Washington's Anatoxin-a Experience

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EPA Region 9 HABs Meeting
April 26, 2017
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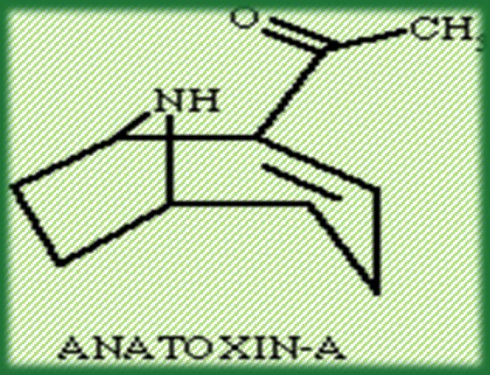
Overview

- Background / History
- Freshwater Algae Control Program
  - Partners
  - Analyses
  - Outreach and Education
- Lessons Learned
- Remaining Questions

Clear Lake, Pierce County

Background – Anatoxin-a

- Primary target - nerve synapse
- Mimics acetylcholine and binds to the nicotinic acetylcholine receptor – binding is irreversible
- Rapid acting poison (VFDF)
- Symptoms: staggering, paralysis, twitching, gasping, convulsions, death
- Sublethal – no chronic effects



Background – Anatoxin-a

- Produced by *Anabaena (Dolichospermum), Planktothrix (Oscillatoria), Aphanizomenon, Cylindrospermum,* and *Microcystis spp.*
- More likely to be present in free water than MCs or nodularin
- Degrades readily: half-life typically < 24 hrs in lakes (about 5 days in laboratory)

Chemical formula: $C_{10}H_{15}NO$

Anabaena sp.

Most Common Toxic Genera in WA

- *Anabaena (Dolichospermum)* - anatoxin-a, microcystins, saxitoxins
- *Aphanizomenon* - anatoxin-a, saxitoxins, cylindrospermopsin
- *Microcystis* - microcystins
- *Oscillatoria* – microcystins, anatoxin-a, aplysiatoxins
- *Gloeotrichia* - microcystins

By: Robin Matthews

Most Common Genera Producing Anatoxin-a in WA

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Animal Deaths in Washington

- 1976 – Four dogs in Spokane County died after drinking water during a toxic *Anabaena* bloom
- 1982 – Two hunting dogs died, Moses Lake
- 1989 – Five cats died, American Lake, Pierce County
- 2006 – Two dogs died, Anderson Lake, Jefferson County
- 2007 – Two hunting dogs died, Potholes Reservoir
- 2015 – Dog died, Lake Washington

History

- 2005 – Legislative funding established
  - Funded through boat registration fees ($1/license)
- ECY and DOH held workshops around the state
  - Algae ID and Toxicity Testing
  - 2007 - Small Grants Program - $50,000
  - 2008 – MC and anatoxin-a (provisional) recreational standards
  - 2011 – Saxitoxins and cylindrospermopsin (provisional) recreational standards
- Annual Funds?

Freshwater Algae Control Program: Partners

- State Agencies: Ecology, DOH
  - Fish and Wildlife
- Close partnership with Local Health Jurisdictions/Counties
- Universities
  - Researchers (Public Health, Environmental Health)
  - Students
- WALPA – Lakes Protection Association

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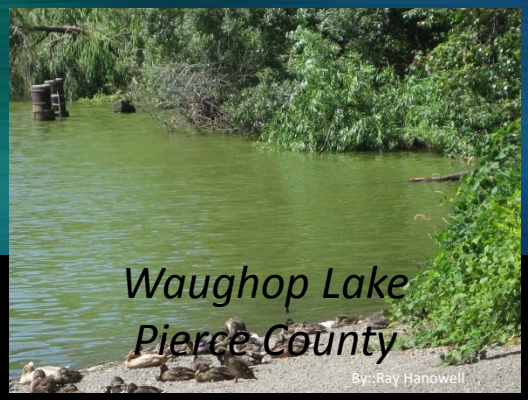
Freshwater Algae Control Program: Analyses

- King County Environmental Laboratory
- Anatoxin-a: LC/MS-MS
  - HPLC coupled to a triple quadrupole MS
  - Samples concentrated by SPE before analyses
  - Oehrle SA, Southwell B, Westrick J. 2010
- Drinking waters are analyzed for Anatoxin-a and Cylindrospermopsins by EPA Method 545
- MCs, Cylindrospermopsin, Saxitoxins: ELISA

Saxitoxin and Cylindrospermopsin in WA Lakes

Saxitoxin Total N = 836	Cylindrospermopsin Total N = 914
1 sample > 75 µg/L	0 samples > 4.5 µg/L
6 samples < 75 µg/L and > 1 µg/L	4 samples < 4.5 µg/L and > MDL
65 samples < 1 µg/L and > MDL	910 samples < MDL
765 samples < MDL	
* 75 µg/L WA saxitoxin recreational GV	* 4.5 µg/L WA cylindro recreational GV



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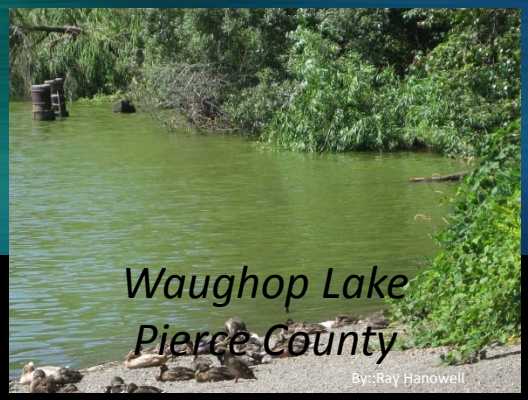
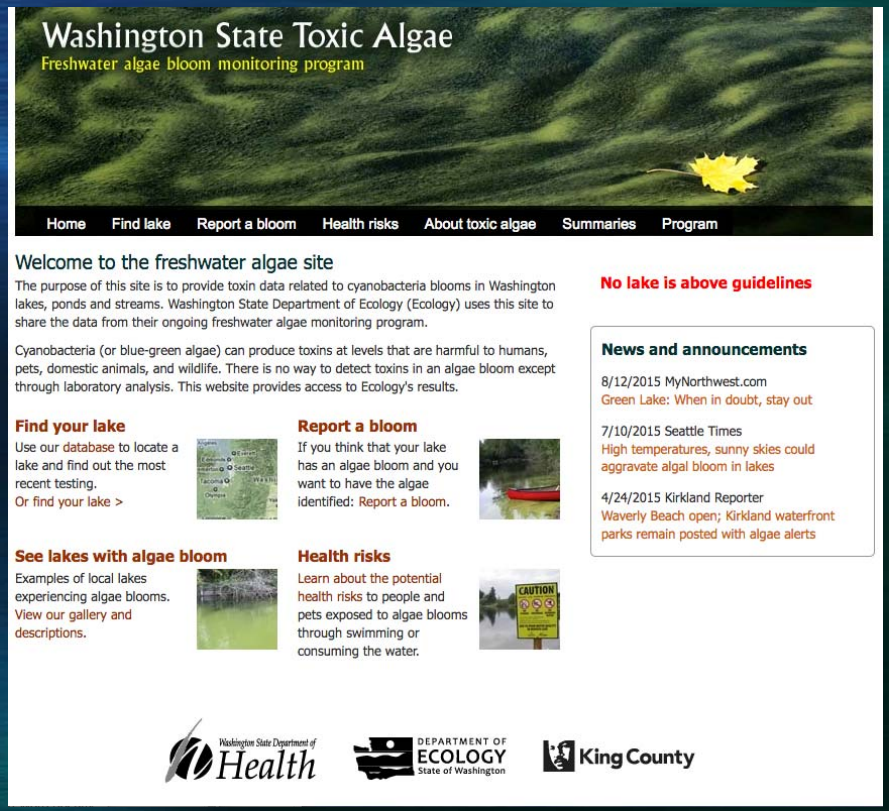


