Harmful Algae 59 (2016) 59–66

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Evidence of freshwater algal toxins in marine shellfish: Implications for human and aquatic health

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ARTICLE INFO

Article history:
Received 12 May 2016
Received in revised form 26 September 2016
Accepted 26 September 2016
Available online 3 October 2016

Keywords:
Microcystis aeruginosa
Microcystin
Mytilus californianus
Mytilus trossulus
Mytilus galloprovincialis
Aquaculture

ABSTRACT

The occurrence of freshwater harmful algal bloom toxins impacting the coastal ocean is an emerging threat, and the potential for invertebrate prey items to concentrate toxin and cause harm to human and wildlife consumers is not yet fully recognized. We examined toxin uptake and release in marine mussels for both particulate and dissolved phases of the hepatotoxin microcystin, produced by the freshwater cyanobacterial genus *Microcystis*. We also extended our experimental investigation of particulate toxin to include oysters (*Crassostrea* sp.) grown commercially for aquaculture. California mussels (*Mytilus californianus*) and oysters were exposed to *Microcystis* and microcystin toxin for 24 h at varying concentrations, and then were placed in constantly flowing seawater and sampled through time simulating riverine flushing events to the coastal ocean. Mussels exposed to particulate microcystin purged the toxin slowly, with toxin detectable for at least 8 weeks post-exposure and maximum toxin of 39.11 ng/g after exposure to 26.65 μg/L microcystins. Dissolved toxin was also taken up by California mussels, with maximum concentrations of 20.74 ng/g after exposure to 7.74 μg/L microcystin, but was purged more rapidly. Oysters also took up particulate toxin but purged it more quickly than mussels. Additionally, naturally occurring marine mussels collected from San Francisco Bay tested positive for high levels of microcystin toxin. These results suggest that ephemeral discharge of *Microcystis* or microcystin to estuaries and the coastal ocean accumulate in higher trophic levels for weeks to months following exposure.

1. Introduction

While *Microcystis aeruginosa* blooms and associated toxins have long been recognized as a problem for freshwater systems, recent studies have identified significant and severe impairment in coastal receiving waters (Miller et al., 2010; Gibble and Kudela, 2014). Production of microcystin toxin is influenced by nutrient supply, light levels, and temperature, and although it is not regularly monitored in the marine environment, *M. aeruginosa* is somewhat tolerant of saltwater conditions and some microcystin toxins can be persistent in saline and freshwater ecosystems (Zehnder and Gorham, 1960; Tsuji et al., 1994; Jacoby et al., 2000; Welker and Steinburg, 2000; Robson and Hamilton, 2003; Ross et al., 2006; Tonk et al., 2007; Paerl and Huisman, 2008; Davis et al., 2009; Paerl and Otten, 2013; Gibble and Kudela, 2014). Microcystin is a known hepatotoxin and exposure to this toxin has impacted different marine trophic levels, including small planktonic invertebrates, fish, and large vertebrates (Demott and Moxter, 1991; Malbrouck and Kestemont, 2006; Richardson et al., 2007; Miller et al., 2010). The emergence of this toxin as both stable in marine receiving waters and harmful for upper marine trophic levels, including apex predators and humans, highlights the need for better understanding of trophic transfer to and effects on humans and wildlife health alike.

Marine bivalves, such as California mussels (*Mytilus californianus*), are particularly useful in assessing accumulation of toxins related to Harmful Algal Blooms (HABs) because they are widespread, are important prey of birds and marine mammals, and are also consumed by humans. Their unique life history traits both impact and increase their toxin accumulation ability. Because they are very active filter feeders and detritivores, these organisms have the ability to consume large quantities of cyanobacteria, and concentrate their toxins (Christoffersen, 1996). Several studies

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http://dx.doi.org/10.1016/j.hal.2016.09.007

have documented microcystin accumulation in freshwater and saltwater invertebrates (Vasconcelos, 1995; Williams et al., 1997; Amorim and Vasconcelos, 1998; Dionisio Pires et al., 2004; Kvitek and Bretz, 2005; Miller et al., 2010). However, trophic level interactions and vulnerability of organisms to toxins at different trophic levels has not been well defined (Turner and Tester, 1997; Lefebvre et al., 1999; Shumway et al., 2003; Kvitek and Bretz, 2005; Smith and Haney, 2006). If microcystin is transferred up the food chain, there may be several detrimental impacts for intermediate and apex predators, as well as for humans.

