to the health of sea otters feeding near ocean outflows of freshwater systems (Miller et al., 2010). *M. aeruginosa* is fairly salt-tolerant and microcystin toxins can be stable and environmentally persistent in both saltwater and freshwater habitats (Robson and Hamilton, 2003; Ross et al., 2006; Tonk et al., 2007; Miller et al., 2010). In addition to direct toxic effects, exposure of aquatic organisms to elevated concentrations of microcystins may negatively affect all levels of the food web (Demott and Moxter, 1991; Malbrouck and Kestemont, 2006; Richardson et al., 2007; Miller et al., 2010).

In 2007, numerous sea otters were found dead in Monterey Bay with signs of liver failure (Miller et al., 2010). Biochemical testing confirmed the presence of microcystin toxin with associated lesions in the livers of 21 otters. Because the occurrence of phytoplankton derived biotoxins are a common phenomenon in Monterey Bay, the otters were evaluated for domoic acid, okadaic acid, nodularin, yessotoxin and anatoxin-A. Otters that were found

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positive for microcystin toxin were negative for all other toxins in the tissues. A few of the microcystin positive otters were also found to have low levels of domoic acid in the urine. However, this is a common finding during necropsy of stranded sea otters from this region, due to domoic acid being broadly dispersed in the sediments of Monterey Bay (Goldberg, 2003; Miller et al., 2010). Freshwater to marine transfer of microcystins was confirmed in areas where sea otters had been recovered, and uptake of microcystins by marine invertebrates and environmental persistence in seawater were demonstrated experimentally (Miller et al., 2010). At this time, potential population-level impacts of these biotoxins on otters and other coastal wildlife remains undetermined. The freshwater to marine transfer of microcystin to the Monterey Bay National Marine Sanctuary waters described by Miller et al. (2010) has the potential to cause major environmental harm. The stability of microcystin allows it to accumulate (van der Oost et al., 2003), and microcystin toxin has been shown to biomagnify and persist in the environment and the food web (Sivonen and Jones, 1999; Dionisio Pires et al., 2004; Kozlowsky-Suzuki et al., 2012; Poste and Ozersky, 2013). Despite the confirmation of microcystin poisoning in marine mammals, the source of these toxins is unclear. Pinto Lake, California was identified as a "hotspot" for toxin production and subsequent transfer to the coastal ocean but this source was not consistent with the location of many of the otters (Miller et al., 2010), which were distributed throughout Monterey Bay, suggesting other, less obvious, sources of toxin to the coastal environment.

We took a wide ranging watershed-based approach to identify the potential pathways leading to microcystin contamination in coastal ecosystems in and around Monterey Bay, CA. Since initial surveys of other potential "hotspots" for toxin production were unsuccessful (Miller et al., 2010) via grab sampling, we deployed Solid Phase Adsorption Toxin Tracking (SPATT) samplers throughout the Monterey Bay area to provide a temporally integrated assessment of potential freshwater sources (Kudela, 2011). Because toxin frequency of occurrence, persistence, and associated environmental drivers may potentially be propelling this freshwater toxin into a sensitive and protected marine sanctuary, our overarching goals were to identify the freshwater sources of microcystin to the Monterey Bay ecosystem, and to identify the underlying environmental drivers influencing toxin production in this area.

2. Materials and Methods

2.1. Initial survey

We surveyed 21 freshwater, estuarine, and marine locations in and around the Monterey Bay area at the land–sea interface (June 2010–July 2011) for microcystin toxin presence and concentration (Fig. 1A). Sites included small and large rivers, estuaries, and nearshore marine locations traversing the six watersheds that surround Monterey Bay (Fig. 1A). Each site was sampled monthly using SPATT (Kudela, 2011). SPATT bags were constructed using 3 g DIAION© HP-20 resin (Sorbent Technologies Inc., Georgia, USA) placed between two 3 inch × 3 inch squares of 100 μM Nitex bolting cloth (Wildlife Supply Company, Product No. 24-C34), and secured in a Caron Westex 2.5 in flex embroidery hoop (Caron International, Ontario, Canada). SPATT was activated by soaking each bag in 100% MeOH, for 48 h, and then rinsed with de-ionized water (Milli-Q), and stored in fresh Milli-Q until deployment (Mackenzie et al., 2004; Lane et al., 2010). When deployed at the beginning of each month, SPATT bags were suspended below the surface of the water, and secured with twine to a stake near the edge of the water. This allowed each bag to be suspended in the water, while being weighed down by the ring so that it remained below the surface. Toxin concentration values are reported as nanogram toxin per gram resin. SPATT toxin concentration levels are not directly comparable to grab sample values (ppb, or μg/L), but previous studies suggest a rough correspondence of 10:1 for SPATT to grab samples (Kudela, 2011), i.e. 10 ng/g SPATT is equivalent to an average concentration of 1 ppb microcystin during SPATT deployment.