Freshwater Algae Control Program

- Freshwater algae listserv
- Searchable online database for algal ID and toxicity
- www.nwtoxicalgae.org



Freshwater Algae Control Program

The pins on the map represent the center of small lakes, regardless of where the sample was taken. To find more precise location information, download the toxin data and click the "view scum info" link. That is where specific sampling location information will be if it was provided. On larger lakes, (such as Lake Washington, Moses Lake and Potholes) pins represent the location of the sample if provided.

County
--All Counties--

WRIA
--All WRIAs--

Site
--All sites--

Toxin
--All toxins--

Minimum Toxin Concentration

Maximum Toxin Concentration

Start Date (MM/DD/YYYY)

End Date (MM/DD/YYYY)

Lab Sample Number

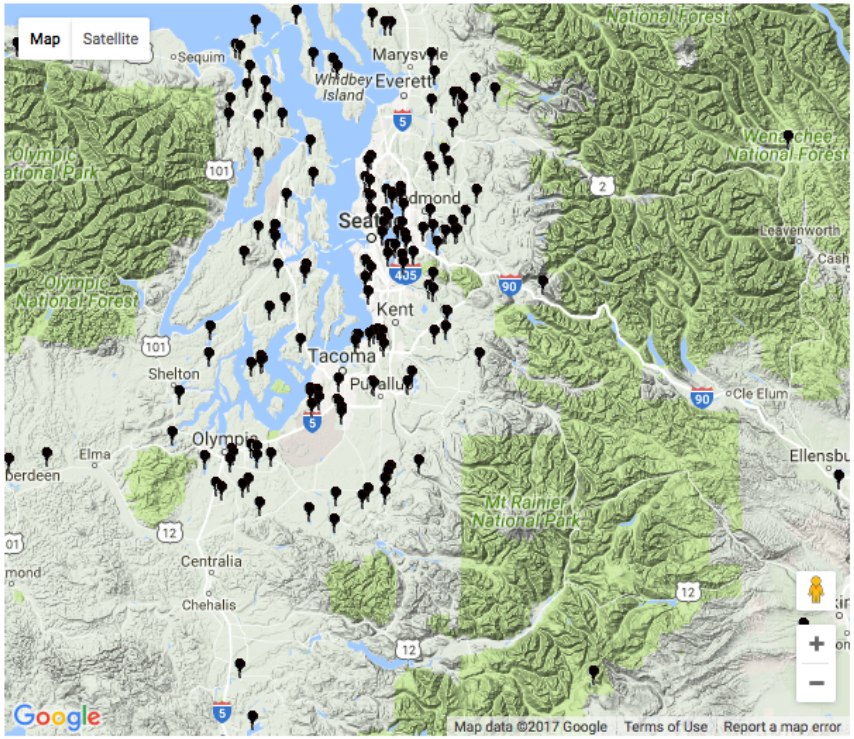
Plot Map
Clear Selections

Get data
Get Toxins
Get Phytoplankton

Export data
Export Toxin Data
Export Phyto Data

Hold **"shift" key** and drag a box around an area or **zoom in** using the slider on the left.

Map Legend: Exceeded state recreation guideline Within state recreation guideline
No data is available for the past 4 weeks.



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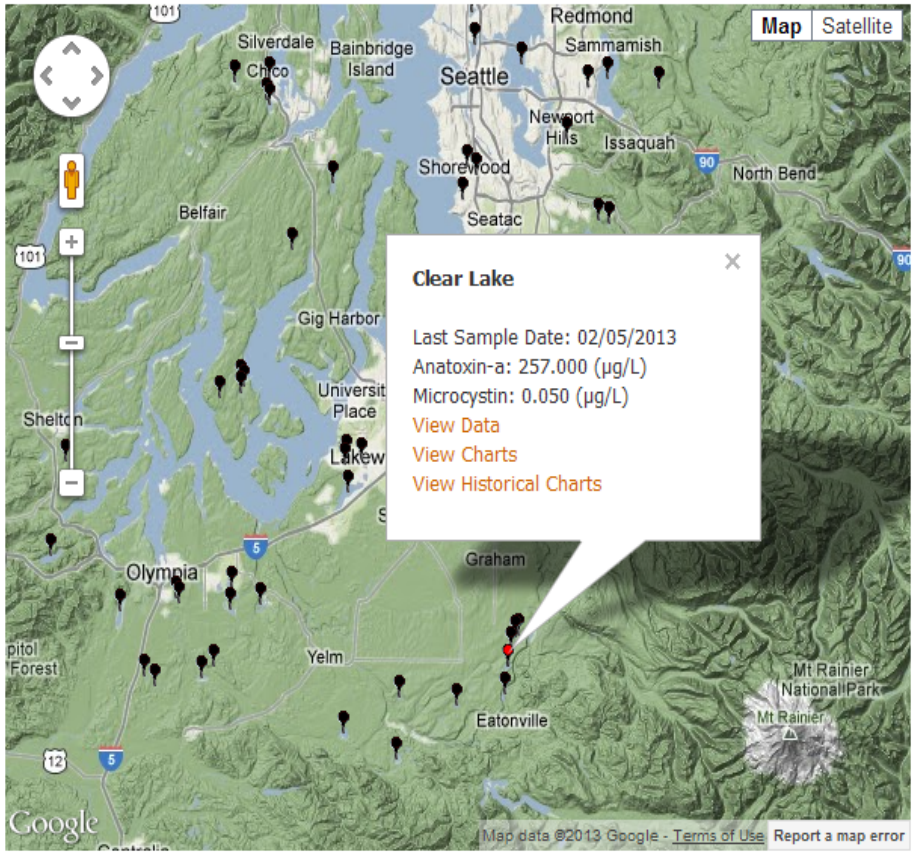

Washington State Toxic Algae

Freshwater algae bloom monitoring program

Home | Find lake | Report a bloom | Health risks | About toxic algae | Summaries | Program

Detailed search for your lake

This database contains the most current toxicity data available. All instances of values above the recreational guidelines are kept as up to date as possible, but values below the guidelines may be somewhat delayed in entry.Since there is a lag time from the date of sample to the date of analysis, be sure to check the sample date when looking at data or before you use the lake. Remember to use caution and avoid scums. "When in doubt, stay out!"