In California, microcystins have been recorded in the estuarine and near-shore marine environments in the Monterey Bay area (Miller et al., 2010; Gibble and Kudela, 2014), and more recently evidence for widespread contamination of California's watersheds by multiple toxins has been documented (Fetscher et al., 2015). San Francisco Bay is another large impacted bay, that displays chronic impairment, including poor water quality, eutrophication, and increases in harmful algal bloom activity (Cloern and Jassby, 2012). This is especially true in the upper estuarine environment where microcystins have been found to impact the food web (Lehman et al., 2005, 2008, 2010; Baxa et al., 2010). Despite significant upstream concentrations and the demonstrated ability for these toxins to be transferred through the marine food web (Miller et al., 2010), there is no routine monitoring of marine invertebrates for freshwater toxins in California. Recent attention to expanding microcystin occurrence (Gibble and Kudela, 2014; Fetscher et al., 2015) generates a need to help address this deficiency.

Aquaculture has been ongoing since the 1800's in Marin County, CA but has been closed in San Francisco Bay since the middle of the 1900's due to poor water quality. Tomales Bay, located immediately north in Marin County, CA, continues to have a vibrant aquaculture business (Carlsen et al., 1996). Historically this bay has been known as a pristine ecosystem, but because it is downstream of 3 major tributaries (Lagunitas, Olema, and Walker Creeks), it also has the potential to be impacted by downstream transport of freshwater toxins, particularly given the known impacts within the San Francisco estuary (Fischer et al., 1996; Lehman et al., 2005; Ger et al., 2009, 2010).

We investigated the rate of toxin accumulation and subsequent loss in the California mussel to determine the length of time it takes for mussels to clear toxin after a marine exposure event. This organism was chosen since it is both recreationally harvested for human consumption, and a common prey item for wildlife. In addition to laboratory experiments, mussels of the same genus (Bay mussel, *M. trossulus* and Mediterranean mussel, *M. galloprovincialis*) collected from San Francisco Bay were analyzed for microcystin LR, YR, RR, and LA to determine if microcystins are present in a tidally influenced estuarine food web. This work led us to examine the presence of microcystins in commercially raised oysters (*Crassostrea* sp.) to compare rates and levels of toxin uptake and loss between a species highly used by wildlife and a species highly used by humans. Because there is no current monitoring for microcystin in either aquaculture raised shellfish in California or recreationally harvested shellfish, there is concern that contamination of oysters sold for human consumption and contaminated naturally-occurring mussels may be going unnoticed.

2. Materials and methods

2.1. Tank experiments

To investigate depuration of both particulate and dissolved microcystin by California mussels, 3 separate tank experiment trials were performed. The first trial involved particulate microcystin toxin in low concentrations (average of 5.6 μg/L per tank); the second trial involved particulate microcystin toxin in higher concentration (average of 26 μg/L per tank); the third trial involved dissolved microcystin toxin in moderate concentration (average of 7.73 μg/L per tank). A naturally occurring bloom of *M. aeruginosa* was collected from Pinto Lake, in Watsonville, CA a well-known "hot spot" for microcystin toxin production in the Monterey Bay area (Miller et al., 2010) mixed with saline water (Instant Ocean, Spectrum Brand, Virginia) and administered to 3 separate tanks per trial. The total salinity in each tank was approximately 33–36 ppt to mimic ocean conditions, and all tanks were placed in a water-table filled with constantly flowing fresh seawater to maintain ambient temperature and exposure to typical coastal seawater. For the dissolved toxin experiment the Pinto Lake water was filtered through a 0.2 μm capsule filter to remove particulates.

A total of 120 California mussels used for the tank experiment trials were collected from Davenport Landing Beach in Monterey Bay, California. Once collected, mussels were acclimatized in constantly flowing filtered seawater for 24 h before the start of the trial. Three representative control mussels were sampled for microcystin toxin concentration via liquid chromatography mass spectrometry (LCMS) and the remaining mussels were divided into 3, 38 L tanks (39 mussels per tank). For each of 3 experimental trials, microcystin water was added to each tank, and mussels were placed in each tank for 24 h. For particulate microcystin trials, Pinto Lake water with associated toxic *Microcystis* biomass was used; testing prior to the experiments indicated that the majority of the toxin was intracellular. For dissolved trials, 0.2 μm filtered Pinto Lake water with known concentration of dissolved microcystins was used. At the start of each trial, whole water and cell counts were collected. After the 24-h immersion period, mussels were transferred to constantly flowing filtered seawater and sampled through time. At each time point 3 individual mussels and one whole water sample were taken from each tank and were assessed for levels of microcystin in the laboratory. Mussels from each tank were subsampled in intervals (24 h, 36 h, 48 h, 72 h, 96 h post initial exposure). After the 96 h time point, mussels in all tanks were sampled at weekly intervals.

