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Short communication

Co-occurring dissolved algal toxins observed at multiple coastal sites in southern California via solid phase adsorption toxin tracking

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ABSTRACT

Algal toxins (domoic acid, saxitoxin, okadaic acid) were monitored at seven locations off southern California using Solid Phase Adsorption Toxin Tracking. At least two types of toxins were found at all locations, with co-occurrence of two and three toxins in 12% and 10% of samples, respectively. This study expands our limited understanding of the simultaneous presence of multiple algal toxins along the coast and raises questions regarding the potential health ramifications of such co-occurrences.

The occurrence of harmful algal blooms (HABs) and their toxins have increased globally in aquatic environments over the last several decades (Van Dolah, 2000; Brooks et al., 2017), with recent reports noting multiple, co-occurring algal toxin suites (Peacock et al., 2018). Algal toxins can bioaccumulate in marine food webs resulting in harm to marine wildlife, human health and commercial/recreational fisheries, therefore it is crucial to understand the temporal and spatial distributions of these compounds. Climate change and other anthropogenic activities have been implicated in modifying and sometimes expanding the ranges of HAB taxa (Wells et al., 2015; Glibert, 2017; Gobler et al., 2017), increasing the risk of co-occurring algal toxins within a region.

Multiple HAB genera are known to occur in the waters of the Southern California Bight (SCB) (Caron et al., 2010), which is one of the most densely populated coasts in the United States with an estimated 129 million beach visits each year (Dwight et al., 2007). The most chronic HAB issue in the region is caused by species within the diatom genus *Pseudo-nitzschia,* which produces the neurotoxin domoic acid (DA) (Smith et al., 2018). Dinoflagellates within the genera *Alexandrium*, *Dinophysis*, and *Prorocentrum* are also commonly present in the SCB (Shipe et al., 2008; Garneau et al., 2011). *Alexandrium* is known to produce saxitoxin (STX), one of the most lethal marine toxins, and a suite of other paralytic shellfish toxins (Burkholder et al., 2006). Production of okadaic acid (OA) and other lipophilic diarrheic shellfish toxins have been attributed to some species of *Dinophysis* and benthic *Prorocentrum* (Zhou and Fritz, 1994; Jester et al., 2009; Li et al., 2012).

Solid Phase Adsorption Toxin Tracking (SPATT) is a passive sampling method that provides greater temporal resolution and increased toxin detection capabilities (e.g. greater sensitivity and detection of multiple toxins) relative to discrete water samples (MacKenzie et al., 2004; Lane et al., 2010; Kudela, 2017). We utilized SPATT bags for algal toxin monitoring on an ad-hoc basis across the SCB at seven different locations from 2012 to 2016. All SPATT bags were deployed for approximately one week, and study periods ranged from 4 to 122 weeks depending on the location (Table 1). Dissolved DA and STX were monitored at three locations: Redondo Beach Pier (Redondo), Catalina Island at the USC Wrigley Marine Science Center pier (Catalina), and Cabrillo Marina (Cabrillo). Dissolved DA, STX and OA were monitored at Newport Beach Pier (Newport) and from three moorings located 0.25 km, 2.5 km and 6.0 km offshore of Newport (Table 1). SPATT bags were deployed at 2 depths on the moorings. The sites monitored were between approximately 30 km and 55 km apart and represent a variety of environments within the SCB. Redondo, Newport and Cabrillo are primarily utilized for recreation and are located along highly urbanized coastline, while the Catalina site is surrounded by a marine protected area. Monitoring at the three offshore moorings was conducted as part of a larger monitoring effort related to a short-term, nearshore wastewater

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diversion (Howard et al., 2017).

Ancillary data (temperature, chlorophyll *a*, and phytoplankton abundances) were collected at Newport, Redondo and Cabrillo. Ancillary data was not collected at Catalina or at the offshore moorings. Potentially toxigenic taxa at these stations were identified to genera, and remaining phytoplankton cells were grouped into "other diatoms" and "other dinoflagellates". Ancillary data were collected and analyzed as described in Seubert et al. (2013). Correlations between toxin concentrations and ancillary data were determined at Newport, Redondo and Cabrillo using Spearman's Rank Order correlation test (significant at $p < 0.05$) in R using the Hmisc Package.

