Title:

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

Authors:

Corinne M. Gibble[1], Melissa B. Peacock[1,2,3], Raphael M. Kudela[1]

Author Affiliation:

[1]Ocean Sciences Department, 1156 High Street, University of California, Santa Cruz, CA 95064, USA, Email: cgibble@ucsc.edu, kudela@ucsc.edu

[2]San Francisco Estuary Institute, 4911 Central Avenue, Richmond Ca 94804, USA, Email: mpeacock@nwic.edu

[3]Native Environmental Science, Northwest Indian College, 2522 Kwina Rd, Bellingham, Wa, 98226, USA, Email: mpeacock@nwic.edu

Corresponding Author:

C.M. Gibble, University of California, Santa Cruz, Kudela Laboratory, 1156 High Street, Santa Cruz, CA 95064, Tel: +1 831-419-8169, Email: cgibble@ucsc.edu

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 hours 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 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; Baxa et al., 2010; Lehman 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; Lehman et al., 2010, 2013).

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

109 experiments, mussels of the same genus (Bay mussel, *M. trossulus* and Mediterranean
110 mussel, *M. galloprovincialis*) collected from San Francisco Bay were analyzed for
111 microcystin LR, YR, RR, and LA to determine if microcystins are present in a tidally
112 influenced estuarine food web. This work led us to examine the presence of microcystins
113 in commercially raised oysters (*Crassostrea sp.*) to compare rates and levels of toxin
114 uptake and loss between a species highly used by wildlife and a species highly used by
115 humans. Because there is no current monitoring for microcystin in either aquaculture
116 raised shellfish in California or recreationally harvested shellfish, there is concern that
117 contamination of oysters sold for human consumption and contaminated naturally-
118 occurring mussels may be going unnoticed.
119
120 **2. Materials and Methods**
121 *2.1 Tank Experiments.*
122 To investigate depuration of both particulate and dissolved microcystin by California
123 mussels, 3 separate tank experiment trials were performed. The first trial involved
124 particulate microcystin toxin in low concentrations (average of 5.6 µg/L per tank); the
125 second trial involved particulate microcystin toxin in higher concentration (average of 26
126 µg/L per tank); the third trial involved dissolved microcystin toxin in moderate
127 concentration (average of 7.73 µg/L per tank). A naturally occurring bloom of *M.*
128 *aeruginosa* was collected from Pinto Lake, in Watsonville, CA a well-known "hot spot"
129 for microcystin toxin production in the Monterey Bay area (Miller et al. 2010) mixed
130 with saline water (Instant Ocean, Spectrum Brand, Virginia) and administered to 3

separate tanks per trial. The total salinity in each tank was approximately 33-36ppt 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 hours 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 liter 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 hours. 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-hour 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. (1998), 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) ~4g of homogenized mussel tissue was extracted using 20mL acid methanol, then sonicated for 30 seconds using a sonic dismembrator (Model 100; Thermo Fisher Scientific, Massachusetts, USA) at ~10 W. Samples were centrifuged for 10 minutes 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 40mL 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 minute 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,

196 Thornwood, NY) at a magnification of 400x and individual cells were counted.

197

198 *2.2 Field Experiments*

199 Bay mussels and Mediterranean mussels, an invasive species, are sympatric, inhabit the
200 same region of San Francisco Bay, and are known to hybridize (McDonald and Koehn,
201 1988; Sarver and Foltz, 1993; Suchanek et al., 1997). Differentiation between species
202 often requires the use of molecular methods. Because of this difficulty in identification,
203 mussels collected in San Francisco Bay are conservatively identified as
204 Bay/Mediterranean mussels. To relate our tank experiment results to naturally occurring
205 assemblages, 8 Bay/Mediterranean mussels were collected monthly from 4 locations in
206 San Francisco Bay: Point Isabel, Point Potrero, Berkeley Marina, Alameda Island from
207 April 2015-September 2015, and from a fifth location, Romberg-Tiburon Center from
208 August 2015-September 2015 (Fig. 4). Salinities at these sites vary from between 27 and
209 32 ppt (USGS 2016). Mussels were prepared for LCMS analysis following the methods
210 described in *2.1* but were examined individually rather than pooled to further investigate
211 individual variability.