In the laboratory, mussels were shucked and the entire mass of tissue was collected to simulate consumption. Mussels were homogenized, and body burden was evaluated via LCMS following the procedures adapted from and outlined in Vasconcelos (1995), Amorim and Vasconcelos (1998), Eriksson et al. (1989), and Mekebri et al. (2009). To address individual variation in toxin uptake, mussels from each tank and timepoint were homogenized using a BioHomogenizer (Model M133/1281-0, Biospec Products Inc., Oklahoma, USA) ~4 g of homogenized mussel tissue was extracted using 20 mL acid methanol, then sonicated for 30 s using a sonic dismembrator (Model 100; Thermo Fisher Scientific, Massachusetts, USA) at ~10 W. Samples were centrifuged for 10 min at 3400 rpm (Model IEC Centra CL2; Thermo Fisher Scientific, Massachusetts, USA) and then prepped for analysis using solid phase extraction as described by Mekebri et al. (2009)

Microcystin-LR, RR, YR, and LA in mussel tissue was analyzed by LCMS with electrospray ionization (ESI) and selected ion monitoring (SIM) on an Agilent 6130 with a Phenomenex Kinetix (100 × 2.10) C18 column. Whole water collected from the tanks at each time point were analyzed in the lab using 3 mL of whole water mixed with 3 mL 50% methanol. Samples were then sonicated using a sonic dismembrator (Model 100; Thermo Fisher Scientific, Massachusetts, USA) for 30 s at ~10 W, filtered (0.2 μm nylon syringe filter), and analyzed by direct injection of 50 μL onto the LCMS column, following Mekebri et al. (2009) but modified to account for use of SIM rather than tandem mass-spectrometry (Kudela, 2011). A gradient-elution method was used with HPLC water (solvent A) and LCMS acetonitrile (solvent B), both acidified with 0.1% formic acid, as the mobile phase. The gradient was as described in Mekebri et al. (2009), starting with 95:5 solvent A:B

and ending with 25:75 at 19 min, held for 1 min, then followed by a 5 min equilibration at initial conditions prior to injection of the next sample. Samples were calibrated with standard curves (for each batch of samples) using pure standards (Fluka 33578 and Sigma–Aldrich M4194). Standards were run again at the end of the run for sample runs lasting more than 8 h. For a subset of mussel samples, standard addition was used to verify peak identification. The Minimum Detection Limit (MDL) for particulate and dissolved toxins was 0.10 μg/L. For shellfish samples the MDL depends on the amount of mussel tissue analyzed and was 0.10 ng/g for 1 g tissue (sample sizes were typically 1–4 g).

Water samples collected for cell counts were preserved with 10 mL 25% glutaraldehyde to 40 mL of sample (5% final concentration). Preserved samples were sonicated using a sonic dismembrator (Model 100; Thermo Fisher Scientific, Massachusetts, USA) at ~4 W for 1 min to break up colonies. 1 mL was then filtered using a 0.2 μm black polycarbonate (PCTE) membrane filter (Poretics Corp, Livermore, CA). Filters were examined via epifluorescence microscopy (Zeiss Axioskop, Zeiss Microscopy, Thornwood, NY) at a magnification of 400 x and individual cells were counted.

2.2. Field experiments

Bay mussels and Mediterranean mussels, an invasive species, are sympatric, inhabit the same region of San Francisco Bay, and are known to hybridize (McDonald and Koehn, 1988; Sarver and Foltz, 1993; Suchanek et al., 1997). Differentiation between species often requires the use of molecular methods. Because of this difficulty in identification, mussels collected in San Francisco Bay are conservatively identified as Bay/Mediterranean mussels. To relate our tank experiment results to naturally occurring assemblages, 8 Bay/Mediterranean mussels were collected monthly from 4 locations in San Francisco Bay: Point Isabel, Point Potrero, Berkeley Marina, Alameda Island from April 2015-September 2015, and from a fifth location, Romberg-Tiburon Center from August 2015-September 2015 (Fig. 4). Salinities at these sites vary from between 27 and 32 ppt (USGS, 2016). Mussels were prepared for LCMS analysis following the methods described in *2.1* but were examined individually rather than pooled to further investigate individual variability.

2.3. Commercial oyster tank experiments

To determine whether invertebrate species used for aquaculture in Tomales Bay were impacted by microcystin, we purchased oysters from a commercial aquaculture vendor in Tomales Bay for immediate testing. Additional oysters were purchased from the same vendor for use in tank experiments at a later date. The standard distribution protocol for the supplier includes placing oysters in a tank of fresh filtered flowing water for five days prior to release for public purchase and consumption. Additionally, oysters were cultivated and harvested under 28500–28519.5 of the California State Health and Safety Code.