2.2. Time-series

In years two and three (August 2011–August 2013) sampling locations were reduced to four major affected watersheds in the Monterey Bay area: the Big Basin watershed, Pajaro River watershed, Salinas River watershed, and the Carmel River

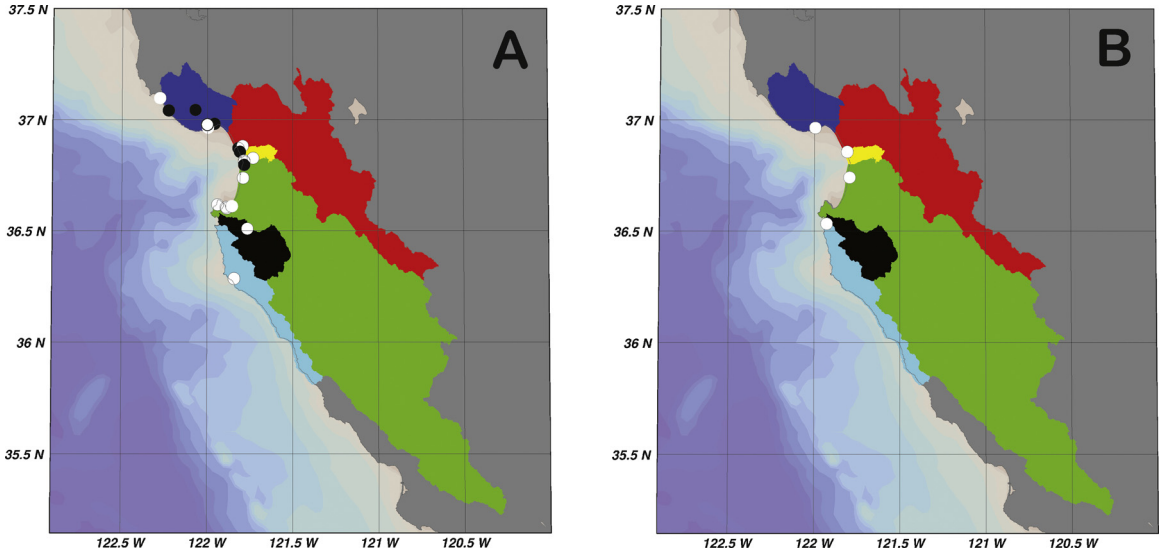

Fig. 1. Map of Monterey Bay, California, USA. (A) Sampling locations in year one (2010–2011) and sampling locations affected by microcystin toxin in year one. White symbols represent sites that were positive for microcystin, black symbols represents sites that were sampled but negative for microcystin toxin. (B) Sampling locations in year two (2011–2013). The watersheds, from north to south, are: Big Basin (dark blue), Pajaro River (red), Bolsa Nueva (yellow), Salinas River (green), Carmel River (black), Santa Lucia (light blue). Ocean bathymetry is indicated with shading. Maps created using Ocean Data View (ODV) and Exelis Visual Information Solutions (ENVI).

watershed. These four sampling locations were determined by the initial year one survey to be highly impacted by microcystin toxin. SPATT was deployed weekly at each site and whole water was collected and analyzed for temperature, ammonium, urea, nitrate, phosphate, silicate, and total toxin, with the whole water samples corresponding to the deployment and recovery dates for SPATT. With the exception of alkalinity, which was monitored only in year three (August 2012–August 2013), all other variables were measured in both years two and three. Temperature was monitored using Hobo Pendant® Temperature/Light Data Loggers (8K-UA-002-08; Onset Computer Corporation, Massachusetts, USA). When data loggers were unavailable for use due to theft or loss, field thermometers were employed in situ (−10/110 °C; Enviro-safe®; HB Instrument Company, Pennsylvania, USA). Ammonium, urea, nitrate, phosphate and silicate samples were collected in the field, immediately filtered (0.7 μM GF/F filter), into 25 mL Falcon™ centrifuge tubes, and were stored frozen until processing. The average time until processing was less than one month. Ammonium was analyzed using the OPA method and RFU values were obtained via fluorometer (TD-700; Turner Designs, California, USA) as described by Holmes et al. (1999). Urea was analyzed using a Varian Cary 50 Bio UV/Visible Spectrophotometer (Varian Medical Systems, California, USA) following methods described by Mulvenna and Savidge (1992). Nitrate, phosphate, and silicate were analyzed using a Lachat QuikChem 8500 Flow Injection Analyst System and Omnion 3.0 software (Lachat Instruments; Hach Company, Colorado, USA). Alkalinity was determined using Total Alkalinity Test Strips, 0–240 mg/L (Hach Company, Colorado, USA) in the field. Whole water was also collected in the field.