212

213 *2.3 Commercial Oyster Tank Experiments*

214 To determine whether invertebrate species used for aquaculture in Tomales Bay were
215 impacted by microcystin, we purchased oysters from a commercial aquaculture vendor in
216 Tomales Bay for immediate testing. Additional oysters were purchased from the same
217 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 hours 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 hours, 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-hour shock period. Following the 24-hour shock period, constantly flowing seawater did not contain

240 microcystin (< minimum detection limit, MDL), and no mussel mortality was observed.
241 Control mussels collected from nearby Monterey Bay and analyzed before the start of the
242 trials were negative for microcystin (<MDL). Mussels used in the trials took up
243 microcystin toxin during the 24-hour shock period (Fig.1), reaching tissue concentrations
244 similar to the water they were placed in. Mussels retained toxin for longer than eight
245 weeks after a single 24 h exposure. Toxin levels slowly decreased after the 24-hour
246 exposure with a noticeable drop in toxin 3 weeks post exposure. At the end of the eight
247 week trials, microcystin toxin was low, but still detectable in mussel tissue.
248
249 *3.2 Particulate Microcystin Trials - High Microcystin*
250 Cell count results indicated that average water per tank contained approximately
251 7,045,229 cells/ mL, and whole water samples indicated that tanks had an average of
252 26.65 µg/L total microcystin during the 24-hour shock period. Post shock period,
253 constantly flowing seawater did not contain microcystin (<MDL), and no mussel
254 mortality was observed. Control mussels analyzed before high microcystin particulate
255 trials were negative for microcystin (<MDL). Mussels again took up particulate
256 microcystin toxin at levels similar to the water they were placed in (Fig. 2). Mussels
257 again retained toxin for longer than eight weeks. However, toxin levels remained high for
258 much longer. There was a prominent drop in toxin 3 to 4 weeks post exposure. At the end
259 of the eight week trials, microcystin toxin was again low, but still detectable in mussel
260 tissue.
261

3.3 Dissolved Microcystin Trials

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

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-hour 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 hours. 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 hours 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.

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; Davis et al., 2009; Paerl and Huisman, 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

306 from freshwater to marine receiving waters. When in contact with seawater, *M.*
307 *aeruginosa* lyses and releases microcystin toxin within 48 hours (Miller et al., 2010)
308 making both particulate and dissolved microcystin potential concerns for shellfish
309 consumption. Our results indicate that both forms of toxin are concentrated by shellfish
310 and consequently, are a concern for human and wildlife health, and for the aquaculture
311 and fishing industries. We have shown experimentally that California mussels
312 bioaccumulate toxin quickly in both particulate and dissolved forms, at high and low
313 levels of exposure, and they release toxin slowly, allowing for rapid detection in real time
314 and conservative estimates for clearance of toxin. Further investigation by examination of
315 shellfish species can ultimately illuminate temporal and spatial patterns and identify long-
316 term trends in microcystin occurrence, which may better aid in future monitoring
317 practices. However, perhaps more importantly, this toxin could be posing a real and
318 unrecognized threat to human health globally.
319
320 Particulate uptake of microcystin toxin by animal cells, and toxicology of microcystin
321 toxin has been investigated in the past (Eriksson et al., 1990; Dawson, 1998; Campos and
322 Vasconcelos, 2010). As a hepatotoxin, microcystin targets the liver cells specifically,
323 where subsequent to hepatocellular uptake, it inhibits protein phosphatases by binding to
324 enzymes, which later causes the failure of liver cells (Eriksson et al., 1990; Dawson,
325 1998; Campos and Vasconcelos, 2010). However, routes of ingestion of microcystin
326 toxin have been less well defined. The currently accepted assumption is that the main
327 mode for uptake of cyanobacterial toxins is through consumption of particulate forms