Oysters purchased for immediate testing were processed following methods described above for mussels. Oysters purchased subsequently for tank experimentation were acclimated in constantly flowing filtered seawater for 24 h before experimentation. Three control oysters were collected and processed before the start of the trials, and the remaining oysters were separated equally into 3 tanks. *Microcystis* was again collected from Pinto Lake, CA mixed with saline water (Instant Ocean, Spectrum Brand, Virginia) and administered to 3 separate tanks with a concentration of 7.71 μg/L microcystin. Cell counts were collected and analyzed as described above. Oysters in all 3 tanks were exposed to microcystin for 24 h, after which they were placed in a water table with constantly flowing filtered seawater for the remainder of the trial. Oysters were sampled and processed as described in *2.1*.

3. Results

3.1. Particulate microcystin trials—low microcystin

Cell count results indicated that seawater tanks contained an average of 1,815,640 cells/mL, with an average of 5.6 μg/L total microcystin during the 24-h shock period. Following the 24-h shock period, constantly flowing seawater did not contain microcystin (<minimum detection limit, MDL), and no mussel mortality was observed. Control mussels collected from nearby Monterey Bay and analyzed before the start of the trials were negative for microcystin (<MDL). Mussels used in the trials took up microcystin toxin during the 24-h shock period (Fig. 1), reaching tissue concentrations similar to the water they were placed in. Mussels retained toxin for longer than eight weeks after a single 24 h exposure. Toxin levels slowly decreased after the 24-h exposure with a noticeable drop in toxin 3 weeks post exposure. At the end of the eight week trials, microcystin toxin was low, but still detectable in mussel tissue.

3.2. Particulate microcystin trials—high microcystin

Cell count results indicated that average water per tank contained approximately 7,045,229 cells/mL, and whole water samples indicated that tanks had an average of 26.65 μg/L total microcystin during the 24-h shock period. Post shock period, constantly flowing seawater did not contain microcystin (<MDL), and no mussel mortality was observed. Control mussels analyzed before high microcystin particulate trials were negative for microcystin (<MDL). Mussels again took up particulate microcystin toxin at levels similar to the water they were placed in (Fig. 2). Mussels again retained toxin for longer than eight weeks. However, toxin levels remained high for much longer. There was a prominent drop in toxin 3 to 4 weeks post exposure. At the end of the eight week trials, microcystin toxin was again low, but still detectable in mussel tissue.

3.3. Dissolved microcystin trials

Whole water samples taken before the start of the 24-h shock period indicated an average of 7.74 μg/L total microcystin. Preceding the 24-h shock period, constantly flowing seawater did not contain microcystin (<MDL), and no mussel mortality occurred. Control mussels analyzed preceding the 24-h shock period were negative for microcystin (<MDL). Mussels took up dissolved toxin within 24 h (Fig. 3), at levels similar to the water they were placed in during the 24-h shock. Mussels in the trials immediately decreased in toxin at 36 h and fell to levels below the MDL by 72 h post exposure (36 h after being removed from exposure; Fig. 3).

3.4. Field experiments

Naturally occurring mussels were harvested from multiple sites in San Francisco Bay. We detected microcystin toxins in at least one individual every month except for August 2015 at Romberg-Tiburon Center. Naturally occurring mussels exhibited a large range of toxin concentrations, as is evident in September for individual mussels collected from Berkeley Marina, where the toxins ranged from <MDL −416.23 ng/g (n = 8). September was the most toxic month for all sites, except for the northernmost site, Point Potrero, which was most toxic in August (Fig. 4).

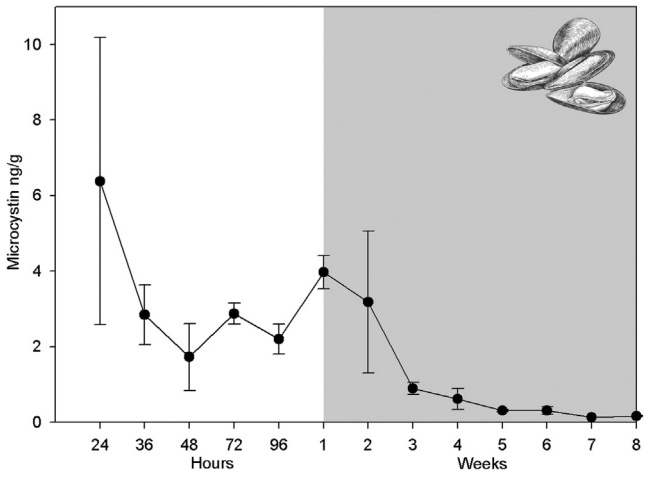

Fig. 1. *M. californianus* and particulate microcystin toxin in low concentrations experimental trials. X-axis begins at 24 h denoting removal from water containing microcystins.

