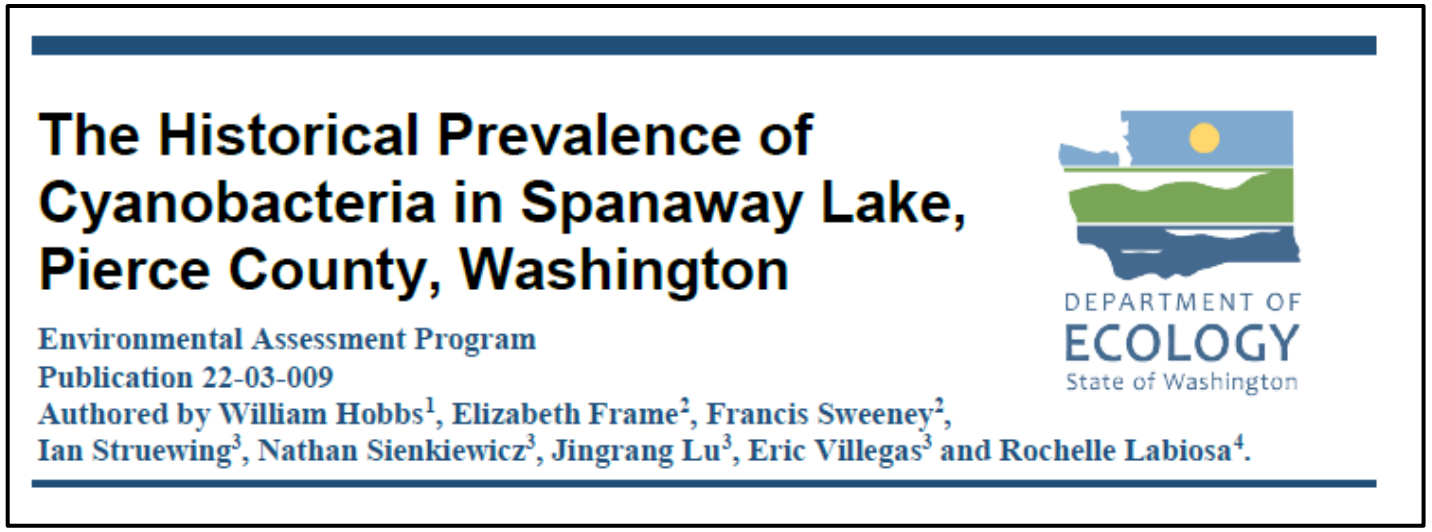
Seasonal and long-term toxin production in a South Sound lake

William Hobbs
Environmental Assessment Program

DEPARTMENT OF ECOLOGY
State of Washington

Two Studies over Different Timescales

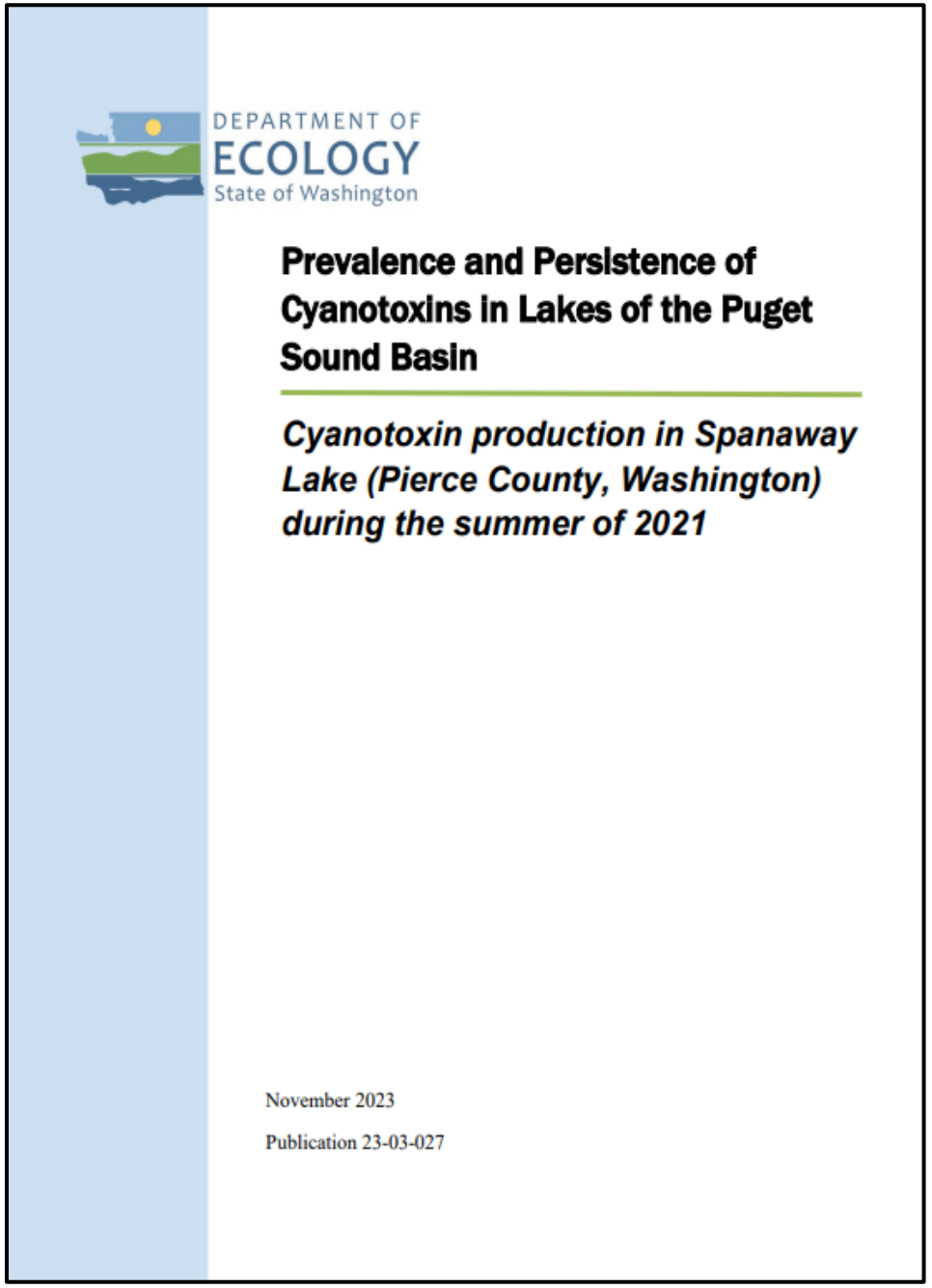
1. Cyanobacteria communities and toxin production during summer 2021
2. Prevalence of Cyanobacteria in Spanaway Lake over the last 150 years



The Historical Prevalence of Cyanobacteria in Spanaway Lake, Pierce County, Washington

DEPARTMENT OF ECOLOGY
State of Washington

Environmental Assessment Program
Publication 22-03-009
Authored by William Hobbs[1], Elizabeth Frame[2], Francis Sweeney[2], Ian Struewing[3], Nathan Sienkiewicz[3], Jingrang Lu[3], Eric Villegas[3] and Rochelle Labiosa[4].



DEPARTMENT OF ECOLOGY
State of Washington

Prevalence and Persistence of Cyanotoxins in Lakes of the Puget Sound Basin

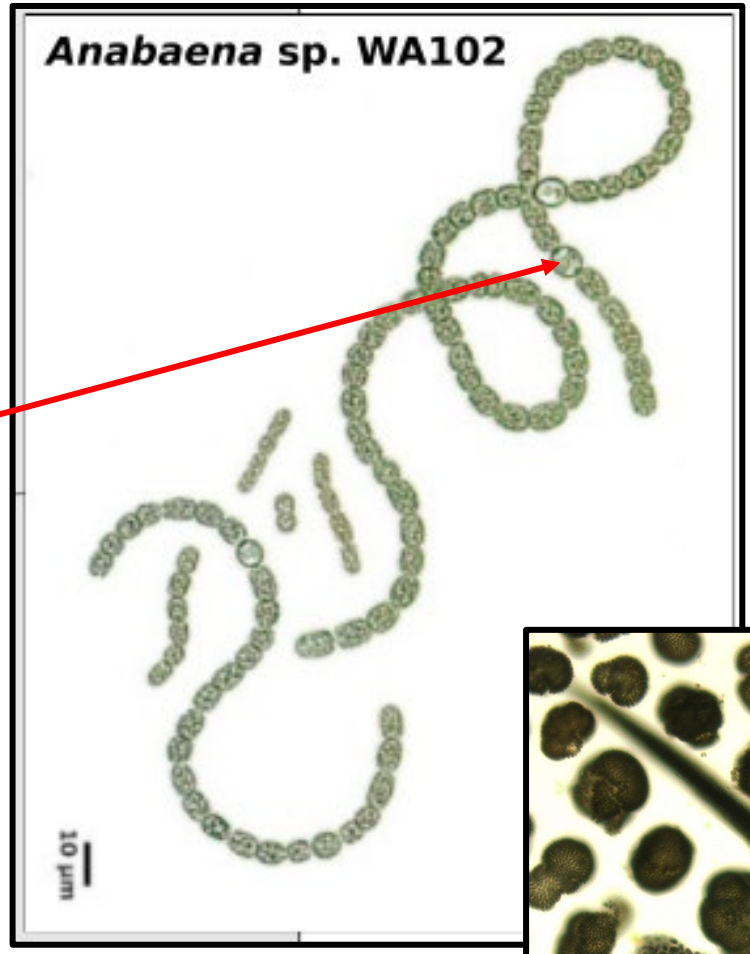
Cyanotoxin production in Spanaway Lake (Pierce County, Washington) during the summer of 2021

November 2023

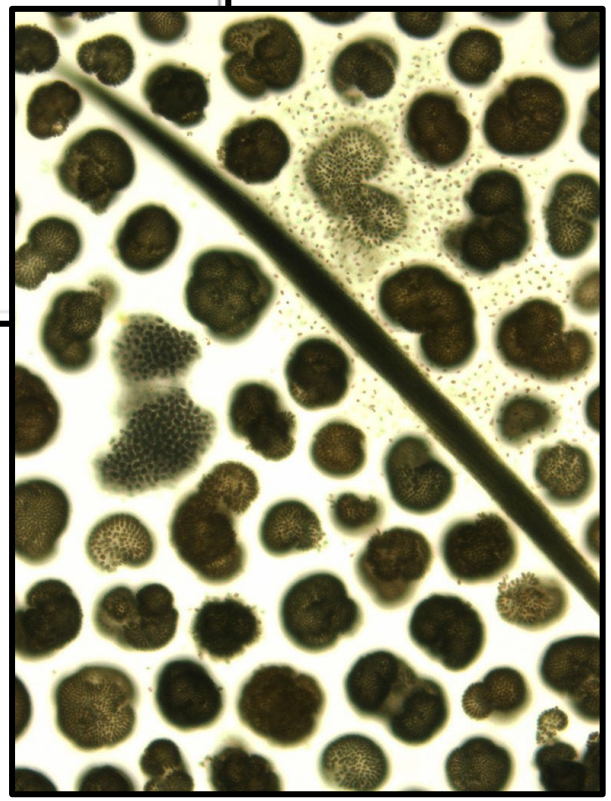
Publication 23-03-027

Some General Ecological Traits

- Photosynthetic bacteria, not algae
- Possible nitrogen fixation (heterocytes)
- Colony formation inhibits predation (grazing)
- Phosphorus and nitrogen storage mechanisms
- Gas vesicles for vertical migrations
- Akinete formation (“spores”)
- Toxin production



Dolichospermum sp. WA102 (formerly known as *Anabaena*)



Woronchinia, Microcystis and Aphanizomenom

Toxicity and Exposure Guidelines

Cyanotoxins	Acute Health Effects in Humans	WADOH recreational guideline	EPA recreational guideline
Microcystin-LR	Abdominal pain, headache, sore throat, vomiting and nausea, dry cough, diarrhea, blistering around the mouth, and pneumonia	8 µg/L	8 µg/L
Anatoxin-a group	Tingling, burning, numbness, drowsiness, incoherent speech, salivation, respiratory paralysis leading to death (experimental animals)	1 µg/L	NA
Cylindrospermopsin	Fever, headache, vomiting, bloody diarrhea	4.5 µg/L	15 µg/L
Saxitoxin	Tingling, numbness, drowsiness, incoherent speech, salivation, respiratory paralysis leading to death. "Paralytic Shellfish Poisoning" in the marine	75 µg/L	NA