Your local jurisdiction may have more specific information about your lake. Questions? Contact Lizbeth Seebacher at Department of Ecology.

If a lake is not listed, it has not been tested for toxic algae through the Ecology program.

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Toxin:

County	WRIA Number	Site	Lab Sample Number	Collect Date	Parameter	Toxin Conc. (µg/L)	MDL (µg/L)	Above State Guideline	Additional Information
King	9	Lake Wilderness	L66625-1	11/14/2016	Microcystin	<MDL	0.200	No	View
King	9	Lake Wilderness	L66625-2	11/14/2016	Microcystin	0.296	0.200	No	View
King	9	Lake Wilderness	L66523-1	10/31/2016	Microcystin	0.322	0.200	No	No
King	9	Lake Wilderness	L66477-3	10/23/2016	Microcystin	0.223	0.200	No	No
King	9	Lake Wilderness	L66433-1	10/18/2016	Microcystin	146.000	0.200	Yes	View
King	9	Lake Wilderness	L66358-2	10/10/2016	Microcystin	11.400	0.200	Yes	No
King	9	Lake Wilderness	L66309-2	10/03/2016	Microcystin	<MDL	0.160	No	No
King	9	Lake Wilderness	L66191-1	09/19/2016	Anatoxin-a	<MDL	0.010	No	View
King	9	Lake Wilderness	L66191-1	09/19/2016	Microcystin	10.000	0.160	Yes	View

Plot Map

Clear Selections

Get data

Get Toxins

Get Phytoplankton

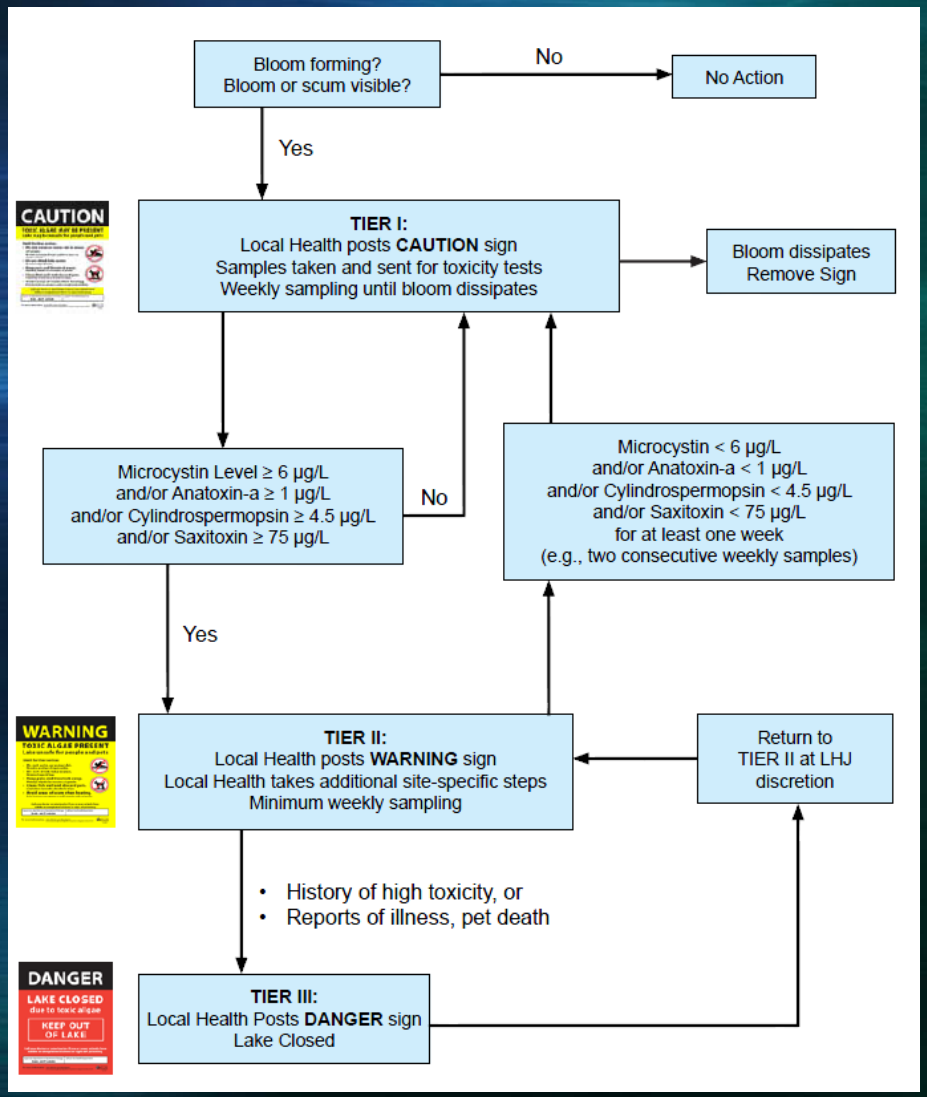
Export data

Export Toxin Data

Export Phyto Data

Freshwater Algae Control Program

- Lake Management Protocol
- Three-tiered approach
- Implemented by LHJs and lake managers



Freshwater Algae Control Program

- Outreach/education
- Veterinary focus
- Survey ID'd preferences
  - Clinic Posters
  - Differential diagnosis cards

Blue-Green Algae Exposure and Clinical Information - There are no antidotes to these toxins. Medical care is supportive.