3.5. Commercial oyster tank experiments

Oysters that were evaluated immediately upon purchase tested positive for low levels of microcystin (3.42 +/− 2.24 ng/g, n = 6). However, a second batch of oysters purchased at a later date and used for tank experiments were negative for microcystin (<MDL). At the beginning of the experimental trials, cell count results indicated that average cells per tank were 1,068,023 cells/mL, and whole water samples indicated that tanks had an average of 7.71 μg/L total microcystin during the 24-h shock period. Post shock period, constantly flowing seawater did not contain microcystin (<MDL), and no oyster mortality was observed. During experimentation, oysters took up microcystin toxin within 24 h. In contrast to the mussels, toxin in the oysters was lower on average than concentrations in the shock treatment tanks (4.88 ng/g; Fig. 5). Oysters in the trials decreased in toxin content at 36 h and maintained a somewhat steady level of toxin until 4 weeks post exposure when there was another decrease. Eight weeks post exposure, oyster samples still contained low but detectable levels of toxin.

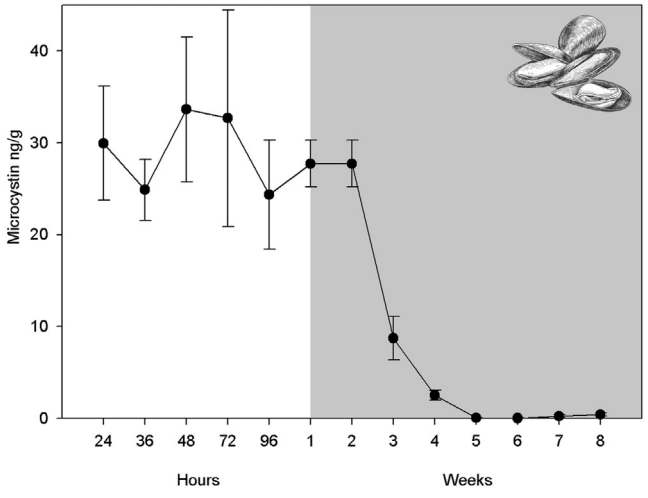

Fig. 2. *M. californianus* and particulate microcystin toxin in high-concentration experimental trials. X-axis begins at 24 h denoting removal from water containing microcystins.

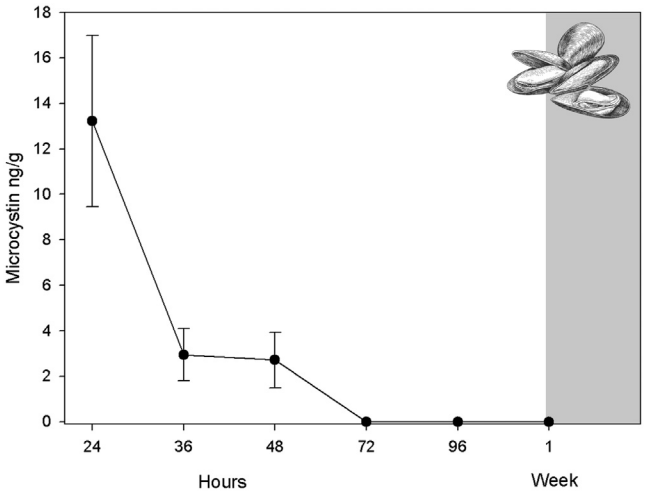

Fig. 3. *M. californianus* and dissolved microcystin toxin experimental trials. X-axis begins at 24 h denoting removal from water containing microcystins.

4. Discussion

Toxic marine and freshwater HAB occurrence is a worldwide problem exacerbated by the deterioration of ecosystem health, eutrophication, and increasingly warmer climate due to human activities (Zehnder and Gorham, 1960; Welker and Steinburg, 2000; Guo, 2007; Paerl and Huisman, 2008, 2009; Davis et al., 2009; Kudela, 2011). Unfortunately, the coastal ocean, which is already affected by marine HAB occurrence is now also influenced by the transport of freshwater toxin to the marine environment, which ultimately impacts the marine food web. As a relevant example, Preece et al. (2015) deployed caged mussels (*M. trossulus*) in Puget Sound, WA to document transfer of microcystins from a nearby lake into marine waters, and also demonstrated transfer from freshwater to marine receiving waters. When in contact with seawater, *M. aeruginosa* lyses and releases microcystin toxin within 48 h (Miller et al., 2010) making both particulate and dissolved microcystin potential concerns for shellfish consumption. Our results indicate that both forms of toxin are concentrated by shellfish and consequently, are a concern for human and wildlife health, and for the aquaculture and fishing industries. We have shown experimentally that California mussels bioaccumulate toxin quickly in both particulate and dissolved forms, at high and low levels of exposure, and they release toxin slowly, allowing for rapid detection in real time and conservative estimates for clearance of toxin. Further investigation by examination of shellfish species can ultimately illuminate temporal and spatial patterns and identify long-term trends in microcystin occurrence, which may better aid in future monitoring practices. However, perhaps more importantly, this toxin could be posing a real and unrecognized threat to human health globally.