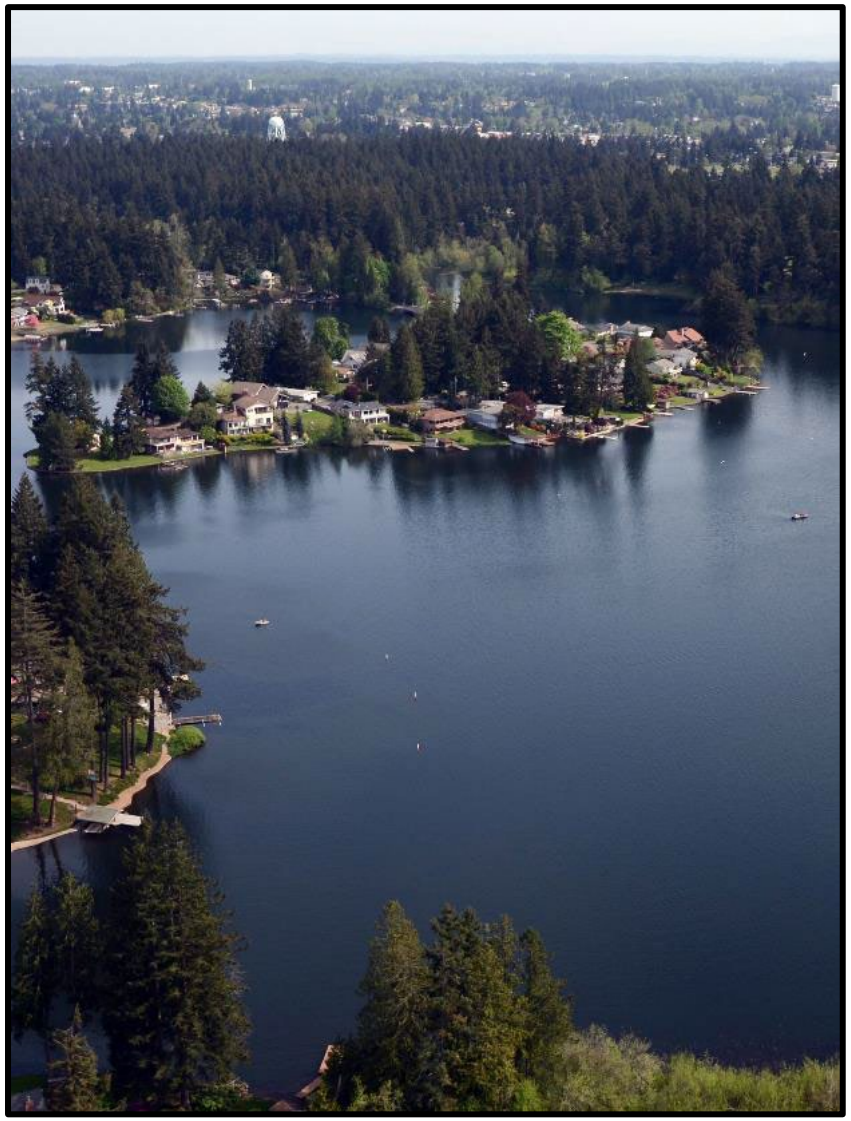
Note: Ecology is currently pursuing rule-making for surface water criteria of toxins

Spanaway Lake, Washington State



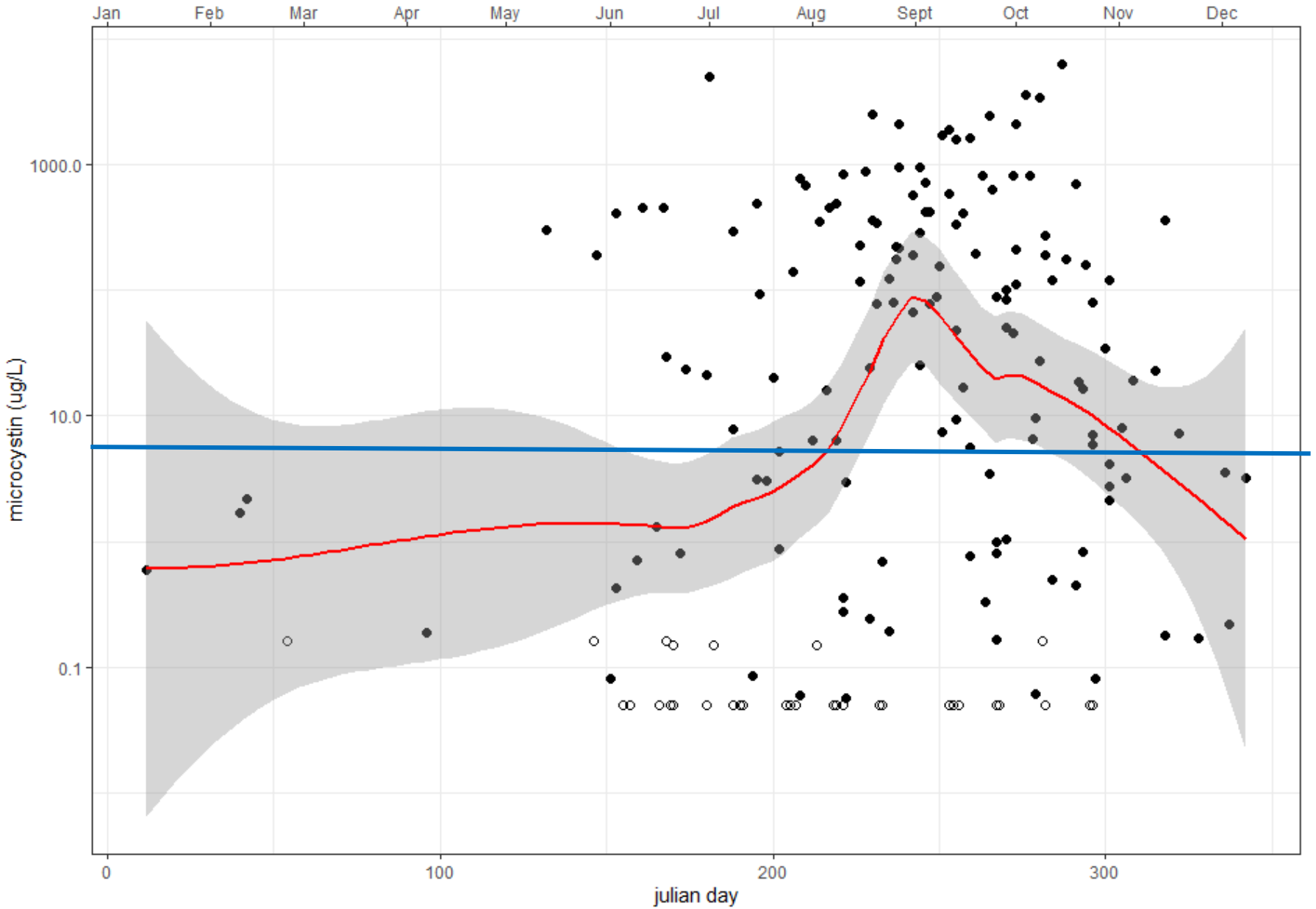






Microcystin (MC)





2007-2022 samples from shoreline accumulations at Spanaway Lake

Study Goals: Cyanobacteria communities and toxin production during the summer of 2021

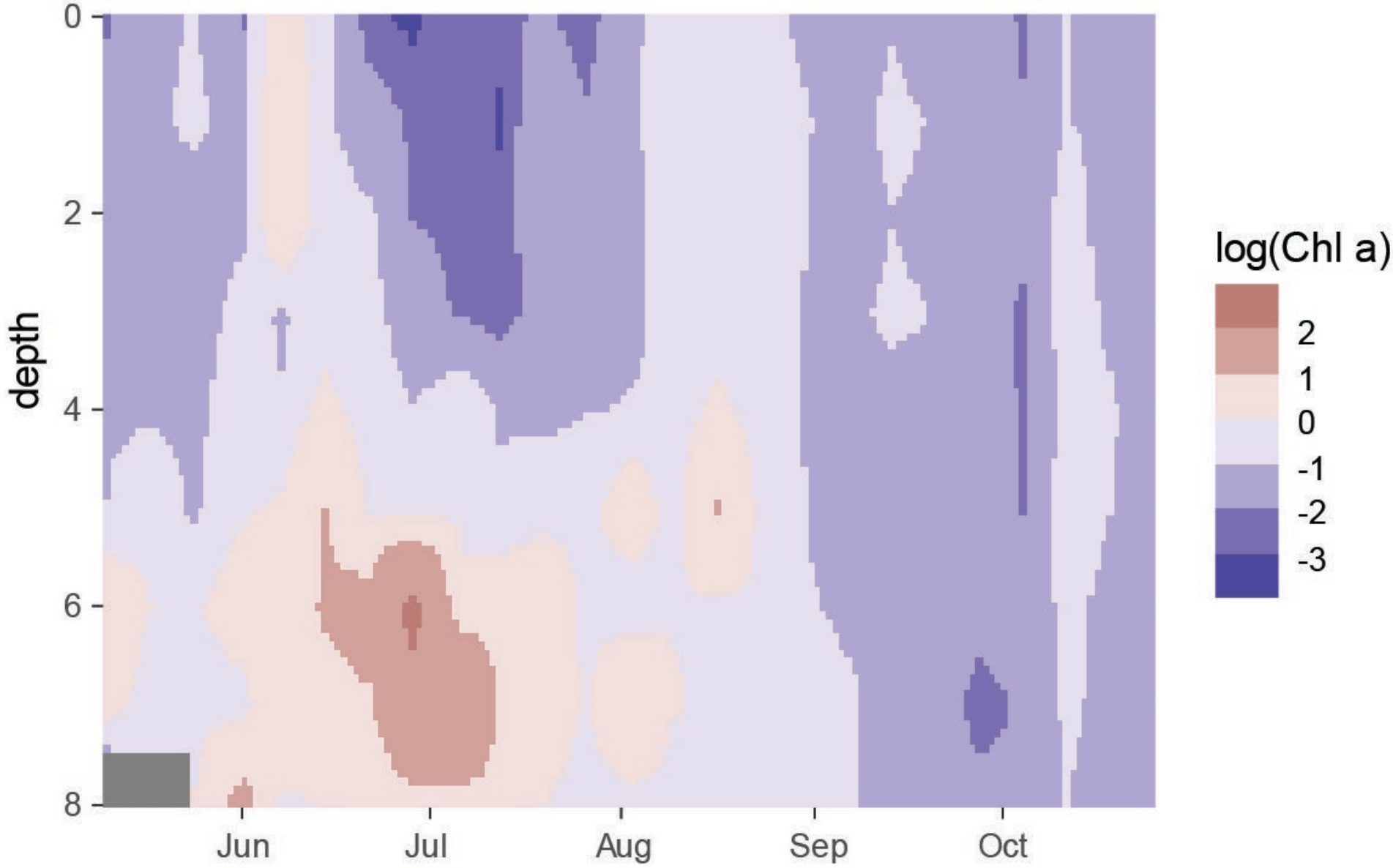


1. Document the succession of cyanobacteria from May-October
2. Measure toxins and toxin-producing genes in the water column
3. Explore associations among nutrients, cyanobacteria communities, and cyanotoxin production