2.3. Toxin analysis

Microcystin-LR, RR, YR, LA was analyzed by liquid chromatography/mass spectrometry (LCMS) 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 LCMS/SIM instead of tandem mass spectrometry (Kudela, 2011). Briefly, a gradient-elution method was used with HPLC water (solvent A) and LCMS acetonitrile (solvent B), both acidified with 0.1% formic acid, as the mobile phase. The gradient was as described in Mekebri et al. (2009), starting with 95:5 solvent A:B and ending with 25:75 at 19 min, held for 1 min, then followed by a 5 min equilibration at initial conditions prior to injection of the next sample. Samples were calibrated with standard curves (for each batch of samples) using pure standards (Fluka 33578 and Sigma–Aldrich M4194). Standards were run again at the end of the run for sample runs lasting more than 8 h.

Whole water was collected in the field, returned to the lab, where 3 mL of whole water was mixed with 3 mL of 10% methanol. Samples were then sonicated using a sonic dismembrator (Model 100; Thermo Fisher Scientific, Massachusetts, USA) for 30 s at ~10 W, filtered (0.2 μM nylon syringe filter), and analyzed by direct injection of 50 μL onto the LCMS. SPATT samples were processed as described by Kudela (2011). Briefly, SPATT were recovered from the field and stored frozen until processing. The resin was transferred to a disposable chromatography column and sequentially extracted with 10, 20, 20 mL 50% methanol. Each extract (50 μL) was analyzed by LCMS and total toxin was determined by summing the individual extracts. Values are reported as μg/L (=ppb) for whole water and ng/g resin for SPATT. The Minimum Detection Limit (MDL) was 0.05 ng/g for SPATT and 0.10 ng/mL (ppb) for whole water. Values <MDL were considered non-detect (zero) for statistical analysis. While it is possible that compound other than microcystins could be falsely identified, it would require the compounds to exhibit the same mass and retention time as the standards making false positives unlikely.

2.4. Statistics

Microcystin toxin presence, concentration, and persistence were evaluated at each sampling location in year one, and for each watershed during years two and three. Data for both the discrete grab samples and the SPATT toxin concentrations were pooled for the analysis. The relationship between environmental variables (date, temperature, nitrate, phosphate, silicate, ammonium, urea) and microcystin were evaluated graphically and statistically. Because there was multicollinearity within the data from years two to three, a PCA was run to account for this, and the variables were grouped into components for further analysis (Zar, 1999; Quinn and Keough, 2002). A mixed effects model was chosen to account for autocorrelation caused by the seasonality component. Because of this autocorrelation the components (date and temperature) that comprised seasonality were removed from the PCA and added back into the model independently. When the model was run, components from the PCA (PC1 and PC2), and temperature were run against microcystin toxin. Date was added back into the model and was set as the random effect, with microcystin toxin concentration set as the fixed effect. This model is appropriate due to the nature of the flexibility it provides for correlated data (Quinn and Keough, 2002; Seltman, 2013). Variables used in the model were transformed via square root transformation to meet assumptions of normality, and $\alpha = 0.05$. From the results of the model, both negative and positive relationships between environmental variables and microcystin concentration were examined, and statistical significance was obtained. PCA and mixed effect model statistical tests were conducted using Systat 13.1 (Systat Software Inc., Chicago, Illinois, USA). The relationship between microcystin concentration and alkalinity was investigated via simple bivariate correlation using IBM SPSS Statistics 21 (IBM Corporation, Armonk, New York, USA).

To investigate any statistical relationship between river discharge and toxin presence, microcystin toxin concentration was compared to river discharge data (USGS, 2014) for each of the four watersheds. Data for the comparison were obtained from the United States Geological Survey (USGS) National Water Information System Web Interface database, and river discharge data were reported in cubic feet per second. Data were analyzed via simple bivariate correlations using IBM SPSS Statistics 21 and the significance was set at $p = 0.05$. Cross correlation function analysis (CCF) was evaluated using Systat 13.1 for effectiveness of introducing temporal lags into the data.

3. Results

3.1. Survey results (2010–2011)

In year one, 15 out of 21 locations surveyed in the Monterey Bay area were positive for microcystin toxin concentration (Fig. 1A; Table 1). There were noticeably high levels of toxin in the autumn season, and at some sites, such as the Carmel River and Salinas River, there were also noticeable spring season peaks in toxin concentration (Fig. 2). From our first year of data, four watersheds (Big Basin, Pajaro River, Salinas River, Carmel River) were identified as persistently toxic; this directed our sampling in years two and three. Toxin concentration values varied from undetectable to 20 ng/g.

Table 1
Survey data for microcystin toxin from 21 locations in and around the Monterey Bay area in year one (2010–2011).

