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Transfer of microcystin from freshwater lakes to Puget Sound, WA and toxin accumulation in marine mussels (*Mytilus trossulus*)

E.P. Preece [a,*], Barry C. Moore [a], F. Joan Hardy [b]

[a] Washington State University, School of the Environment, PO Box 646410, Pullman, WA 99164, USA
[b] Washington State Department of Public Health, Office of Shellfish and Water Protection, PO Box 47890, Olympia, WA 98504, USA

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ABSTRACT

Many eutrophic inland freshwater lakes in the Puget Sound Washington region produce toxic cyanobacteria blooms annually. While such blooms in lakes tend to be viewed as a localized phenomenon, there is significant potential for downstream export of toxins to freshwater streams, and marine and brackish water environments. However, monitoring for cyanotoxins typically associated with freshwaters, such as the hepatotoxin, microcystin (MC) in marine receiving waters is rare. In 2013 we studied four eutrophic Puget Sound area lakes to assess both toxin transport to marine waters and its potential accumulation in marine shellfish, specifically mussels. Shellfish beds are extensive throughout Puget Sound, and recreational harvest occurs downstream of our study lakes, so a study goal was to also assess if shellfish consumption poses a human health risk for MC exposure. We confirm, for the first time, freshwater to marine transfer of MCs in Puget Sound with subsequent bioaccumulation of MC by mussels. ELISA analysis estimated maximum MC concentrations in source lakes of 2700 μg/L, up to 0.34 μg/L in marine waters and 6.5 μg/kg in mussels. Confirmatory analyses by LC-MS/MS on water and mussel samples identified MC-LA as the major toxin. Although we found relatively low MC levels in mussels, our study implies that potential concern for human food safety is justified and warrants further investigation.

1. Introduction

Harmful algae blooms (HABs) caused by cyanobacteria (or blue green algae) constitute a persistent worldwide water quality problem. Cultural eutrophication creates conditions that favor HABs in aquatic systems. Climatic changes are expected to exacerbate HAB growth (Paerl and Huisman, 2009) and at the same time expanding human populations will exert greater demands for water resources. Recent closures of water supplies due to HABs in communities around the Great Lakes, such as Toledo, Ohio, point to growing water quality problems in the US. In freshwater environments, cyanobacteria are the most prolific HAB organisms and cyanotoxins can kill or sicken people, livestock, and wildlife, disrupt aquatic food webs, reduce fishery productivity, and cause severe esthetic impacts (Metz et al., 1997, Azevedo et al., 2002, Ibelings and Chorus, 2007, Chen et al., 2009, Handeland and Østensvik, 2010).

The most common and dangerous group of cyanotoxins are the potent liver toxins microcystins (MC) (Chorus and Bartram, 1999), a group with over 90 known variants (Welker and Von Döhren, 2006). Most MC poisonings occur from drinking contaminated water (Falconer et al., 1999). Over the past decade, numerous studies have also shown that MC can accumulate in aquatic food webs (e.g. Poste et al., 2011, Sotton et al., 2014, Lance et al., 2014) including seafood consumed by humans (Ibelings and Chorus, 2007). Concerns associated with consumption of MC-contaminated food sources led the World Health Organization (WHO) to set a provisional tolerable daily intake (TDI) for seafood at 0.04 μg/kg of MC-LR body weight/day (WHO, 1998). Despite this concern, most MC exposure scenarios do not consider potential exposure through seafood consumption.

Until recently cyanotoxins such as MC were primarily associated with freshwater habitats. However, MCs are stable and can be environmentally persistent in both freshwater and marine ecosystems (Tonk et al., 2007, Miller et al., 2010). Recent studies show that MC contaminated freshwater can deliver toxins into marine environments (Miller et al., 2010, De Pace et al., 2014, Gibble and Kudela, 2014) raising concerns that biota may accumulate and potentially transfer MC through marine food webs (Kozlowsky-Suzuki et al., 2012). In 2007, waters in Monterey Bay National Marine Sanctuary tested positive for MC after receiving inflow from three rivers draining freshwater lakes with HABs. A group of 21 federally threatened southern sea otters in the Bay subsequently died from MC poisoning (Miller et al., 2010).

* Corresponding author.
E-mail address: eppreece@wsu.edu (E.P. Preece).

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Environmental persistence of MC in marine shellfish and marine receiving waters was demonstrated in laboratory work and investigators concluded that sea otters were exposed to MC through consumption of contaminated shellfish (Miller et al., 2010).

In Washington State, eutrophication problems have increased over the past few decades and HABs have been observed more frequently in lakes that flow into Puget Sound, an estuarine inlet of the Pacific Ocean (Jacoby and Kann, 2007). Transfer of MC from freshwater sources raises concerns that Puget Sound shellfish may also accumulate MCs which would not only put wildlife at risk but also humans who harvest and consume local shellfish. There is concern that people who rely on local seafood as a major source of dietary protein may be at particularly high risk for MC exposure. MC concentrations in bivalves are generally highest in the hepatopancreas and viscera (Chen and Xie, 2005), so contaminated marine shellfish may pose a significant MC risk for human exposure since the entire animal is usually consumed.

In a 2012 pilot study, we determined that Puget Sound mussels accumulate MCs with the likely sources being HABs in inland lakes that drain into the Sound (Preece et al., 2015a). However, due to the exploratory nature of the initial project, we were unable to establish a direct relationship between cyanobacteria blooms in nearby lakes and MCs in marine mussels. We conducted further research in 2013, expanding the pilot study to include four inland lakes and their respective outflows. Our ultimate goal was to elucidate potential health risks for humans whose diets include significant consumption of Puget Sound shellfish.