328 (Yokoyama and Park, 2003; White et al., 2006; Ibelings and Chorus, 2007). Some studies
329 speculate that dissolved toxin is somewhat inconsequential to organisms, citing that
330 levels of dissolved toxin may not be high in the natural environment, or uptake of
331 dissolved toxin may be unimportant (Ozawa et al., 2003; Ibelings and Chorus, 2007).
332 However, Karjalainen et al. (2003) showed that planktonic grazers can take up dissolved
333 nodularin toxin, and our study shows the substantial uptake of dissolved microcystin
334 toxin in marine mussels, which emphasizes the fact that both types of ingestion play a
335 role in food web transport and bioaccumulation, and should be investigated further.
336 Currently the mechanism for concentrating dissolved microcystin in shellfish remains
337 unknown.
338
339 The fact that marine shellfish species take up and incorporate dissolved toxin into tissues
340 may provide an even greater likelihood that prey species down stream of *Microcystis*
341 bloom activity will encounter microcystin toxin at some time during the year. Because
342 microcystin toxin is both stable and environmentally persistent, it remains in the
343 environment for extended periods of time (Cousins et al., 1996; Robson and Hamilton,
344 2003; Ross et al., 2006; Tonk et al., 2007). Dissolved microcystin may also be
345 transported further and extend into more distant environments than particulate forms,
346 which increases the possibility of exposure downstream of bloom formation and toxin
347 production. There is a general assumption that because *Microcystis* lyses within 48 hours
348 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

371 more surprising that toxin was detected in mussels during a period of catastrophic
372 drought. During dry seasons, there is a tendency for reduced toxin occurrence (Paerl and
373 Huisman, 2008; Miller et al., 2010; Gibble and Kudela, 2014), because nutrients
374 necessary to sustain blooms are often delivered to bodies of water containing *M.*
375 *aeruginosa* through both groundwater and surface runoff from land (Paerl and Huisman,
376 2008). Drought periods theoretically should make detecting microcystin in organisms that
377 are downstream of *M. aeruginosa* colony and bloom formation less likely. However,
378 Lehman et al. (2013) report that warm, dry conditions such as occurred during our
379 sampling period, in a multi-year drought, could lead to westward (estuarine) expansion of
380 *Microcystis*, leading to longer duration of blooms, greater spatial extent, and more
381 impacts to higher trophic levels in response to predicted climate change. Our findings
382 support the theory that downstream in the estuary, rain events following periods of higher
383 bloom activity may increase dispersal of toxin found in mussels collected in San
384 Francisco Bay. Our results also suggest that ephemeral discharge to the coastal ocean
385 could have the potential to carry toxin to higher trophic levels for weeks to months
386 following exposure.
387
388 Our results also indicated that oysters used in aquaculture in the adjacent Tomales Bay
389 are at risk for microcystin toxin exposure. Given the various agricultural practices and
390 sources of nutrient loading upstream of Tomales Bay's main tributaries, toxin produced
391 higher in the watershed may be traveling from these freshwater sources (Lewis et al.,
392 2005). Oysters purchased from a local commercial vendor and prepared for public

393 consumption contained low but detectable levels of microcystin toxin. In addition, results
394 of experimentation in this study also indicate that while oysters did not become as toxic
395 as California mussels, oysters did accumulate microcystins within 24 hours of contact,
396 and exhibited low but detectable levels of microcystin toxin at 8 weeks post exposure.
397 Shellfish aquaculture practices vary globally. While most counties have initiated
398 extensive monitoring programs to prevent human illness from harmful algal blooms,
399 many developing countries do not have this type of organization (Shumway, 1990).
400 Because it is a freshwater toxin, microcystin is not frequently monitored for in marine
401 environments where aquaculture operations exist. It is even less likely that this toxin
402 would be monitored for in countries where less advanced aquaculture practices are used
403 (Shumway 1990). Two other studies (De Pace et al., 2014; Preece et al., 2015),
404 documented presence of microcystins close to or above the World Health Organization
405 (WHO) Tolerable Daily Intake (TDI; 24 µg/kg wet weight) in marine mussels. Here we
406 documented levels routinely exceeding California Office of Environmental Health and
407 Hazard Assessment (OEHHA; 10 ng/g wet weight for microcystins per week in seafood)
408 and WHO guidelines. From our environmental mussel samples, between 8% and 16% of
409 the individual mussels at each site exceeded the OEHHA guideline for weekly fish
410 consumption, and in the month of September, four mussels from Berkeley Marina and
411 Alameda Island had > 230 ng/g of microcystin toxins, with one mussel at 416.23 ng/g of
412 toxin, more than 40-fold the suggested weekly intake for consumption of fish (Fig. 4).
413

