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

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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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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