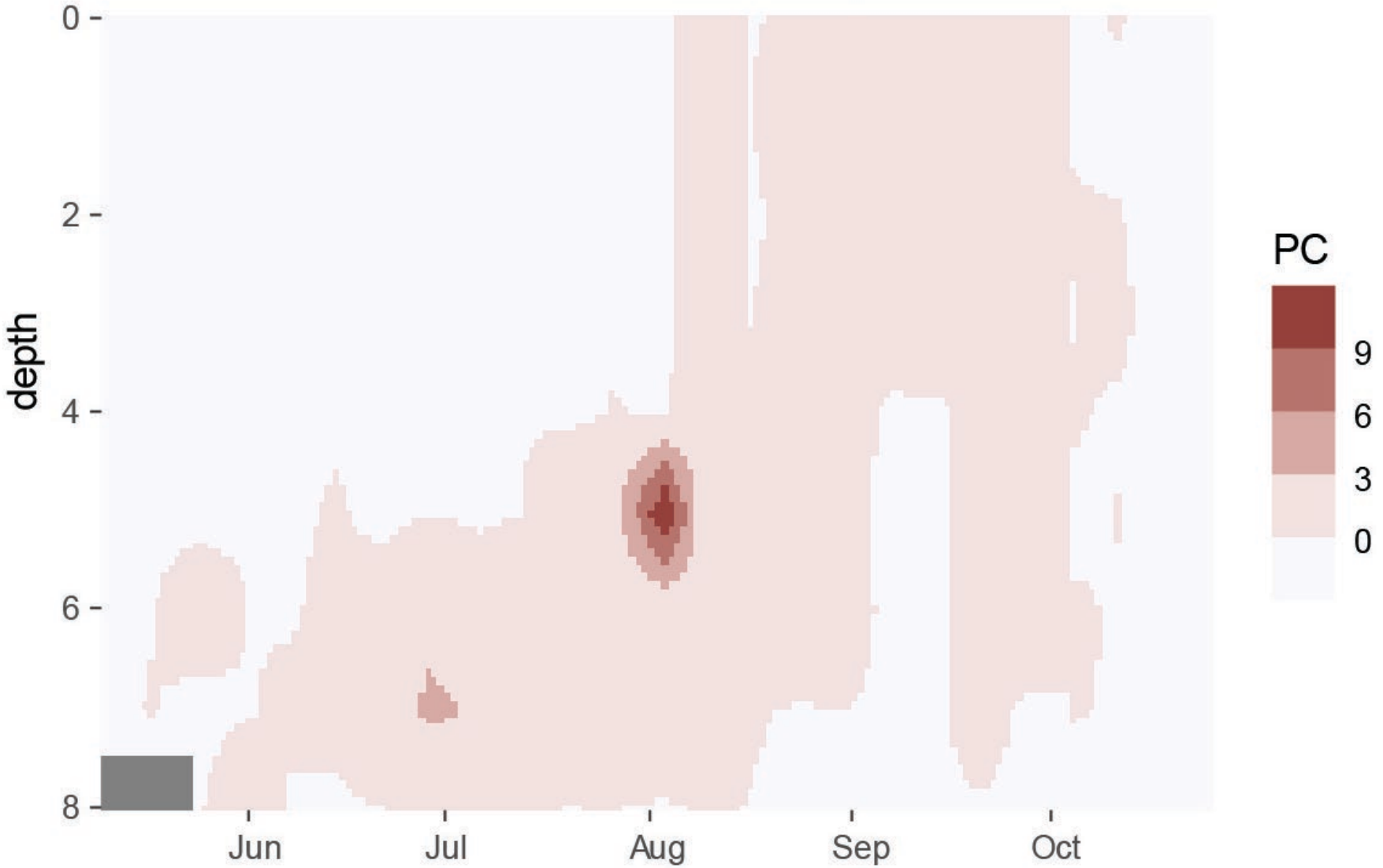
In situ total chlorophyll profile

- Late spring (early June) phytoplankton bloom
- Beginning of deep chlorophyll maximum (DCM) in late June and a peak in early to mid-July



In situ total phycocyanin (PC) profile

- Early summer cyanobacteria growth in bottom waters
- Late-July and early August PC concentrations peak at 5 m, then throughout water column until October



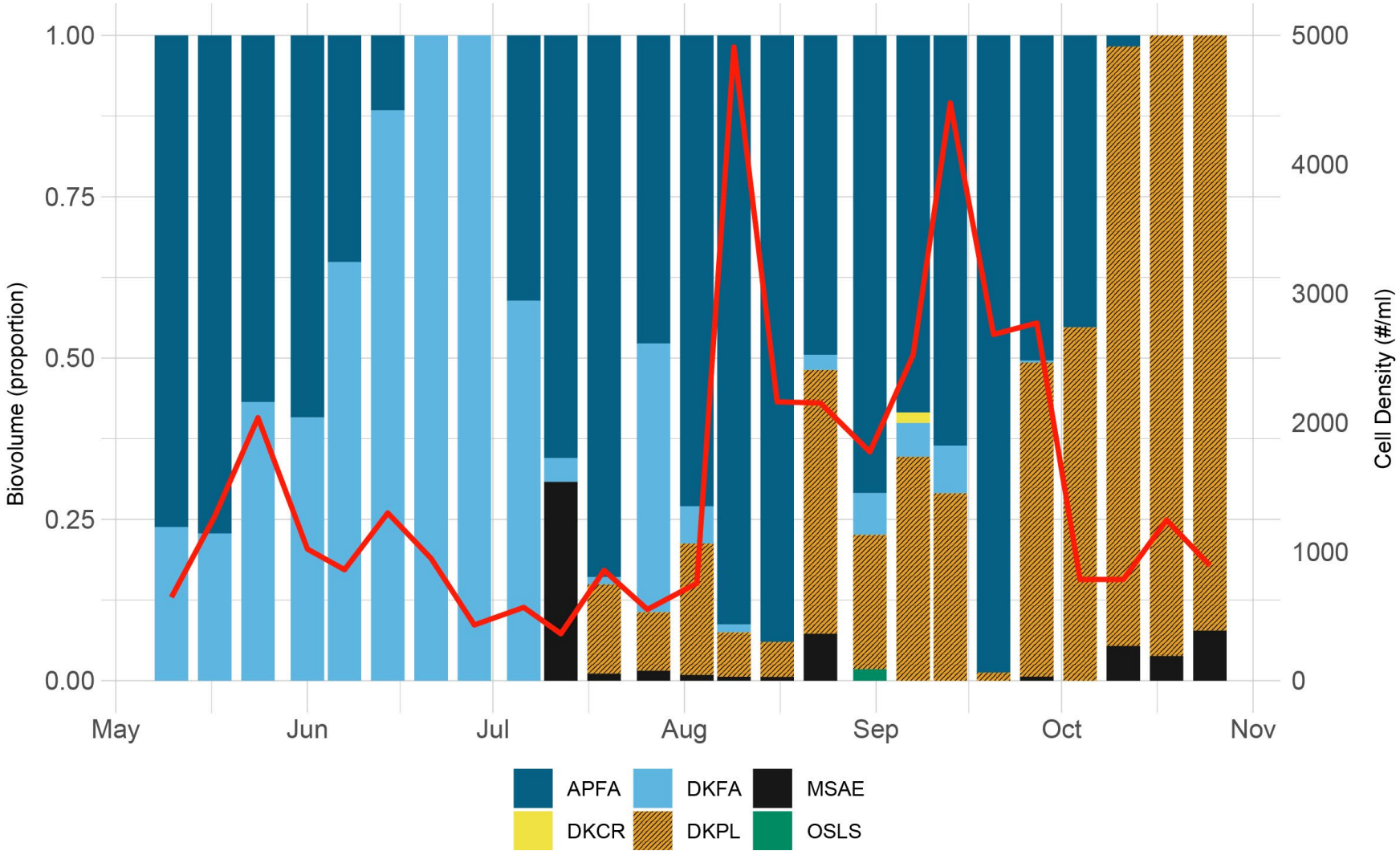
Cyanobacteria Succession

Nostocales (APFA; DKFA; DKPL)

- Filamentous
- Some fix dissolved nitrogen gas
- Very few strains contain genes to produce microcystin

***Microcystis* sp (MSAE)**

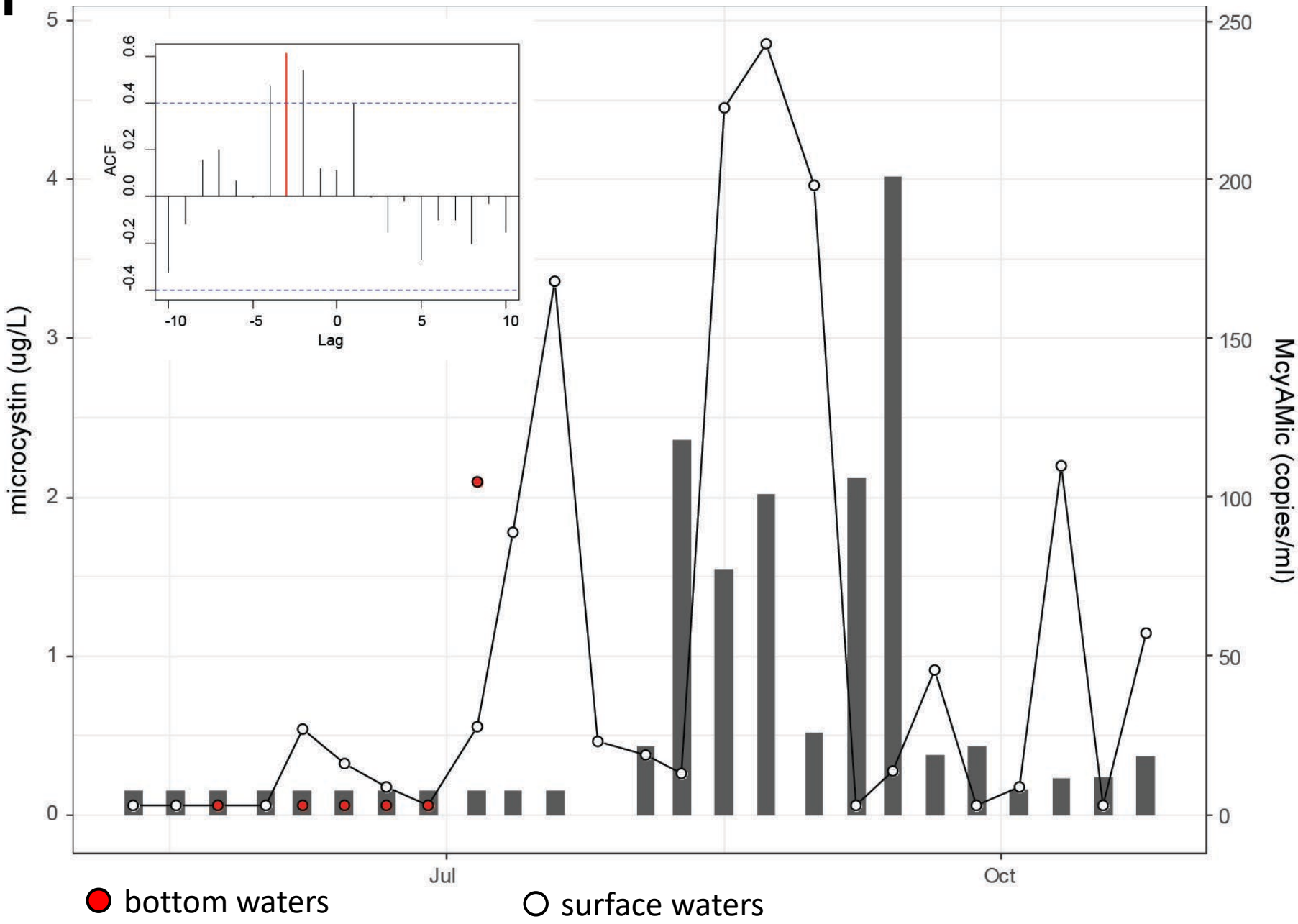
- Colonial, spherical
- Widespread microcystin-producing species



Take Home: *Microcystis* sp. are low abundance but likely producing microcystin

Toxin Production

- Microcystin-producing genes (*line graph*) precede measurable microcystin accumulation (*bar graph*)
- ~2-3 week lag and significant correlation



