US EPA ARCHIVE DOCUMENT

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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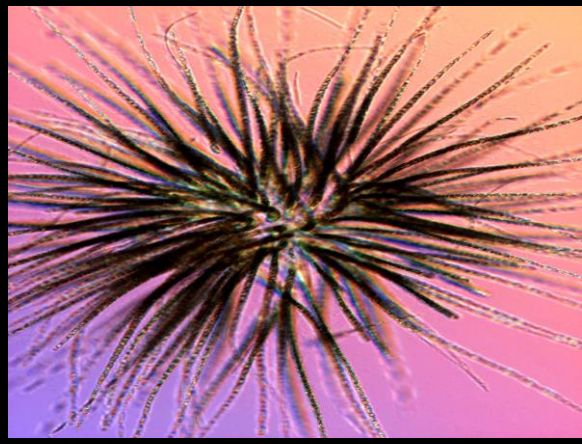
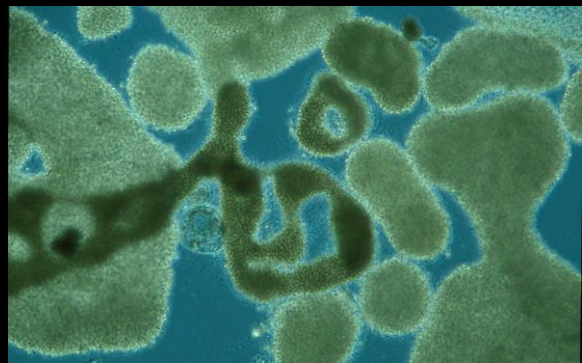
Cyanobacteria (Blue Green Algae)