Particulate uptake of microcystin toxin by animal cells, and toxicology of microcystin toxin has been investigated in the past (Eriksson et al., 1990; Dawson, 1998; Campos and Vasconcelos, 2010). As a hepatotoxin, microcystin targets the liver cells specifically, where subsequent to hepatocellular uptake, it inhibits protein phosphatases by binding to enzymes, which later causes the failure of liver cells (Eriksson et al., 1990; Dawson, 1998; Campos and Vasconcelos, 2010). However, routes of ingestion of microcystin toxin have been less well defined. The currently accepted assumption is that the main mode for uptake of cyanobacterial toxins is through consumption of particulate forms (Yokoyama and Park, 2003; White et al., 2006; Ibelings and Chorus, 2007). Some studies speculate that dissolved toxin is somewhat

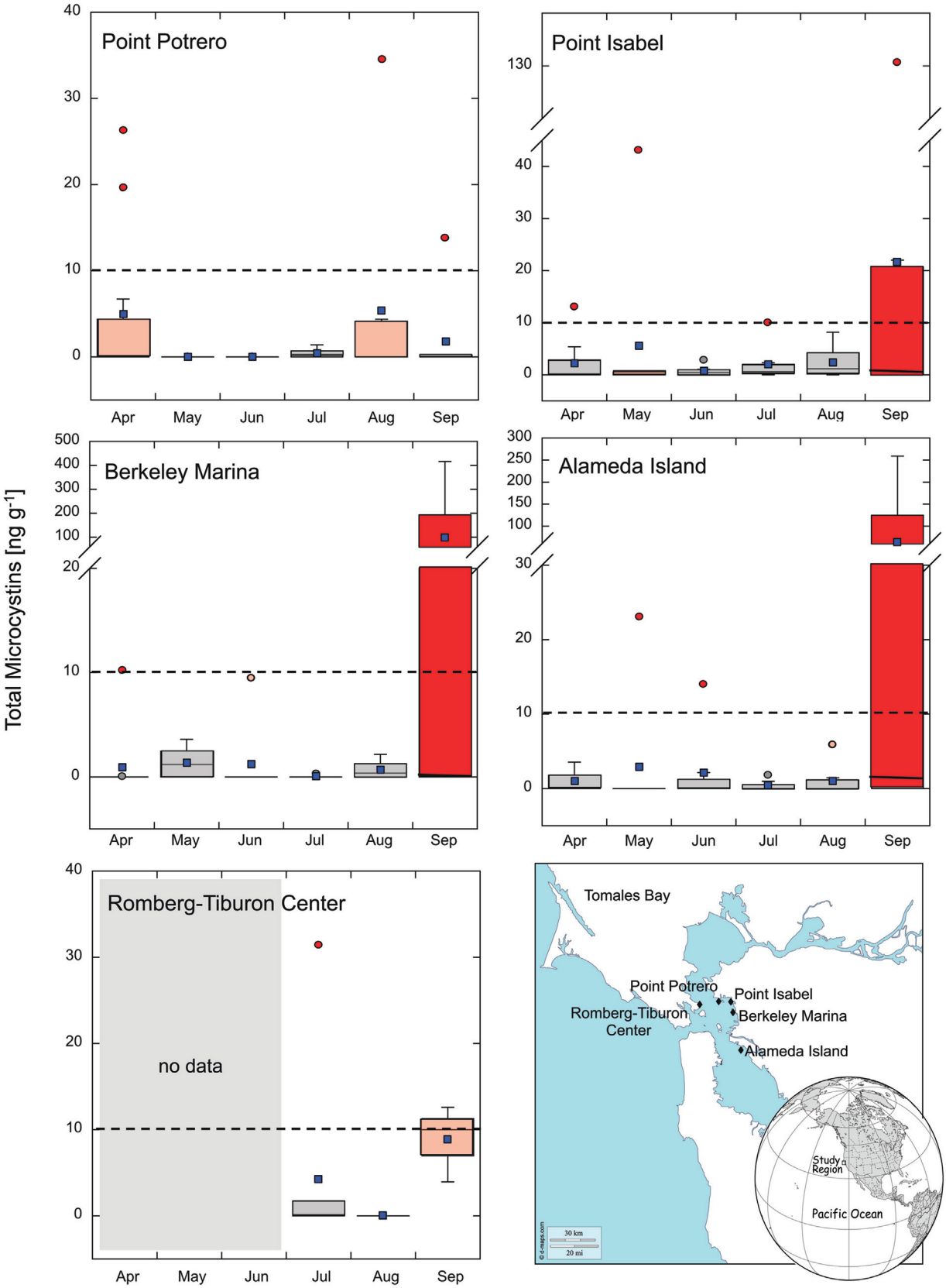

Fig. 4. Environmental samples of mussels collected monthly from central San Francisco Bay for April to September 2015. Boxes are the 25th and 75th percentile, and whiskers indicate 1.5 IQR. Dots are statistical outliers, blue squares are the means, and the black dotted line indicates the OEHHA action level for fish at 10 ng/g. Bars and outliers are colored via the following scheme: levels of toxin <5 ng/g are grey, levels of toxin between 5 and 10 ng/g are light red, and levels of toxin >10 ng/g are bright red. The outliers also follow that scheme, so just the > 10 ng/g outliers are bright red.

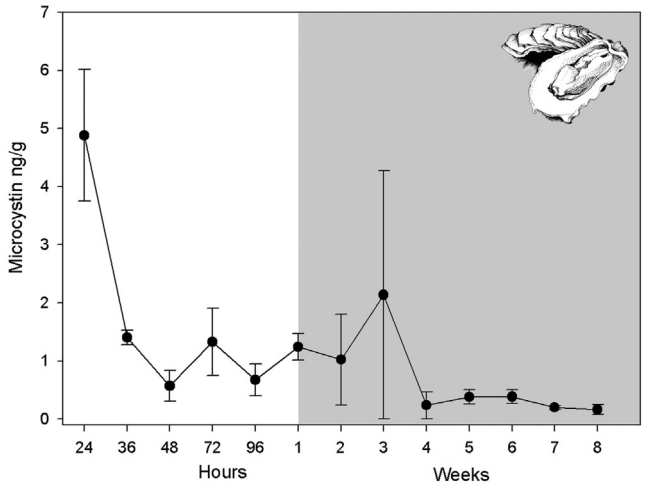

Fig. 5. Commercial oyster and microcystin toxin experimental trials. X-axis begins at 24 h denoting removal from water containing microcystins.

inconsequential to organisms, citing that levels of dissolved toxin may not be high in the natural environment, or uptake of dissolved toxin may be unimportant (Ozawa et al., 2003; Ibelings and Chorus, 2007). However, Karjalainen et al. (2003) showed that planktonic grazers can take up dissolved nodularin toxin, and our study shows the substantial uptake of dissolved microcystin toxin in marine mussels, which emphasizes the fact that both types of ingestion play a role in food web transport and bioaccumulation, and should be investigated further. Currently the mechanism for concentrating dissolved microcystin in shellfish remains unknown.