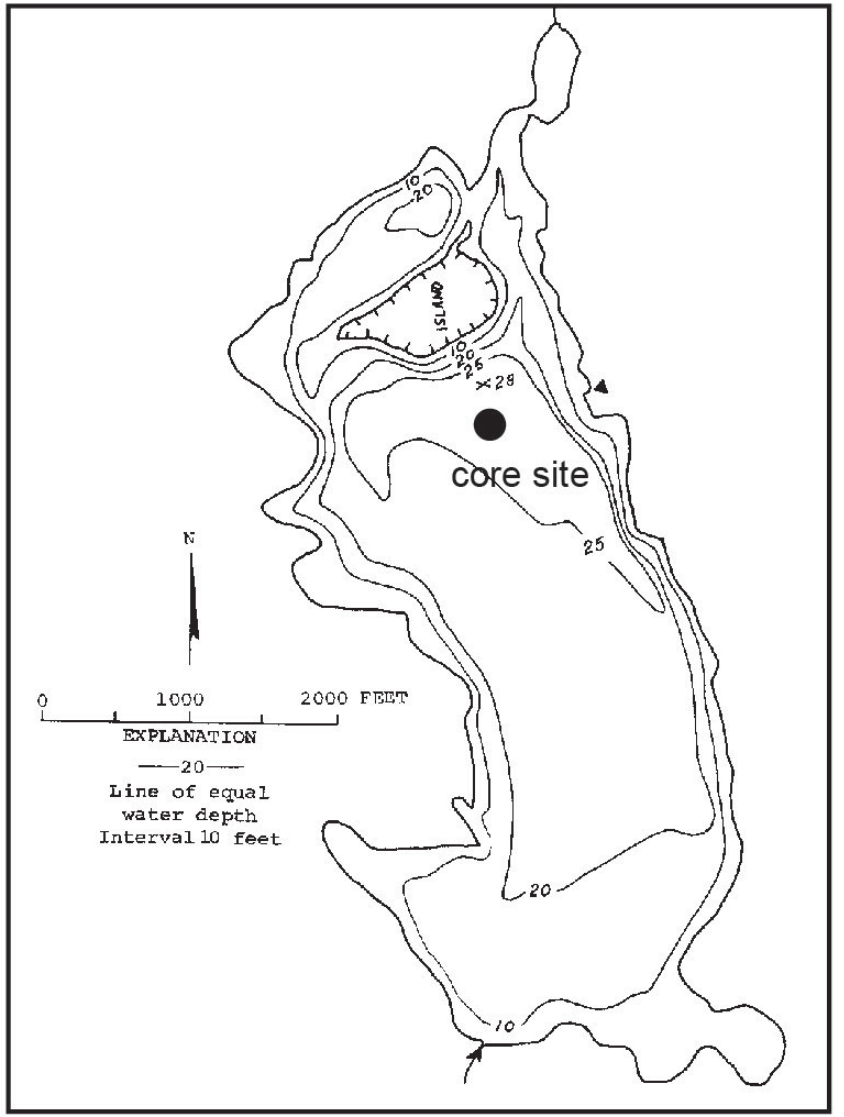
The Prevalence of Cyanobacteria in Spanaway Lake over the last 150 years

1. Have cyanobacteria been a dominant part of the lake phytoplankton historically (pre-settlement)?
2. Was microcystin produced historically?

Lake Sediment Core

- Gravity box corer preserved sediment-water interface





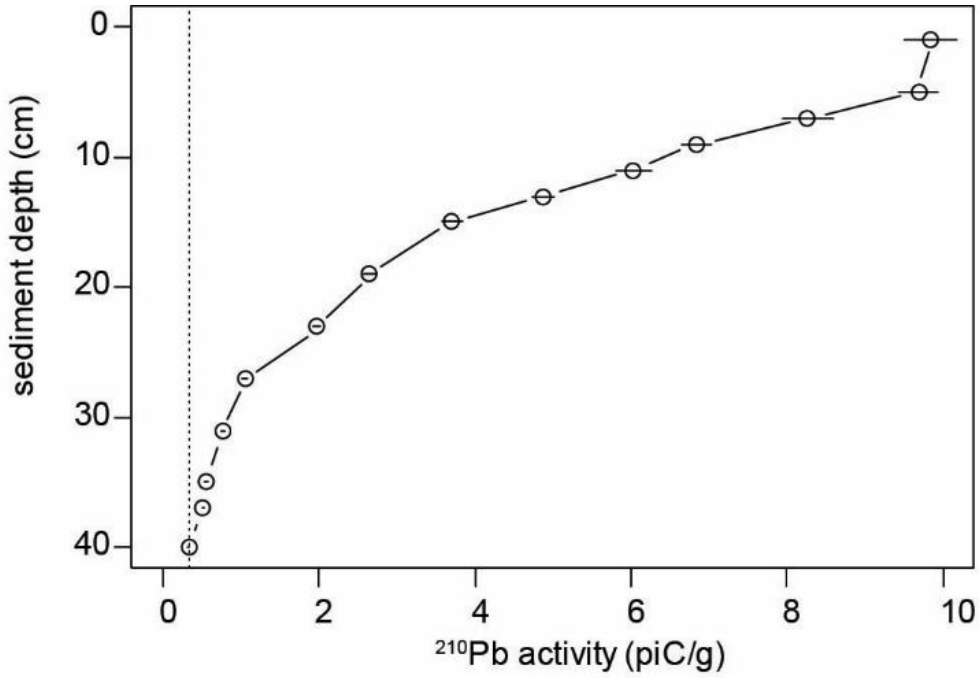
Core Dating

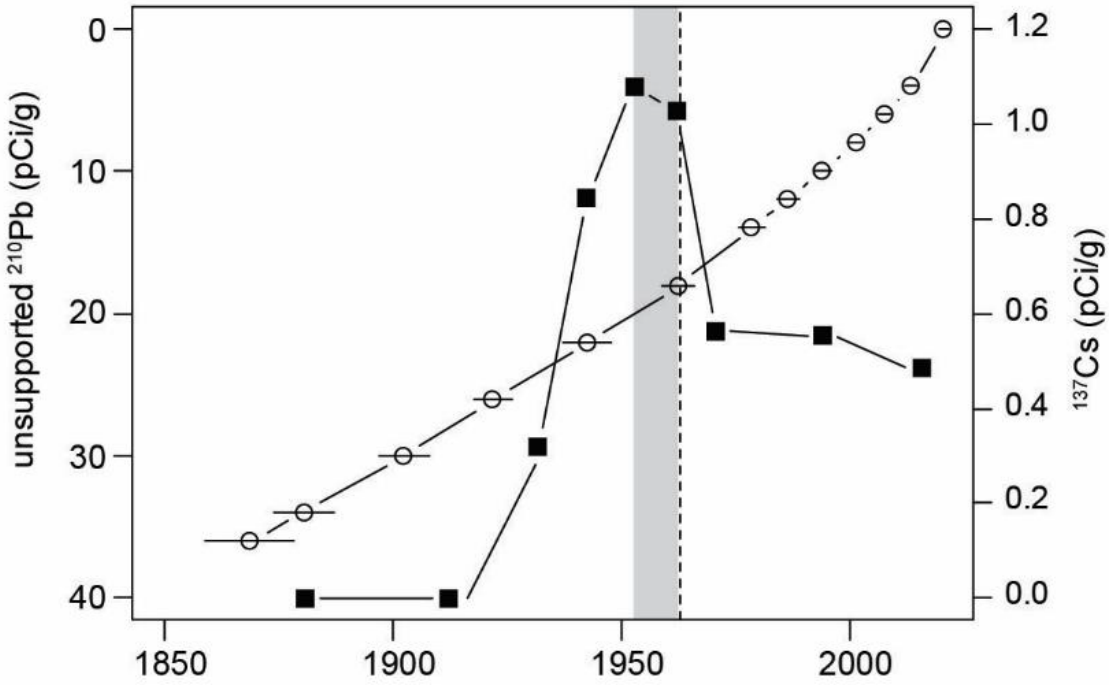


2020

1960

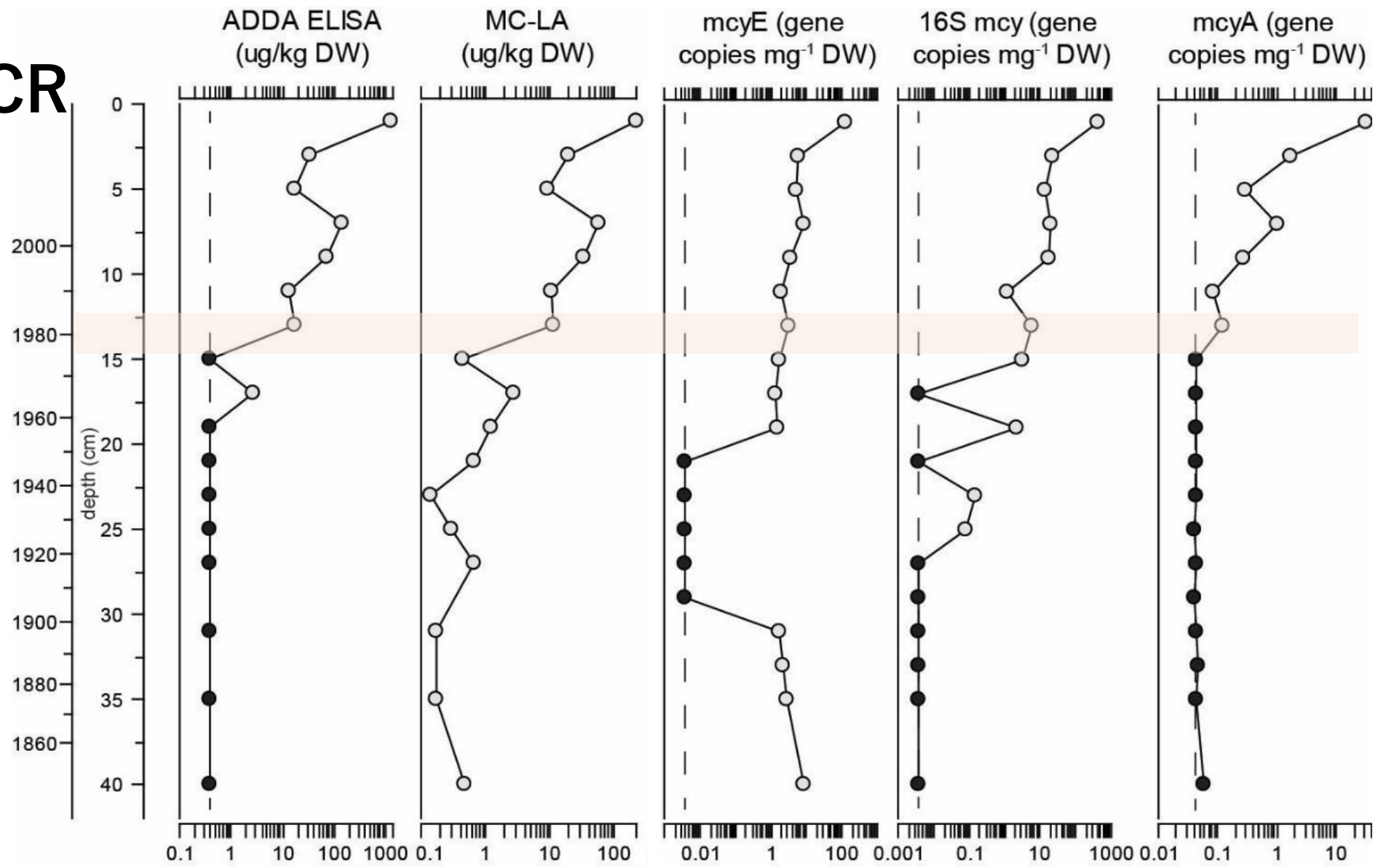
~1845





Microcystins (MCs) and qPCR

- MC-LA measured throughout core
- *Microcystis* 16S rRNA not detected throughout core



- Microcystin genes specific to *Microcystis* (mcyA) detected post-1985

Concluding Observations

- Early summer growth of cyanobacteria in bottom waters; late summer growth peaked at 5 m
- Dominant cyanobacteria was filamentous, but *M. aeruginosa* produced the microcystin
- Microcystin concentration positively correlated to McyA gene expression with 2-3 week lag
- Physical, and likely chemical, setting naturally supports a microcystin-producing cyanobacteria community
- Post-1985 characterized by a dominance of cyanobacteria production and presence of microcystin-producing genes

Acknowledgements

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Elizabeth Frame and Francis Sweeney, King County Environmental Laboratory

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- Julia Dolan

Washington State Department of Ecology

- Siana Wong
- Chad Larson
- Jessica Archer
- James Medlen
- Callie Mathieu
- Kuba Bednarek



Extra Slides

Cyanobacteria Monitoring at Ecology

- Opportunistic program relying on citizens and county stakeholders (local health authorities)

- Freshwater Algae Control Program (WQP)
  - Partnership of Ecology, DOH and King Co Environmental Lab.
  - nwtoxicalgae.org – 48 hr turnaround of toxin results
  - Local health authorities decide on limiting access to the beach or lake.