- 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

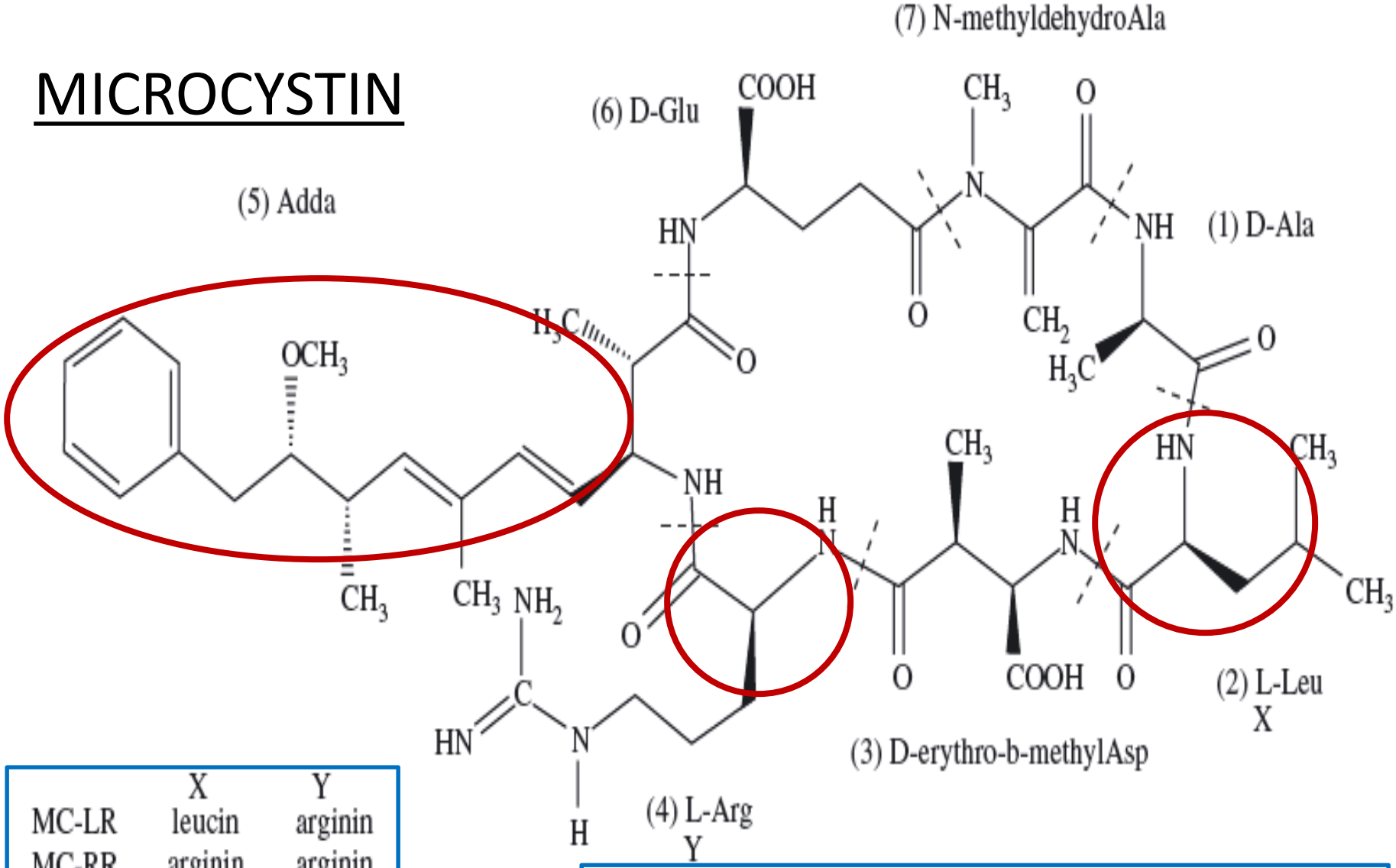
Microcystin

- 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





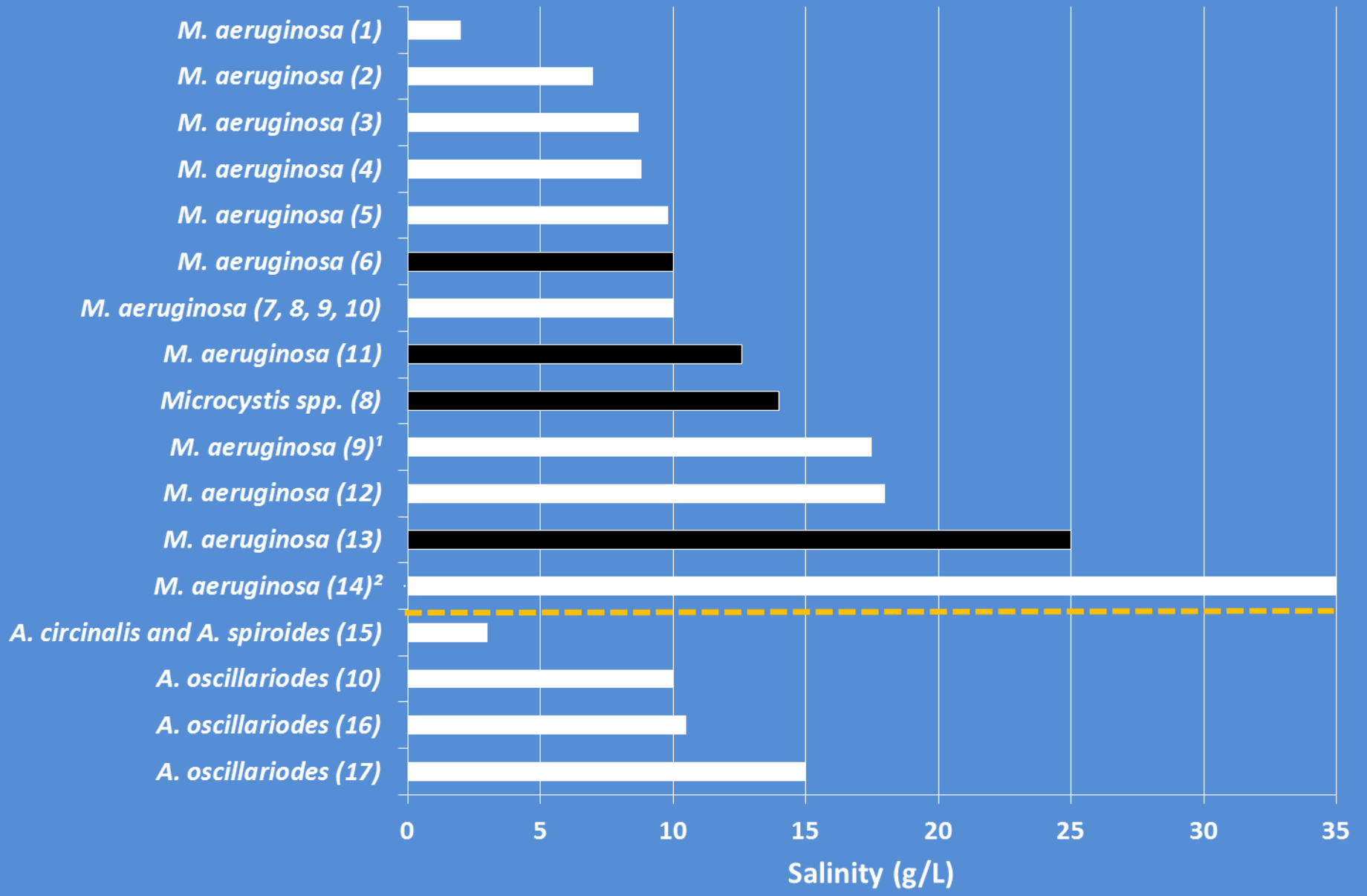
MICROCYSTIN



	X	Y
MC-LR	leucin	arginin
MC-RR	arginin	arginin
MC-LY	leucin	tyrosin
MC-YR	tyrosin	arginin
MC-WR	tryptophan	arginin
MC-FR	phenylanalin	arginin

- **Cyclic heptapeptide**
- **90+ variants** (Sivonen and Jones 1999)
- **LR most common/toxic**

Salinity Tolerance of *Microcystis* and *Anabaena spp.*

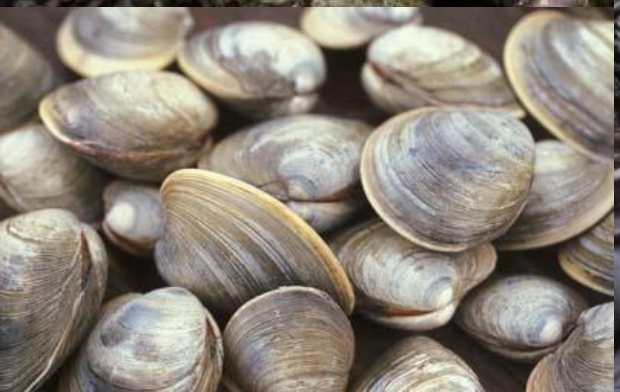


MC in Marine Waters

from Contaminated Freshwater Discharge

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)

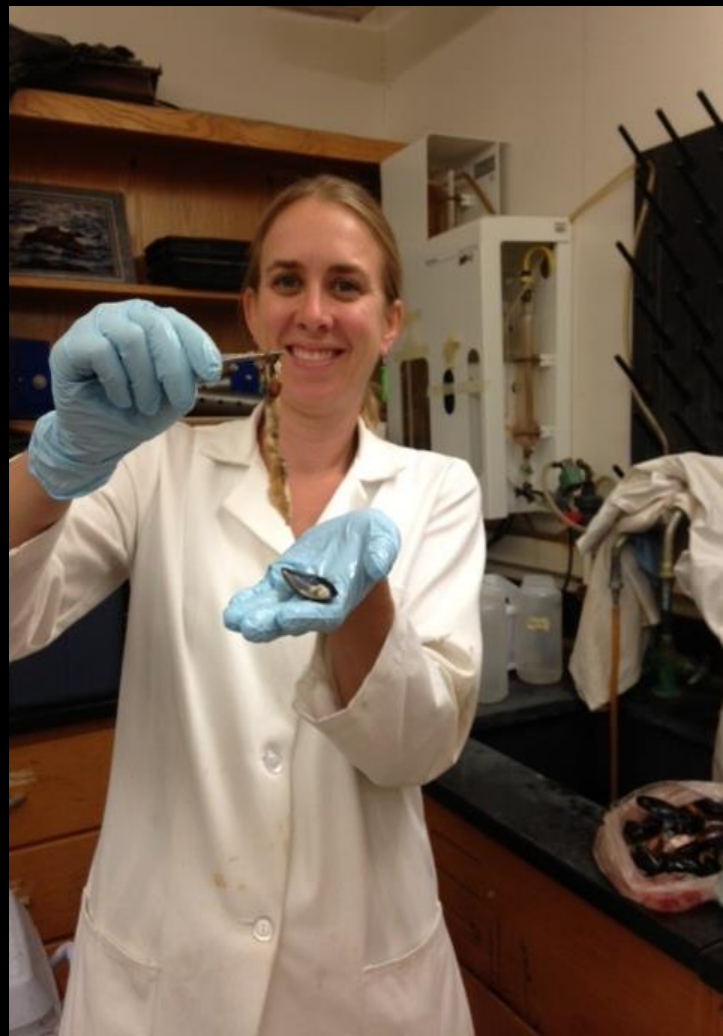
- Mussels shucked
  - Frozen until analysis