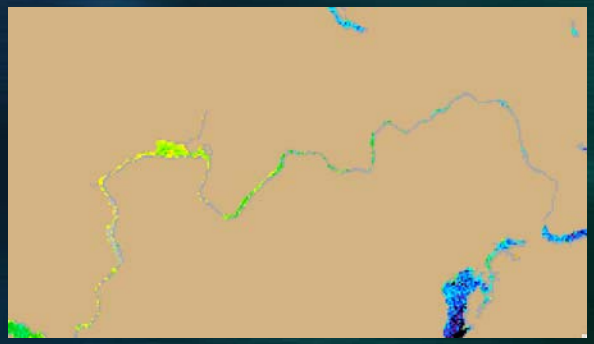
Exposure Route	Likely Signs	Onset to Signs	Differential Diagnosis	Possible Laboratory or Other Findings
Swallowing water with toxic blue-green algae (cyanobacteria) or other toxins **Licking fur or hair** contaminated with toxic blue-green algae	**Hepatotoxins** - Acute depression - Weakness & incoordination - Loss of appetite - Excess drooling - Vomiting and diarrhea - Abdominal tenderness - Jaundice - Dark urine	One or two hours, or more	Acetaminophen, nonsteroidal anti-inflammatories, aflatoxin, mushrooms, copper, zinc, iron, xylitol, sago palm	- Elevated bile acids & liver enzymes - Hypoglycemia - Prolonged clotting times - Proteinuria - Presence of toxin in clinical specimens (liver, gastrointestinal contents) collected from animals
	Neurotoxins - Excess drooling - Apprehension & anxiousness - Vomiting - Muscle twitching - Seizures - Respiratory failure	Minutes to hours	Organophosphate and carbamate insecticides, strychnine, metaldehyde, pyrethrins, moldy foods, chlorinated hydrocarbon pesticides, bromethalin, mushrooms	- Presence of toxin in clinical specimens from stomach contents taken from animals that became ill
Skin contact with toxic blue-green algae or other toxin(s)	**Dermal Toxins** - Rash, hives, allergic reaction	Minutes to hours	Other dermal allergens	- Blue-green staining of fur or hair

Monogastric animals appear less sensitive than ruminants or birds; however, the dose-response curve is very steep in dogs – up to 90% of a lethal dose may elicit no clinical signs. Surviving animals have a good chance for recovery. While therapies for cyanobacterial poisonings have not been investigated in detail, activated charcoal slurry is likely to be of benefit. Health effects from exposure are derived from reports of animal poisonings. For more information see Department of Health (www.doh.wa.gov/algae) or the Merck Veterinary Manual (www.vetmanual.com).

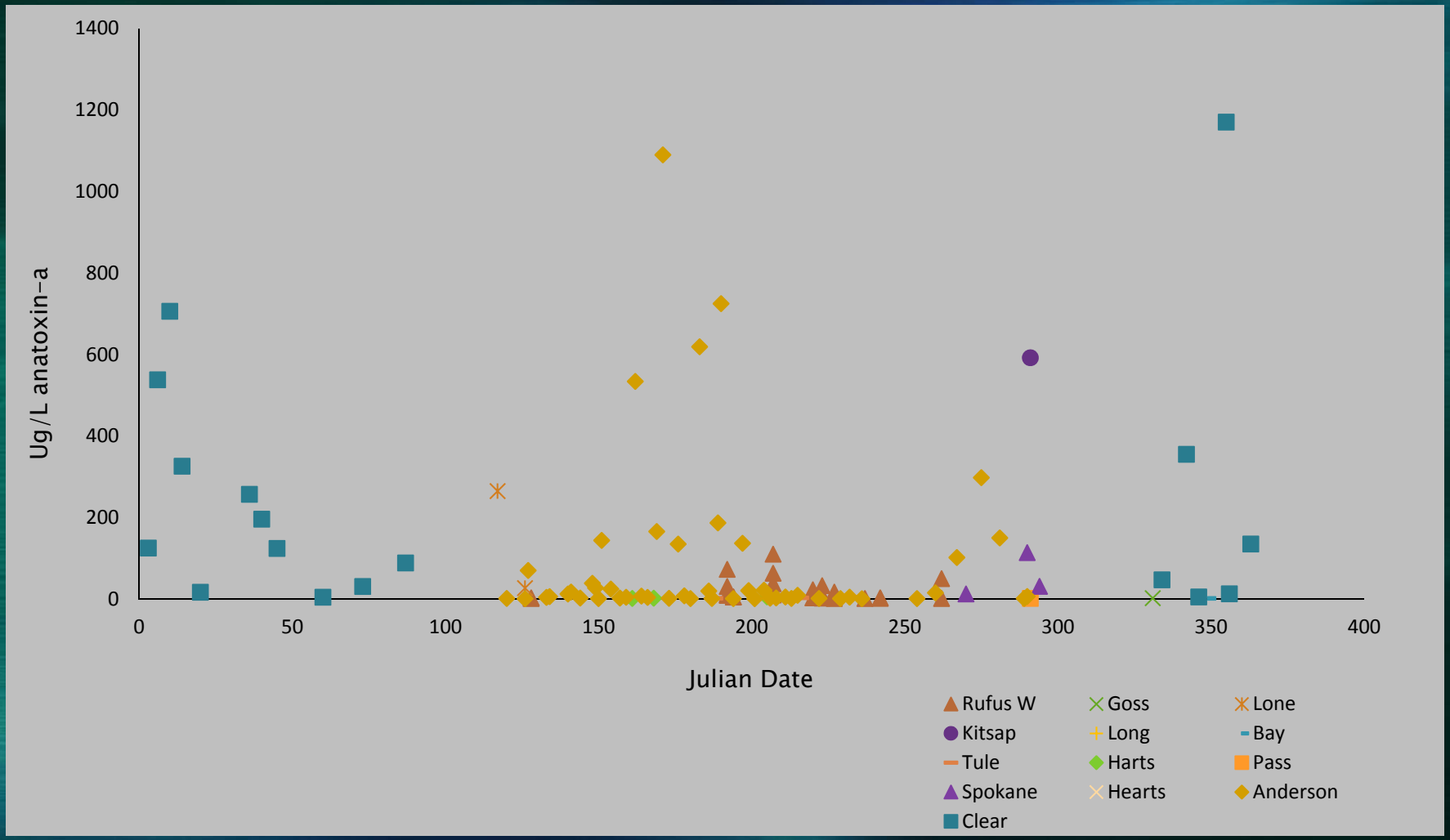
Anatoxin-a in WA Lakes

Year	# Lakes	# Samples above Std.	Maximum Conc. (µg/L)
2009	4	21	144
2010	5	14	538
2011	8	32	1,170
2012	7	40	706
2013	6	25	257
2014	5	15	991
2015	9	27	7,951
2016	7	19	1,300