414 The results of our study elucidate an extensive unrecognized problem for environmental
415 health, wildlife and human health alike. Cyanobacterial blooms with toxin production can
416 have debilitating effects on ecosystems, leading to eutrophication, light limitation of
417 other species, reduction of food web efficiency, and mortality of species living in the
418 environment (Jones, 1987; Paerl and Ustach, 1982; Havens 2008). Such impacts are
419 detectable in both marine and freshwater environments in California at many different
420 trophic levels (Miller et al., 2010). The implications of environmental degradation and
421 trophic transfer of microcystin toxin is important not only for California, but also strongly
422 suggest that communities reliant on aquaculture and wild-harvest mussels for sustenance
423 worldwide are at risk. A combined real-time monitoring process including water quality
424 and biomarker species monitoring is recommended to control detrimental effects and to
425 better understand the breadth of impacts caused by this suite of toxins. We advocate
426 immediate use of bivalves for monitoring and management, especially for aquaculture
427 practices where low levels of toxins were identified in commercially sold shellfish. The
428 structure to implement these recommendations presently exists and auxiliary testing
429 could easily compliment current monitoring practices.
430

431 **Acknowledgements**

432 We would like to thank Kendra Hayashi, Rosemary Romero, and Regina Radan for lab
433 and field support, and Betsy Steele for facilities support. This work was completed with
434 salary and tuition assistance from the NOAA Nancy Foster Scholarship program (CMG)
435 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.

Figure Captions

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

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

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

Figure 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 5-10 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.

458

459 Figure 5. Commercial oyster and microcystin toxin experimental trials. X-axis begins at
460 24 hours denoting removal from water containing microcystins.

461

462 **Highlights**

463 • Shellfish were exposed to microcystin toxin to simulate riverine flushing to the
464 coastal ocean.
465 • Mussels exposed to particulate microcystin purged toxin slowly.
466 • Dissolved toxin was also taken up by mussels, but was purged more rapidly.
467 • Commercially raised oysters took up particulate toxin but purged it more quickly.
468 • Naturally occurring marine mussels tested positive for high levels of microcystin.

469

470 **References**

471 Amorim, A., Vasconcelos, V., 1998. Dynamics of microcystins in the mussel *Mytilus*
472 *galloprovincialis*. Toxicon. 37,1041-1052.
473
474 Baxa, D.V., Kurobe, T., Ger, K.A., Lehman, P.W., The, S.J., 2010. Estimating the
475 abundance of toxic Microcystis in the San Francisco Estuary using quantitative real-time
476 PCR. Harmful Algae. 9(3),342-349.
477
478 Campos, A., Vasconcelos, V., 2010. Molecular mechanisms of microcystin toxicity in
479 animal cells. Int. J. Mol. Sci. 11(1),268-287.
480
481 Carlsen, S.K., De Grasse, A., Powell, A., Sauber, A., HSU, C., Warren, J., 1996. Annual
482 Crop Report. Livestock and Agricultural Crop Report. San Rafael, CA: Marin County

Dept. of Agriculture, Agricultural Commissioner, Director of Weights and Measures. Print. Available online: http://www.marincounty.org/~/media/files/departments/ag/crop-reports/1996.pdf?la=en

Christoffersen, K., 1996. Ecological implications of cyanobacterial toxins in aquatic food webs. Phycologia. 35(6S),42-50.

Cloern, J.E., 1996. Phytoplankton bloom dynamics in coastal ecosystems: A review with some general lessons from sustained investigation of San Francisco Bay, California. Rev. Geophys. 34(2),127–168.

Cloern, J.E., Jassby, A.D., 2012. Drivers of change in estuarine-coastal ecosystems: Discoveries from four decades of study in San Francisco Bay. Rev. Geophys. 50(4),1-33.

Cousins, I.T., Bealing, D.J., James, H.A., Sutton, A., 1996. Biodegradation of microcystin-LR by indigenous mixed bacterial populations. Water Res. 30(2),481-5.

Davis, T.W., Berry, D.L., Boyer, G.L., Gobler, C.J., 2009. The effects of temperature and nutrients on the growth dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacterial blooms. Harmful Algae. 8,715-725.

Dawson, R.M., 1998. The toxicology of microcystins. Toxicon. 36(7),953-962.