- **Policy 1-11 amendment** (2023): Lakes can now be listed based on cyanoHABs and toxins

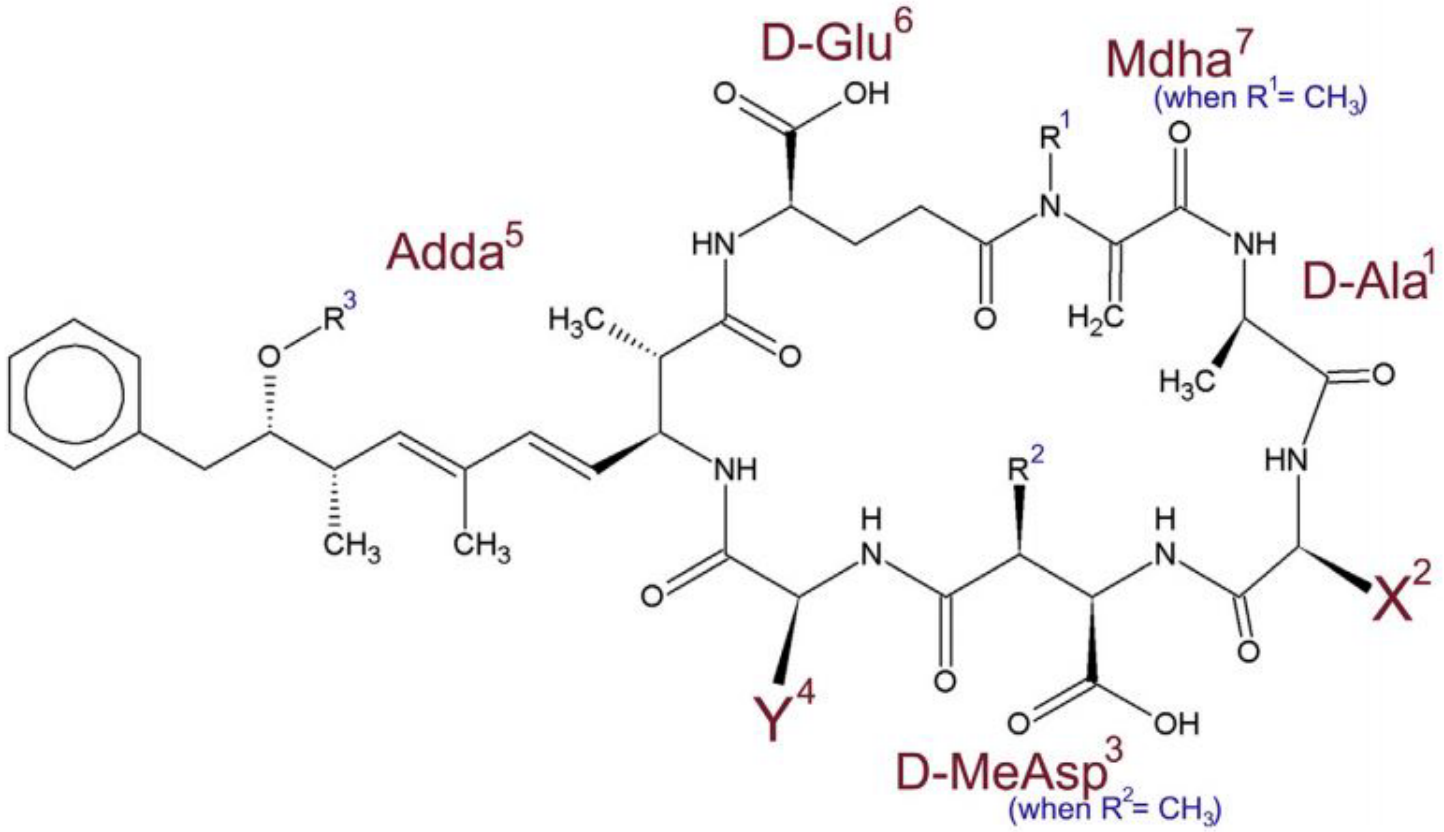
- **Surface Water Quality Criteria** development: being developed with nutrient criteria for surface waters.





Microcystin (MC)

- Over 150 variants or congeners
- MCs are stable and slow to degrade

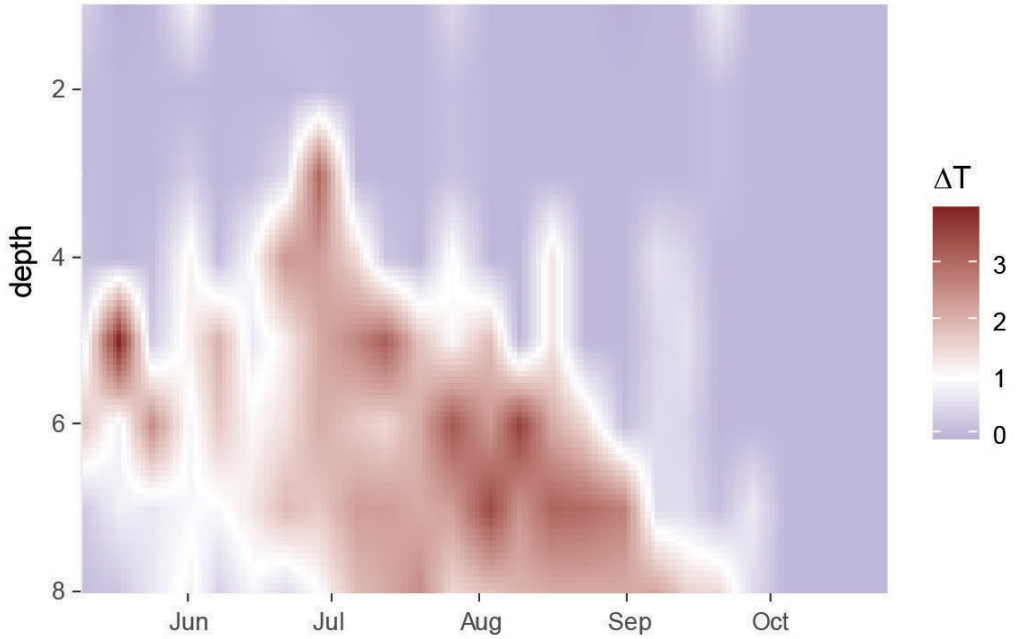


Foss and Aubel, 2015. Toxicon 104:91

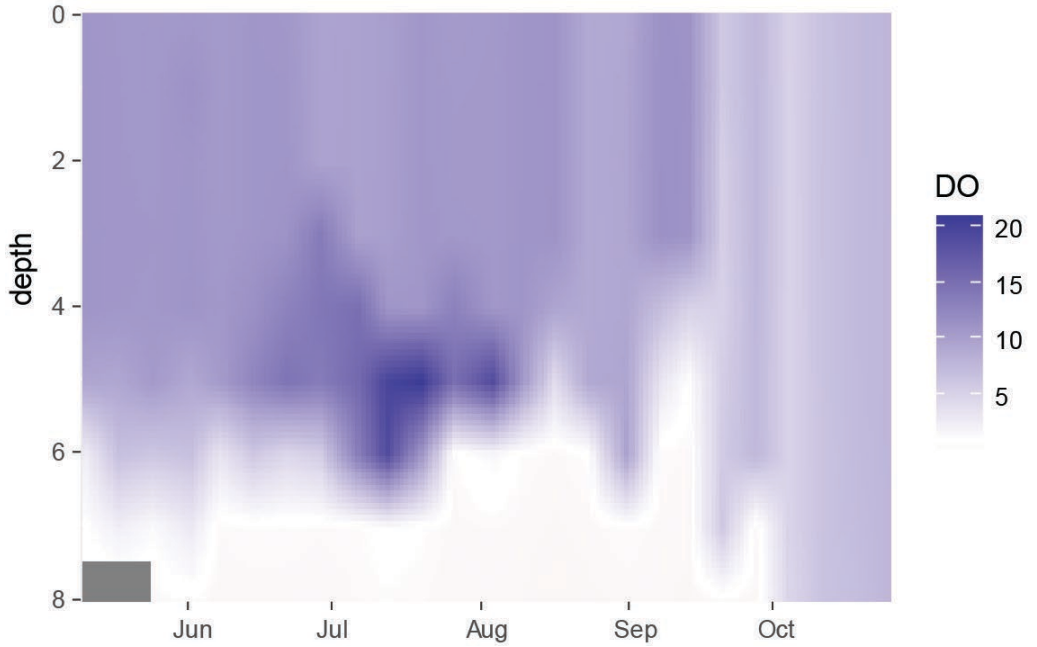
- Most of the total MC is composed of 7 variants, analyzed by LC-MS/MS
- MC-LR is a common congener and the most toxic tested so far; often used for instrument calibration

Water Column Profiles

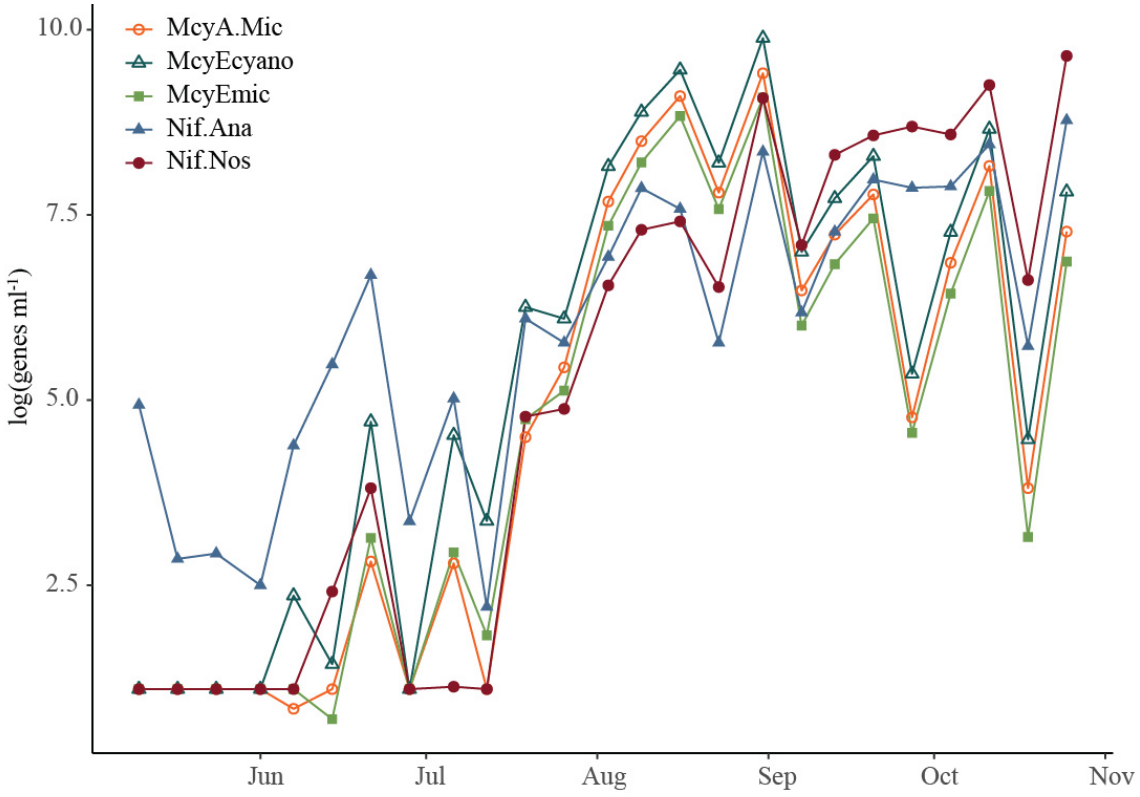
- early June water column strongly stratified
- thermocline increases as summer progresses