2. Methods

2.1. Site description

Puget Sound is a deep, fjord-type estuarine complex with extensive habitats suitable for shellfish. Shellfish from this region sustain recreational, sustenance and commercial harvests. We identified shellfish areas in Puget Sound subject to inflows from lakes with frequent HABs. Four of these sites deemed to be at particularly high risk for MC contamination were selected for the study (Fig. 1A):

Site 1: Bay Lake (Fig. 1B) is a suburban–rural lake located on the Key Peninsula of Pierce County. Bay Lake drains into Mayo Creek, which flows into Mayo Cove (distance, 0.7 km, Penrose Point State Park).

Site 2: Lake Steilacoom (Fig. 1C) is an urban lake, located in Lakewood, Washington. The outlet stream, Chambers Creek, flows into Chambers Bay (distance 6.5 km).

Site 3: Long Lake is a shallow eutrophic lake located just southeast of Port Orchard, Washington. The lake drains via Curly Creek to Yukon Harbor (distance 8 km).

Site 4: Kitsap Lake is surrounded by dense residential development in Bremerton, Washington. The lake drains via Chico Creek to Chico Bay in the southwestern corner of Dyes inlet (distance 4 km).

Site 5. Penn Cove, a major aquaculture farm with no upstream freshwater sources and no documented occurrences of MC was selected as a control site.

2.2. Mussel and water collection and processing

Mussel (*Mytilus trossulus*) cages with 100 organisms per cage were placed in marine receiving waters of each study site. Cages were suspended from existing docks for sampling access at the mouth of each respective inlet at a distance of about 0.3 m from shore and in water sufficiently deep to remain covered at low tide. The cages were deployed shortly after MC was detected in each source lake but prior to any storm events that would increase outflow of lake water to the marine sites. Environmental parameters (temperature, salinity and tidal conditions, Table 1) were similar at all sites.

Mussel collections were initiated one week after cage deployment and continued weekly until one month after the last visible scum was observed. For Chambers Bay and Mayo Cove, this led to mussel collection from September to December 2013. Chico Bay mussels were collected from October through November and Yukon Harbor mussels were only collected in October. Weekly collections included five mussels, all the same age and similarly sized (1.5–3.0 in.). Immediately after collection, soft parts of the mussel were removed and shells were discarded. Collected mussel bodies were rinsed with deionized water, individually bagged, and frozen at −20 °C until processed for MC extraction.

Water samples were collected weekly from each source lake, corresponding drainage stream, and respective shellfish sites (Figs. 2 and 3) for phytoplankton identification and MC

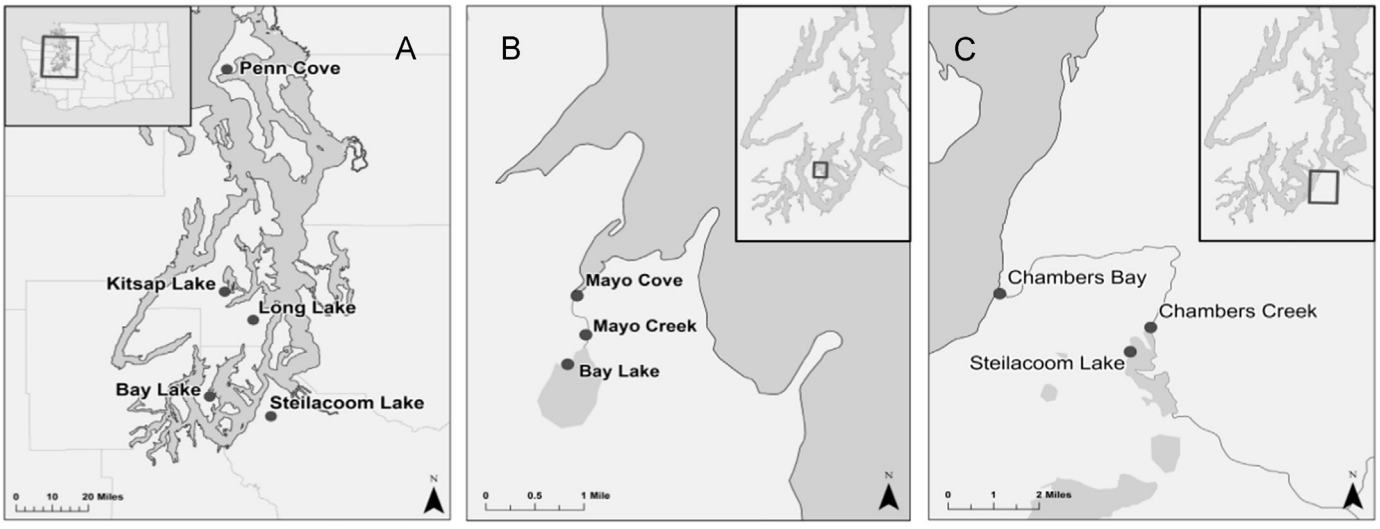

Fig. 1. (A) Map of Puget Sound, WA showing study lakes and Penn Cove where control mussels were collected, (B) Map of Bay Lake and inflow through Mayo Creek to Mayo Cove. Locations of water sample collection are indicated by black circles. Mayo Cove circle shows location of mussel cage. (C) Map of Steilacoom Lake and inflow through Chambers Creek to Chambers Bay. Locations of water sample collection are indicated by black circles. Chambers Bay circle shows location of mussel cage.