The fact that marine shellfish species take up and incorporate dissolved toxin into tissues may provide an even greater likelihood that prey species down stream of *Microcystis* bloom activity will encounter microcystin toxin at some time during the year. Because microcystin toxin is both stable and environmentally persistent, it remains in the environment for extended periods of time (Cousins et al., 1996; Robson and Hamilton, 2003; Ross et al., 2006; Tonk et al., 2007). Dissolved microcystin may also be transported further and extend into more distant environments than particulate forms, which increases the possibility of exposure downstream of bloom formation and toxin production. There is a general assumption that because *Microcystis* lyses within 48 h in the marine environment, the expected encounter and exposure rates of this toxin in saline water is minimal. However, our results strongly contradict this assumption, with demonstrated bioaccumulation of both particulate and dissolved toxins.

During field sampling of naturally occurring mussels, we found microcystin toxin in mussels at all sites, during all months except for August at the most oceanic site. This result was somewhat unexpected because field sampling occurred during a 6-month period when large blooms of *Microcystis* are less likely to occur (Lehman et al., 2005, 2008) and freshwater input is minimal (Cloern, 1996). The variation in toxin load among tested mussels was substantial (<MDL—416.23 ng/g) leading us to us to further elucidate the potential bias in examining individual organisms. Individuals were pooled during tank experimentation to deal with this bias. The differential concentrations of toxin shown in Fig. 4 may have been caused by fluctuating concentrations of particulate and dissolved microcystin to different areas of the nearshore marine environment, or may have been caused by differential uptake and retention rates by individuals.