- Rufus Woods
- Summit Lake



Anatoxin-a: Seasonal Distribution



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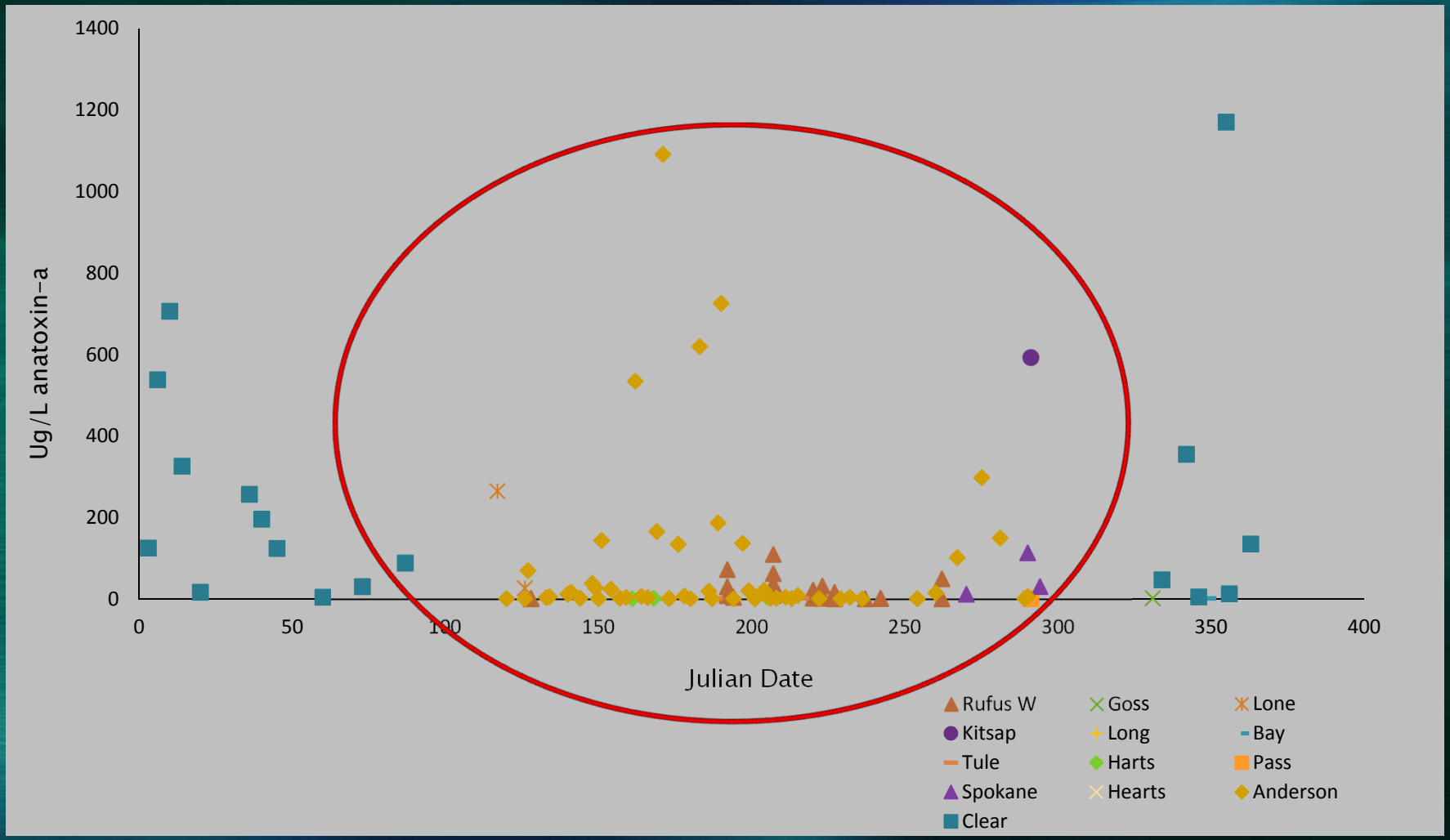