Demott, W.R., Moxter, F., 1991. Foraging cyanobacteria by copepods: responses to chemical defense and resource abundance. Ecology. 72(5),1820-1834.

De Pace, R., Vita, V., Bucci, M.S., Gallo, P., Bruno, M., 2014. Microcystin Contamination in Sea Mussel Farms from the Italian Southern Adriatic Coast following Cyanobacterial Blooms in an Artificial Reservoir. J. Ecosys. 2014,1-11.

Dionisio Pires, L.M., Karlsson, K.M., Meriluoto, J.A.O., Kardinaal, E., Visser, P.M., Siewertsen, K., Van Donk, E. and Ibelings, B.W., 2004. Assimilation and depuration of microcystin–LR by the zebra mussel, *Dreissena polymorpha*. Aquat. Toxicol. 69,385–396.

Eriksson, J.E., Meriluoto, J.A.O., Lindholm, T., 1989. Accumulation of a peptide toxin from the cyanobacterium *Oscillatoria agardhii* in the freshwater mussel *Anadonta cygnea*. Hydrobiologia. 183(3),211-216.

Eriksson, J.E., Toivola, D., Meriluoto, J.A., Karaki, H., Han, Y.G., Hartshorne, D., 1990. Hepatocyte deformation induced by cyanobacterial toxins reflects inhibition of protein phosphatases. Biochem. Biophys. Res. Commun. 173(3),1347-1353.

Fetscher, A.E., Howard, M.D., Stancheva, R., Kudela, R.M., Stein, E.D., Sutula, M.A., Busse, L.B., Sheath, R.G., 2015. Wadeable streams as widespread sources of benthic cyanotoxins in California, USA. Harmful Algae. 49,105-116.

Fischer, D.T., Smith, S.V., Churchill, R.R., 1996. Simulation of a century of runoff across the Tomales watershed, Marin County, California. J. Hydrol. 186(1),253-273.

Ger, K.A., The, 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(17),4852-4857.

Ger, K.A., The, 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." Freshwater Biol. 55(7),548-1559.

541 Gibble, C.M., Kudela, R.M., 2014. Detection of persistent microcystin toxins at the
542 land–sea interface in Monterey Bay, California. Harmful Algae 39,146-153.
543
544 Guo, L., 2007. Ecology: doing battle with the green monster of Taihu Lake. *Science*
545 317:1166.
546
547 Havens, K.E., 2008. Cyanobacteria blooms: effects on aquatic ecosystems. In: Hudnell,
548 H.K. (Ed.), Cyanobacterial harmful algal blooms: state of the science and research needs.
549 Springer, New York, pp. 733-747.
550
551 Ibelings, B.W., Chorus, I., 2007. Accumulation of cyanobacterial toxins in freshwater
552 "seafood" and its consequences for public health: a review. Environ. Pollut. 150(1),177-
553 92.
554
555 Jacoby, J.M., Collier, D.C., Welch, E.B., Hardy, F.J., Crayton, M., 2000. Environmental
556 factors associated with a toxic bloom of *Microcystis aeruginosa. Can. J. Fish. Aquat.*
557 *Sci.* 57,231-240.
558
559 Jones, B.I., 1987. Lake Okeechobee eutrophication research and management. Aquatics.
560 9,21–26.
561
562 Karjalainen, M., Reinikainen, M., Lindvall, F., Spoof, L., Meriluoto, J.A., 2003. Uptake
563 and accumulation of dissolved, radiolabeled nodularin in Baltic Sea zooplankton.
564 Environ. Toxicol. 18(1),52-60.
565
566 Kudela, R.M., 2011. Characterization and deployment of Solid Phase Adsorption Toxin
567 Tracking (SPATT) resin for monitoring of microcystins in fresh and saltwater. Harmful
568 Algae. (11),117–125.
569