Extraction

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

Analysis

- LC-MS/MS

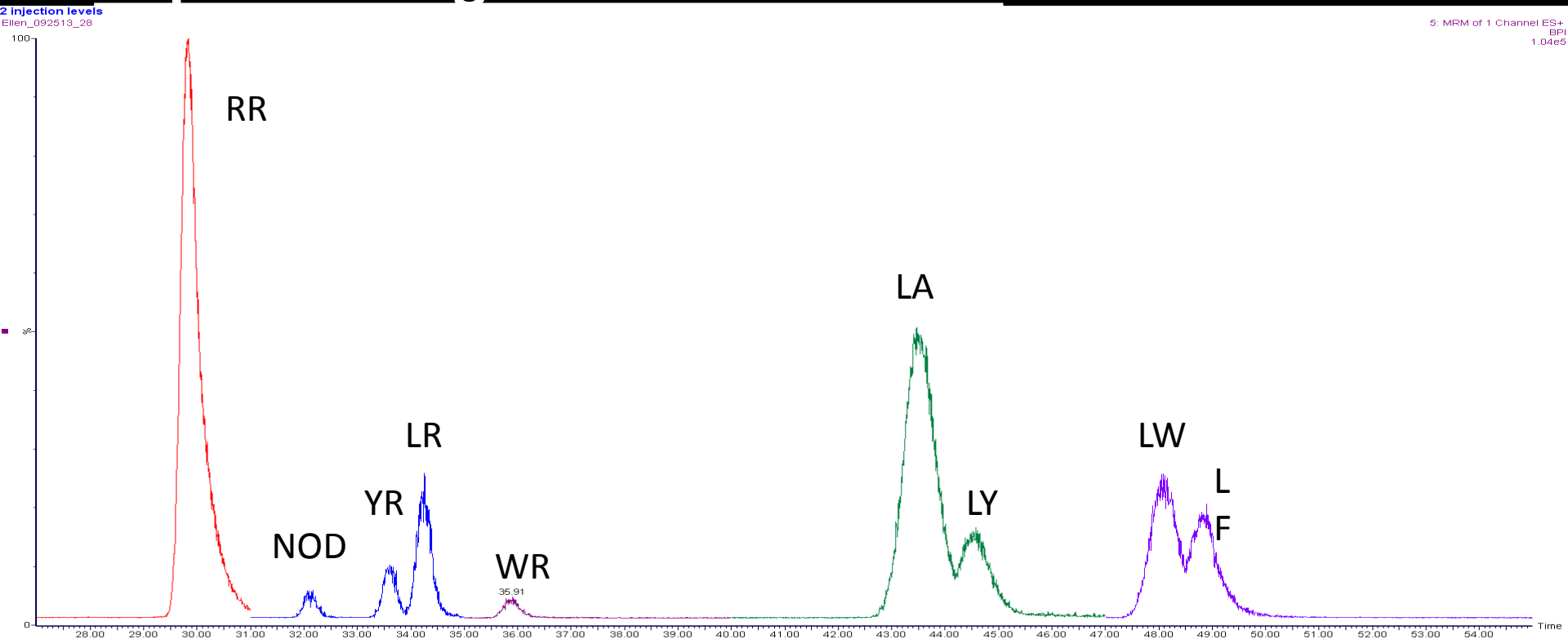


Methods (Laboratory)

