

## Blurred lines: Multiple freshwater and marine algal toxins at the land-sea interface of San Francisco Bay, California



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### ABSTRACT

San Francisco Bay (SFB) is a eutrophic estuary that harbors both freshwater and marine toxigenic organisms that are responsible for harmful algal blooms. While there are few commercial fishery harvests within SFB, recreational and subsistence harvesting for shellfish is common. Coastal shellfish are monitored for domoic acid and paralytic shellfish toxins (PSTs), but within SFB there is no routine monitoring for either toxin. *Dinophysis* shellfish toxins (DSTs) and freshwater microcystins are also present within SFB, but not routinely monitored. Acute exposure to any of these toxin groups has severe consequences for marine organisms and humans, but chronic exposure to sub-lethal doses, or synergistic effects from multiple toxins, are poorly understood and rarely addressed. This study documents the occurrence of domoic acid and microcystins in SFB from 2011 to 2016, and identifies domoic acid, microcystins, DSTs, and PSTs in marine mussels within SFB in 2012, 2014, and 2015. At least one toxin was detected in 99% of mussel samples, and all four toxin suites were identified in 37% of mussels. The presence of these toxins in marine mussels indicates that wildlife and humans who consume them are exposed to toxins at both sub-lethal and acute levels. As such, there are potential deleterious impacts for marine organisms and humans and these effects are unlikely to be documented. These results demonstrate the need for regular monitoring of marine and freshwater toxins in SFB, and suggest that co-occurrence of multiple toxins is a potential threat in other ecosystems where freshwater and seawater mix.

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### 1. Introduction

Harmful Algal Blooms (HABs) cause health problems for humans and wildlife, as well as persistent ecosystem damage. Both frequency and intensity of HABs are increasing (Anderson et al., 2002; Anderson, 2009; Hallegraeff, 1993; Wells et al., 2015). Impacts from human induced-nutrient inputs from agriculture and urban runoff, global warming, and drought events increase the likelihood that HABs and their impacts will continue to expand (Lehman et al., 2017; Paerl and Paul, 2012). This expansion is occurring in both freshwater and marine environments, but to

date, there have been few studies examining the co-occurrence of freshwater and marine toxins in estuaries (Lopes and Vasconcelos, 2011; Miller et al., 2010; Rita et al., 2014; Vasconcelos, 1995), in part because management and monitoring of HABs are often partitioned as either freshwater or marine (Gobble et al., 2016; Preece et al., 2017).

Both marine and freshwater toxigenic HAB species have been documented in San Francisco Bay (SFB) for the last three decades (Cloern and Dufford, 2005; Kudela et al., 2008; Lehman et al., 2010; Nejad et al., 2017). Marine HABs likely enter SFB from the Pacific Ocean (Horner et al., 1997), while freshwater HABs are dominant upstream and are likely transported into the estuary during high-flow events (Lehman et al., 2005), though SFB and the surrounding environs, such as the South Bay salt ponds, also contain resident populations of HAB organisms (Thébault et al., 2008). In part because of legacy anthropogenic contamination, there are few commercial

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fisheries within SFB, but recreational and subsistence shellfish harvesting is common (Davis et al., 2002; Gobble et al., 2016). Acute exposures to toxigenic algae can have severe and deadly impacts on higher trophic levels (Lefebvre et al., 2012; Shumway et al., 2003; Van Dolah et al., 2002). Sub-lethal chronic doses may also produce serious health compromises in humans (Capper et al., 2013; Ferriss et al., 2017), but chronic exposure to algal toxins has been poorly documented, and is an emerging concern (Brooks et al., 2012; Ger et al., 2010, 2009; Preece et al., 2017).

Although multiple HAB species are present in SFB, the toxins most commonly documented in the food web are domoic acid, produced by the diatom genus *Pseudo-nitzschia*, and microcystins, produced by the cyanobacteria genus *Microcystis* (Gobble et al., 2016; Lehman et al., 2010; McHuron et al., 2013). Human and wildlife acute exposure to domoic acid presents as Amnesic Shellfish Poisoning (ASP; Lefebvre et al., 2012), and is regulated by the California Department of Public Health (CDPH) for commercial and recreational shellfish harvests in coastal waters following federal guidelines (Wekell et al., 2004). Microcystins are a family of strong hepatotoxins causing liver damage in humans and wildlife (Carmichael et al., 2001), and have been documented in both estuarine and marine waterways, including SFB (Gobble et al., 2016; Gobble and Kudela, 2014; Lehman et al., 2005; Miller et al., 2010). Microcystins have been detected in marine mussels within SFB (Gobble et al., 2016), further documenting the occurrence of freshwater toxins in the marine environment (Gobble et al., 2016; Gobble and Kudela, 2014; Miller et al., 2010; Preece et al., 2017). To date, current monitoring has been effective at preventing ASP in humans, though mortality events in marine wildlife are common (McCabe et al., 2016; McKibben et al., 2017). The CDPH does not routinely monitor for microcystins, though episodic sampling does take place when blooms are documented.

In addition to microcystins and domoic acid, other algal toxins are likely to occur in SFB but are not routinely monitored. This includes the paralytic shellfish toxins (PSTs), causative agents for Paralytic Shellfish Poisoning (PSP) in wildlife and humans and primarily associated with the marine dinoflagellate genus *Alexandrium* (Horner et al., 1997). A fourth toxin suite evaluated in this study is produced by the marine dinoflagellate genus *Dinophysis*, comprised of the lipophilic toxins okadaic acid and derivatives (i.e., dinophysistoxin-1 and -2) collectively referred to as *Dinophysis* Shellfish Toxins (DSTs). The DSTs are the causative agent for Diarrhetic Shellfish Poisoning (DSP) in humans (Reguera et al., 2012; Yasumoto et al., 1978). In SFB *Dinophysis* is rare and when present does not dominate the phytoplankton community (Cloern et al., 1985; Cloern and Dufford, 2005), but even at low abundances *Dinophysis* has been linked to DSP occurrences (Rundberget et al., 2009). The CDPH routinely monitors coastal shellfish with an actionable regulatory limit for PSTs (Wekell et al., 2004), but there is currently no routine monitoring for *Dinophysis* or DSTs for either coastal California or SFB.

Here, this study documents co-occurrence of all four toxin suites in the marine mussel *Mytilus californianus* during 100-day deployments in 2012 and 2014, and in the hybridized Bay/Mediterranean marine mussel (*M. trossulus* and *M. galloprovincialis*) during a 5-month period in 2015. The occurrence of domoic acid and microcystins in both particulate and dissolved phases throughout SFB from 2011 to 2016 are also recorded.

## 2. Methods

### 2.1. Study area

The SFB is the largest estuary in California, consisting of six sub-embayments defined by their characteristic ranges of water-quality variables (Jassby et al., 1997). Spatial patterns in this estuary are controlled by the salinity gradient and its seasonal

variability (Cloern et al., 2017). Most freshwater flow into SFB comes from the Sacramento and San Joaquin Rivers draining 40% of California's landscape. Runoff from the agricultural Central Valley, urban sources, and treated sewage and storm-water contribute to high nutrient inputs. As a result, dissolved nutrient concentrations in SFB equal or exceed those in other estuaries where nutrient enrichment has degraded water and habitat quality (Cloern and Jassby, 2012). Thus, the potential for HAB development is high in this enriched estuary.

This study encompasses a period of severe drought (2012–2016), with record high temperatures and salinity in water year 2015 (October 1, 2014 to September 30, 2015; Work et al., 2017), and record low flows (Lehman et al., 2017). These conditions increased the intensity and duration of *Microcystis* blooms in the SFB/Delta system (Lehman et al., 2017). At the same time, unusual conditions along the open coast associated with the Pacific Warm Anomaly (Bond et al., 2015), including a massive *Pseudo-nitzschia* bloom with correspondingly high levels of domoic acid (McCabe et al., 2016) are expected to drive both physical and biological changes inside SFB through its connectivity with the open coast (Cloern et al., 2017; Raimonet and Cloern, 2017).

### 2.2. Field samples

#### 2.2.1. Particulate grab (filter) samples

Water samples were collected during USGS water quality cruises within SFB from November 2011 to June 2016 roughly twice monthly. Surface water (1–2 m) was filtered under low-vacuum pressure to collect particulate toxins. The same grab sample filter was used to analyze both domoic acid and microcystins. Ancillary data from these stations are available from the United States Geological Survey (USGS), including phytoplankton microscopy samples (Cloern and Dufford, 2005; Nejad et al., 2017).