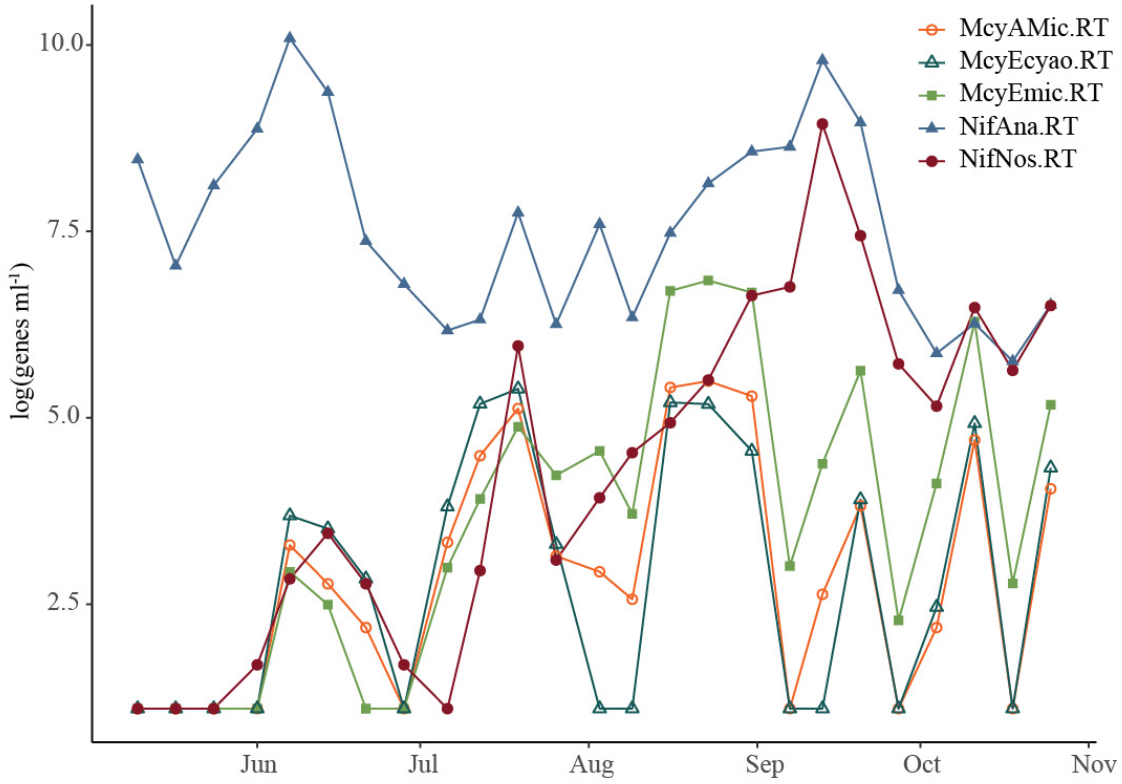


- bottom waters contained very little oxygen from June to fall turnover in late-September
- mid-July and early August very high concentrations of DO at a depth of ~5 - 6m; likely reflecting the presence of photosynthetic activity from algal growth



Toxin Production



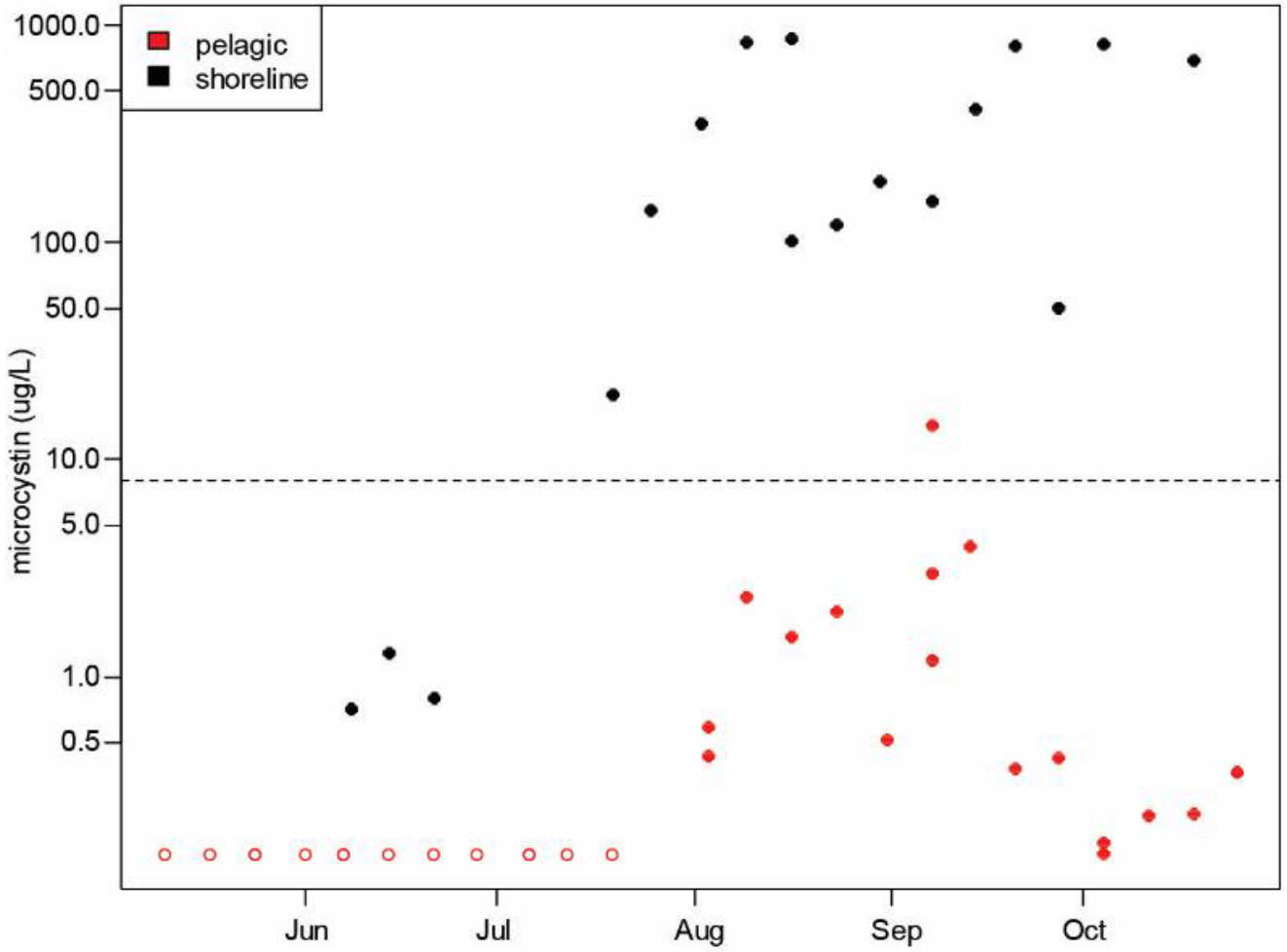


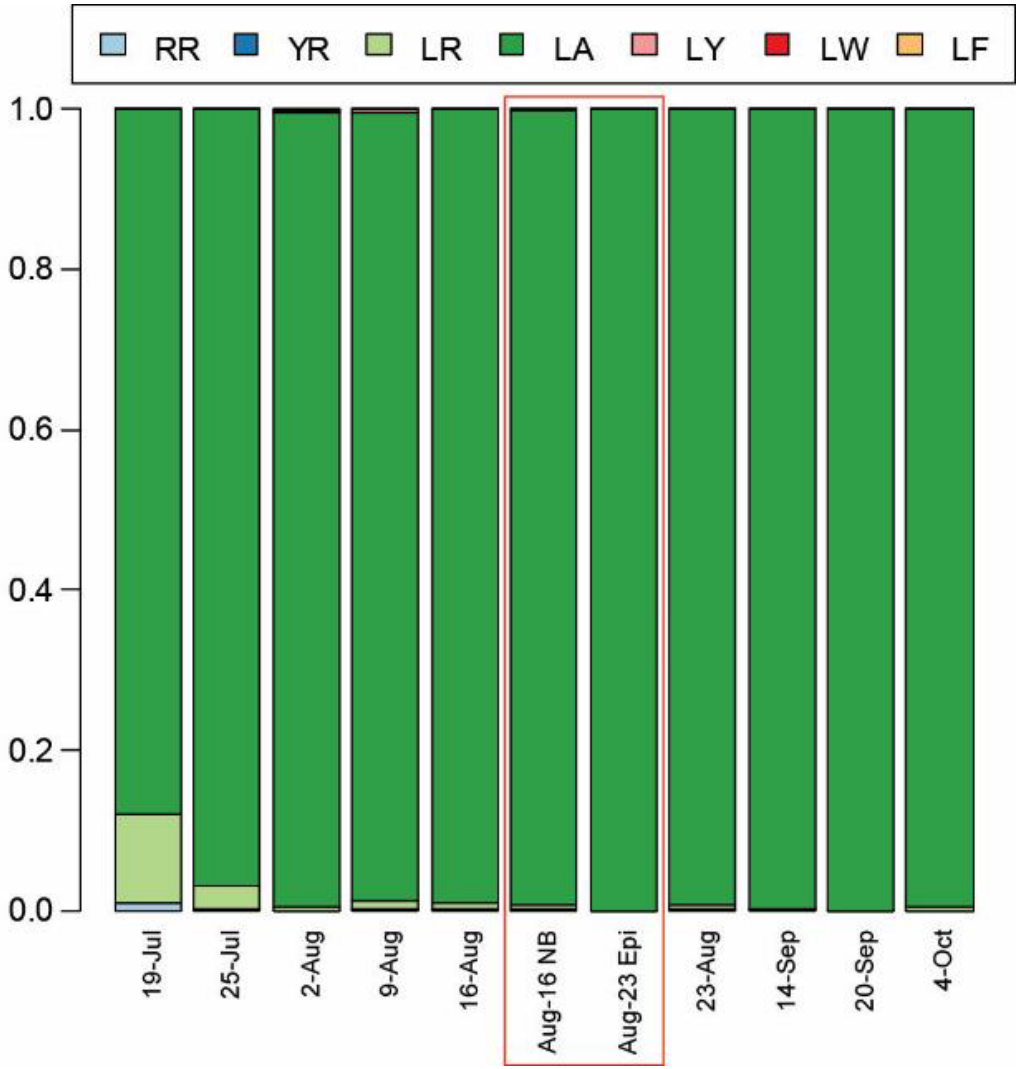
- ***Total cDNA***: increase through the month of July, peaking in August of microcystin genes (Mcy) and nitrogen (N_2) fixation genes (Nif)

- ***Reverse transcription qPCR***: expression of Mcy genes between July and September dominated by microcystin-producing genera (McyE) and genes attributable to *Microcystis* (McyA).

Microcystin

- Higher concentrations in samples collected from shoreline accumulations (Pierce Co data)
- Spanaway Lake is dominated by the variant MC-LA

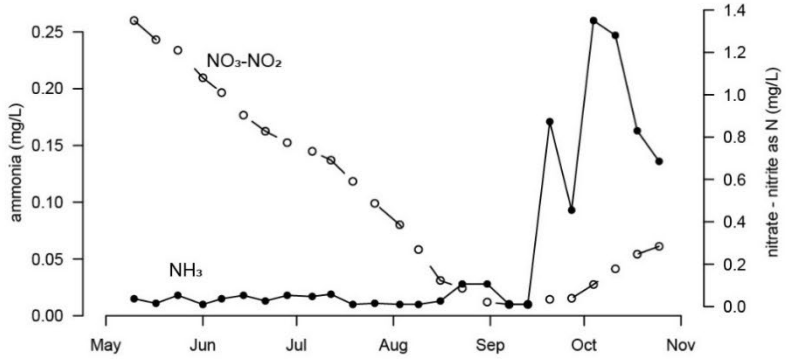


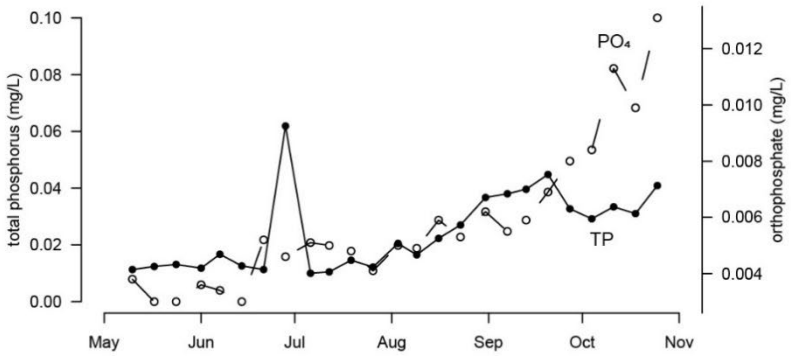


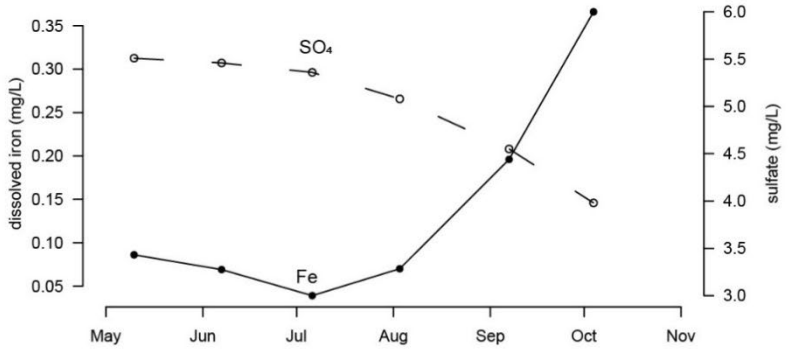
Water Chemistry

- Steady decrease of dissolved N over the summer; ammonia peak at depth in late-august
- P concentrations steadily increased over summer in upper waters
- Ortho-P concentrations low, but follow similar trend to TP
- Release from sediments evident: decrease in nitrate, increase in SO_4 reduction = iron, ammonia and P released from the sediments