SPATT bags were constructed with Diaion HP20 resin (Sorbtech; Norcross, GA) and 100 μm mesh (Wildco; Yulee, FL), activated in 100% MeOH at 4 °C for 24 h, then rinsed and stored in ultrapure water at 4 °C until use (Lane et al., 2010). After collection, SPATT bags were stored at −20 °C until extraction. Toxin extraction was conducted as described in Lane et al. (2010), resulting in 3 extracts from each individual SPATT bag. Extract 1 was eluted with 10 mL of 50% MeOH (v/v) and Extract 2 and Extract 3 were eluted with 1 M ammonium acetate in 50% MeOH for extract volumes of 10 mL and 20 mL, respectively. The final concentration extracted from the bag was calculated by summing the concentrations of all 3 extracts.

All SPATT extracts were analyzed via Enzyme-Linked Immunosorbent Assay (ELISA). SPATT extracts were analyzed for DA using Mercury Science Inc. DA ELISA kits (Limit of Detection (LOD): 3.3 ng g^{-1}) and for STX using BiooScientific MaxSignal STX ELISA kits (LOD: 1.2 ng g^{-1}) at all locations except Newport. SPATT extracts from Newport were analyzed for STX using Mercury Science Inc Total STX ELISA kits (LOD: 3.3 ng g^{-1}), due to the greater affordability and ease of use. Extracts were analyzed for OA using BiooScientific MaxSignal OA ELISA test kits (Austin, TX) (LOD: 16.9 ± 8.3 ng g^{-1}, see below). Extracts were analyzed following the manufacturer's guidelines for each ELISA kit. For all analytes, extracts were diluted 1:10 in the manufacturer supplied dilution buffers to reduce the MeOH and ammonium acetate concentration before analysis. The Mercury Science DA and STX kits did not display signal suppression/enhancement when tested with standards spiked into each extraction solvent and diluted 1:10 in dilution buffer prior to loading onto the plate. The BiooScientific MaxSignal OA ELISA kits showed some signal suppression in standard spikes that contained ammonium acetate, even when diluted 1:10 in dilution buffer. Therefore, the limit of detection was set conservatively at 80% B/B_0 ([mean absorbance of standard or sample]/[mean absorbance of negative control]) to compensate for the reduced sensitivity in extracts that contained ammonium acetate. BiooScientific Max Signal STX ELISA kits were not tested for matrix effects due to limited resources in 2012–2013 for the ad-hoc sampling effort.

Both DA and STX were present in 18% of the bags deployed in Redondo, but the toxins never co-occurred (Fig. 1B). Detectable DA concentrations ranged from 9.2 to 37 ng g^{-1} and detectable STX concentrations ranged from 1.3 to 5.3 ng g^{-1}. DA was detected primarily in the spring, while STX occurred sporadically. Neither toxin showed significant correlations to any of the collected ancillary data. Algal toxins were rarely observed at Cabrillo, with DA and STX present in 2% and 5% percent of samples, respectively and detectable concentrations of both toxins were ≤11 ng g^{-1} (Fig. 1C). DA was positively correlated ($\rho = 0.34$, $p = 0.037$) with large size-class (>3 μm cell width) *Pseudo-nitzschia* abundances and temporally coincided with a large *Pseudo-nitzschia* bloom in the central Bight (Smith et al., 2017).

SPATT bags deployed at Catalina revealed a surprisingly regular occurrence of STX (Fig. 1D). 41% of samples had measurable concentrations of STX. Concentrations of detectable STX ranged from 2.2 to 13 ng g^{-1}. STX was detected sporadically throughout summer-late autumn 2012, and then quite frequently in winter-late spring 2013. Only 5% of samples detected DA, and detectable concentrations ranged

Table 1
Summary of monitoring locations along the coast of southern California, dates of study period, the ratio of bags deployed to those recovered, toxin occurrence, and deployment details. SPATT monitoring at each location was conducted on an ad-hoc basis and the study periods at each location were linked to the duration of pre-existing efforts at each site.

Location	Study Period	Bags Recovered/ Weeks Deployed	Domoic Acid Detected	Saxitoxin Detected	Okadaic Acid Detected	Co-occurrence of two toxins	Co-occurrence of three toxins	Deployment Strategy and Nominal Depth
Redondo Beach Pier[a]	Mar 5, 2012–Nov 12, 2013	28/37	18%	18%	Not measured	0%	Not applicable	Northern most point of pier, 2 m
Catalina Island Wrigley Marine Science Center	Mar 13, 2012–July 12, 2013	64/69	5%	41%	Not measured	2%	Not applicable	Floating Dock, 1 m
0.25 km Mooring	Sept 9, 2012–Oct 9, 2012	4/4	75%	75%	25%	75%	25%	Offshore Mooring, 1 m
0.25 km Mooring	Sept 9, 2012–Oct 9, 2012	4/4	75%	75%	75%	75%	75%	Offshore Mooring, 7 m
2.5 km Mooring	Sept 9, 2012–Oct 9, 2012	3/4	66.7%	66.7%	33.3%	66.7%	33.3%	Offshore Mooring, 1 m
2.5 km Mooring	Sept 9, 2012–Oct 9, 2012	4/4	50%	100%	50%	100%	0%	Offshore Mooring, 7 m
6.0 km Mooring	Sept 9, 2012–Oct 9, 2012	4/4	75%	75%	25%	75%	25%	Offshore Mooring, 1 m
6.0 km Mooring	Sept 9, 2012–Oct 9, 2012	4/4	75%	100%	75%	100%	50%	Offshore Mooring, 7 m
Cabrillo Marina[a]	Nov 19, 2012–Sept 24, 2013	41/45	2%	5%	Not measured	0%	Not applicable	Floating Dock, 1 m
Newport Beach Pier[a]	July 7, 2014–Oct 31, 2016	100/122	29%	0%	34%	10%	0%	End of Pier, 2 m