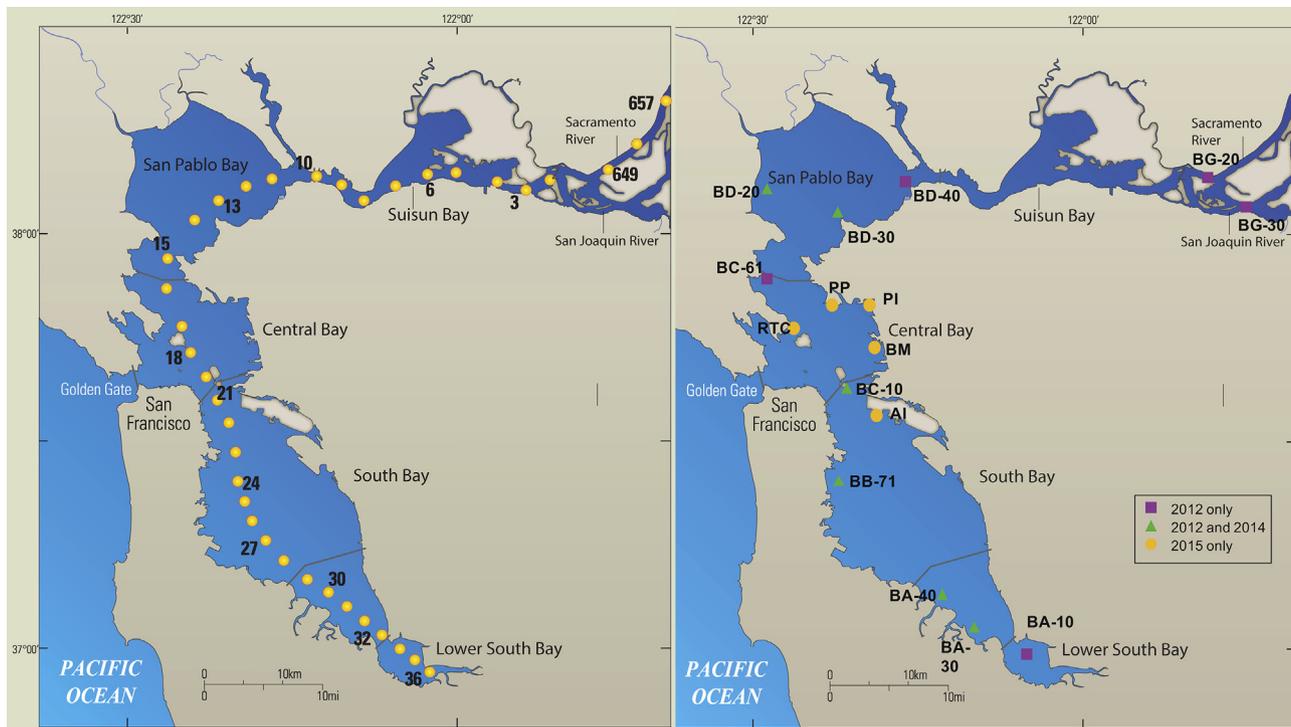
#### 2.2.2. Solid Phase Adsorption Toxin Tracking (SPATT) samplers

In addition to analyses of particulate toxins, an indicator of integrated dissolved toxin along subembayment transects was also used. Sample bags of SPATT were constructed and activated with 3 g (dry weight) DIAION HP20 (Sorbert Technologies) in 100  $\mu$ m nitex (Wildlife Supply Company) mesh bags (Kudela, 2011; Lane et al., 2010). The SPATT bags were clamped into plastic embroidery hoops and secured in a container with continuous underway flow from 0.5 m using a through-hull pumping system and were deployed on five transect lines. Fan et al. (2014) demonstrated that SPATT with HP20 exhibit non-linear effects on adsorption of okadaic acid and dinophysistoxin-1 in laboratory studies. Given the salinity gradients in San Francisco Bay, salinity probably introduced variability in the SPATT data but it is not possible to correct for this effect without better characterization of this effect in field-deployed samples for a full range of toxins.

#### 2.2.3. Mussel collections

*M. californianus* were collected from Bodega Head, CA (Fig. 1) in May 2012 and 2014 and held in filtered seawater tanks for one month to allow depuration of toxins (Bricelj and Shumway, 1998). Cages with mussels were attached to moorings throughout SFB (June 2012 and 2014), at all stations marked on Fig. 1. Mussels were harvested for analysis in September each year, after 100 days deployment. Mussels were homogenized for analysis (60 mussels, 2012; 110 mussels, 2014) and 5–20 g, a function of the size distribution of the available mussels, of homogenized tissue was stored frozen until toxin analysis.

Environmental hybridized *M. trossulus* and *M. galloprovincialis* were collected in SFB monthly from April to September 2015 at four locations: Point Isabel, Point Potrero, Berkeley Marina, and Alameda Island, with 15 individual mussels collected per site and sampling



**Fig. 1.** Map of San Francisco Bay. Left panel shows the particulate (filter) and dissolved (SPATT) stations, and the right panel shows the mussel collection or placement stations. Placed mussels are indicated as squares (2012 only) and triangles (2012 and 2014) and natural mussel collection sites (2015) are indicated as circles.

event. Romberg-Tiburon Center was included from July to September 2015 (Fig. 1). Salinities at these sites varied between 27 and 32 at collection time (Cloern and Schraga, 2016). The microcystin data has been published (Gibble et al., 2016). For this study a randomly selected subset of 3 of the 15 mussels were chosen for toxin analysis, as described below. There was insufficient biomass to test for all toxins, so sample sizes ranged from  $n = 34$  to  $n = 81$ .

### 2.3. Domoic acid analysis

Particulate grab (filter) samples were analyzed via Liquid Chromatography Mass Spectrometry (LC/MS) for domoic acid (Lane et al., 2010). 1.5 mL of the extract was stored ( $-80^{\circ}\text{C}$ ) for processing of microcystins. Samples were purified on BondElut C18 Solid Phase Extraction (SPE) columns (Agilent, USA; Sison-Mangus et al., 2016) with an elution volume of 3.0 mL.

For extraction of domoic acid, SPATT were rinsed with Milli-Q water and processed (Lane et al., 2010) with the following modification: bags were cut open and the resin was extracted out of bag without vortex mixing. Following the first 10 mL 50% MeOH extraction, a second extraction with 10 mL of 1 M ammonium acetate in 50% MeOH, and a third extraction with 20 mL of 1 M ammonium acetate in 50% MeOH was completed. Extracts were analyzed separately and summed for total toxin, then normalized to the resin dry weight to provide units of  $\mu\text{g}$  domoic acid per kg resin ( $\mu\text{g kg}^{-1}$ ; Lane et al., 2010).

Mussel samples for domoic acid analysis were prepared as follows: 1 g of homogenized mussel from each individual was added to 10 mL of 50% MeOH and disrupted by ultrasonic probe for 30 s with output power of  $<10\text{ W}$ . Extracts were clarified through a  $0.22\ \mu\text{m}$  PTFE filter and 3 mL was cleaned using Biotage ISOLUTE SAX SPE columns pre-conditioned with 6 mL of 100% MeOH, 3 mL of Milli-Q, and 3 mL of 50% MeOH. The mussel sample was added to the column, followed by 3 mL of acetonitrile: MilliQ: formic acid in (10:88:2 by volume) to elute the toxin, which was analyzed by LC/MS (Lane et al., 2010). The Method Detection Limit (MDL) was

$0.30\ \mu\text{g kg}^{-1}$  for tissue,  $7.50\ \text{ng L}^{-1}$  for particulate samples, and  $0.87\ \mu\text{g kg}^{-1}$  for SPATT. Domoic acid is reported as the sum of domoic acid and *epi*-domoic acid, and quantified by peak area and retention time using an external standard curve and NRC-Canada Certified Reference Material (NRC-CCRM).

### 2.4. Microcystin toxins analysis

Particulate grab samples were processed using the set aside 1.5 mL of domoic acid preparation (see above) that had been stored at  $-80^{\circ}\text{C}$ , and re-disruption of the filter following Lane et al., 2010, but with a finished extract concentration of 90% acidified MeOH before cleaning using methods of Mekebri et al., 2009 for saltwater mussels. Briefly, a  $375\ \mu\text{L}$  aliquot of the previously prepared domoic acid extract (in 10% MeOH) was set aside (labeled here as extract A), and  $750\ \mu\text{L}$  of the remaining 10% MeOH extract with the addition of the disrupted filter was re-disrupted as above and extracted in 6 mL of 100% MeOH. Extract was filtered through a  $0.22\ \mu\text{m}$  PTFE filter. 1 mL of 90% MeOH extract was set aside for archival purposes, and extract A was added to produce  $6.125\ \mu\text{L}$  of total extract (combined and labeled here as extract B) for a final concentration of 90% MeOH. Extract B was then acidified with  $6\ \mu\text{L}$  of trifluoroacetic acid (Mekebri et al., 2009). This process allowed particulate samples to be analyzed for both domoic acid (in 10% MeOH) and microcystins (in 90% acidified MeOH). Before analysis, microcystin samples were then cleaned using BAKER-BOND™ C18 SPE disposable columns and the collected elution was analyzed by LC/MS (Gibble et al., 2016). Analysis of extract A and B separately by LC/MS indicated that differences were within the method error for replicates, as were replicates which were not first extracted in 10% MeOH for domoic acid.