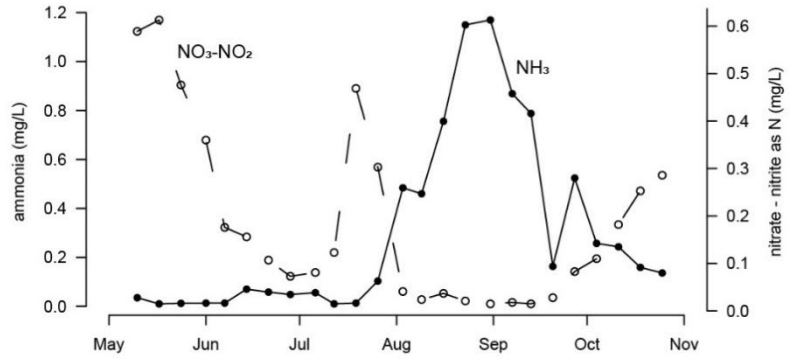
Epilimnion (0-2m)

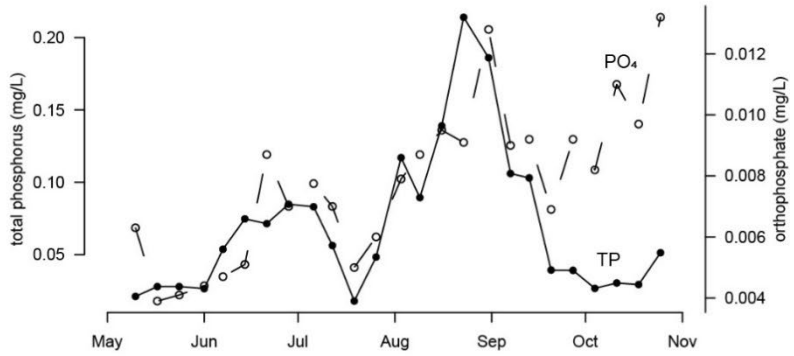


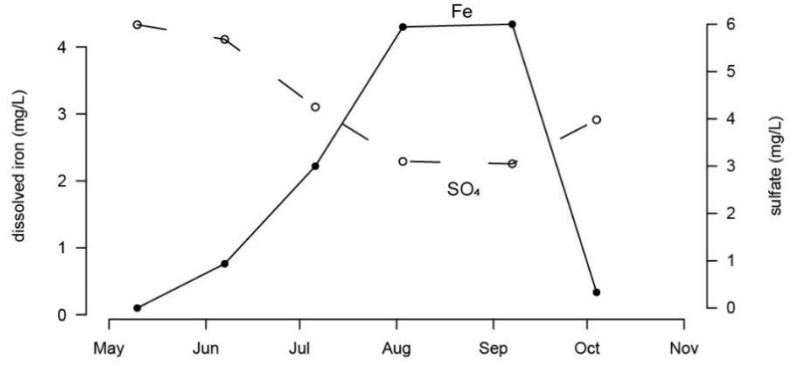




Hypolimnion (7m)

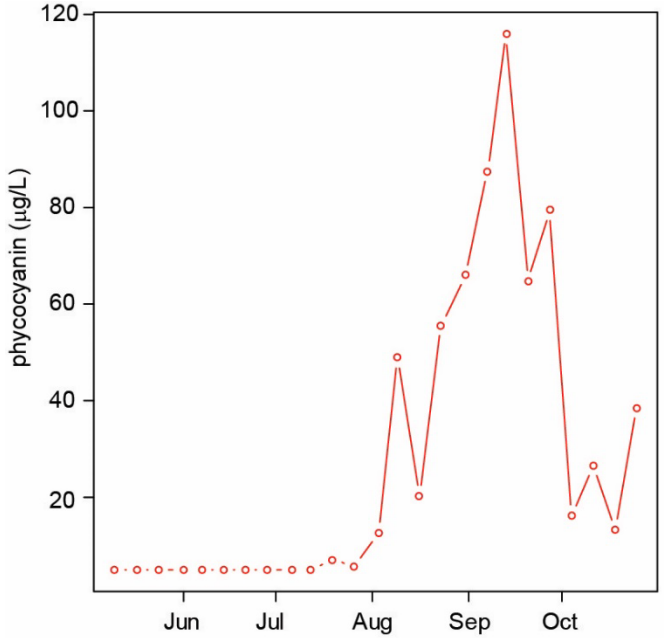


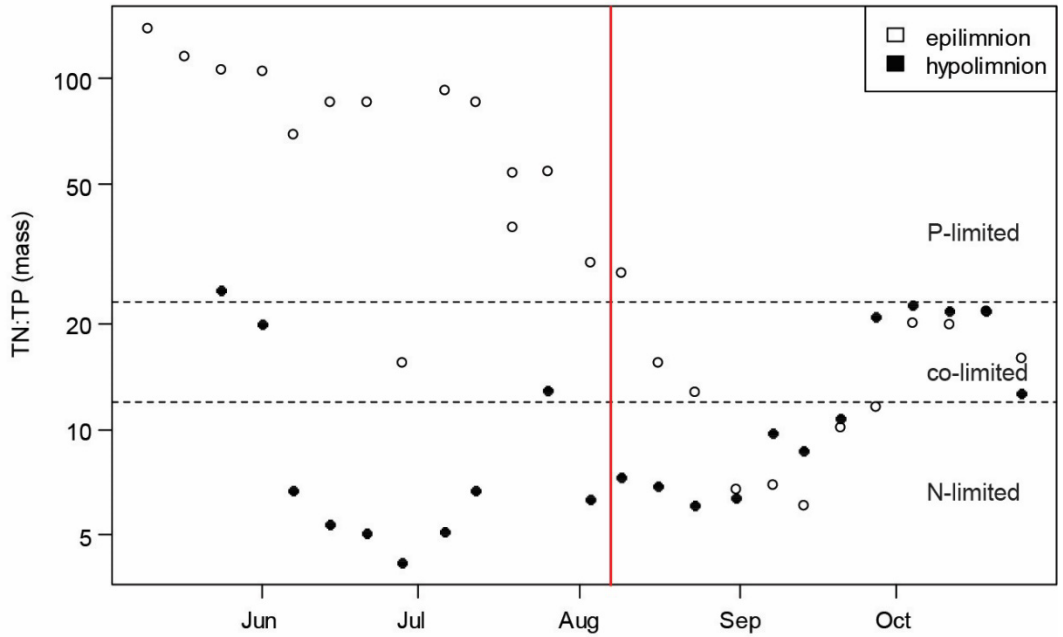




Nutrient Limitations

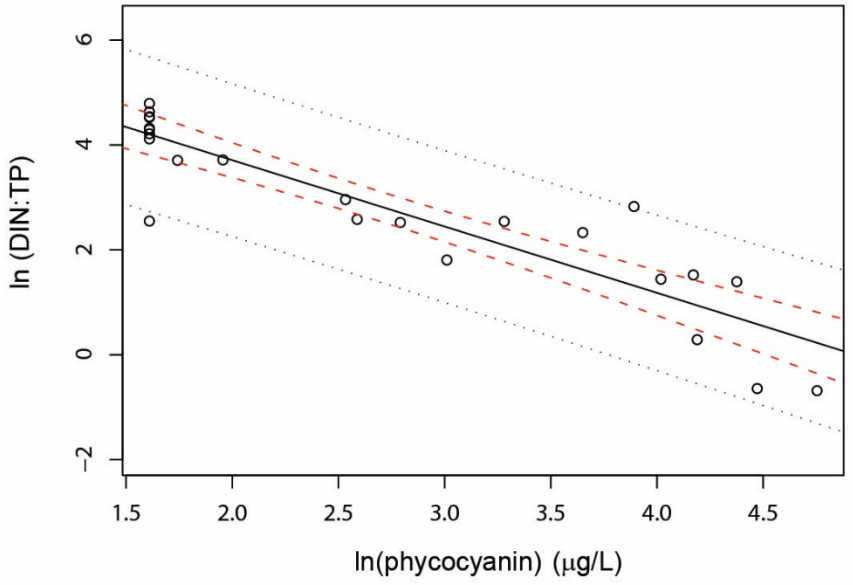
- Greater PC concentrations under N-limitation (PC vs DIN:TP; r = -0.91; p<0.001)
- Greater MC concentrations with higher PC (cyanobacteria growth) (r = 0.82; p<0.001)

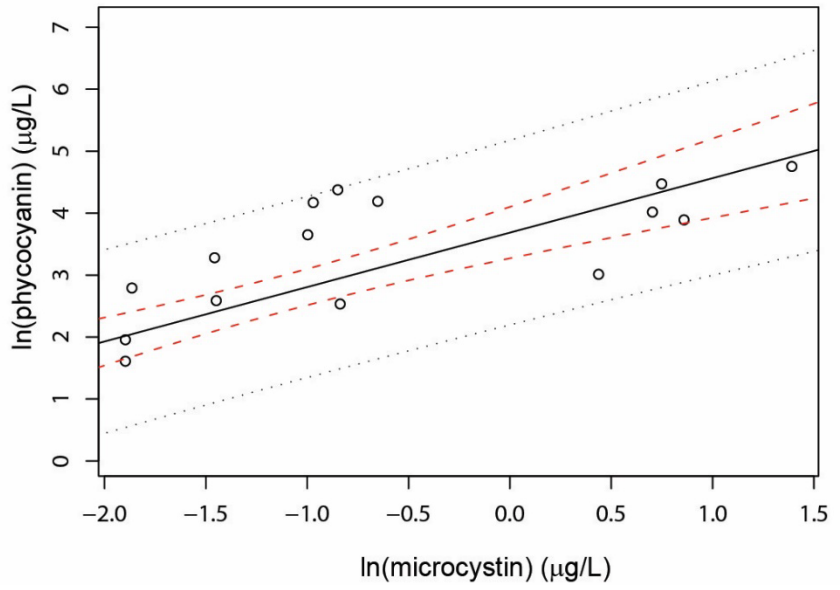




Nitrogen is important

- microcystin compounds are N-rich, requiring available inorganic N to synthesize
- Mcy gene regulated by N supply; expression of genes highest under N-limitation





History of Spanaway Lake

- **1863** – Puget Sound Agricultural Company (Hudson's Bay Company subsidiary) farm and raise cattle and sheep on the south and east lake shore
- **1891** – Lake Park Railway established with the intent of making Spanaway Lake a resort community. Flume constructed to take Spanaway Lake water to the City of Tacoma.
- **1910** – first structures on the lake; dance hall, boat house, shooting gallery, restaurant, swimming pier and float
- Late **1950s** /early 1960's - Fish and Wildlife fish kill in the lake to get rid of the bottom fish and stocked it with rainbow trout.
- **1980's** – last significant freezes
- **1993** – Spanaway Park Clubhouse hooked to sewers
- **1998** - Spanaway Golf Course Club house and other Park facilities were connected to the sanitary sewer system.
- **1999** – Sanitary sewers were extended to the Coffee Creek Condominiums to remedy the failing community drainfield system
- **2006** – Beginning of annual spraying of south end of lake for invasive plants