Location	OBS	POS	Range	Mean	SD
Waddell Creek	8	1	0–1.800	0.138	0.499
Scott Creek	12	0	0	0	0
San Lorenzo River	13	0	0	0	0
Santa Cruz Harbor	10	4	0–4.025	0.495	1.147
Twin Lakes State Beach	12	3	0–0.990	0.142	0.311
Soquel Creek	13	0	0	0	0
Pajaro River	13	1	0–2.930	0.225	0.813
Pajaro Lagoon	13	0	0	0	0
Watsonville Slough	13	0	0	0	0
Bennet Slough	12	1	0–0.58	0.045	0.161
Moss Landing Harbor	11	3	0–7.097	0.987	2.315
Strawberry Pond	13	3	0–2.960	0.403	0.892
Moro Cojo	13	0	0	0	0
Salinas River	13	3	0–4.700	0.472	1.298
Laguna Grande	13	1	0–0.165	0.013	0.046
Lake El Estero	12	1	0–0.414	0.032	0.115
Monterey Coast Guard Pier	11	2	0–8.109	0.867	2.345
Fisherman's Wharf	10	2	0–5.382	0.500	1.548
Asilomar Creek	11	2	0–0.0810	0.115	0.281
Carmel River	12	7	0–19.564	2.905	5.517
Big Sur River	11	1	0–0.1837	0.014	0.051

The number of observations where SPATT was deployed and also recovered, is represented by OBS, the number of months positive for microcystin toxin is measured in ng/g and is represented by POS. The range, mean, and standard deviation represented by SD, are also provided.

3.2. Time series (2011–2013)

In year two, all four watersheds (Fig. 1B) exhibited an increase in microcystin toxin presence compared to year one (Table 2). In year three, all watersheds again exhibited similar or increased occurrences of microcystin toxin. As was seen in year one, high values of microcystin toxin concentration were observed in both autumn and spring seasons (Fig. 3), but in years two and three this seasonal pattern was evident in all four watersheds.

The PCA produced three significant principal components (Table 3). Principal component one (PC1) was comprised of ammonium and urea (21.02% variance explained), principal component two (PC2) was comprised of temperature and date (19.97% variance explained), and principal component three (PC3) was comprised of nitrate, phosphate and silicate (19.83% variance explained). All of the variables loaded positively with the exception of silicate. These PCA components were used in place of direct environmental variables to account for multicollinearity within the data. The seasonality component (date and temperature) produced an autocorrelation within the data. Seasonality was therefore removed and the PCA was re-run; two significant components were produced and grouped similarly. PC1 was comprised of ammonium and urea (29.36% variance explained), and PC2 was comprised of nitrate, phosphate, and silicate (27.25% variance explained). PC1, PC2 and temperature were then run in a mixed effects model against the presence and amount of microcystin toxin with date set as the random effect. The model showed that microcystin toxin concentration had a statistically significant relationship to all tested variables ($p < 0.05$; Table 3). Nitrate, phosphate, ammonium and urea were negatively associated with microcystin concentration within the model. Because silicate loaded negatively in the PCA, it was considered positively associated with microcystin in the model, while temperature was negatively associated with microcystin in the model (Table 3). The results of a CCF analysis indicated significant correlations between toxin concentration and individual environmental factors with temporal lags of three weeks. However, when the data were lagged, the overall model was greatly weakened. Microcystin toxin concentration remained significantly related to all tested variables ($p < 0.05$). For this reason we present the statistical results without lags.