LC-MS/MS

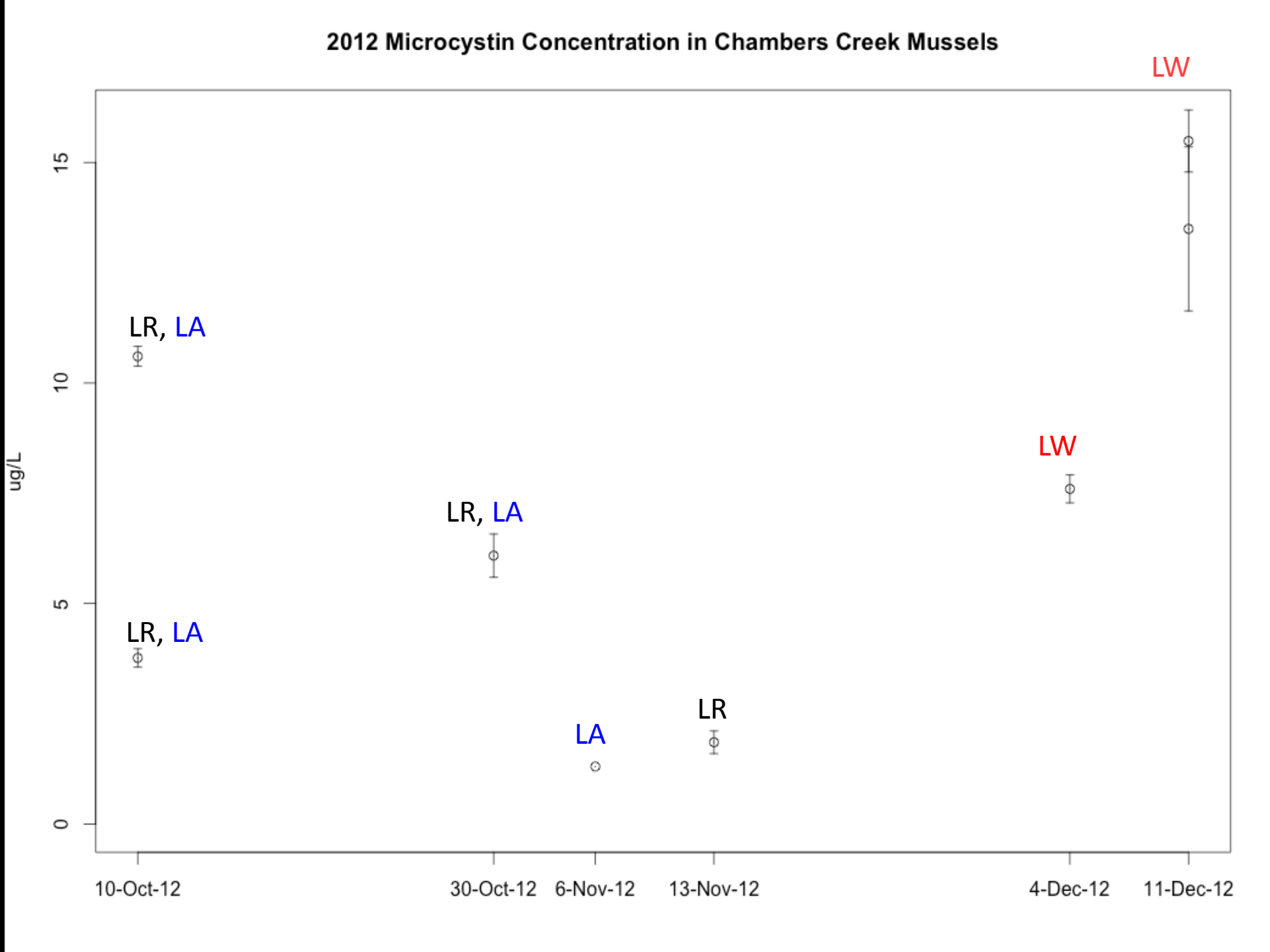
- Identify/quantify MC variants

MC-LR, RR, YR & LA listed by EPA as most important algal toxins in the US

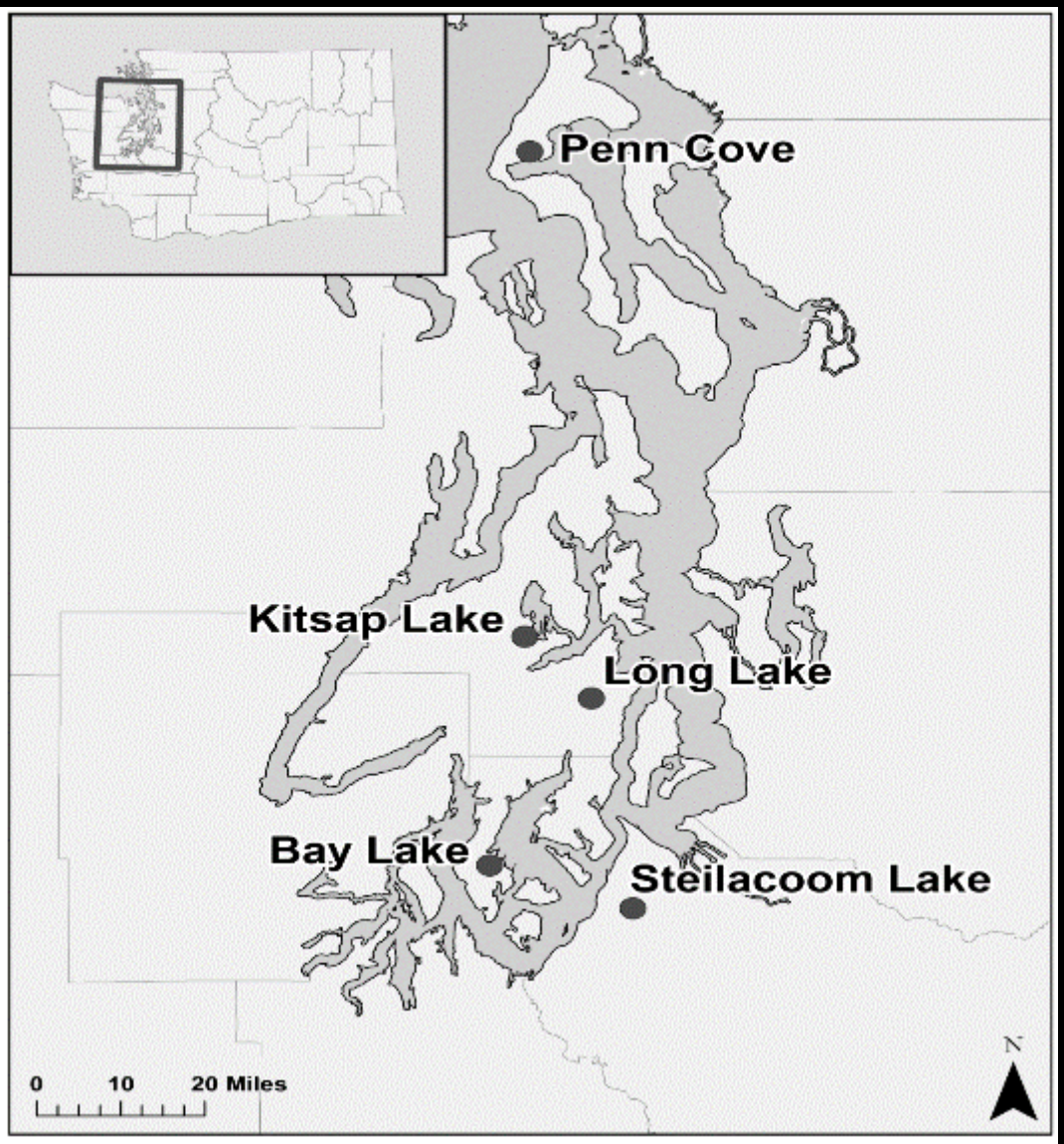


2012 Pilot Study Results

- 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

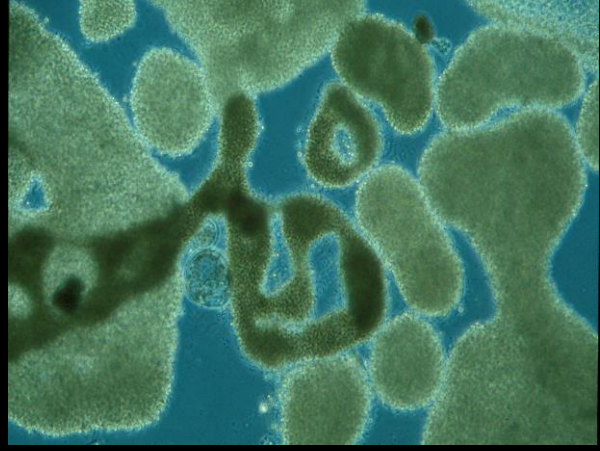

2012 Microcystin Concentration in Chambers Creek Mussels
ug/L
0
5
10
15
LR, LA
LR, LA
LR, LA
LA
LR
LW
LW
10-Oct-12
30-Oct-12
6-Nov-12
13-Nov-12
4-Dec-12
11-Dec-12