570 Kvitek, R., Bretz, C., 2005. Shorebird foraging behavior, diet, and abundance vary with
571 harmful algal bloom toxin concentrations in invertebrate prey. Mar. Ecol. Prog. Ser. 293,
572 303-309.
573
574 Lefebvre, K.A., Powell, C.L., Busman, M., Doucette, G.J., Moeller, P.D., Silver, J.B.,
575 Miller, P.E., Hughes, M.P., Singaram, S., Silver, M.W. and Tjeerdema, R.S., 1999.
576 Detection of domoic acid in northern anchovies and California sea lions associated with
577 an unusual mortality event. Nat Toxins. 7(3),85-92.
578
579 Lehman, P.W., Boyer, G., Hall, C., Waller, S., Gehrts, K., 2005. Distribution and toxicity
580 of a new colonial *Microcystis aeruginosa* bloom in the San Francisco Bay Estuary,
581 California. Hydrobiologia. 541,87-99.
582
583 Lehman, P.W., Boyer, G., Satchwell, M., Waller, S., 2008. The influence of
584 environmental conditions on the seasonal variation of Microcystis cell density and
585 microcystins concentration in San Francisco Estuary. Hydrobiologia. 600,187-204.
586
587 Lehman, P.W., Teh, S.J., Boyer, G.L., Nobriga, M.L., Bass, E. and Hogle, C., 2010.
588 Initial impacts of *Microcystis aeruginosa* blooms on the aquatic food web in the San
589 Francisco Estuary. Hydrobiologia. 637(1),229-248.
590
591 Lehman, P.W., Marr, K., Boyer, G.L., Acuna, S., The, S.J., 2013. Long-term trends and
592 causal factors associated with Microcystis abundance and toxicity in San Francisco
593 Estuary and implications for climate change impacts. Hydrobiologia. 718(1),141-158.
594
595 Lewis, D.J., Atwill, E.R., Lennox, M.S., Hou, L., Karle, B., Tate, K.W., 2005. Linking
596 on-farm dairy management practices to storm-flow fecal coliform loading for California
597 coastal watersheds. *Environ. Manage. Asses.* 107(1-3),407-25.
598

599 Malbrouck, C., Kestemont, P., 2006. Effects of microcystins on fish. Environ. Toxicol.
600 Chem. 25(1),72-86.
601
602 Mekebri, A., Blondina, G.J., Crane, D.B., 2009. Method validation of microcystins in
603 water and tissue by enhanced liquid chromatography tandem mass spectrometry. J.
604 Chromatogr. 1216(15),3147-3155.
605
606 McDonald, J.H., Koehn, R.K., 1988. The mussels *Mytilus galloprovincialis* and *M.*
607 *trossulus* on the Pacific coast of North America. Mar. Biol. 99(1),111-118.
608
609 Miller, M.A., Kudela, R.M., Mekebri, A., Crane, D., Oates, S.C., Tinker, M.T., Staedler,
610 M., Miller, W.A., Toy-Choutka, S., Dominik, C. and Hardin, D., 2010. Evidence for a
611 novel marine harmful algal bloom: cyanotoxin (microcystin) transfer from land to sea
612 otters. PLoS One. 5:e12576.
613
614 Ozawa, K., Yokoyama, A., Ishikawa, K., Kumagai, M., Watanabe, M.F., Park, H.D.,
615 2003. Accumulation and depuration of microcystin produced by the cyanobacterium
616 Microcystis in a freshwater snail. Limnology. 4(3),131-8.
617
618 Paerl, H.W., Huisman, J., 2008. Climate: blooms like it hot. Science. 320,57–58.
619
620 Paerl, H.W., Huisman, J., 2009. Climate change: a catalyst for global expansion of
621 harmful cyanobacterial blooms. Environ. Microbiol. 1,27–37.
622
623 Paerl, H.W., Otten, T.G., 2013. Harmful cyanobacterial blooms: causes, consequences,
624 and controls. Microb. Ecol. 65,995-1010.
625
626 Paerl, H.W., Ustach, J.F., 1982. Blue–green algal scums: an explanation for their
627 occurrence during freshwater blooms. Limnol. Oceanogr. 27,212–217.