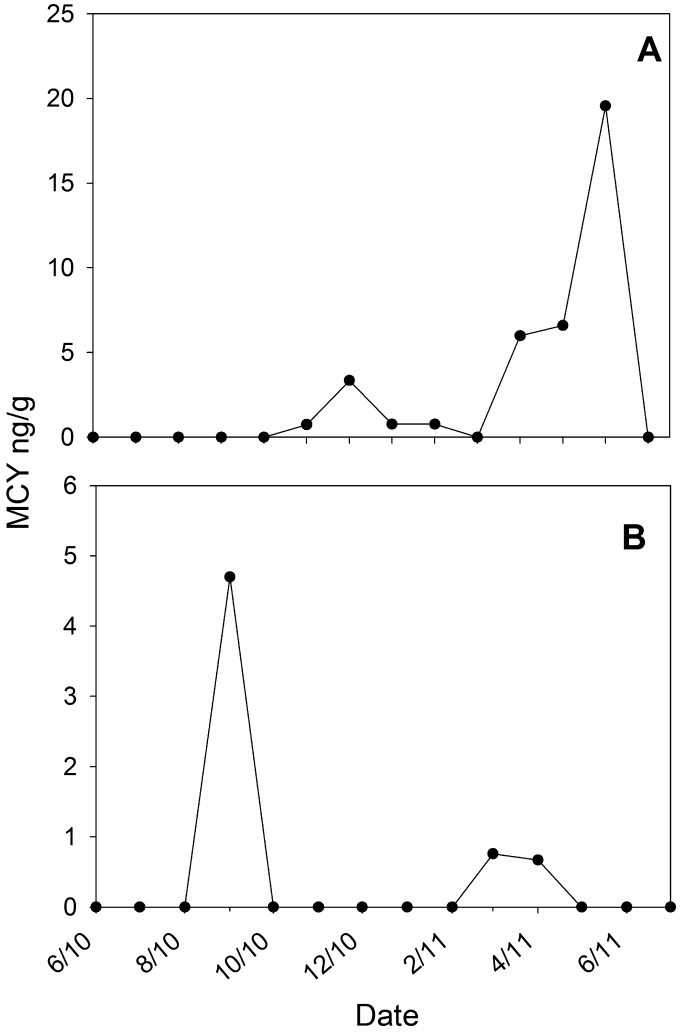

Fig. 2. Microcystin toxin (MCY) time series from SPATT samplers (ng toxin per gram resin) for two locations (A). Carmel River (B). Salinas River, during year one (June 2010–July 2011).

Bivariate correlations showed negative correlation with alkalinity and positive correlation with river flow. Alkalinity had a significant negative correlation ($p < 0.05$) with microcystin concentration (Fig. 4). River discharge and microcystin toxin concentration were significantly positively correlated for Big Basin, Pajaro River, and Carmel River watersheds ($p < 0.05$). Salinas River exhibited a weak, non-significant ($p > 0.05$) correlation. The results of CCF analysis indicated that a lag of four weeks may better align the data and increase the strength of the correlation. When the data were lagged all watersheds again were positively correlated; however, only the Pajaro River and Carmel River watersheds exhibited statistically significant correlations ($p < 0.05$).

4. Discussion

The results from this study show a serious condition at the nearshore interface in the Monterey Bay area, consistent with previous reports (Miller et al., 2010). In year one, approximately half of all tested locations were positive for microcystin toxins at some time during the year. We believe these toxins are being produced by

Table 2
Survey data for microcystin toxin and environmental variables from four watershed locations at the land–sea interface in Monterey Bay in years two and three (2011–2013).

Location	Variable	Range	Mean	SD
Big Basin Watershed	Microcystin SPATT	0–8.22	0.749	1.61
	Microcystin water	0–12.85	0.17	1.31
	Ammonium	0.07–30.37	5.56	7.13
	Urea	0.13–18.42	1.98	2.77
	Nitrate	0–92.37	11.14	19.25
	Phosphate	0.30–51.91	11.224	10.273
	Silicate	0.91–543.23	143.37	125.33
	Alkalinity	40–240	117.29	46.46
	Temperature	6.21–21.92	15.35	4.27
Pajaro River Watershed	Microcystin SPATT	0–8.97	0.59	1.42
	Microcystin water	0–1.09	0.03	0.14
	Ammonium	0.05–32.1	3.06	4.33
	Urea	0.15–8.28	0.94	0.96
	Nitrate	1.75–1257.10	318.96	199.36
	Phosphate	0–66.16	4.54	8.46
	Silicate	10.37–668.72	129.38	102.30
	Alkalinity	120–240	231.86	26.03
	Temperature	7.75–22.00	15.72	4.09
Salinas River Watershed	Microcystin SPATT	0–62.71	1.12	6.83
	Microcystin water	0–1.02	0.02	0.12
	Ammonium	0.03–93.95	3.79	12.01
	Urea	0.10–4.47	0.93	0.77
	Nitrate	0.74–1311.12	504.22	287.47
	Phosphate	0.11–56.16	11.93	8.92
	Silicate	13.45–805.44	196.88	108.86
	Alkalinity	180–240	211.77	30.25
	Temperature	7.50–25.00	15.82	4.62
	Microcystin	0–62.71	1.11	6.79
Carmel River Watershed	Microcystin SPATT	0–104.31	7.91	16.70
	Microcystin water	0–0.90	0.04	0.16
	Ammonium	0–2.93	0.28	0.42
	Urea	0–3.52	0.40	0.57
	Nitrate	0.40–28.65	4.03	3.53
	Phosphate	0.01–3.59	0.46	0.45
	Silicate	27.00–856.00	292.79	106.53
	Alkalinity	40–240	131.19	73.52
	Temperature	7.00–20.66	13.41	2.98
	Microcystin	0–104.31	7.78	16.69

Microcystin toxin found in SPATT is measured in ng/g, microcystin toxin found in water samples is measured in ppb. The measured environmental variables: ammonium (μM), urea (μM), nitrate (μM), phosphate (μM), silicate (μM), alkalinity (mg/L), temperature (°C) are shown. The range, mean, and standard deviation represented by SD, are also provided.