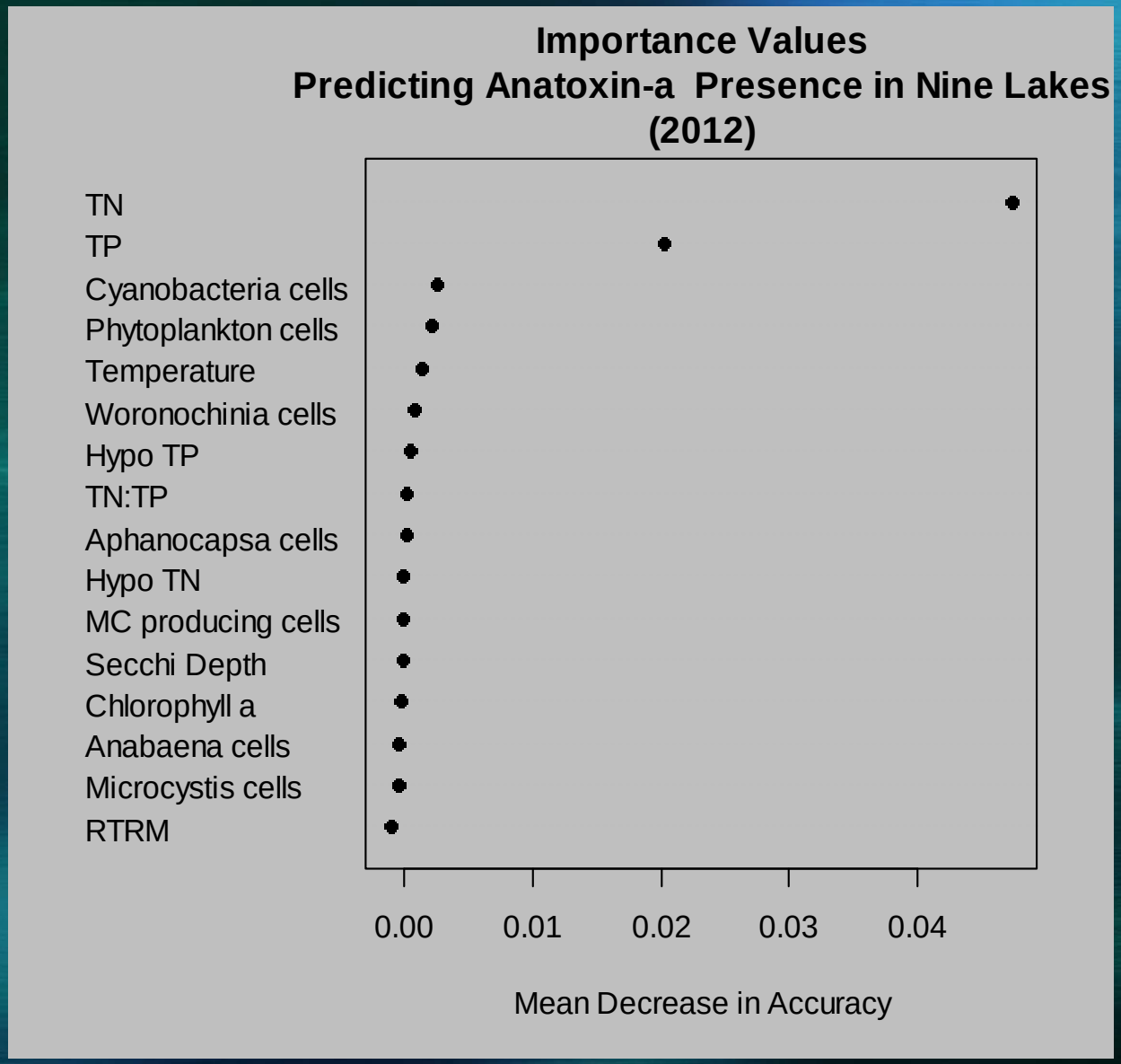
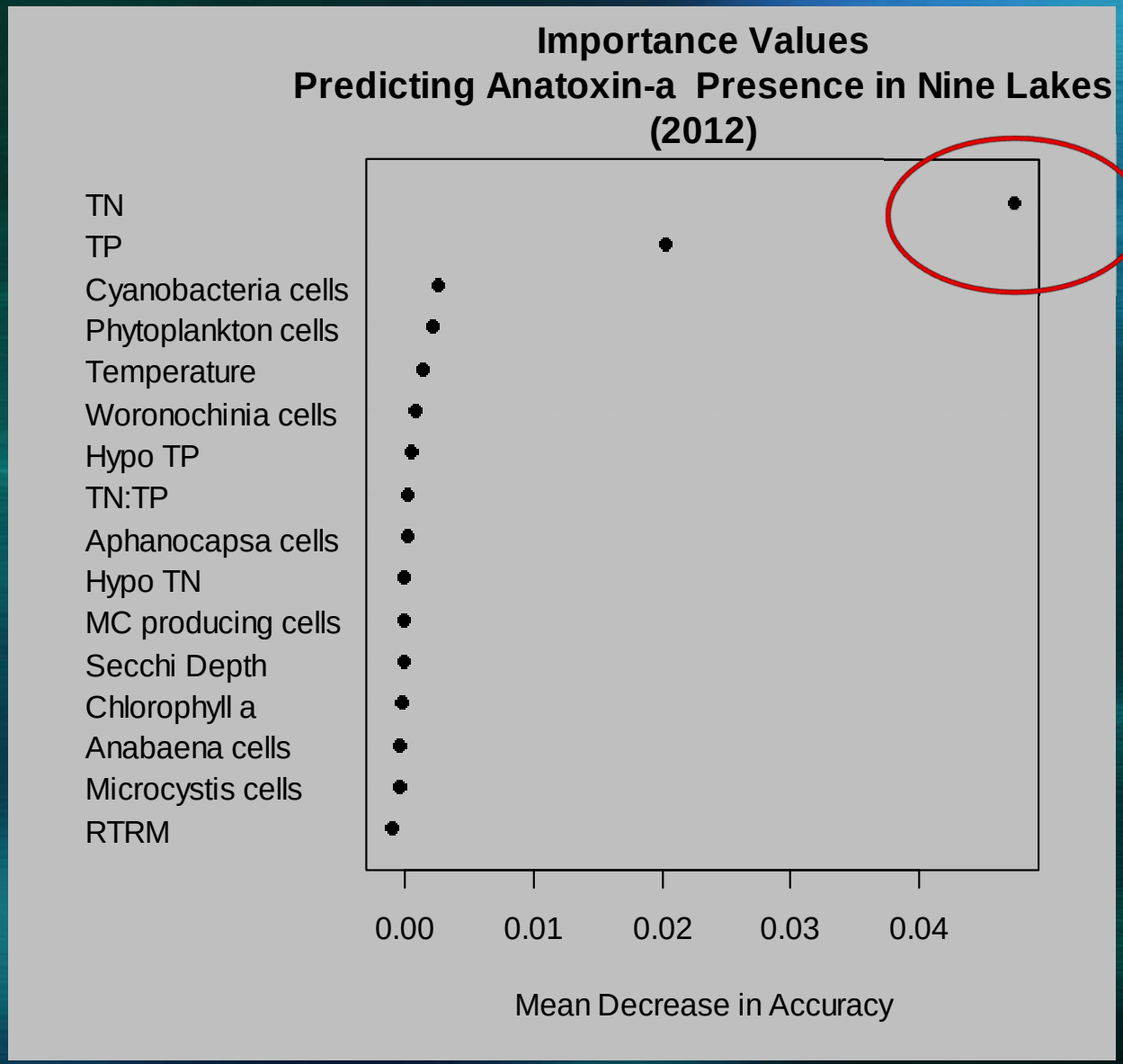
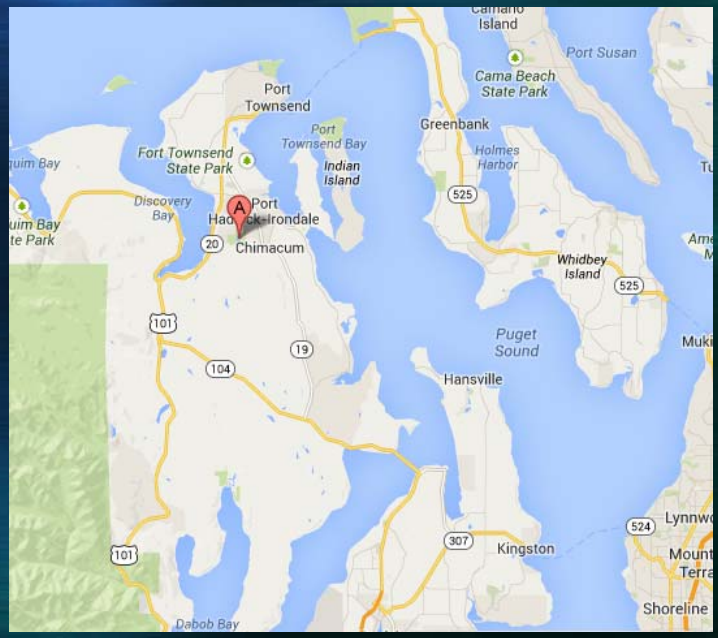