2013 Study



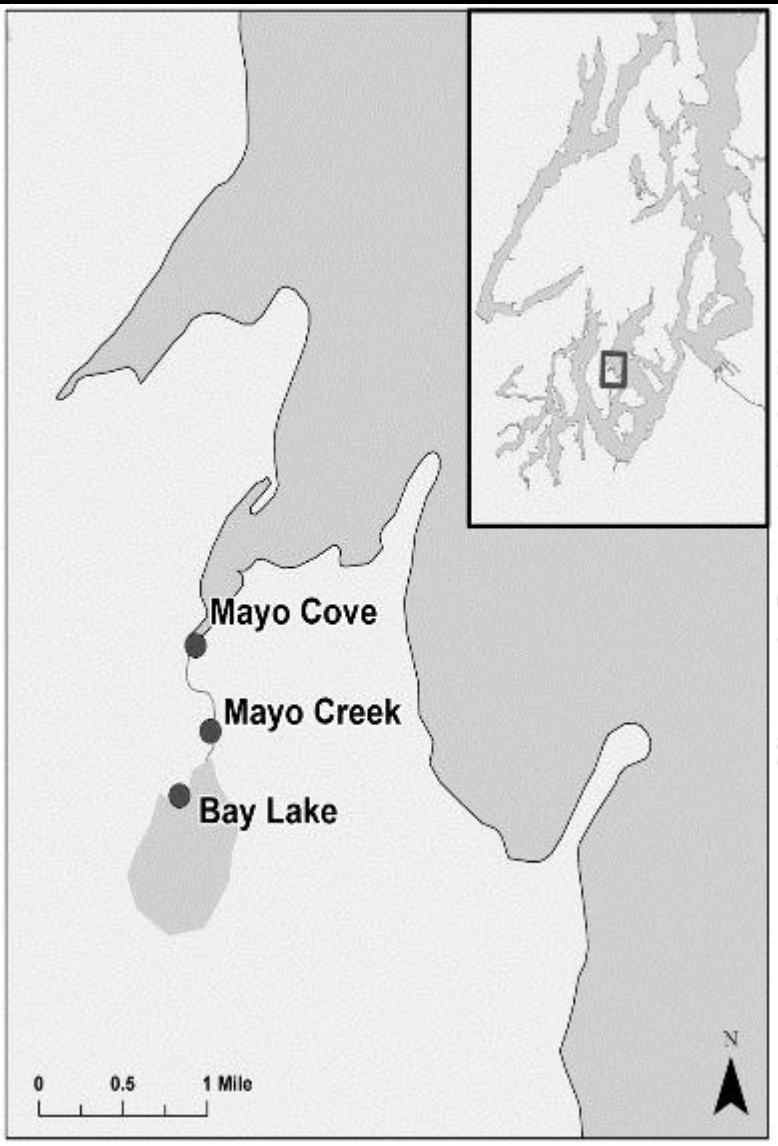
- More comprehensive study
  - 4 Puget Sound Sites
  - Stepwise sampling of water