Microcystis aeruginosa in the nearshore freshwater environment and have the potential to be subsequently transported to the marine environment. Miller et al. (2010) found that cells lysed in seawater after 48 h; there is also possibility for some cells to be carried to the marine environment, lyse, and then release toxin. Monterey Bay is at high risk for this type of problem due to the nature of the surrounding land which is highly populated and widely used for agriculture. However, we believe this may be a phenomenon in other near-shore marine systems that have not been monitored for this particular toxin, and therefore, have gone unnoticed.

The use of SPATT technology allowed us to access time integrative toxin survey data simultaneously at many different locations, thus providing more than a "snapshot" of information such as would be obtained with intensive surveying. While SPATT was originally developed to mimic shellfish toxicity, its use has proven to be more beneficial and easy to use for toxin monitoring as compared to other popular monitoring methods like the use of shellfish testing, rote phytoplankton surveys, and whole water sampling (Mackenzie et al., 2004; Lane et al., 2010; Mackenzie, 2010; Kudela, 2011).

The occurrence of two dominant peaks, in spring and autumn, indicate an unexpected seasonal pattern of microcystin toxin for all primary watersheds in the Monterey Bay area. It is widely accepted that bloom formation is largely driven by light and nutrient availability, and often water stagnation (Zehnder and Gorham, 1960; Webb and Walling, 1992; Tsuji et al., 1994; Jacoby et al., 2000; Welker and Steinburg, 2000; Jeong et al., 2003; Paerl and Huisman, 2008; Davis et al., 2009; Paerl and Otten, 2013a). This often leads to a seasonal pattern, with optimal conditions for bloom formation occurring in summer and autumn seasons. (Reynolds et al., 1981; Paerl, 1988; Lehman et al., 2008; Moisander et al., 2009). This seasonal characteristic has similarly been identified in nearby waterways like the San Francisco Estuary (Lehman et al., 2005, 2008; Moisander et al., 2009). Additionally, Miller et al. (2010) reported that the Monterey Bay area generally experiences increases in microcystin presence and concentration in freshwater lakes and rivers during autumn. The patterns of microcystin presence and concentration observed during this study suggest that microcystins are likely present throughout the year. In years two and three microcystin toxin increased or remained elevated at all locations and the spring/autumn peaks persisted. While only three years in duration, these results suggest that microcystin production and subsequent transfer to the coastal environment has the potential to be a persistent issue in the Monterey Bay area.

The statistical analysis exhibited a distinct delineation between variables. Ammonium, urea, nitrate, and phosphate all exhibited a negative association with toxin in the model. We infer that the negative relationship was caused by biological drawdown of nutrients; toxin is produced by cells which are stimulated by the high nutrient levels, but toxin concentrations can become