History of Spanaway Lake

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- 1910 – first structures on the lake dance hall, boat house, shooting gallery, restaurant, swimming pier and float
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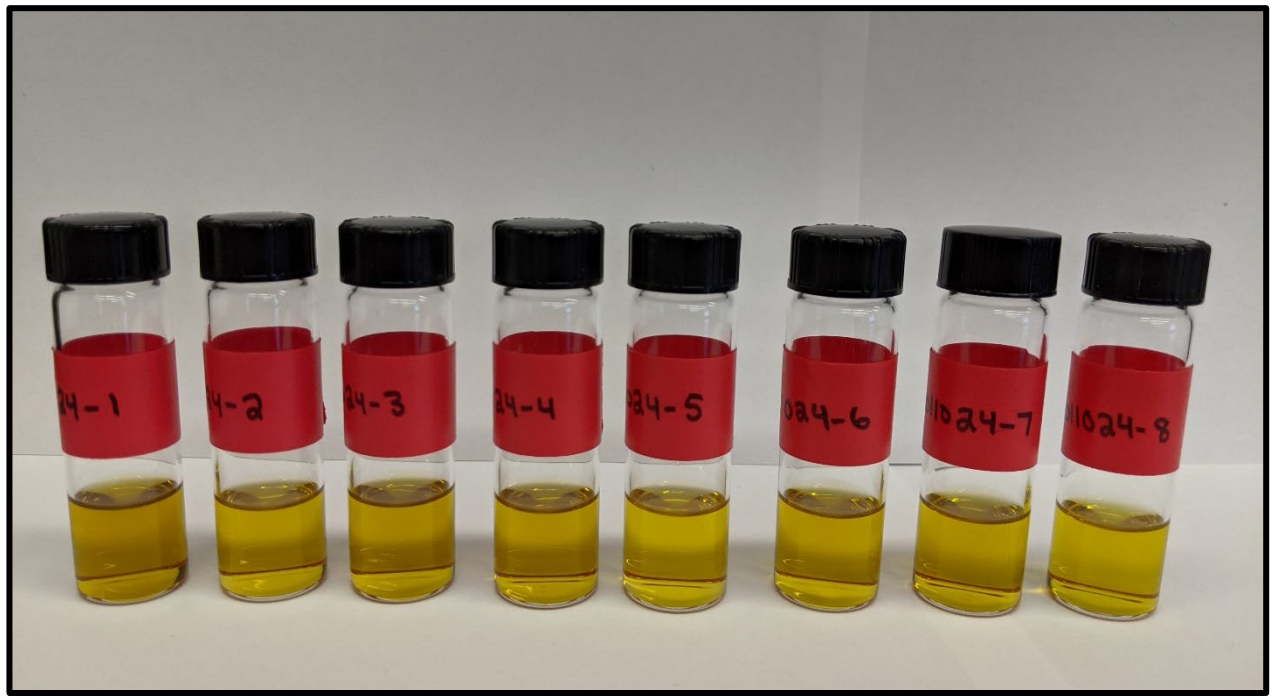
Possible shifts in algal community

Climate driver for post-1985 rise of MC production?

Nutrient inputs (septic) from the basin throughout the 1900s

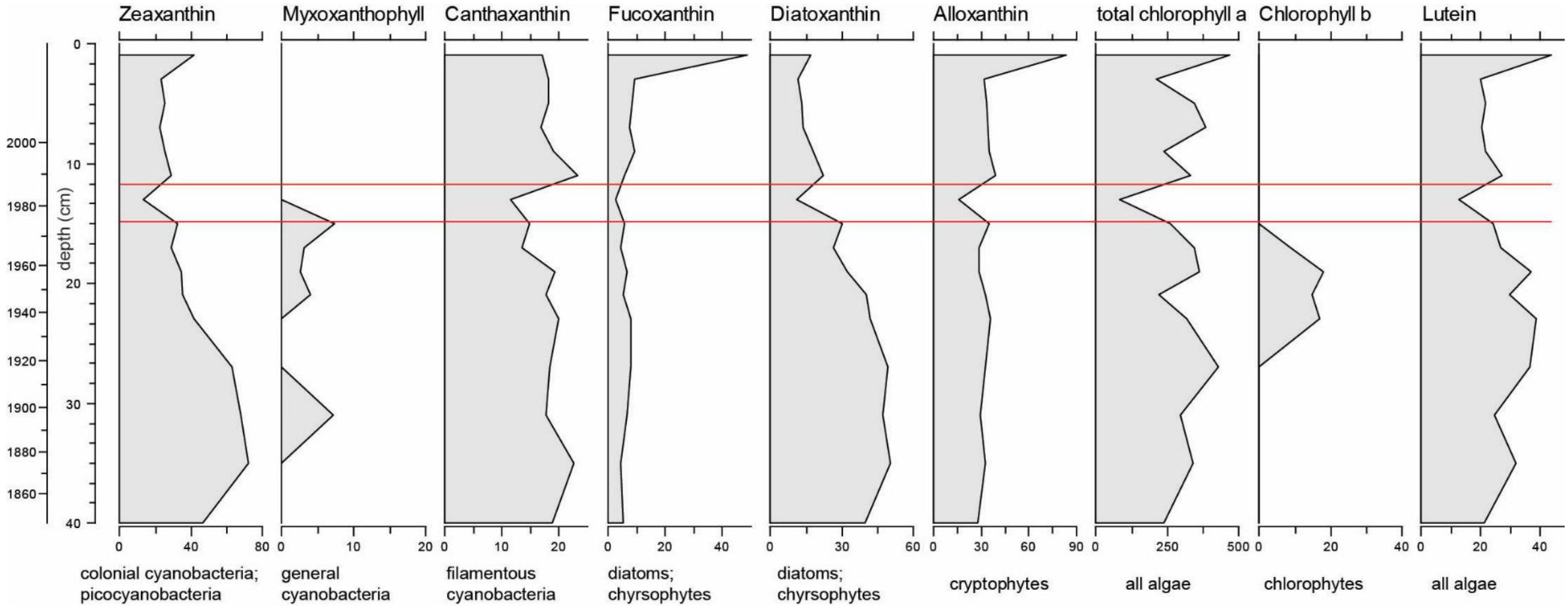
Algal Pigments

- Primary pigments - chlorophylls
- Secondary metabolites - carotenoids
- Some carotenoids specific to cyanobacteria
- Measured using HPLC



Pigment	Affinity
b,b-carotene	Plantae, Algae, some phototrophic bacteria
b,a-carotene	Cryptophyta, Chrysophyta, Dinophyta, some Chlorophyta
b-isorenieratene	Chlorobiaceae (green sulfur bacteria)
isorenieratene	Chlorobiaceae (brown varieties)
alloxanthin	Cryptophyta
fucoxanthin	Dinophyta, Bacillariophyta, Chrysophyta
diatoxanthin	Bacillariophyta, Dinophyta, Chrysophyta
diadinoxanthin	Dinophyta, Bacillariophyta, Chrysophyta, Cryptophyta
dinoxanthin	Dinophyta
peridinin	Dinophyta
echinenone	Cyanobacteria
zeaxanthin	Cyanobacteria
canthaxanthin	colonial Cyanobacteria, herbivore tissues
myxoxanthophyll	colonial Cyanobacteria
scytonemin	colonial Cyanobacteria
oscillaxanthin	Cyanobacteria (Oscillatoriaceae)
aphanizophyll	nitrogen-fixing Cyanobacteria (Nostocales)
lutein	Chlorophyta, Euglenophyta, Plantae
neoxanthin	Chlorophyta, Euglenophyta, Plantae
violaxanthin	Chlorophyta, Euglenophyta, Plantae
okenone	purple sulfur bacteria
astaxanthin	invertebrates, nitrogen-limited Chlorophyta
chlorophyll a	Plantae, Algae
chlorophyll b	Plantae, Chlorophyta, Euglenophyta
pheophytin a	chlorophyll a derivative (general)
pheophytin b	chlorophyll b derivative (general)
pheophorbide a	chlorophyll a derivative (grazing, senescent diatoms)
pyro-pheo (pigments)	derivatives of a- and b-phorbins
chlorophyll c	Dinophyta, Bacillariophyta, Chrysophyta

Algal pigments



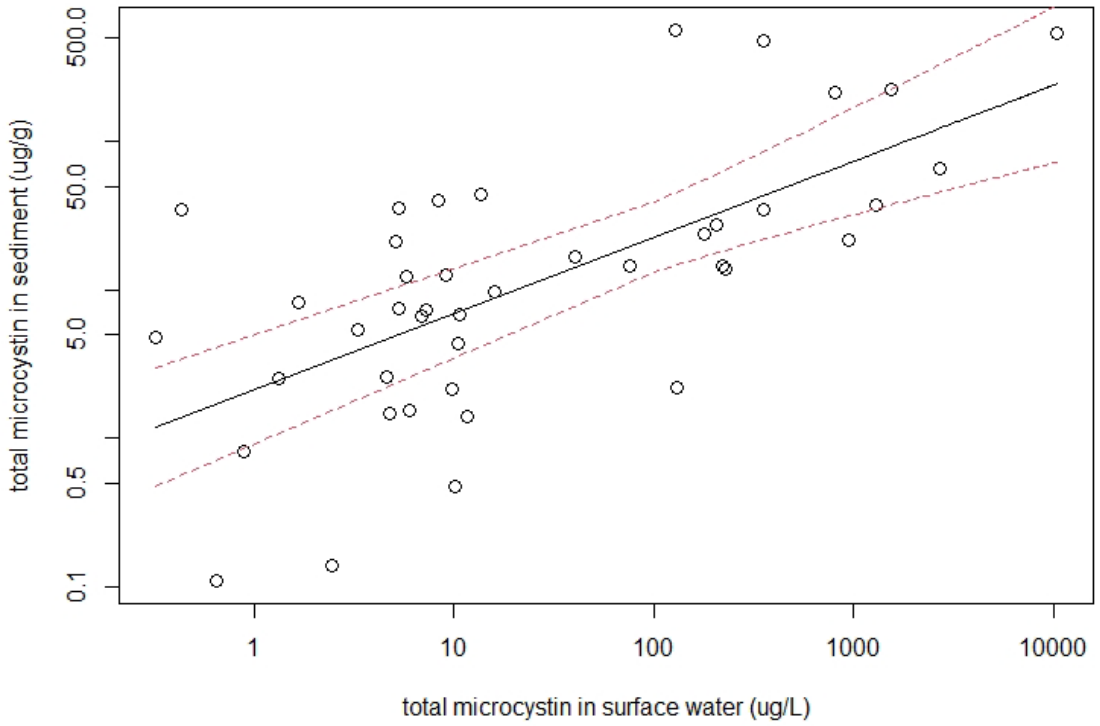
1850-1975: periods of cyanobacteria producing myxoxanthophylls; picocyanobacteria abundance are high; filamentous cyanobacteria are abundant

1975-1985: a period of transition; algae production is the lowest for the sediment core.

~1985-present: cyanobacteria abundance is high, relative to other types of algae

Microcystin adsorption to sediment

- Other studies relying on similar methods
- Partition coefficients established for MC-RR > MC-YR > MC-LR > MC-LA.
- Relationship between MCs in littoral sediment and water



LAKE AND RESERVOIR MANAGEMENT
2017, VOL. 33, NO. 4, 444–451
https://doi.org/10.1080/10402381.2017.1362491

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Distribution and flux of microcystin congeners in lake sediments

Arthur Zastepa[a], France R. Pick[b], and Jules M. Blais[b]

J Paleolimnol (2015) 54:87–101
DOI 10.1007/s10933-015-9839-1

CrossMark

ORIGINAL PAPER

Temporal trends in cyanobacteria revealed through DNA and pigment analyses of temperate lake sediment cores

S. Pal · I. Gregory-Eaves · F. R. Pick

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Prevalence and persistence of microcystin in shoreline lake sediments and porewater, and associated potential for human health risk

Ellen P. Preece [a, *], William Hobbs [b], F. Joan Hardy [c, **], Lenford O'Garro [c], Elizabeth Frame [d], Francis Sweeney [d]