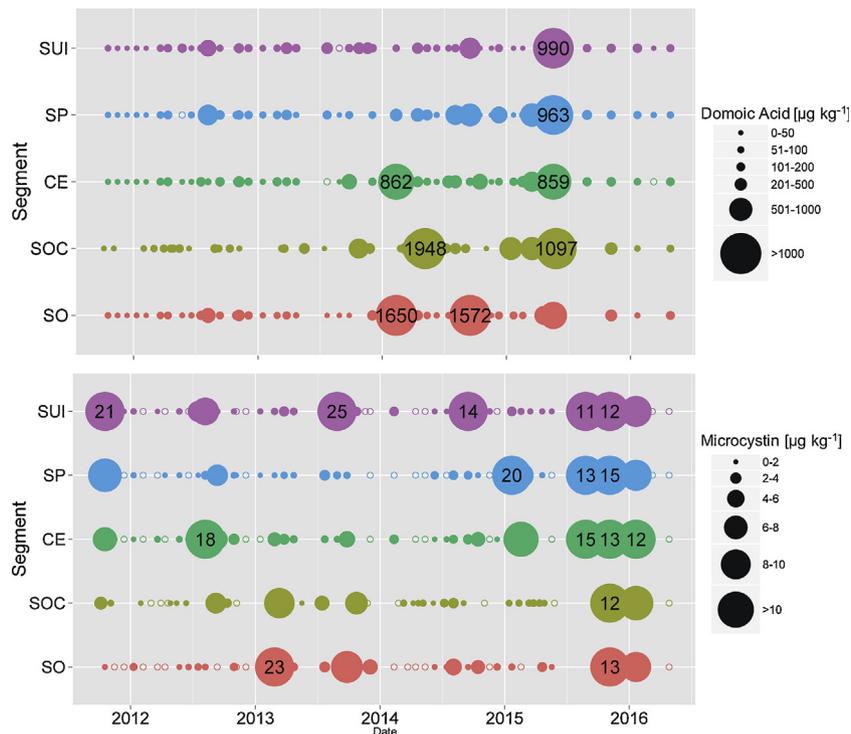
The SPATT bags were rinsed with deionized water and processed (Kudela, 2011). Mussel analysis for microcystins follows the field sample method analysis of Gibble et al. (2016), with the addition of a 45 min sonicating water bath following probe sonication using a Fisher Scientific Sonic Dismembrator Model 100 at 8 W power for 30 s.



**Table 1**

Particulate toxin concentrations in San Francisco Bay, Ca, 2011–2015. Stations correspond to Fig. 1, data is displayed graphically in Fig. 2.

Particulate toxin	Domoic acid [ $\text{ng L}^{-1}$ ]					Microcystin toxins [ $\text{ng L}^{-1}$ ]				
	Station	min	max	mean	median	n=	min	max	mean	median
657	bdl	88.7	3.9	0.0	44	bdl	461.6	18.5	2.9	44
6	bdl	32.9	1.6	0.0	44	bdl	53.3	3.7	1.1	44
13	bdl	19.3	0.7	0.0	44	bdl	25.6	3.9	1.8	44
18	bdl	316.7	12.9	0.0	75	bdl	51.1	3.7	0.0	74
22	bdl	84.4	3.8	0.0	44	bdl	9.6	1.2	0.0	31
27	bdl	44.0	1.9	0.0	69	bdl	70.7	4.6	0.2	68
32	bdl	16.0	1.0	0.0	28	bdl	10.0	1.7	0.0	28
34/36	bdl	90.3	2.0	0.0	72	bdl	22.0	3.8	0.2	72

**Fig. 3.** Dissolved toxin SPATT samples taken during USGS cruises from November 2011 to April 2016. Top panel is domoic acid concentrations, bottom panel is microcystins concentrations. SUI = Suisun Bay, SP = San Pablo Bay, CB = Central Bay, SOC = South-Central Bay, and SB = South Bay.**Table 2**

Solid Phase Adsorption Toxin Tracking (SPATT) samples in San Francisco Bay, Ca, 2011–2016. Stations correspond to Fig. 1, data is displayed graphically in Fig. 3.

SPATT Toxin	Domoic acid [ $\mu\text{g kg}^{-1}$ ]					Microcystin toxins [ $\mu\text{g kg}^{-1}$ ]				
	Segment	min	max	mean	median	n=	min	max	mean	median
Suisun Bay	bdl	990.7	115.8	79.8	44	bdl	25.5	2.8	0.5	44
San Pablo Bay	bdl	963.6	139.2	81.3	44	bdl	20.1	2.1	0.3	43
Central Bay	bdl	862.5	133.0	83.7	41	bdl	18.0	2.5	0.6	41
South-Central Bay	4.7	1948.0	236.2	97.1	41	bdl	13.0	1.5	0.5	41
South Bay	5.5	1650.5	174.0	62.5	41	bdl	23.0	1.9	0.4	41

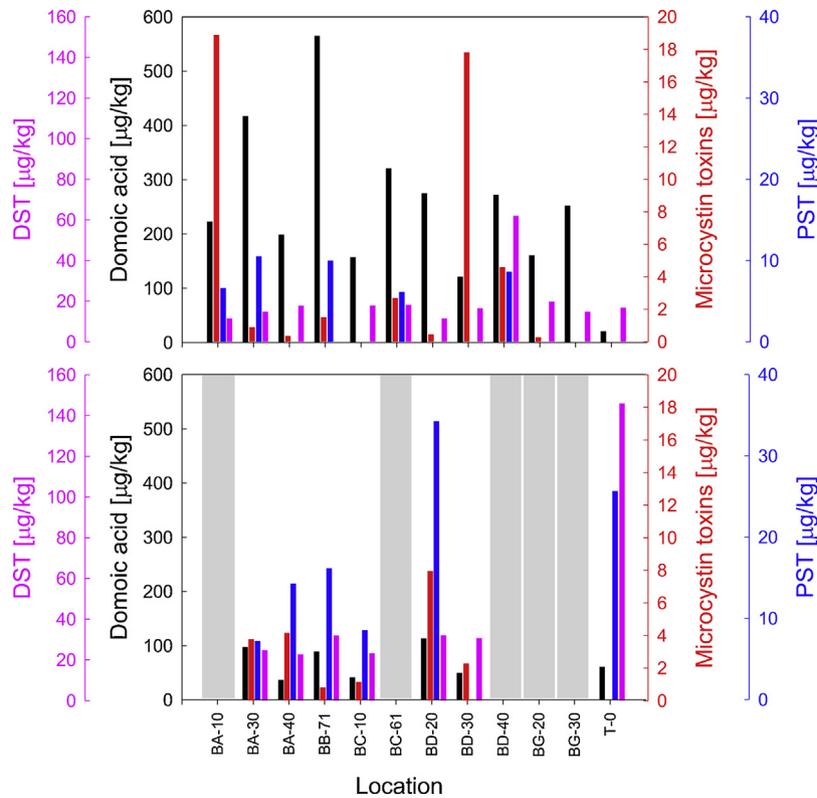
$62.1 \mu\text{g kg}^{-1}$ , and the Bodega Head samples had  $16.7 \mu\text{g kg}^{-1}$  (2012) and  $146 \mu\text{g kg}^{-1}$  (2014). Nearly half (47%) of the mussel samples collected after placement in SFB had detectable levels of all four toxins (Fig. 4).