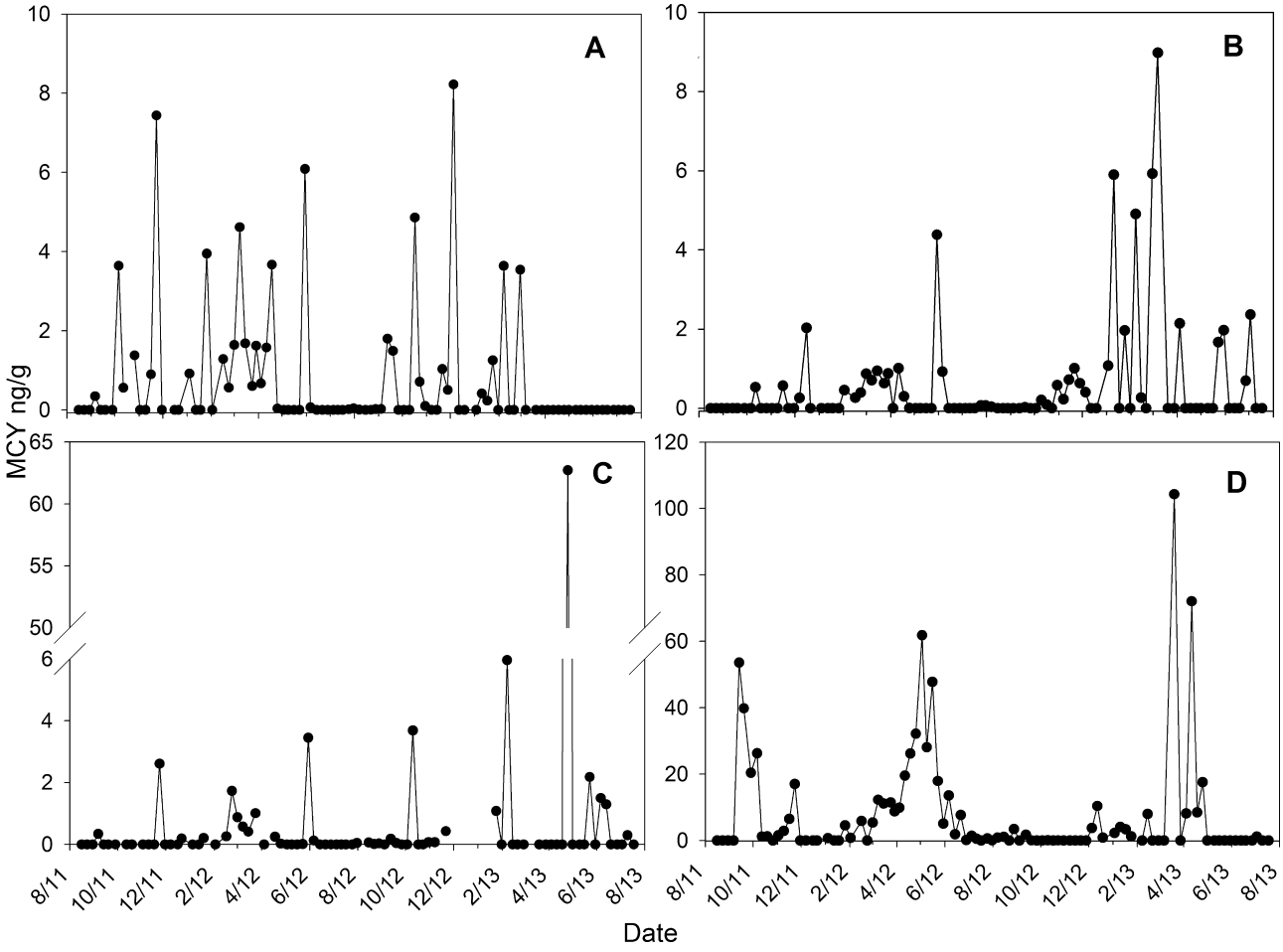

Fig. 3. Microcystin toxin from SPATT (MCY; ng/g) time series from August 2011 to August 2013. (A) Big Basin watershed; (B) Pajaro River watershed; (C) Salinas River watershed; (D) Carmel River watershed.

uncoupled from cell growth and nutrient concentrations due to cell lysis and differences between cell toxin quota and cell growth. Given the likely disconnect between toxin production and detection (SPATT were deployed for one week to one month), there is consistency with nutrient enrichment leading to increased algal biomass, with subsequent toxin production. Low nutrient levels would then be correlated with elevated toxins due to the time lag. This theory could be tested by identifying lagged correlations between nutrients and toxins, but our toxin data are integrative (SPATT) while the nutrients were collected at the time of SPATT recovery (with a coarse time scale relative to nutrient dynamics), precluding such an analysis. The assertion we present is consistent with the relationship between microcystin concentrations and silicate concentrations identified in the model. Silicate was positively associated with microcystin. Silicate is not utilized by *Microcystis aeruginosa* and consequently remained in the environment while other nutrients were presumably biologically drawn down in the absence of diatom blooms. The link between

Table 3
Results from the mixed effects model evaluating principal components, and temperature versus microcystin toxin.

Variable	Estimate number	*p*-value
PC1		
Urea	−0.1240	0.0520
Ammonium		
PC2		
Nitrate	−0.9200	0.0060
Phosphate		
Silicate		
Temperature	−0.0480	0.0020

The first principle component is represented by PC1 and contains the variables urea and ammonium. The second principal component is represented by PC2 and contains variables nitrate, phosphate and silicate. The associated estimate numbers and *p*-values from the model are provided.

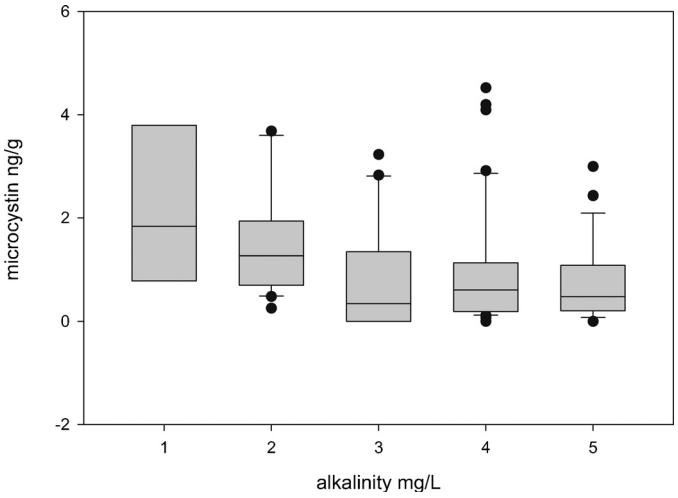

Fig. 4. Alkalinity versus microcystin toxin box plots. Data for all locations from August 2012 to August 2013 was pooled, zeros were removed and microcystin toxin data was square root transformed.

macronutrients, particularly ammonium and urea, and toxin concentrations, and the relatively high nutrient concentrations point to anthropogenic loading as a significant driver of toxin accumulation in these watersheds (Kudela et al., 2008), consistent with other studies (Paerl et al., 2001; Paerl, 2008; Schindler and Vallentyne, 2008; Wilhelm et al., 2011).

