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

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\textbf{A R T I C L E   I N F O}

Article history:
Received 5 September 2017
Received in revised form 10 February 2018
Accepted 10 February 2018
Available online xxx

Keywords:
Amnesic shellfish poisoning
Paralytic shellfish poisoning
Diarrhetic shellfish poisoning
Microcystin toxins
Shellfish
Chronic toxin exposure

\textbf{A B S T R A C T}

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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\textbf{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; Hallegaard, 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; Paele 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 (Gibble 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...
fisheries within SFB, but recreational and subsistence shellfish harvesting is common (Davis et al., 2002; Gibble 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* (Gibble 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 (Gibble et al., 2016; Gibble and Kudela, 2014; Lehman et al., 2005; Miller et al., 2010). Microcystins have been detected in marine mussels within SFB (Gibble et al., 2016), further documenting the occurrence of freshwater toxins in the marine environment (Gibble et al., 2016; Gibble 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 subembayments 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 Jasby, 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 (Sorben Technologies) in 100 μm 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
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 Schrarga, 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 °C) for processing of microcysts. 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 µg domoic acid per kg resin (µg 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 W. Extracts were clarified through a 0.22 µm PTFE filter and 3 mL was cleaned using Biotage ISOLUTEX 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 µg kg⁻¹ for tissue, 7.50 ng L⁻¹ for particulate samples, and 0.87 µg 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 °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 µL aliquot of the previously prepared domoic acid extract (in 10% MeOH) was set aside (labeled here as extract A), and 750 µL 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 µm PTFE filter. 1 mL of 90% MeOH extract was set aside for archival purposes, and extract A was added to produce 6.125 µL of total extract (combined and labeled here as extract B) for a final concentration of 90% MeOH. Extract B was then acidified with 6 µL 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.
Mussel samples for microcystin analysis were prepared according to Gibble et al., 2016. Grab, SPATT, and mussel samples were analyzed by LC/MS (Gibble et al., 2016; Mekebri et al., 2009). The MDL for microcystins was 0.10 μg kg⁻¹ for tissue, 0.10 ng L⁻¹ for particulate samples, and 0.05 μg kg⁻¹ for SPATT. Microcystins are reported as the sum of four congeners: MC-LR, MC-RR, MC-LA, and MC-YR. This follows the recommended guidelines for the California Office of Environmental Health Hazard Assessment (Butler et al., 2009).

2.5. PSTs analysis

Mussels were prepared for PST analysis following MaxSignal® Saxitoxin (PSP) Enzyme-Linked Immunosorbant Assay (ELISA) Test Kit Manual instructions (BIOO Scientific, USA). The reported detection limit from the manufacturer is 3 μg kg⁻¹ for mussel tissue as saxitoxin equivalents, with reported cross-reactivity varying from 4 to 100% for the various derivatives, and optimized for saxitoxin and decarbamoyl saxitoxin.

2.6. DSTs analysis

Mussels were prepared for DST analysis following extraction and hydrolysis by Villar-González and Rodríguez-Velasco (2008). Samples were quantified for DTX-1, DTX-2, and okadaic acid, using an external standard curve with NRC-CCRM standards. The MDL was 3.44 μg kg⁻¹ tissue. Concentrations of DSTs are reported as the sum of DTX-1, DTX-2, and okadaic acid.

3. Results

3.1. Particulate toxins in grab samples

Fig. 1 provides an overview of sampling locations, while Fig. 2 shows spatial and temporal patterns of toxin variability measured in particulate grab samples collected in SFB. The minimum, maximum, mean, median, and number of samples for each station are provided in Table 1. Eleven percent (n = 420) of grab samples had measurable domoic acid, and 51% (n = 405) had detectable microcystins. In 3% of samples there was detection of both domoic acid and microcystins. There was no correlation between presence of toxin and phytoplankton cell counts of toxigenic organisms for either toxin group.

3.2. Dissolved toxins in Solid Phase Adsorption Toxin Tracking (SPATT) samples

Toxin was detectable in 97% (n = 211) of SPATT in varying concentrations for domoic acid, and was present in both the marine and brackish/freshwater segments of SFB. Concentrations were elevated across all segments in the fall of 2014 through the spring of 2015. Microcystins were detected in 76% of SPATT (n = 210), most often in the freshwater Northern Estuary of SFB, and frequently in the marine portions. Combined, 100% of SPATT were positive for either domoic acid or microcystins (Fig. 3, Table 2), with 73% positive SPATT for both toxins.