Toxic Gene Presence

- Puget Sound Lowland Lakes (2012 season)
- Anderson Lake, Jefferson County
  - Very high anatoxin-a concentrations



Anabaena flos-aquae-like morphotype is a major anatoxin-a producer in Anderson Lake

(9 July 2012 and 24 June 2013)

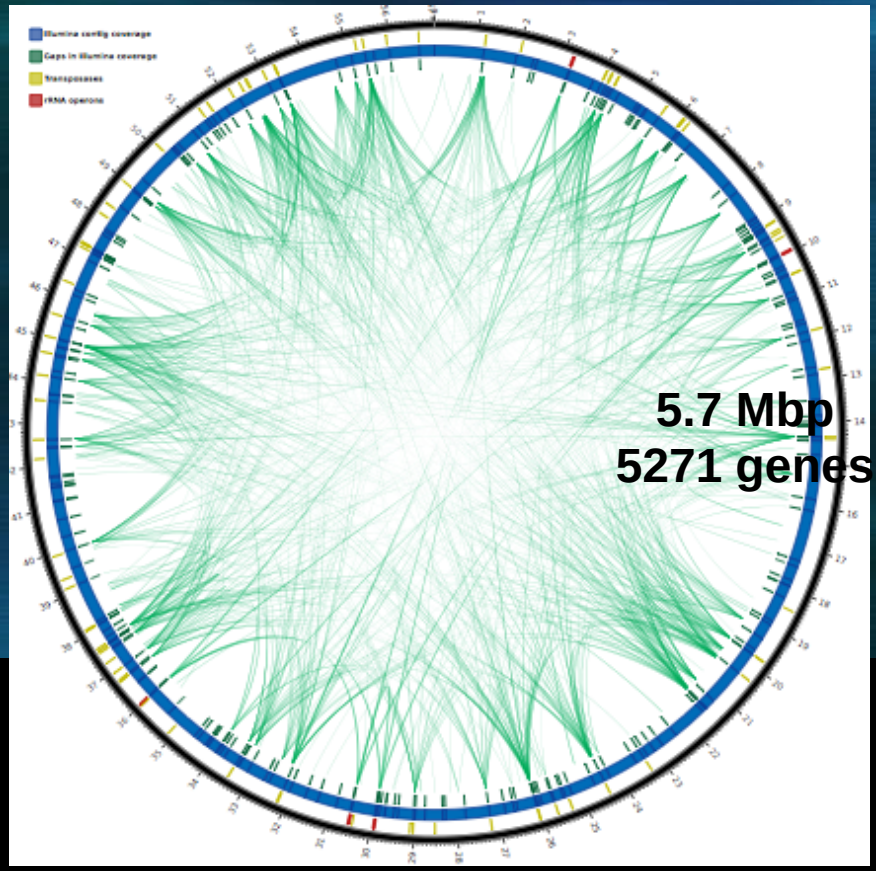
Oregon State University OSU

Anabaena sp. WA102

Culture from 5/20/2013 (@ 12.5 µg/L ana-a)

Grown in BG-11 medium without N

Tested positive for anatoxin-a production

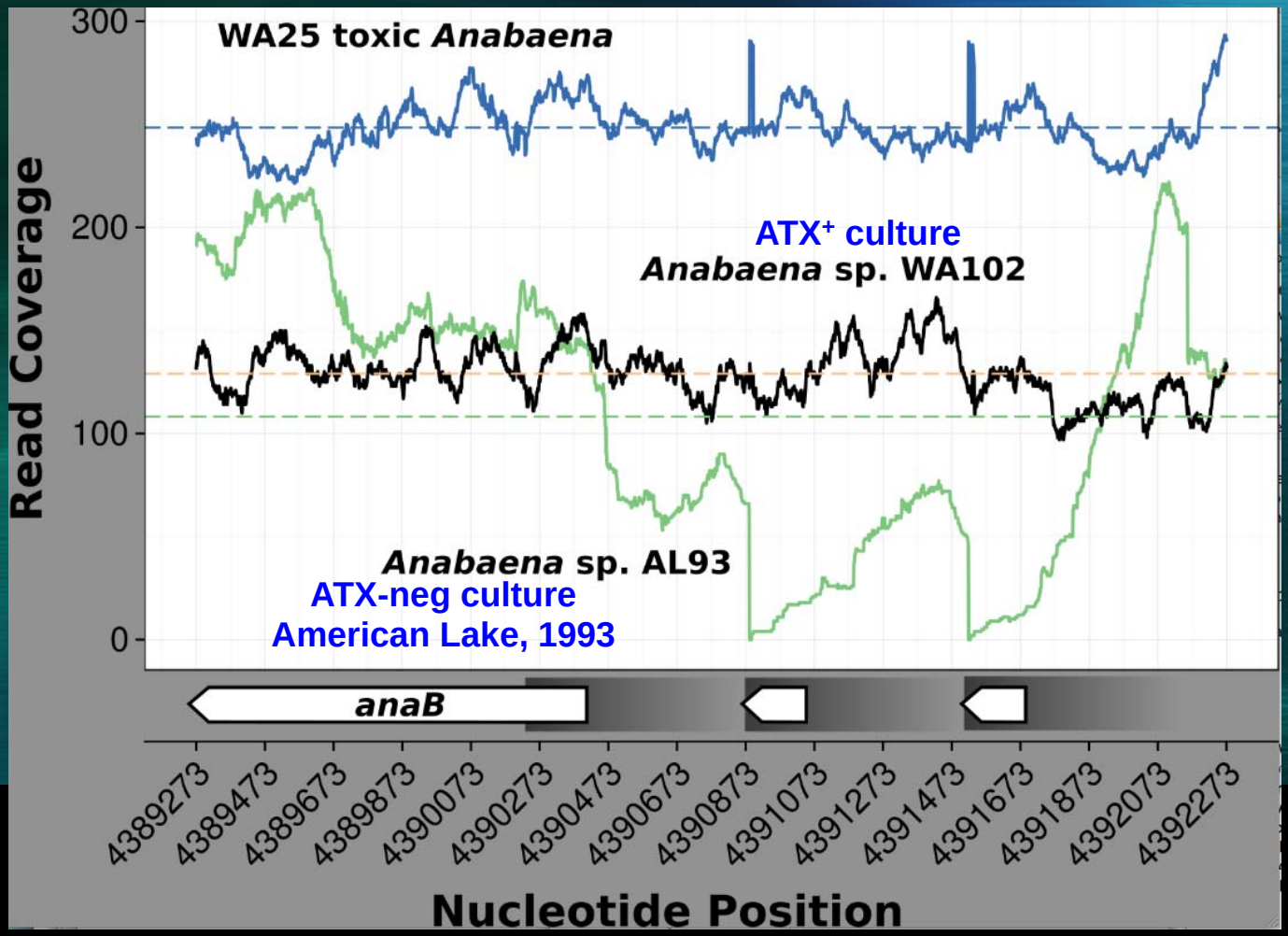


Structural and Functional Analysis of the Finished Genome of the Recently Isolated Toxic Anabaena sp. WA102. 2016, BMC Genomics NM Brown, RS Mueller, JW Shepardson ZC Landry, JT Morre, CS Maier, FJ Hardy, TW Dreher

Oregon State University OSU

Anatoxin-a biosynthetic gene clusters

Anderson Lake 9 July 2012 metagenome



Triplicated gene control region: is *Anabaena* sp. WA102 an ATX super producer?

