Transfer of Microcystin from Freshwater Lakes to Puget Sound, WA and Toxin Accumulation in Marine Mussels

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REGION 10 HAB WORKSHOP
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Cyanobacteria (Blue Green Algae)

- Number of competitive advantages over other algal species
  - Tolerant of a wide range of water quality condition
  - Outcompete other algal species in polluted waters
- Increasing in prevalence
  - Climate change
  - Anthropogenic eutrophication
- CyanoHABs
  - 60% of blooms toxic
    - Neurotoxins
    - Liver Toxins
    - Skin irritants
Microcystin

- Most common and dangerous cyanotoxin
  - Targets the liver
  - Over 90 variants
- Cause illness and deaths in
  - Livestock, Pets, Wildlife, Humans
- Pose a serious problem to human health
  - Drinking water
  - Consuming contaminated seafood
MICROCYSTIN

- Cyclic heptapeptide
- 90+ variants (Sivonen and Jones 1999)
- LR most common/toxic
MCs in Estuarine and Marine Environments

1. Marine species forming MCs – Synechoccus
2. CyanoHABs forming in coastal waters – Microcystis and Anabaena spp.
3. Discharge of MC contaminated freshwater to saltwater

Salinity Tolerance of Microcystis and Anabaena spp.

- M. aeruginosa (1)
- M. aeruginosa (2)
- M. aeruginosa (3)
- M. aeruginosa (4)
- M. aeruginosa (5)
- M. aeruginosa (6)
- M. aeruginosa (7, 8, 9, 10)
- M. aeruginosa (11)
- Microcystis spp. (8)
- M. aeruginosa (9)
- M. aeruginosa (12)
- M. aeruginosa (13)
- M. aeruginosa (14)
- A. circinalis and A. spiroides (15)
- A. oscillarioides (10)
- A. oscillarioides (16)
- A. oscillarioides (17)
MC in Marine Waters from Contaminated Freshwater Discharge

1. Adriatic sea, Italy
2. Ariake Bay, Japan
3. Puget Sound, WA
4. Klamath Estuary, CA
5. San Francisco Estuary, CA
6. Monterey Bay, CA
MC Contaminated Freshwater Discharge in Puget Sound?

• Ocean discharge of MCs confirmed for 3 rivers flowing into Monterey Bay National Marine Sanctuary

Miller et al. 2010

http://www.seaotterresearch.org
MCs in Puget Sound Mussels?

• Extensive habitats for shellfish
  – Recreational and commercial harvest

• Certain populations depend on collecting local shellfish for food

3 years of research to determine if Puget Sound mussels accumulate MCs
2012 Pilot Study

Sites located in recreational shellfish areas
Methods (Field)

• Cages of mussels (*Mytilus trossulus*) deployed at mouths of creeks discharging from lakes w/toxic blooms

• Mussels collected weekly while bloom present
  - 10/10/12-12/11/12

• Control mussels
  - Collected from Puget Sound, areas not near MC discharge
Methods (Laboratory)

• Mussels shucked
  – Frozen until analysis

**Extraction**

• Freeze dried
• Extracted with 75% acidified MeOH for 24 hrs. (Wilson et al. 2008)

**Analysis**

• LC-MS/MS
Methods (Laboratory)

**LC-MS/MS**

- Identify/quantify MC variants

**MC-LR, RR, YR & LA listed by EPA as most important algal toxins in the US**
2012 Pilot Study Results

- All results determined by LC-MS/MS analysis
- No MC detected in control mussels (n=20)
- No MC detected in mussels exposed to Bay Lake Discharge (n=10)
  - Bay Lake Max. MC = 10.8 ppb
- Positive MC in mussels exposed to Lake Steilacoom discharge (n=21, 20 +MC)
  - Steilacoom Lake Max. MC = 52.4 ppb
2012 Microcystin Concentration in Chambers Creek Mussels

LR, LA

LR, LA

LR, LA

LA

LR

LW

10-Oct-12 30-Oct-12 6-Nov-12 13-Nov-12 4-Dec-12 11-Dec-12
2013 Study

- More comprehensive study
  - 4 Puget Sound Sites
  - Stepwise sampling of water
Methods

• Mussels
  – Freeze dried
  – Extracted with acidified 90% MeOH 3x
  – 3X hexane rinse

• Water and mussels screened/analyzed with DM ELISA

• LC-MS/MS used to determine variants/confirm presence
  – 10 MC variants
Bay Lake → Mayo Cove = 1 km

Map showing locations of Bay Lake and Mayo Cove.

Graph showing microcystin concentration over time for Bay Lake, Mayo Creek, Mayo Cove Water, and Mayo Cove Mussels.

MC-LA
MC-LR
Mayo Cove Mussels

[Graph showing microcystin concentration over time from 21-Oct-13 to 3-Dec-13]
Steilacoom Lake ➔ Chambers Bay = 6.5 km

Graph showing microcystin concentration over time with data points labeled "Chambers Creek" and "Chambers Bay Mussels."
Chambers Bay Mussels
2013 Results Summary

• Results determined by LC-MS/MS and ELISA
  – ELISA for concentration
  – LC-MS/MS for variant identification
• No MC detected in mussels exposed to Kitsap or Long Lakes
• MC detected in Mayo Cove mussels on 5 dates
  – MC detected in Mayo Creek and Mayo Cove
• MC detected in Chambers Bay mussels on 3 dates
• MC-LR and MC-LA most prevalent
2015 Study

• Same study lakes as 2013
  – Kitsap, Bay, Long and Steilacoom lakes
• Only have preliminary results
  – From water samples
• Mussels and sediment samples also collected
2015 Study Results

- Results determined by LC-MS/MS and ELISA
  - 6 variants
- Mayo Creek
  - 0.09 (LC-MS/MS)
  - 1.70 (ELISA)
- Mayo Cove
  - 0.15 ppb (LC-MS/MS)
- Chico Culvert
  - 0.40 (LC-MS/MS)
    - 0.27 (ELISA)
- Primarily MC-LR and MC-LA detected
Elucidate potential health risks for humans whose diets include significant consumption of Puget Sound shellfish
<table>
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<tr>
<th>95th percentile Shellfish Consumption Rate (g/day)</th>
<th>Microcystin TDI (WHO) or Chronic exposure criteria value (EPA) µg/kg/dy</th>
<th>Body Weight</th>
<th>Calculated Criteria Value µg/kg (ppb)</th>
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<td>Suquamish: 499</td>
<td>WHO: 0.04</td>
<td>70</td>
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Summary

• Primary MC variants MC-LR and MC-LA
  – MC-RR and MC-LW also detected
• Freshwater derived MCs are polluting the land sea interface of Puget Sound
• MC from freshwater sources are accumulating in Puget Sound shellfish
• Certain Puget Sound populations are likely exposed to MC through consumption of contaminated seafood
Broader Impacts

• Connectivity between marine and freshwaters will play an important role in spread of cyanotoxins

• Better management of freshwater systems may help decrease exposure of marine systems to cyanotoxins
  – develop mitigation practices to reduce pollution entering aquatic ecosystems
Acknowledgements
Questions?