[a] Indicates a location indicates ancillary data on potentially toxic genera, temperature and chlorophyll *a* were collected.

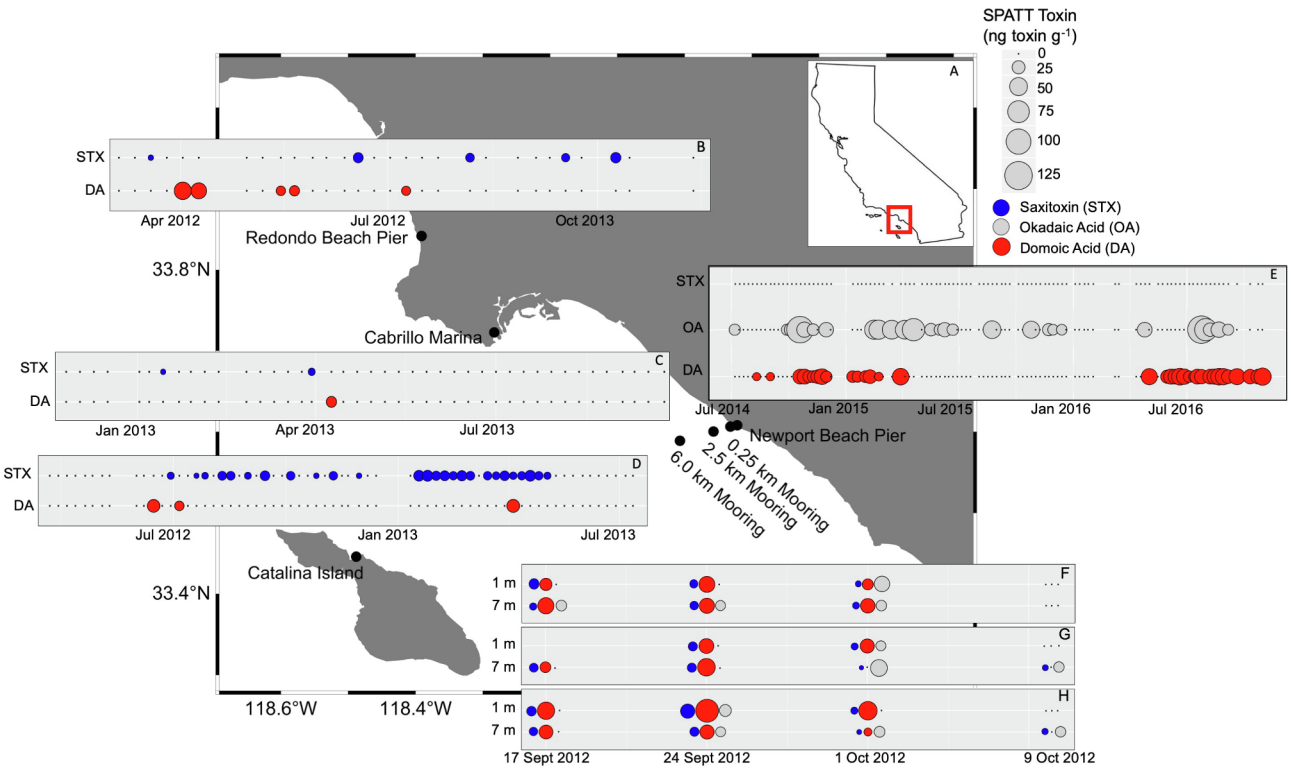

Fig. 1. Concentrations of domoic acid (red), saxitoxin (blue) and okadaic acid (grey) from solid phase adsorption toxin tracking (SPATT) monitoring conducted in the southern California Bight, highlighted in the red box in panel A. Results from Redondo Beach Pier (B), Cabrillo Marina (C), Wrigley Marine Science Center on Catalina Island (D), Newport Beach Pier (E), two depths at 0.25 km off shore of Newport Beach Pier (F), two depths at 2.5 km off shore of Newport Beach Pier (G), and two depths at 6.0 km (H) off shore of Newport Beach Pier.

from 9.7 to 19 ng g^{-1}. Both toxins co-occurred in 2% of samples.