As previously reported in Gobble et al. (2016), microcystins were detected in mussels sampled from April to October 2015 for 56% of individual mussels ( $n = 223$ ). Here are the described results for the subset of all mussels tested for multiple toxins. For that subset, 61% had detectable microcystins ( $n = 81$ ), ranging from <MDL to  $416 \mu\text{g kg}^{-1}$ . Domoic acid was detected in 98%, or all but two, of the

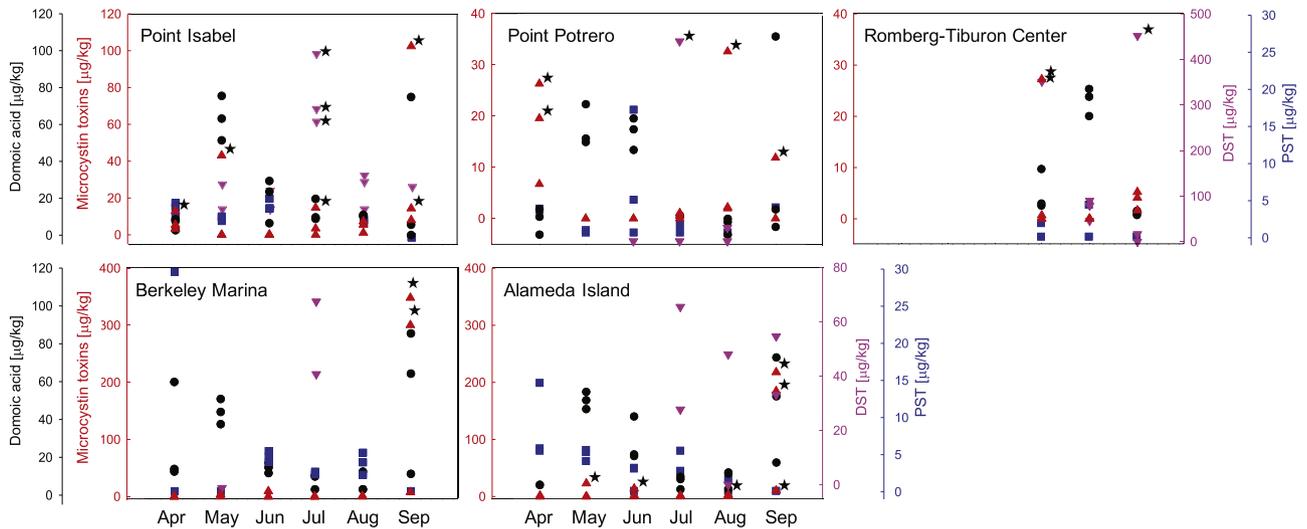
individual mussels ( $n = 81$ ). The concentration of domoic acid was very low and ranged from <MDL to  $107 \mu\text{g kg}^{-1}$ . The PSTs were detected in 59% of mussels ( $n = 73$ ), ranging from <MDL to  $29.4 \mu\text{g kg}^{-1}$ . The DSTs were detected in 71% of mussels ( $n = 34$ ) ranging from <MDL to  $430 \mu\text{g kg}^{-1}$  (Fig. 5).

#### 4. Discussion

This study is the first to report both freshwater and marine toxins in the same environmental mussel samples, and is the first



**Fig. 4.** Toxin concentrations in mussel homogenates at each station, from left to right: domoic acid, microcystins, PSTs, and DSTs. Samples were collected from Bodega Head, CA, the open coast, just north of SFB (T-0) in May and placed at mooring locations throughout the Bay in 2012 (top panel) and 2014 (bottom panel) for June–September. Sample locations are indicated on Fig. 1. Gray bars indicate no samples. Regulatory limits are as follows: 20,000  $\mu\text{g kg}^{-1}$  (20 ppm) for domoic acid, 10  $\mu\text{g kg}^{-1}$  (10  $\text{ng g}^{-1}$ ) for microcystins, 800  $\mu\text{g kg}^{-1}$  (80  $\mu\text{g per 100 kg}$ ) for PSTs, and 160  $\mu\text{g kg}^{-1}$  (0.16 ppm) for DSTs.



**Fig. 5.** Toxin concentrations in mussels for (circle) domoic acid, (triangle) microcystins, (inverted triangle) PSTs, and (square) DSTs. All samples were collected from April–September 2015 from within Central Bay. Locations are indicated on graphs and can be seen in Fig. 1. Each toxin is represented by its own y-axis. \* Indicates a sample that was above the regulatory limit. Regulatory limits are as follows: 20,000  $\mu\text{g kg}^{-1}$  (20 ppm) for domoic acid, 10  $\mu\text{g kg}^{-1}$  (10  $\text{ng g}^{-1}$ ) for microcystins, 800  $\mu\text{g kg}^{-1}$  (80  $\mu\text{g per 100 kg}$ ) for PSTs, and 160  $\mu\text{g kg}^{-1}$  (0.16 ppm) for DSTs.

report providing evidence for accumulation and exposure to four distinct classes of HAB toxins in the marine food web. Co-occurrence of HAB toxins in marine shellfish has been described (MacKenzie et al., 2002) and often involves DSTs and other lipophilic toxins including pectenotoxins and yessotoxins. Domoic acid and PSTs are strongly polar, hydrophilic toxins, and are not commonly associated with DSP events (Dominguez et al., 2010). In 2008 though, during a dolphin stranding event in Texas, USA, there

was documented co-occurrence of three HAB toxins: domoic acid, DSTs, and brevetoxins (Fire et al., 2011), where toxins found in feces and gastric contents were associated with the presence of known HAB species (Fire et al., 2011; Swanson et al., 2010). This unique combination of sampling modalities, including particulate samples, SPATT, and accumulation by mussels provides insight into chronic toxin exposure that would not be readily apparent with single methods. This could be augmented with information about

bloom toxicity, duration, and depuration rates for each of the four toxins, but we lack information at suitably high concurrent temporal resolution to document exposure of the mussels. Application of depuration models or detoxification rates is also difficult given the lack of information about depuration of (e.g.) microcystins in mussels (Gibble et al., 2016) as well as the lack of data on exposure time and concentration. Notably, no correlation was found between the presence of HAB species and toxins, suggesting that traditional phytoplankton monitoring is not an adequate substitute for toxin testing.

In 2015 there was an unprecedented toxic *P. australis* bloom along the west coast of North America (McCabe et al., 2016; McKibben et al., 2017; Ryan et al., 2017). This HAB was associated with the Pacific Warm Anomaly (Bond et al., 2015; Di Lorenzo and Mantua, 2016), and was responsible for the suspected domoic acid toxicosis of at least 229 sea lions (2014–2015). Considering that SFB is an estuary closely coupled with the open coast Pacific Ocean (Raimonet and Cloern, 2017), there was an expectation that evidence of the domoic acid event would be apparent within SFB, despite reported fast depuration rates for domoic acid (Blanco et al., 2002; Novaczek et al., 1992). The bloom was not apparent based on particulate grab samples (Fig. 2) or cell counts, but is represented as dissolved toxin in SPATT. While there is no 1:1 conversion of SPATT toxin concentration to toxin in mussels, the regulatory limit of 20,000  $\mu\text{g kg}^{-1}$  (20 ppm; Wekell et al., 2004) is roughly equivalent to 150  $\mu\text{g kg}^{-1}$  of SPATT resin (Lane et al., 2010). In May 2015, the peak of the open coast event (McCabe et al., 2016), the highest dissolved domoic acid concentrations throughout the Bay were observed with concentrations between 618–990  $\mu\text{g kg}^{-1}$ , suggesting that there was increased dissolved domoic acid and potential for trophic transfer of domoic acid within SFB (Fig. 3). At the same time, mussels collected from Central Bay where coastal exchange is expected to be influential (Raimonet and Cloern, 2017) were considerably less toxic than those collected along the open coast (McCabe et al., 2016; Fig. 5). Only 120 km south in Santa Cruz, CA, mussel concentrations of  $>75,000 \mu\text{g kg}^{-1}$  in May and June 2015 (McCabe et al., 2016) clearly documented an acute toxicity that was not present in SFB mussels. Yet, even with the very low concentrations of domoic acid, all sites had elevated concentrations in May and June when domoic acid peaked in SFB, which follows the temporal pattern of the bloom event along the open coast. Thus, the temporal sequence of increasing domoic acid in spring and summer 2015 is consistent with the open coast bloom, but something inhibited toxin accumulation within SFB. One possibility is that the increased domoic acid seen in SFB was the result of advection from the coast, and that temperatures within SFB were not conducive to growth of *P. australis*. McCabe et al. (2016) documented maximum growth rates of *P. australis* isolates from Monterey Bay, CA at  $\sim 16^\circ\text{C}$ , followed by a decline at higher temperatures. The SFB exhibited mean temperatures of  $\sim 16\text{--}20^\circ\text{C}$  for the 2015 water year with peak (record-breaking) temperatures in Central Bay of  $>20^\circ\text{C}$  in August 2015 (Work et al., 2017). While unproven, the authors of this study hypothesize that domoic acid was transported into SFB primarily as dissolved toxin (captured by SPATT), with more limited transport of intact cells (captured by mussels) and likely with temperature-based suppression of growth within the SFB during that summer.