Because our sampling locations were in San Francisco Bay, a brackish environment, and *Microcystis* does not grow in salinities above 5 for this region (Lehman et al., 2005), it is likely that mussels encountered microcystin originating in the upper San Francisco Estuary. *Microcystis* laden freshwater runoff has been documented in both San Francisco Bay and Monterey Bay in the nearshore environment, and runoff is known to carry microcystin toxin to downstream areas (Lehman et al., 2005; Paerl and Huisman, 2008; Miller et al., 2010; Gibble and Kudela, 2014). Because of these factors, it is perhaps even more surprising that toxin was detected in mussels during a period of catastrophic drought. During dry seasons, there is a tendency for reduced toxin occurrence (Paerl and Huisman, 2008; Miller et al., 2010; Gibble and Kudela, 2014), because nutrients necessary to sustain blooms are often delivered to bodies of water containing *M. aeruginosa* through both groundwater and surface runoff from land (Paerl and Huisman, 2008). Drought periods theoretically should make detecting microcystin in organisms that are downstream of *M. aeruginosa* colony and bloom formation less likely. However, Lehman et al. (2013) report that warm, dry conditions such as occurred during our sampling period, in a multi-year drought, could lead to westward (estuarine) expansion of *Microcystis*, leading to longer duration of blooms, greater spatial extent, and more impacts to higher trophic levels in response to predicted climate change. Our findings support the theory that downstream in the estuary, rain events following periods of higher bloom activity may increase dispersal of toxin found in mussels collected in San Francisco Bay. Our results also suggest that ephemeral discharge to the coastal ocean could have the potential to carry toxin to higher trophic levels for weeks to months following exposure.

Our results also indicated that oysters used in aquaculture in the adjacent Tomales Bay are at risk for microcystin toxin exposure. Given the various agricultural practices and sources of nutrient loading upstream of Tomales Bay's main tributaries, toxin produced higher in the watershed may be traveling from these freshwater sources (Lewis et al., 2005). Oysters purchased from a local commercial vendor and prepared for public consumption contained low but detectable levels of microcystin toxin. In addition, results of experimentation in this study also indicate that while oysters did not become as toxic as California mussels, oysters did accumulate microcystins within 24 h of contact, and exhibited low but detectable levels of microcystin toxin at 8 weeks post exposure. Shellfish aquaculture practices vary globally. While most counties have initiated extensive monitoring programs to prevent human illness from harmful algal blooms, many developing countries do not have this type of organization (Shumway, 1990). Because it is a freshwater toxin, microcystin is not frequently monitored for in marine environments where aquaculture operations exist. It is even less likely that this toxin would be monitored for in countries where less advanced aquaculture practices are used (Shumway, 1990). Two other studies (De Pace et al., 2014; Preece et al., 2015), documented presence of microcystins close to or above the World Health Organization (WHO) Tolerable Daily Intake (TDI; 24 μg/kg wet weight) in marine mussels. Here we documented levels routinely exceeding California Office of Environmental Health and Hazard Assessment (OEHHA; 10 ng/g wet weight for microcystins per week in seafood) and WHO guidelines. From our environmental mussel samples, between 8% and 16% of the individual mussels at each site exceeded the OEHHA guideline for weekly fish consumption, and in the month of September, four mussels from Berkeley Marina and Alameda Island had >230 ng/g of microcystin toxins, with one mussel at 416.23 ng/g of toxin, more than 40-fold the suggested weekly intake for consumption of fish (Fig. 4).

The results of our study elucidate an extensive unrecognized problem for environmental health, wildlife and human health alike. Cyanobacterial blooms with toxin production can have debilitating effects on ecosystems, leading to eutrophication, light

limitation of other species, reduction of food web efficiency, and mortality of species living in the environment (Jones, 1987; Paerl and Ustach, 1982; Havens 2008). Such impacts are detectable in both marine and freshwater environments in California at many different trophic levels (Miller et al., 2010). The implications of environmental degradation and trophic transfer of microcystin toxin is important not only for California, but also strongly suggest that communities reliant on aquaculture and wild-harvest mussels for sustenance worldwide are at risk. A combined real-time monitoring process including water quality and biomarker species monitoring is recommended to control detrimental effects and to better understand the breadth of impacts caused by this suite of toxins. We advocate immediate use of bivalves for monitoring and management, especially for aquaculture practices where low levels of toxins were identified in commercially sold shellfish. The structure to implement these recommendations presently exists and auxiliary testing could easily compliment current monitoring practices.

Acknowledgements

We would like to thank Kendra Hayashi, Rosemary Romero, and Regina Radan for lab and field support, and Betsy Steele for facilities support. This work was completed with salary and tuition assistance from the NOAA Nancy Foster Scholarship program (CMG) NOAA award number: NA10SEC4810031. Collection of samples was completed under California Department of Fish and Wildlife Scientific Collecting Permit #12147, and Monterey Bay National Marine Sanctuary Permit #MBNMS-2012-005.[CG]

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