628

629 Preece, E.P., Barry, C., Moore, F., Hardy, J.F., Deobald, L.A., 2015. First detection of
630 microcystin in Puget Sound, Washington, mussels (*Mytilus trossulus*). Lake Reserve.
631 Manage. 31(1),50-54.
632
633 Richardson, L.L., Sekar, R., Myers, J.L., Gantar, M., Voss, J.D., Kaczmarsky, L.,
634 Remily, E.R., Boyer, G.L. and Zimba, P.V., 2007. The presence of the cyanobacterial
635 toxin microcystin in black band disease in corals. FEMS Microbiol. Lett. 272,182-187.
636
637 Robson, B.J., Hamilton, D.P., 2003. Summer flow event induces a cyanobacterial bloom
638 in a seasonal Western Australian estuary. Mar. Freshwater. Res. 54,139-151.
639
640 Ross, C., Santiago-Vazquez, L., Paul, V., 2006. Toxin release in response to oxidative
641 stress and programmed cell death in the cyanobacterium *Microcystis aeruginosa*. Aquat.
642 Toxicol. 78,66-73.
643
644 Sarver, S.K., Foltz, D.W., 1993. Genetic population structure of a species' complex of
645 blue mussels (*Mytilus spp.).* Mar. Biol. 117(1),105-112.
646
647 Shumway, S.E., 1990. A review of the effects of algal blooms on shellfish and
648 aquaculture. J. World. Aquacult. Soc. (2),65-104.
649
650 Shumway, S.E., Allen, S.M., Boersma, P.D., 2003. Marine birds and harmful algal
651 blooms: sporadic victims or under-reported events? Harmful Algae. 2,1-17.
652
653 Smith, J.L., Haney, J.F., 2006. Foodweb transfer, accumulation, and depuration of
654 microcystins, a cyanobacterial toxin, in pumpkinseed sunfish (*Lepomis*
655 *gibbosus*). Toxicon. 48(5),580-589.
656

657 Suchanek, T.H., Geller, J.B., Kreiser, B.R., Mitton, J.B., 1997. Zoogeographic
658 distributions of the sibling species *Mytilus galloprovincialis* and *M. trossulus* (Bivalvia:
659 Mytilidae) and their hybrids in the North Pacific. Biol. Bull. 193(2),187-194.
660
661 Tonk. L., Bosch, K., Visser, P.M., Huisman, J., 2007. Salt tolerance of the harmful
662 cyanobacterium *Microcystis aeruginosa*. Aquat. Microb. Ecol. 46,117-123.
663
664 Tsuji, K., Nalto, S., Kondo, F., Ishikawa, N., Watanabe, M.F., Suzuki, M., Harada, K.I.,
665 1994. Stability of microcystins from cyanobacteria: effect of light on decomposition and
666 isomerization. Environ. Sci. Technol. 1094(28),173-177.
667
668 Turner, J.T., Tester, P.A., 1997. Toxic marine phytoplankton, zooplankton grazers, and
669 pelagic food webs. Limnol. Oceanogr. 42(5)2,1203-1213.
670
671 [USGS] United States Geographic Survey. 2016. Continuous Monitoring in the San
672 Francisco Bay and Delta Database. Accessed: 9/10/2016.
673
674 Vasconcelos, V.M., 1995. Uptake and depuration of the heptapeptide toxin microcystin-
675 LR in *Mytilus galloprovincialis.* Aquat. Toxicol. 32(2),227-237.
676
677 Welker. M., Steinburg. C., 2000. Rates of humic substance photosensitized
678 degradation of Microcystin-LR in natural waters. Environ. Sci. Technol. 34,3415-
679 3419.
680
681 White, S.H., Duivenvoorden, L.J., Fabbro, L.D., Eaglesham, G.K., 2006. Influence of
682 intracellular toxin concentrations on cylindrospermopsin bioaccumulation in a freshwater
683 gastropod *(Melanoides tuberculata*). Toxicon. 47(5)497-509.
684

685 Williams DE, Dawe, S.C., Kent, M.L., Andersen, R.J., Craig, M. and Holmes, C.F.,
686 1997. Bioaccumulation and clearance of microcystins from salt water mussels, *Mytilus*
687 *edulis*, and *in vivo* evidence for covalently bound microcystins in mussel tissues.
688 Toxicon. 35(11),1617-1625.
689
690 Yokoyama, A., Park, H.D., 2003. Depuration kinetics and persistence of the
691 cyanobacterial toxin microcystin-LR in the freshwater bivalve *Unio douglasiae*. Environ.
692 Toxicol. 18(1),61-7.
693
694 Zehnder, A., Gorham, P.R., 1960. Factors influencing the growth of *Microcystis*
695 *aeruginosa*. Can. J. Microbiol. 6,645-660.
696
697