3.3. Toxins in mussels

In 2012 (n = 11) and 2014 (n = 6) mussel homogenates from transplanted mussels obtained from Bodega Head, CA, were tested for all four toxin suites, including reference samples from Bodega Head. Domoic acid was detected in 100% of mussels for 2012 and 2014, including Bodega Head, ranging from 20.5 to 565 μg kg⁻¹. Microcystins were detected in 82% (2012) and 100% (2014) of mussel homogenates, but not in the reference mussels from Bodega Head. Concentrations ranged from <MDL to 18.9 μg kg⁻¹. PSTs were detected in 45% (2012) and 83% (2014) of homogenates, as well as in the reference mussels in 2014 (25.7 μg kg⁻¹), ranging from <MDL to 34.3 μg kg⁻¹. DSTs were found in 91% (2012) and 100% (2014) of homogenates, including reference mussels in 2012 and 2014. Concentrations in SFB ranged from <MDL to...
Table 1
Particulate concentrations in San Francisco Bay, Ca, 2011–2015. Stations correspond to Fig. 1, data is displayed graphically in Fig. 2.

<table>
<thead>
<tr>
<th>Particulate toxin</th>
<th>Domoic acid [ng L⁻¹]</th>
<th>Microcystin toxins [ng L⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td>max</td>
</tr>
<tr>
<td>Station</td>
<td></td>
<td></td>
</tr>
<tr>
<td>657</td>
<td>bdl</td>
<td>88.7</td>
</tr>
<tr>
<td>6</td>
<td>bdl</td>
<td>32.9</td>
</tr>
<tr>
<td>13</td>
<td>bdl</td>
<td>19.3</td>
</tr>
<tr>
<td>18</td>
<td>bdl</td>
<td>316.7</td>
</tr>
<tr>
<td>22</td>
<td>bdl</td>
<td>84.4</td>
</tr>
<tr>
<td>27</td>
<td>bdl</td>
<td>44.0</td>
</tr>
<tr>
<td>32</td>
<td>bdl</td>
<td>16.0</td>
</tr>
<tr>
<td>34/36</td>
<td>bdl</td>
<td>90.3</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>SPATT Toxin</th>
<th>Domoic acid [µg kg⁻¹]</th>
<th>Microcystin toxins [µg kg⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min</td>
<td>max</td>
</tr>
<tr>
<td>Suisun Bay</td>
<td>bdl</td>
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</tr>
<tr>
<td>San Pablo Bay</td>
<td>bdl</td>
<td>963.6</td>
</tr>
<tr>
<td>Central Bay</td>
<td>bdl</td>
<td>862.5</td>
</tr>
<tr>
<td>South-Central Bay</td>
<td>4.7</td>
<td>1948.0</td>
</tr>
<tr>
<td>South Bay</td>
<td>5.5</td>
<td>1650.5</td>
</tr>
</tbody>
</table>

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.

62.1 µg kg⁻¹, and the Bodega Head samples had 16.7 µg kg⁻¹ (2012) and 146 µg 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 Gibble 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 µg 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 µg kg⁻¹. The PSTs were detected in 59% of mussels (n = 73), ranging from <MDL to 29.4 µg kg⁻¹. The DSTs were detected in 71% of mussels (n = 34) ranging from <MDL to 430 µg 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
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 (Domínguez 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; Novaczeck 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 μg kg⁻¹ (20 ppm; Wekell et al., 2004) is roughly equivalent to 150 μg 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 μg kg⁻¹, suggesting that there was increased 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 μg 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 ~16 °C, followed by a decline at higher temperatures. The SFB exhibited mean temperatures of ~16–20 °C for the 2015 water year with peak (record-breaking) temperatures in Central Bay of >20 °C in August 2015 (Worke 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 PSTDs, 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 PSTDs, 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 PSTDs, and 94% had DSTs. Only one sample had acute toxin levels (for microcystins; Fig. 4). The concentration of domoic acid and PSTDs 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 μg PSTs per kg shellfish (160 μg kg⁻¹; Lewitus et al., 2012), and 10 ng microcystins per gram of fish (10 μg 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 μg 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 ± 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 μg L⁻¹ microcystins, while WHO identifies low risk at <10 μg 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 to 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 bio-accumulation 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.

Acknowledgements

Funding for this project was provided by NOAA MERHAB [grant A15NOS4780205, SFEI Contract 1084, 1006, and 1220, and NOAA IOOS [grant NA14NOS120148)]. Thank you for field support from Applied Marine Sciences, C. Martin, T. Schraga, E. Novick, E. Nejad, R. Romero, Z. Sylvester, and the captain and crew of the R/V Polaris. Thank you for laboratory support from K. Negrey, T. Schraga, T. Fredrickson, and Z. Sylvester. This is NOAA MERHAB publication MER209. [SS]

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