Oregon State University OSU

Lessons Learned

- Importance of stable funding –toxicity tests and studies
- Importance of partnerships with LHJ – eyes on lakes
- Constant feedback from partners (ex. Signs)
- Hard to manage anatoxin-a in DW without health standard

Clear Lake
196 ug/L anatoxin-a

Remaining Questions/Needs

- Overall: Toxicity studies to set national criteria values
- In-state: Better coordination for tracking animal and human health illnesses
- Importance of benthic mats in lakes?
- Nutrients – TN? Micronutrients?
- Genetic screening in other lakes, annual variability of gene expression in Lake Anderson

Lake Howard, Snohomish County

Questions?

Differential Diagnosis: Neurotoxins

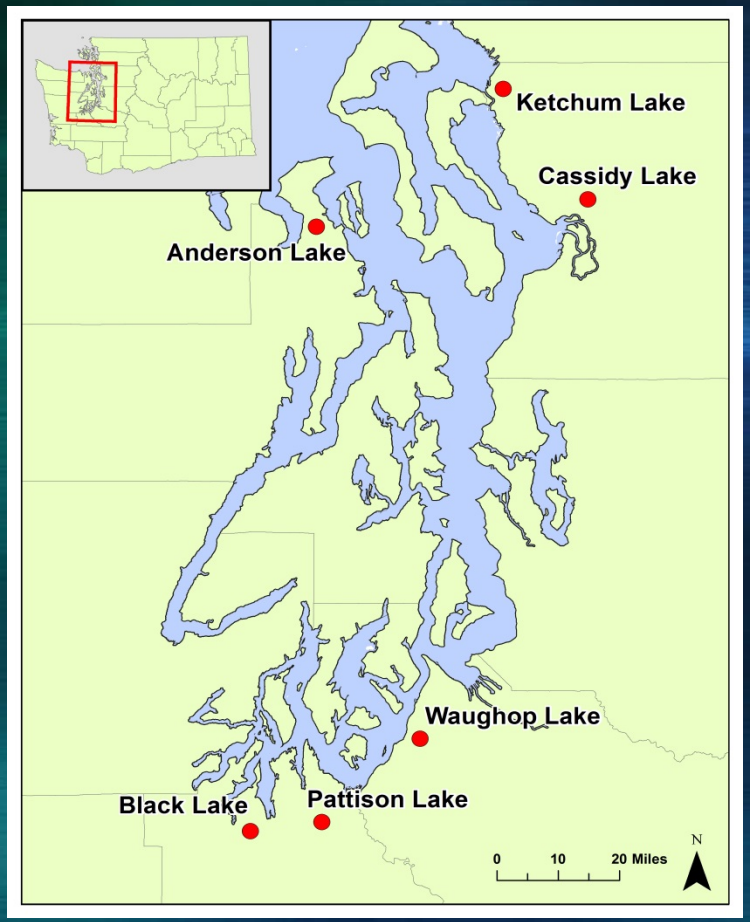
- Organophosphate and carbamate insecticides
- Strychnine
- Metaldehyde
- Pyrethrins
- Moldy foods
- Chlorinated hydrocarbon
  - pesticides
- Bromethalin
- Mushrooms

Confirmed MC in WA Fish

- Methods
  - Used two types of ELISA and LC-MS/MS
- Highest concentrations in the liver:
  - **mean of 50 ug/Kg - 2010 , 64 ug/Kg – 2012 (wet weight)**
- Next highest concentration in gut
- Lowest in muscle tissue:
  - **mean of 5.6 ug/Kg, - 2010, 14 ug/Kg – 2012 (wet weight)**

- *Rainbow Trout*
- *Pumpkinseed*
- *Yellow Perch*
- *Brown Bullhead*
- *Lake Whitefish*
- *Largemouth Bass*
- *Rock Bass*
- *Cutthroat*
- *Largescale sucker*
- *Northern Pikeminnow*

ECY Collected 10 Fish Species



Microcystins in Fish (ADDA ELISA)

Waterbody	Species	Tissue	N =	Microcystins (ug/Kg, wet)	Reference
Western Wash. lakes (5)	4 species	muscle	14	5.6	Present study
		liver	16	50	
Western Wash. lakes (6)	6 species	muscle	20	14	Johnson (2010)
		liver	11	64	
Lago de Patzcuero, Mexico	Carp	muscle	?	5.0	Berry et al. (2011)
		liver	?	94	
Lake Albufera, Spain	Mullet	muscle	103	5.0	Romo et al. (2012)
		liver	103	200	
Greek Lakes (13)	Carp	muscle	130	7.1	Papadimitriou et al. (2010)
		liver	130	124	
Lake Ontario and Lake Erie	17 species	muscle	57	7.8	Poste et al. (2011)

MCs in Fish Muscle Tissue

- Recommend that ELISA be used only to screen fish tissue
- Recommend that LC-MS/MS be used in conjunction with ELISA to confirm results of screening
- Recommend cleaning and discarding guts before eating
- OK to eat muscle tissue, based on present studies

Hardy, FJ, A Johnson, K Hamel, E Preece. 2015. Cyanotoxin bioaccumulation in freshwater fish, Washington State, USA. Environ Monit Assess (2015) 187: 667

Mussels in Puget Sound

Pilot Project - 2012

- Bay Lake – Mayo Cove
- Lake Steilacoom – Chambers Creek
- Kitsap Lake – Chico Creek

Repeated in 2013, 2014

WSU analyzed MCs (E. Preece)

- Found MCs present in mussels associated with lake blooms

MC in Puget Sound Mussels

- Method development
  - Identifying best methods for routine ELISA detection of microcystin in seafood. 2015. Preece et al. Environ Monit Assess 187:12
- Detected MC in mussels
  - First detection of microcystin in Puget Sound, Washington, mussels (*Mytilus trossulus*). 2015. Preece et al. Lake and Reservoir Management, 31:1, 50-54.
- ID'd MC in lake, stream, and mussels
  - Transfer of Microcystin from Freshwater Lakes to Puget Sound, WA and Toxin Accumulation in Marine Mussels (*Mytilus trossulus*). Preece et al. Ecotoxicol Environ Saf 122-98-105.