In 2015 during that same event, 29% of mussels sampled were positive for all four toxins, while 98% had at least one detectable toxin. Both domoic acid and PSTs, which California regulates along the open coast, were well below acute regulatory guidelines for quarantine of marine mussels, but more than half were contaminated with both domoic acid and PSTs, and there were 23 occurrences of acute toxin levels (Fig. 5). In 2012 and 2014 100% of mussel homogenates were contaminated with domoic acid, 88% had microcystins, 59% had PSTs, and 94% had DSTs. Only one

sample had acute toxin levels (for microcystins; Fig. 4). The concentration of domoic acid and PSTs found in the mussels collected for this study would be categorized as low-level; however, the risk of long-term health effects from consumption of these contaminated mussels is unknown. Chronic levels of domoic acid have been linked to a multitude of deleterious effects in marine organisms, including persistent seizures and toxicity syndrome in sea lions (Ferriss et al., 2017; Goldstein et al., 2008; Gulland et al., 2002), increased toxin susceptibility in zebrafish (Lefebvre et al., 2012), and a possible link between chronic domoic acid exposure and memory loss for people regularly exposed to toxin through shellfish consumption (Ferriss et al., 2017; Grattan et al., 2016). Harmful effects from chronic PSTs are similar, with reports of juvenile shellfish mortality (MacQuarrie and Bricelj, 2008), morphological abnormalities in larval fish (Lefebvre et al., 2004; Silva de Assis et al., 2013), and reduced diving capabilities in marine mammals (Durbin et al., 2002).

There is no regulatory limit for chronic exposure to domoic acid or PSTs (Adams et al., 2016; Ferriss et al., 2017), or any marine phycotoxin, and effects from chronic exposure to humans and marine mammals are difficult to ascertain (Visciano et al., 2016). The US regulatory limit for acute exposure is based on the amount of toxin that would reasonably be ingested with a safety factor of 10 for PSP and 12 for ASP assuming typical seafood consumption rates of 100–200 g per serving. A more typical shellfish consumption rate is as high as 500–1000 g per serving (Wekell et al., 2004). Existing guidelines do not directly account for sensitive groups, such as pregnant women, children, recreational harvesters, or Native Americans who collect shellfish for subsistence as well as recreational and commercial purposes, and are likely ingesting even higher levels through repeated consumption (Adams et al., 2016; Ferriss et al., 2017; Grattan et al., 2016). As such, while the low levels of domoic acid and PSTs found in these shellfish will not produce acute toxicosis, the consequences of sub-lethal chronic exposure for both humans and wildlife are poorly documented.

In contrast to domoic acid and PSTs, the concentrations of microcystins and DSTs were variable and on occasion considerably higher than regulatory guidelines of 160  $\mu\text{g DSTs per kg shellfish}$  (160  $\mu\text{g kg}^{-1}$ ; Lewitus et al., 2012), and 10 ng microcystins per gram of fish (10  $\mu\text{g kg}^{-1}$ ; Office of Environmental Health and Hazard Assessment; OEHHA); there is currently no United States or California regulatory guidelines addressing microcystin ingestion through shellfish consumption so it is assumed that the same regulatory guidance can be applied to shellfish consumption. Human symptoms related to DSP are minor compared to the other toxins discussed herein, but do include mild to moderate gastrointestinal symptoms, and can lead to illness or death through dehydration. These symptoms are often not reported or misdiagnosed as pathogen induced gastroenteritis (Lewitus et al., 2012). California does not routinely monitor for DSP, though Baja California, Mexico, and the Pacific Northwest do (Lewitus et al., 2012; Trainer et al., 2013). Of the 34 mussels tested for DSTs in 2015, 15% were above the regulatory guidelines, while 59% of the mussels had detectable DSTs (Fig. 5). In 2014 the mussel homogenate collected from Bodega Head on the open coast (146  $\mu\text{g kg}^{-1}$ ) was just below the regulatory guidelines, and in both 2012 and 2014 there were low toxin levels in 94% of SFB samples. These percentages include mussels transplanted into both the northern Estuary and South Bay (Figs. 1 and 4); while there are no commercial shellfisheries within SFB, recreational and subsistence harvesters regularly collect shellfish in SFB.

As described by Gibble et al. (2016), microcystins were found in locally occurring mussel samples from all sites, during all months in 2015, except August at Romberg-Tiburon Center, the most oceanic site (Figs. 1 and 5). There was no expectation that a predominantly freshwater toxin would be found in mussels placed

in high-salinity regions of SFB. There are precedents for freshwater cyanotoxins within an estuary (Gibble et al., 2016; Lehman et al., 2010; Paerl and Huisman, 2009; Preece et al., 2017), but the conditions during this study were not the “expected” conditions for high concentrations of cyanotoxins within an estuarine environment (Lehman et al., 2013, 2008). There was minimal freshwater seasonal input, and California had been experiencing an unprecedented five-year drought at the time of sampling (Lehman et al., 2017). A full 25% of the individual mussels tested exceeded the recommended regulatory guidance, though there was large variability even among the three mussels tested per site (Fig. 5), and in the larger sample sizes reported in Gibble et al. (2016). The mussels weighed on average  $5.76 \pm 3.5$  g each. This translates to about 17–34 mussels per person consumption, which accurately reflects subsistence and recreational harvester mussel consumption to reach the assumed ingestion of 100–200 g of shellfish used for regulatory purposes. These data indicate that eating just one of the contaminated mussels would exceed the weekly suggested intake of microcystins, and a single meal of mussels could exceed the suggested intake by ~50x. As is common with most biotoxins, visual inspection of the mussels does not indicate contamination, and the wide range of microcystin concentrations in the mussels provides little strategy for mitigation of exposure to the mussels above regulatory limits. For testing purposes, homogenization of multiple mussels would remove some of this individual variability (Gibble et al., 2016), yet offers no additional safety for recreational or subsistence harvesting. In 2014 microcystins were found in all of the homogenized mussel samples, and in all but two samples in 2012 (Fig. 4). Lehman et al. (2017) describes the 2014 SFB *Microcystis* bloom as the largest recorded biomass since 1999, leading to an expanded bloom. While the samples in 2014 were less toxic than samples from 2015, there was a clear pattern of widespread contamination throughout SFB. Consequently, accumulation of this freshwater cyanotoxin in bivalves is cause for alarm in marine environments receiving freshwater runoff.

During this study, the particulate grab samples for microcystins never exceeded regulatory limits (Fig. 2). Regulatory guidelines vary widely, but recent guidance from OEHHA recommend caution when recreational users are exposed to  $0.8 \mu\text{g L}^{-1}$  microcystins, while WHO identifies low risk at  $<10 \mu\text{g L}^{-1}$ ; the highest values were therefore ~2 to 20-fold below those guidance levels. The grab samples may be underrepresenting the total amount of microcystins in the water, since samples were collected from 1 to 2 m depth and *Microcystis* cells typically float at the surface (Mlouka et al., 2004). This is consistent with the absence of *Microcystis* from cell count data, which also collected at 1–2 m depth. Gibble et al. (2016) documented accumulation of dissolved toxins in mussels, highlighting the potential importance of both dissolved and particulate phases, and the SPATT samplers indicate there is chronic, system-wide dissolved microcystins exposure to the food web year-round (Fig. 3).

While toxin production is often suppressed during periods of drought (Gibble and Kudela, 2014; Miller et al., 2010; Paerl and Huisman, 2008), there is some indication that drought conditions in SFB can spread *Microcystis* blooms westward and downstream (Gibble et al., 2016; Lehman et al., 2017, 2013). This may explain the pervasiveness of the dissolved toxin found during this sample period, and the high microcystin concentrations in 2014 and 2015 mussel samples from the marine influenced Central and South Bays (Figs. 4 and 5). But not to be discounted are point-source and urban run-off or smaller freshwater inputs which could be additional, and unmeasured, sources. The bioaccumulation of microcystins in marine mussels suggests the potential for acute as well as chronic risk of toxicosis for marine mammals, marine and estuarine birds (Gibble et al., 2017), and humans, given that concentrations recorded from this study exceeded levels

associated with a 2009 acute marine otter mortality event in Monterey Bay, CA (Miller et al., 2010). Currently, however, there is no regulatory monitoring of the marine food web for freshwater toxins in California.