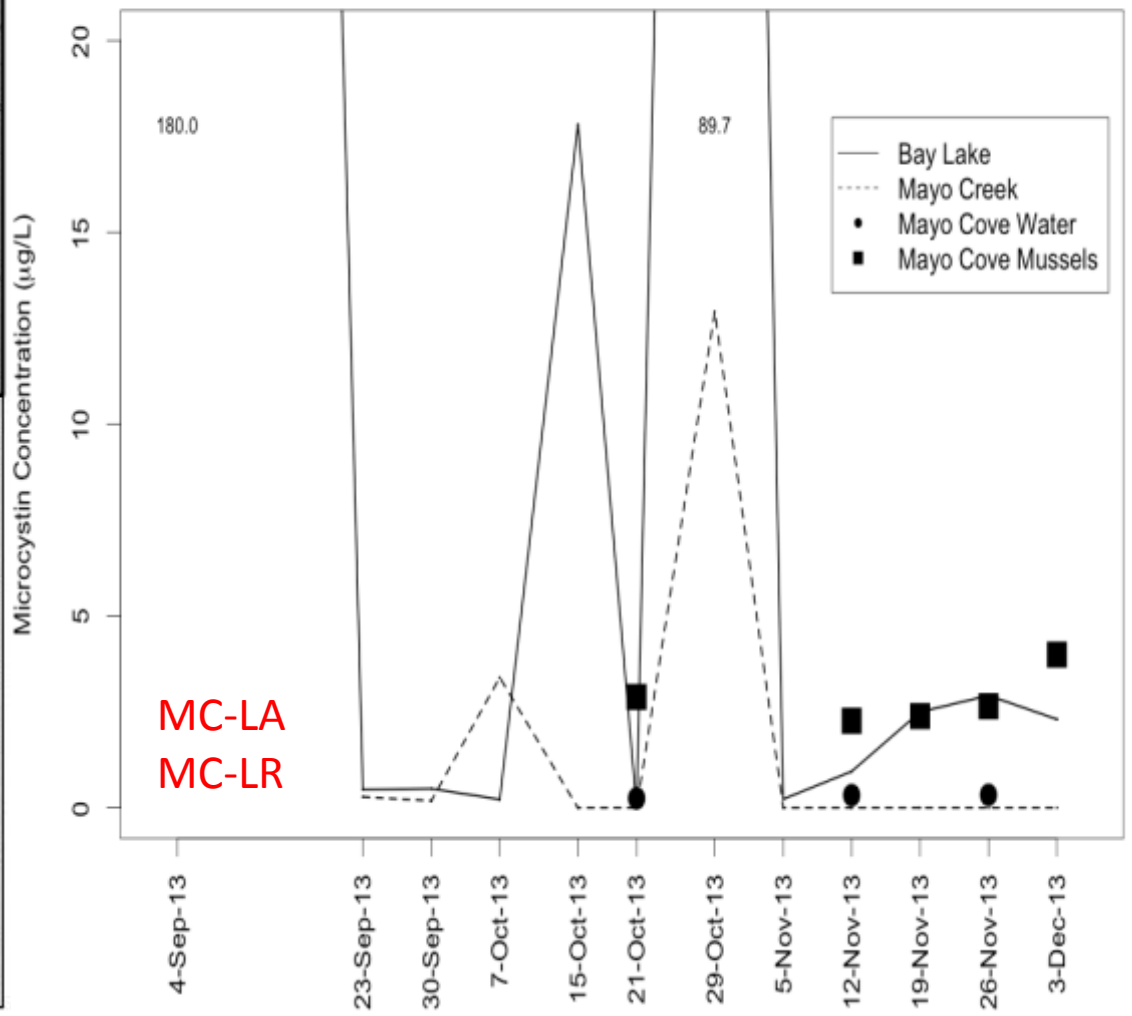
Methods



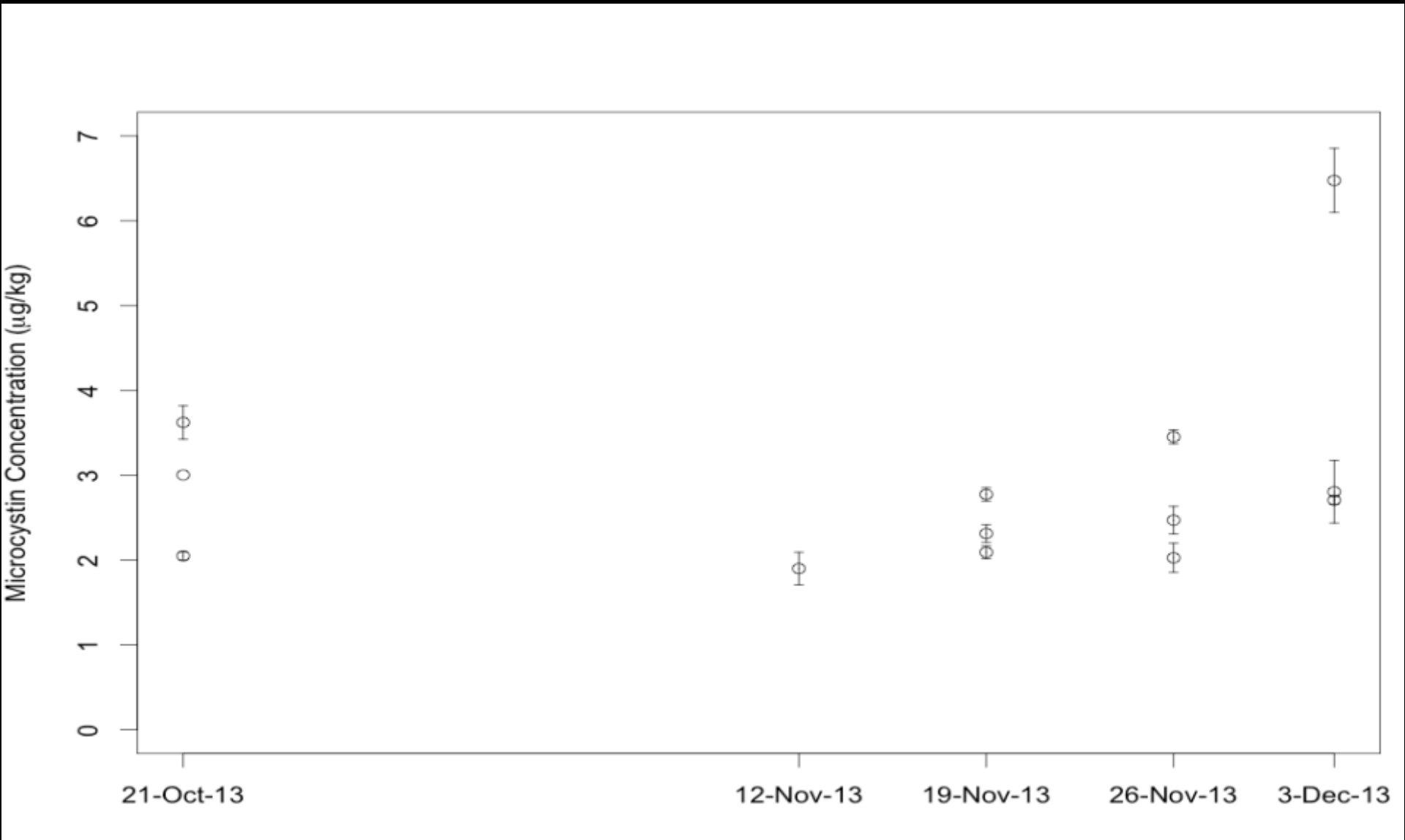
- Mussels
  - Freeze dried
  - Extracted with acidified 90% MeOH 3x
  - 3X hexane rinse
- Water and mussels screened/analyzed with DM ELISA
- LC-MS/MS used to determine variants/confirm presence
  - 10 MC variants