Analysis of SPATT extracts from Newport showed DA and OA were present in 29% and 34% of samples, respectively (Fig. 1E). All extracts were below the detection limit for STX. Concentrations of detectable DA ranged from 9.6 to 47 ng g^{-1} and detectable OA concentrations ranged from 50 to 378 ng g^{-1}. DA and OA co-occurred in 10% of SPATT extracts. DA positively correlated with small size-class (<3 μm cell width) *Pseudo-nitzschia* ($\rho = 0.24$, $p = 0.016$), non-HAB diatoms ($\rho = 0.29$, $p = 0.004$), and, unexpectedly, *Dinophysis* cell abundances ($\rho = 0.23$, $p = 0.024$). OA was weakly, but positively correlated with temperature ($\rho = 0.20$, $p = 0.046$).

Analysis of SPATT extracts from moorings deployed during the wastewater diversion revealed the near-constant presence of one or more algal toxins at both the near-surface (1 m) and subsurface (7 m) depths. SPATT samples from 0.25 km offshore (Fig. 1F) showed the presence of all 3 studied toxins (DA, STX and OA), with DA and STX co-occurring in 75% of the samples and all three toxins in 25% of the samples at 1 m. At the same location at 7 m, 75% of samples showed the co-occurrence of DA, STX and OA. The SPATT collected from 2.5 km offshore (Fig. 1G) had DA and STX in 67% of samples, and all three toxins were detected in 33% of samples at 1 m. At 7 m, DA and OA were observed in 50% of samples and STX in all samples, with two toxins co-occurring in 100% of samples. SPATT bags collected from 6.0 km offshore (Fig. 1H) had DA and STX in 75% of samples and OA, DA, and STX in 25% of samples at 1 m. At 7 m, DA and OA occurred in 75% of samples and STX was detected in all samples. Two toxins were observed in 100% of the samples at 7 m and three toxins were observed in 50% of samples. A general decrease in toxin observations occurred after the diversion ended on 3 October 2012.

DA showed some relationship to the presence of the suspected causative organism but STX and OA did not in this study. *Alexandrium* cells were not detected at Redondo or Cabrillo, despite detection of STX at those locations in SPATT samples. Conversely, STX was not observed at Newport, but *Alexandrium* cells were occasionally observed. Cell abundance data was not collected at Catalina, where STX was most prevalent. Observations of *Alexandrium* abundances are not always clearly related to toxin concentrations, however, and STX concentrations can reach dangerous levels at cell abundances that are undetectable with standard microscopic techniques (Jester et al., 2009). Similarly, *Dinophysis* spp. abundances had no distinct relationship with OA detection. Overall, more work is needed to understand the relationship between SPATT results and putative producers, as well as human and animal health risks.

A majority of algal toxin research and monitoring in the SCB region to date has focused on DA and STX in the plankton (e.g. particulate toxins) and in shellfish tissue. Less is known about the distribution of dissolved toxins in the water, and especially the co-occurrence of multiple toxins in a single locale. Here we present the first, to our knowledge, description of OA in the SCB region, despite regular observations of the causative genera (Seubert et al., 2012). Observations in other regions of the U.S. indicate that OA and other lipophilic diarrheic shellfish toxins are underestimated as a public health threat, with recent studies reporting the presence of these toxins in shellfish (Shultz et al., 2019) and clinical reports of illness due to consumption of contaminated seafood (Trainer et al., 2013). Our results highlight that more work is needed to understand the spatial and temporal patters of OA and other lipophilic diarrheic shellfish toxins in the SCB region.

This work indicated the presence of DA, OA and STX throughout the central SCB, as well as the occurrence of two and sometimes three toxins concurrently. Only marine toxins were considered in the present study but there is emerging evidence for the transport of freshwater toxins from their sources in freshwater ecosystems to coastal environments (Gibble and Kudela, 2014; Gibble et al., 2016; Tatters et al. 2017, 2019; Paerl et al., 2018; Peacock et al., 2018). Future studies should also consider the potential of freshwater toxins in nearshore marine environments in addition to the suite of toxins examined in the present

study.

Ethical statement

No human or animal subjects were used in this study.

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