The concentrations of algal toxins detected in SFB for domoic acid and PSTs were low relative to concentrations associated with acute mortality in marine mammals and humans (Lefebvre and Robertson, 2010; Trainer et al., 2007). The concentrations for DSTs and microcystins were more concerning, as they frequently exceeded the regulatory limits for human consumption (Díaz et al., 2016; Preece et al., 2017). The impact of both chronic and multiple toxin exposure to marine wildlife or humans is unknown (Adams et al., 2016; Capper et al., 2013; Ferriss et al., 2017; Fire et al., 2011). In some cases, studies suggest no or unknown synergistic effects, such as for simultaneous exposure to domoic acid and brevetoxin in bottlenose dolphins (Fire et al., 2011) or brevetoxins, okadaic acid, and saxitoxin in manatees and turtles (Capper et al., 2013). Yet, there is likely a reduction in marine mammal fitness through immunosuppression (Capper et al., 2013). Further study is clearly needed to assess the consequences of multiple, simultaneous toxin exposure in both wildlife and humans.

## 5. Conclusion

San Francisco Bay exhibits simultaneous presence and bioaccumulation of at least four HAB toxin groups. Domoic acid and microcystins are persistent in both time and space within the estuary, while microcystins and DSTs routinely exceed regulatory action levels. The synergistic or additive effects for higher trophic level exposure to these multiple toxin suites are unknown, and are unlikely to be detected given that there has historically been no routine regulatory monitoring within SFB for these toxins, DSTs are not regularly monitored in California shellfish, and there is no requirement for monitoring freshwater algal toxins in marine bivalves. As with other estuaries (Preece et al., 2017), SFB may be acting as a mixing bowl and bioreactor for harmful algal species and toxins endemic to freshwater, brackish, and marine ecosystems. To the extent that SFB is representative of other estuaries, this strongly suggests that the community needs to reassess monitoring and management practices with regard to harmful algae and their toxins at the land-sea interface.

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## References

- Adams, N.G., Robertson, A., Grattan, L.M., Pendleton, S., Roberts, S., Tracy, J.K., Trainer, V.L., 2016. Assessment of sodium channel mutations in Makah tribal members of the U.S. Pacific Northwest as a potential mechanism of resistance to paralytic shellfish poisoning. *Harmful Algae* 57, 26–34. doi:<http://dx.doi.org/10.1016/j.hal.2016.03.008>.
- Anderson, D., Glibert, P., Burkholder, J., 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries* 25 (4), 704–726.
- Anderson, D.M., 2009. Approaches to monitoring, control and management of harmful algal blooms (HABs). *Ocean Coast. Manag.* 52, 342. doi:<http://dx.doi.org/10.1016/j.ocecoaman.2009.04.006>.
- Blanco, J., Arévalo, F., Salgado, C., Morono, Á., 2002. Depuración del ácido domoico de mejillones (*Mytilus galloprovincialis*). *Aquat. Living Resour.* 15, 53–60. doi:[http://dx.doi.org/10.1016/S0990-7440\(01\)01139-1](http://dx.doi.org/10.1016/S0990-7440(01)01139-1).