Bay Lake → Mayo Cove = 1 km

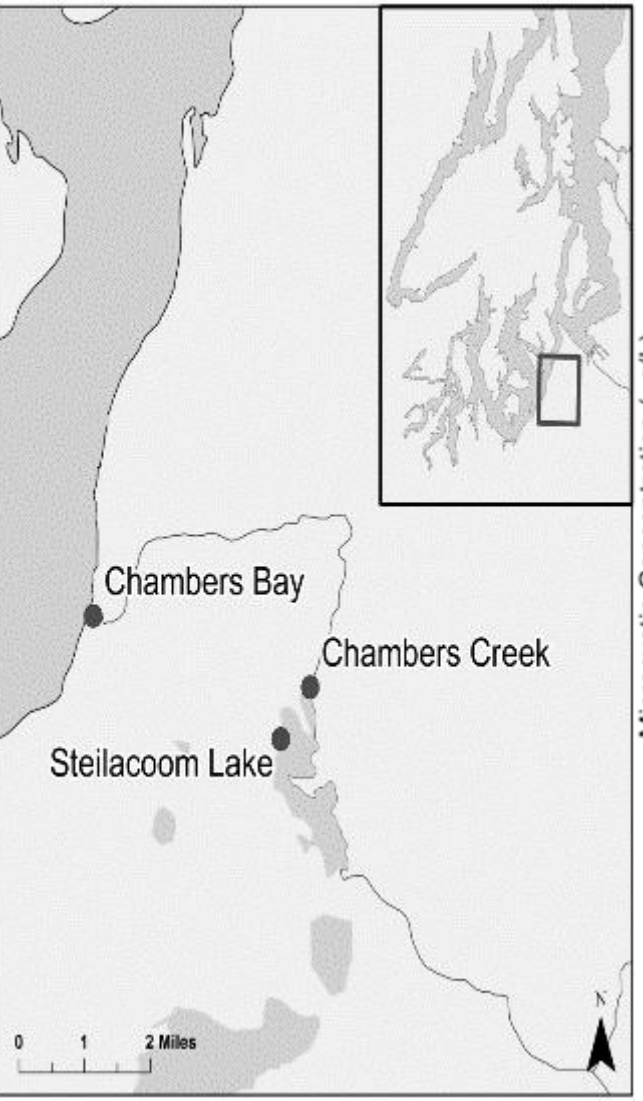


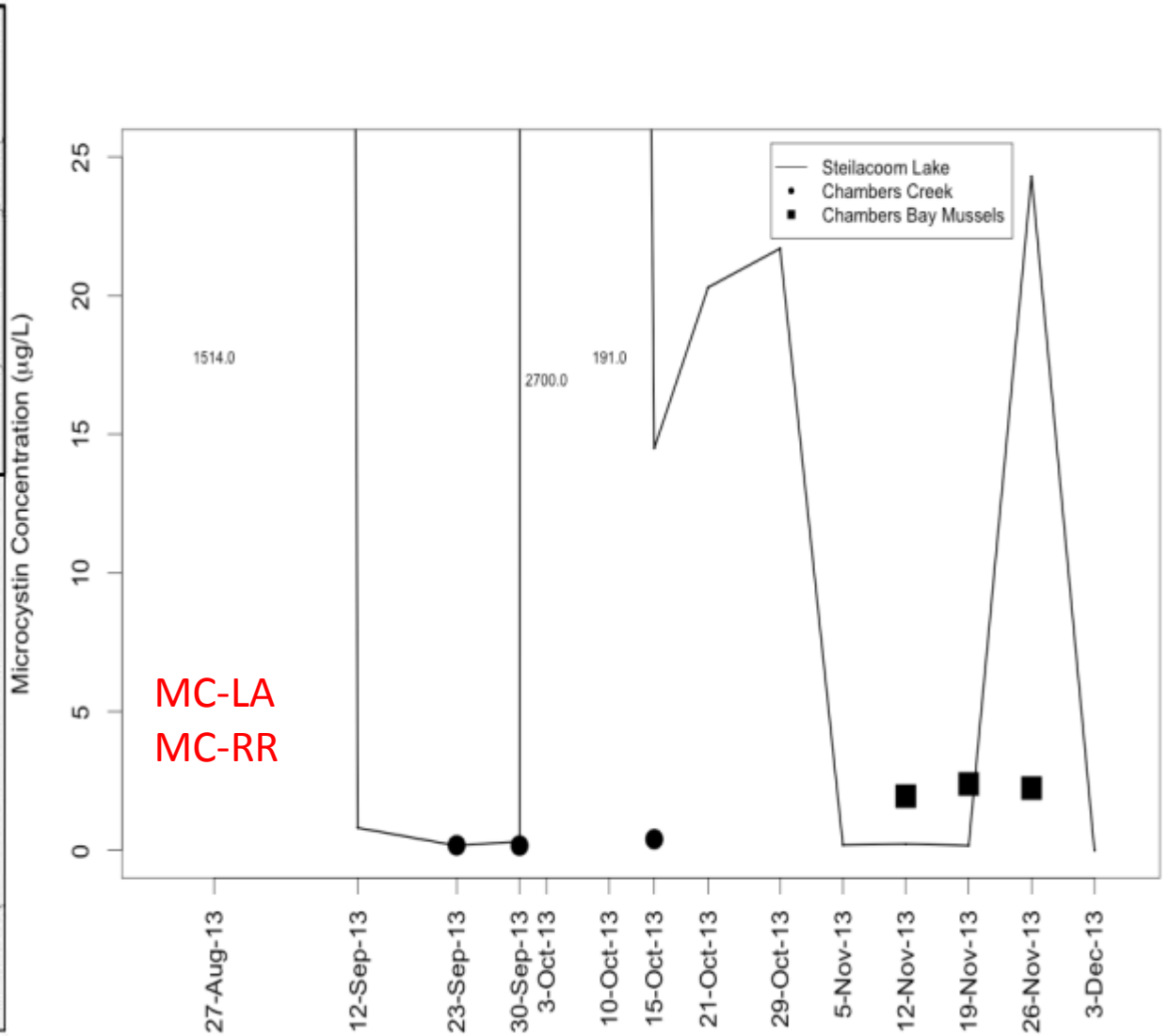


Mayo Cove Mussels

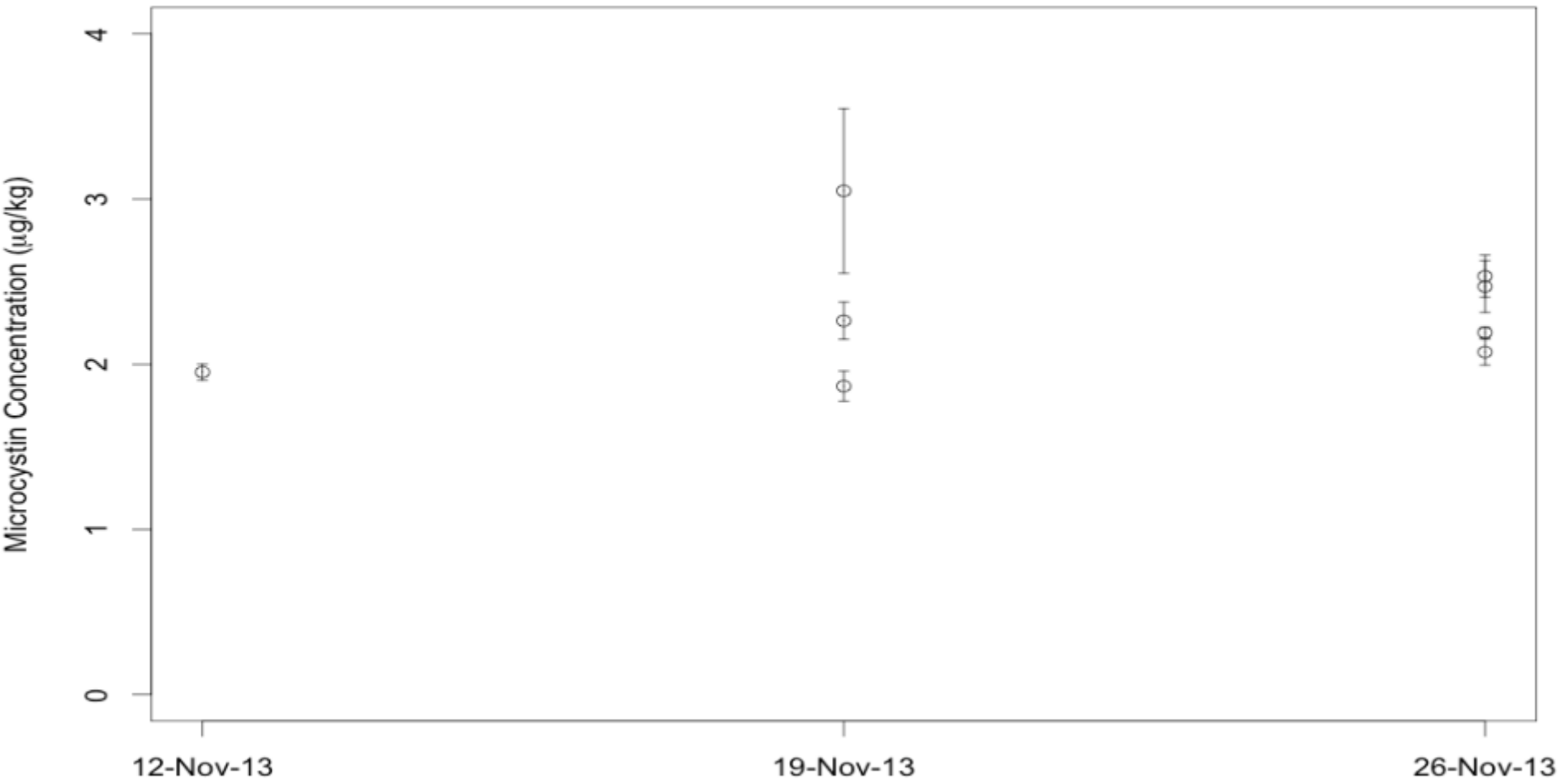


Steilacoom Lake ⟶
Chambers Bay= 6.5 km





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

95th percentile Shellfish Consumption Rate g/day	Microcystin TDI (WHO) or Chronic exposure criteria value (EPA) µg/kg/dy	Body Weight	Calculated Criteria Value µg/kg (ppb)