Temperature, unexpectedly, exhibited a negative association with microcystin toxin within the model. Several studies have demonstrated the link between elevated and increasing temperature and the frequency of toxic blooms (Butterwick et al., 2005; Reynolds, 2006; Paerl and Huisman, 2008, 2009). However, the phenomenon of a non-correlative relationship between microcystin toxin and temperature has been seen previously, where elevated toxin concentrations have been associated with a range of temperatures (van der Westhuizen and Eloff, 1985; Amé and Wunderlin, 2005; Davis et al., 2009; Kudela, 2011). This may be indicative of a non-linear relationship between the two variables, possibly driven by different growth–temperature responses for different toxigenic cyanobacteria (Paerl and Otten, 2013a, 2013b).

The inverse relationship between alkalinity and microcystin was expected. This dynamic has been documented previously (Aboal et al., 2005); additionally alkalinity has been correlated with shifts in algal groups when ammonium is available to provide the source of nitrogen (Brewer and Goldman, 1976). River flow had positive correlations with microcystin toxin before and after time lags. Before the data were lagged there were more significant positive correlations; after lags, all sites were positively correlated, but fewer were significant. Many studies have highlighted that stagnancy of water is associated with increased cell density of *Microcystis* (Christian et al., 1986; Reynolds, 1992; Jeong et al., 2006; Lehman et al., 2008). Lehman et al. (2008) also found that *Microcystis* cell density was positively correlated with flow at some locations in the San Francisco Estuary. Cell density was highest during periods of lowest river flow, and toxin was potentially produced during subsequent stagnant, long retention-time periods. The weak positive correlations identified in this study suggest that while river flow has an effect on toxin concentration, it is not be the dominant effect. Other contributors to toxin abundance with stronger relationships, such as nutrient loading, appear to have a greater influence on toxin levels.

Presence of microcystin toxins is often indicative of an unhealthy ecosystem (Miller et al., 2012). Within the Monterey Bay region, toxins are present and persistent in the four major watersheds flowing into the Monterey Bay National Marine Sanctuary. Despite the persistence of this toxin in California watersheds and the potential negative impacts to humans and wildlife, microcystins are not routinely monitored by federal, state or local management agencies. Because this toxin has the capacity for accumulation, biomagnification, and persistence within food webs, elevated levels within the watershed may increase the possibility for morbidity and mortality of wildlife and humans in terrestrial, estuarine, and marine waters. Additionally there is increasing evidence to support chronic exposure to microcystins as a significant threat to wildlife and humans (Bury et al., 1995; Wiegand et al., 1999; Jacquet et al., 2004; de Figueiredo et al., 2004;Malbrouck and Kestemont, 2006; Wang et al., 2010). Thus, even the low but detectable levels identified in this study may be indicative of a potentially unhealthy ecosystem. It is possible that low levels of microcystins are endemic to California and therefore a natural component of the ecosystem. The lack of baseline studies makes this assertion difficult to test, but this study provides a reasonable baseline for assessing future changes in toxins within the Monterey Bay watersheds.

Cyanobacterial harmful algal bloom events are often intensified by anthropogenic activities such as discharge of sewage, as well as both urban and agricultural practices that cause nutrient rich runoff to flow into local watersheds (Zehnder and Gorham, 1960; Fogg, 1969; Reynolds, 1987; Paerl, 1988; Davis et al., 2009; Paerl et al., 2011). Because cyanobacteria have the capacity to thrive in water with both low and high nutrient concentrations, these organisms have the potential to outcompete other algal groups and dominate affected watersheds (Falconer and Humpage, 2005). The combined effect of high growth response to nutrient input and the ability to outcompete other organisms creates the potential for microcystin toxin to overwhelm affected ecosystems.

The extensive manifestation of microcystin at low to moderate levels throughout the year and throughout all major watersheds in the Monterey Bay area exhibits the potential complication of managing environmental impacts, and ecosystem disruptions. Management agencies have long grappled with the problem of microcystin toxins in inland watersheds. However, the ubiquity of microcystin at the land–sea interface in the Monterey Bay area represents a new management obstacle. Decisions made at the terrestrial level in the proximity of the marine environment may now impact freshwater, estuarine, and marine ecosystems, particularly given the demonstrated capacity for bio-accumulation in commercially harvested shellfish (Miller et al., 2010). New management plans and implementations may now have to regard this freshwater epidemic as an expanding and pervasive problem.

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