- Bond, N., Cronin, M., Freeland, H., 2015. Causes and impacts of the 2014 warm anomaly in the NE Pacific. *Geophys. Res.* 42, 3414–3420.
- Bricej, V., Shumway, S., 1998. Paralytic shellfish toxins in bivalve molluscs: occurrence, transfer kinetics, and biotransformation. *Rev. Fish. Sci.* 6(4), 315–383.
- Brooks, M.L., Fleishman, E., Brown, L.R., Lehman, P.W., Werner, I., Scholz, N., Mitchelmore, C., Lovvorn, J.R., Johnson, M.L., Schlenk, D., van Drunick, S., Drever, J.I., Stoms, D.M., Parker, A.E., Dugdale, R., 2012. Life histories, salinity zones, and sublethal contributions of contaminants to pelagic fish declines illustrated with a case study of San Francisco Estuary, California, USA. *Estuaries Coasts* 35, 603–621. doi:http://dx.doi.org/10.1007/s12237-011-9459-6.
- Butler, N., Carlisle, J.C., Linville, R., Washburn, B., 2009. Microcystins: A Brief Overview of Their Toxicity and Effects, with Special Reference to Fish, Wildlife, and Livestock, 5. California Environmental Protection Agency, Sacramento.
- Capper, A., Flewelling, L.J., Arthur, K., 2013. Dietary exposure to harmful algal bloom (HAB) toxins in the endangered manatee (*Trichechus manatus latirostris*) and green sea turtle (*Chelonia mydas*) in Florida, USA. *Harmful Algae* 28, 1–9. doi:http://dx.doi.org/10.1016/j.hal.2013.04.009.
- Carmichael, W.W., Azevedo, S.M., An, J.S., Molica, R.J., Jochimsen, E.M., Lau, S., Rinehart, K.L., Shaw, G.R., Eaglesham, G.K., 2001. Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. *Environ. Health Perspect.* 109, 663–668.
- Cloern, J., Dufford, R., 2005. Phytoplankton community ecology: principles applied in San Francisco Bay. *Mar. Ecol. Prog. Ser.* 285, 11–28.
- Cloern, J., Jassby, A., 2012. Drivers of change in estuarine-coastal ecosystems: discoveries from four decades of study in San Francisco Bay. *Rev. Geophys.* 50(4) RG4001.
- Cloern, J.E., Schraga, T.S., 2016. USGS Measurements of Water Quality in San Francisco Bay (CA). , pp. 1969–2015. doi:http://dx.doi.org/10.5066/F7TQ5ZPR.
- Cloern, J.E., Cole, B.E., Wong, R.L., Alpine, A.E., 1985. Temporal dynamics of estuarine phytoplankton: a case study of San Francisco Bay. *Temporal Dynamics of an Estuary: San Francisco Bay*, Springer, Dordrecht, pp. 153–176.
- Cloern, J.E., Jassby, A.D., Schraga, T.S., Nejad, E., Martin, C., 2017. Ecosystem variability along the estuarine salinity gradient: examples from long-term study of San Francisco Bay. *Limnol. Oceanogr.* 62(S1), 272–291.
- Díaz, P.A., Ruiz-Villarreal, M., Pazos, Y., Moita, T., 2016. Climate variability and Dinophysis acuta blooms in an upwelling system. *Harmful Algae* 53, 145–159. doi:http://dx.doi.org/10.1016/j.hal.2015.11.007.
- Davis, J., May, M., Greenfield, B., Fairey, R., 2002. Contaminant concentrations in sport fish from San Francisco Bay, 1997. *Mar. Pollut.* 44(10), 1117–1129.
- Dominguez, H.J., Paz, B., Daranas, A.H., Norte, M., Franco, J.M., Fernández, J.J., 2010. Dinoflagellate polyether within the yessotoxin, pectenotoxin and okadaic acid toxin groups: characterization, analysis and human health implications. *Toxicol.* 56, 191–217. doi:http://dx.doi.org/10.1016/j.toxicol.2009.11.005.
- Durbin, E., Teegarden, G., Campbell, R., Cembella, A., Baumgartner, M.F., Mate, B.R., 2002. North Atlantic right whales, Eubalaena glacialis, exposed to paralytic shellfish poisoning (PSP) toxins via a zooplankton vector, Calanus finmarchicus. *Harmful Algae* 1, 243–251. doi:http://dx.doi.org/10.1016/S1568-9883(02)00046-X.
- Fan, L., Sun, G., Qiu, J., Ma, Q., Hess, P., Li, A., 2014. Effect of seawater salinity on pore-size distribution on a poly (styrene)-based HP20 resin and its adsorption of diarrhetic shellfish toxins. *J. Chromatogr. A* 1373, 1–8.
- Ferriss, B.E., Marcinek, D.J., Ayres, D., Borchert, J., Lefebvre, K.A., 2017. Acute and chronic dietary exposure to domoic acid in recreational harvesters: a survey of shellfish consumption behavior. *Environ. Int.* 101, 70–79. doi:http://dx.doi.org/10.1016/j.envint.2017.01.006.
- Fire, S., Wang, Z., Byrd, M., Whitehead, H., Paternoster, J., 2011. Co-occurrence of multiple classes of harmful algal toxins in bottlenose dolphins (*Tursiops truncatus*) stranding during an unusual mortality event in Texas, USA. *Harmful Algae* 10(3), 330–336.
- Ger, K.A., Teh, S.J., Goldman, C.R., 2009. Microcystin-LR toxicity on dominant copepods Eurytemora affinis and Pseudodiaptomus forbesi of the upper San Francisco Estuary. *Sci. Total Environ.* 407, 4852–4857. doi:http://dx.doi.org/10.1016/j.scitotenv.2009.05.043.
- Ger, K.A., Teh, S.J., Baxa, D.V., Lesmeister, S., Goldman, C.R., 2010. The effects of dietary Microcystis aeruginosa and microcystin on the copepods of the upper San Francisco Estuary. *Freshw. Biol.* 55, 1548–1559. doi:http://dx.doi.org/10.1111/j.1365-2427.2009.02367.x.
- Gibble, C.M., Kudela, R.M., 2014. Detection of persistent microcystin toxins at the land-sea interface in Monterey Bay, California. *Harmful Algae* 39, 146–153. doi:http://dx.doi.org/10.1016/j.hal.2014.07.004.
- Gibble, C.M., Peacock, M.B., Kudela, R.M., 2016. Evidence of freshwater algal toxins in marine shellfish: implications for human and aquatic health. *Harmful Algae* 59, 59–66. doi:http://dx.doi.org/10.1016/j.hal.2016.09.007.
- Gibble, C.M., Hayashi, K., Kudela, R., 2017. The use of blood collection cards for assessing presence of microcystin in marine and estuarine birds. *J. Wildl. Rehabil.* 37, 7–12.
- Goldstein, T., Mazet, J., Zabka, T.S., Langlois, G., Colegrove, K.M., Silver, M.W., Bargo, S., Van Dolah, F.M., Leighfield, T.A., Conrad, P.A., Barakos, J., Williams, D.C., Dennison, S., Haulena, M., Gulland, F.M.D., 2008. Novel symptomatology and changing epidemiology of domoic acid toxicosis in California sea lions (*Zalophus californianus*): an increasing risk to marine mammal. *Proc. R. Soc. B* 275. doi:http://dx.doi.org/10.1098/rspb.2007.1221.
- Grattan, L.M., Boushey, C., Tracy, K., Trainer, V.L., Roberts, S.M., Schluterman, N., Morris, J.G., 2016. The association between razor clam consumption and memory in the CoASTAL cohort. *Harmful Algae* 57, 20–25. doi:http://dx.doi.org/10.1016/j.hal.2016.03.011.
- Gulland, F.M.D., Haulena, M., Fauquier, D., Langlois, G., Lander, M.E., Zabka, T., Duerr, R., 2002. Domoic acid toxicity in Californian sea lions (*Zalophus californianus*): clinical signs, treatment and survival. *Vet. Rec.* 150(15), 475–480.
- Hallegraeff, G., 1993. A review of harmful algal blooms and their apparent global increase. *Phycologia* 32(2), 79–99.
- Horner, R.A., Garrison, D.L., Plumley, F.G., 1997. Harmful algal blooms and red tide problems on the U.S. west coast. *Limnol. Oceanogr.* 42, 1076–1088. doi:http://dx.doi.org/10.4319/lo.1997.42.5\_part\_2.1076.
- Jassby, A.D., Cole, B.E., Cloern, J.E., 1997. The design of sampling transects for characterizing water quality in estuaries. *Estuar. Coast. Shelf Sci.* 45, 285–302. doi:http://dx.doi.org/10.1006/ecss.1996.0199.
- Kudela, R.M., Lane, J.Q., Cochlan, W.P., 2008. The potential role of anthropogenically derived nitrogen in the growth of harmful algae in California, USA. *Harmful Algae* 8, 103–110. doi:http://dx.doi.org/10.1016/j.hal.2008.08.019.
- Kudela, R., 2011. Characterization and deployment of Solid Phase Adsorption Toxin Tracking (SPATT) resin for monitoring of microcystins in fresh and saltwater. *Harmful Algae* 11, 117–125.
- Lane, J.Q., Roddam, C.M., Langlois, G.W., Kudela, R.M., 2010. Application of Solid Phase Adsorption Toxin Tracking (SPATT) for field detection of the hydrophilic phycotoxins domoic acid and saxitoxin in coastal California. *Limnol. Oceanogr. Methods* 8(11), 645–660.
- Lefebvre, K.A., Robertson, A., 2010. Domoic acid and human exposure risks: a review. *Toxicol.* 56, 218–230. doi:http://dx.doi.org/10.1016/j.toxicol.2009.05.034.
- Lefebvre, K., Trainer, V., Scholz, N., 2004. Morphological abnormalities and sensorimotor deficits in larval fish exposed to dissolved saxitoxin. *Aquat. Toxicol.* 66(2), 159–170.
- Lefebvre, K.A., Frame, E.R., Kendrick, P.S., 2012. Domoic acid and fish behavior: a review. *Harmful Algae* 13, 126–130. doi:http://dx.doi.org/10.1016/j.hal.2011.09.011.
- Lehman, P., Boyer, G., Hall, C., Waller, S., Gehrts, K., 2005. Distribution and toxicity of a new colonial Microcystis aeruginosa bloom in the San Francisco Bay Estuary, California. *Hydrobiologia* 541, 87–99.
- Lehman, P.W., Boyer, G., Satchwell, M., Waller, S., 2008. The influence of environmental conditions on the seasonal variation of Microcystis cell density and microcystins concentration in San Francisco Estuary. *Hydrobiologia* 600, 187–204. doi:http://dx.doi.org/10.1007/s10750-007-9231-x.
- Lehman, P.W., Teh, S.J., Boyer, G.L., Nobriga, M.L., Bass, E., Hogle, C., 2010. Initial impacts of Microcystis aeruginosa blooms on the aquatic food web in the San Francisco Estuary. *Hydrobiologia* 637, 229–248. doi:http://dx.doi.org/10.1007/s10750-009-9999-y.
- Lehman, P.W., Marr, K., Boyer, G.L., Acuna, S., Teh, S.J., 2013. Long-term trends and causal factors associated with Microcystis abundance and toxicity in San Francisco Estuary and implications for climate change impacts. *Hydrobiologia* 718, 141–158. doi:http://dx.doi.org/10.1007/s10750-013-1612-8.
- Lehman, P.W., Kurobe, T., Lesmeister, S., Baxa, D., Tung, A., Teh, S.J., 2017. Impacts of the 2014 severe drought on the Microcystis bloom in San Francisco Estuary. *Harmful Algae* 63, 94–108. doi:http://dx.doi.org/10.1016/j.hal.2017.01.011.
- Lewitus, A., Horner, R., Caron, D., Garcia-Mendoza, E., 2012. Harmful algal blooms along the North American west coast region: history, trends, causes, and impacts. *Harmful Algae* 19, 133–159.
- Lopes, V.R., Vasconcelos, V.M., 2011. Planktonic and benthic cyanobacteria of European brackish waters: a perspective on estuaries and brackish seas. *Eur. J. Phycol.* 46, 292–304. doi:http://dx.doi.org/10.1080/09670262.2011.602429.
- Di Lorenzo, E., Mantua, N., 2016. Multi-year persistence of the 2014/15 North Pacific marine heatwave. *Nat. Clim. Change* 6(11), 1042–1047.
- MacKenzie, L., Holland, P., McNabb, P., Beuzenberg, V., 2002. Complex toxin profiles in phytoplankton and Greenshell mussels (*Perna canaliculus*), revealed by LC-MS/MS analysis. *Toxicol.* 44(8), 901–918.
- MacQuarrie, S., Bricej, V., 2008. Behavioral and physiological responses to PSP toxins in Mya arenaria populations in relation to previous exposure to red tides. *Mar. Ecol. Prog. Ser.* 366, 59–74.
- McCabe, R.M., Hickey, B.M., Kudela, R.M., Lefebvre, K.A., Adams, N.G., Bill, B.D., Gulland, F.M.D., Thomson, R.E., Cochlan, W.P., Trainer, V.L., 2016. An unprecedented coastwide toxic algal bloom linked to anomalous ocean conditions. *Geophys. Res. Lett.* 43, 10,366–10,376. doi:http://dx.doi.org/10.1002/2016GL070023.
- McHuron, E.A., Greig, D.J., Colegrove, K.M., Fleetwood, M., Spraker, T.R., Gulland, F.M.D., Harvey, J.T., Lefebvre, K.A., Frame, E.R., 2013. Domoic acid exposure and associated clinical signs and histopathology in Pacific harbor seals (*Phoca vitulina richardii*). *Harmful Algae* 23, 28–33. doi:http://dx.doi.org/10.1016/j.hal.2012.12.008.
- McKibben, S.M., Peterson, W., Wood, A.M., Trainer, V.L., Hunter, M., White, A.E., 2017. Climatic regulation of the neurotoxin domoic acid. *Proc. Natl. Acad. Sci. U. S. A.* 114, 239–244. doi:http://dx.doi.org/10.1073/pnas.1606798114.
- Mekebi, A., Blondina, G.J., Crane, D.B., 2009. Method validation of microcystins in water and tissue by enhanced liquid chromatography tandem mass spectrometry. *J. Chromatogr. A* 1216, 3147–3155. doi:http://dx.doi.org/10.1016/j.chroma.2009.01.095.
- Miller, M.A., Kudela, R.M., Mekebi, A., Crane, D., Oates, S.C., Tinker, M.T., Staedler, M., Miller, W.A., Toy-Choutka, S., Dominik, C., Hardin, D., Langlois, G., Murray, M., Ward, K., Jessup, D.A., 2010. Evidence for a novel marine harmful algal bloom: cyanotoxin (Microcystin) transfer from land to sea otters. *PLoS One* 5, e12576. doi:http://dx.doi.org/10.1371/journal.pone.0012576.
- Mlouka, A., Comte, K., Castets, A.-M., Bouchier, C., Tandeau de Marsac, N., 2004. The gas vesicle gene cluster from Microcystis aeruginosa and DNA rearrangements