Suquamish: 499	WHO: 0.04	70	*5.9*
	EPA: 0.003	70	*0.42*
	WHO: 0.04	60	*4.8*
	EPA: 0.003	60	*0.36*
Tulalip: 146	WHO: 0.04	70	18.9
	EPA: 0.003	70	*1.4*
	WHO: 0.04	60	16.4
	EPA: 0.003	60	*1.23*
Squaxin: 51.9	WHO: 0.04	70	53.9
	EPA: 0.003	70	*4.0*
	WHO: 0.04	60	46.2
	EPA: 0.003	60	*3.5*

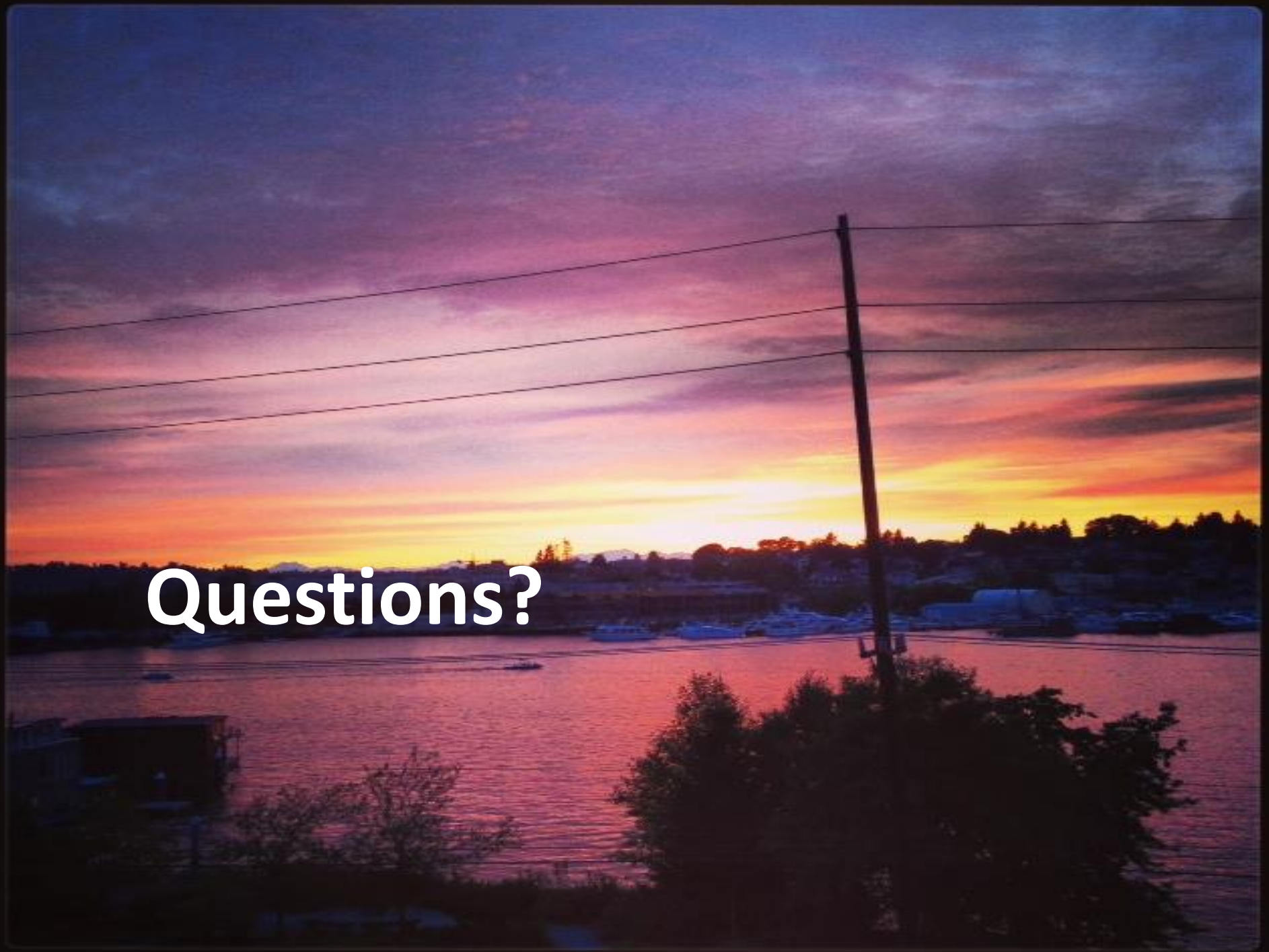
Summary

- 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?