- that lead to loss of cell buoyancy. *J. Bacteriol.* 186, 2355–2365. doi:<http://dx.doi.org/10.1128/JB.186.8.2355-2365.2004>.
- Nejad, E.S., Cloern, J., Schraga, T., 2017. Phytoplankton Species Composition, Abundance and Cell Size in San Francisco Bay: Microscopic Analyses of USGS Samples Collected 1992–2014. doi:<http://dx.doi.org/10.5066/F74F1P6P>.
- Novaczek, I., Madhyastha, M.S., Ablett, R.F., Donald, A., Johnson, G., Nijjar, M.S., Sims, D.E., 1992. Depuration of domoic acid from live blue mussels (*Mytilus edulis*). *Can. J. Fish. Aquat. Sci.* 49, 312–318. doi:<http://dx.doi.org/10.1139/f92-035>.
- Paerl, H., Huisman, J., 2008. Blooms like it hot. *Science* (80–) 320, 57.
- Paerl, H.W., Huisman, J., 2009. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. *Environ. Microbiol. Rep.* 1, 27–37. doi:<http://dx.doi.org/10.1111/j.1758-2229.2008.00004.x>.
- Paerl, H.W., Paul, V.J., 2012. Climate change: links to global expansion of harmful cyanobacteria. *Water Res.* 46, 1349–1363. doi:<http://dx.doi.org/10.1016/j.watres.2011.08.002>.
- Preece, E.P., Hardy, F.J., Moore, B.C., Bryan, M., 2017. A review of microcystin detections in Estuarine and Marine waters: environmental implications and human health risk. *Harmful Algae* 61, 31–45. doi:<http://dx.doi.org/10.1016/j.hal.2016.11.006>.
- Raimonet, M., Cloern, J.E., 2017. Estuary–ocean connectivity: fast physics, slow biology. *Glob. Change Biol.* 23 (6), 2345–2357.
- Reguera, B., Velo-Suárez, L., Raine, R., Park, M.G., 2012. Harmful dinophysis species: a review. *Harmful Algae* 14, 87–106. doi:<http://dx.doi.org/10.1016/j.hal.2011.10.016>.
- Rita, D.P., Valeria, V., Silvia, B.M., Pasquale, G., Milena, B., 2014. Microcystin contamination in sea mussel farms from the Italian southern Adriatic coast following cyanobacterial blooms in an artificial reservoir. *J. Ecosyst.* 2014, 1–11. doi:<http://dx.doi.org/10.1155/2014/374027>.
- Rundberget, T., Gustad, E., Samdal, I.A., Sandvik, M., Miles, C.O., 2009. A convenient and cost-effective method for monitoring marine algal toxins with passive samplers. *Toxicon* 53, 543–550. doi:<http://dx.doi.org/10.1016/j.toxicon.2009.01.010>.
- Ryan, J., Kudela, R., Birch, J., Blum, M., Bowers, H., Chavez, F., Doucette, G., Hayashi, K., Marin III, R., Mikulski, C., Pennington, J., Scholin, C., Smith, G., Woods, A., Zhang, Y., 2017. Causality of an extreme harmful algal bloom in Monterey Bay, California during the 2014–2016 northeast Pacific warm anomaly. *Geophys. Res. Lett.* 44 (11), 5571–5579.
- Shumway, S.E., Allen, S.M., Dee Boersma, P., 2003. Marine birds and harmful algal blooms: sporadic victims or under-reported events? *Harmful Algae* 2, 1–17. doi:[http://dx.doi.org/10.1016/S1568-9883\(03\)00002-7](http://dx.doi.org/10.1016/S1568-9883(03)00002-7).
- Silva de Assis, H.C., da Silva, C.A., Oba, E.T., Pamplona, J.H., Mela, M., Doria, H.B., Guiloski, I.C., Ramsdorf, W., Cestari, M.M., 2013. Hematologic and hepatic responses of the freshwater fish *Hoplias malabaricus* after saxitoxin exposure. *Toxicon* 66, 25–30. doi:<http://dx.doi.org/10.1016/j.toxicon.2013.01.012>.
- Sison-Mangus, M.P., Jiang, S., Kudela, R.M., Mehic, S., 2016. Phytoplankton-associated bacterial community composition and succession during toxic diatom bloom and non-bloom events. *Front. Microbiol.* 7, 1433. doi:<http://dx.doi.org/10.3389/fmicb.2016.01433>.
- Swanson, K., Flewelling, L., Byrd, M., Nunez, A., 2010. The 2008 Texas Dinophysis ovum bloom: distribution and toxicity. *Harmful Algae* 9 (2), 190–199.
- Thébault, J., Schraga, T.S., Cloern, J.E., Dunlavy, E.G., 2008. Primary production and carrying capacity of former salt ponds after reconnection to San Francisco Bay. *Wetlands* 28, 841–851. doi:<http://dx.doi.org/10.1672/07-190.1>.
- Trainer, V.L., Cochlan, W.P., Erickson, A., Bill, B.D., Cox, F.H., Borchert, J.A., Lefebvre, K.A., 2007. Recent domoic acid closures of shellfish harvest areas in Washington State inland waterways. *Harmful Algae* 6, 449–459. doi:<http://dx.doi.org/10.1016/j.hal.2006.12.001>.
- Trainer, V., Moore, L., Bill, B., Adams, N., Harrington, N., Borchert, J., da Silva, D., Eberhart, B.-T., 2013. Diarrhetic shellfish toxins and other lipophilic toxins of human health concern in Washington State. *Mar. Drugs* 11, 1815–1835. doi:<http://dx.doi.org/10.3390/md11061815>.
- Van Dolah, F., Doucette, G., Gulland, F., Rowles, T., Bossart, G., 2002. Impacts of Algal Toxins on Marine Mammals. doi:<http://dx.doi.org/10.1201/9780203165577.ch10>.
- Vasconcelos, V.M., 1995. Uptake and depuration of the heptapeptide toxin microcystin-LR in *Mytilus galloprovincialis*. *Aquat. Toxicol.* 32, 227–237. doi:[http://dx.doi.org/10.1016/0166-445X\(94\)00085-5](http://dx.doi.org/10.1016/0166-445X(94)00085-5).
- Villar-González, A., Rodríguez-Velasco, M., 2008. Assessment of the hydrolysis process for the determination of okadaic acid-group toxin ester: presence of okadaic acid 7-O-acyl-ester derivatives in Spanish shellfish. *Toxicon* 51, 765–773.
- Visciano, P., Schirone, M., Berti, M., Milandri, A., Tofalo, R., Suzzi, G., 2016. Marine biotoxins: occurrence, toxicity, regulatory limits and reference methods. *Front. Microbiol.* 7, 1051. doi:<http://dx.doi.org/10.3389/fmicb.2016.01051>.
- Wekell, J.C., Lefebvre, K.A., Hurst, J., 2004. Impacts of algal toxins on marine mammals. *J. Shellfish Res.* 23, 927–930.
- Wells, M.L., Trainer, V.L., Smayda, T.J., Karlson, B.S.O., Trick, C.G., Kudela, R.M., Ishikawa, A., Bernard, S., Wulff, A., Anderson, D.M., Cochlan, W.P., 2015. Harmful algal blooms and climate change: learning from the past and present to forecast the future. *Harmful Algae* 49, 68–93. doi:<http://dx.doi.org/10.1016/j.hal.2015.07.009>.
- Work, P., Downing-Kunz, M., Livsey, D., 2017. Record-high Specific Conductance and Water Temperature in San Francisco Bay During Water Year 2015. Open-File Report. doi:<http://dx.doi.org/10.3133/ofr20171022>.
- Yasumoto, T., Oshima, Y., Yamacuchi, M., 1978. Occurrence of a new type of shellfish poisoning in the Tohoku district. *Nippon Suisan Gakkaishi* 44, 1249–1255. doi:<http://dx.doi.org/10.2331/suisan.44.1249>.