



# **Lesser Known Toxins – A Review of Emerging Cyanobacterial Peptides of Concern and Re-Emergence of a Potent Neurotoxin**

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# Cyanobacterial Toxins in Freshwater

## Commonly Measured Toxins

- Microcystins
- Anatoxin-a
- Saxitoxins (PSP's)
- Cylindrospermopsins



## Lesser Studied “Toxins”

- A lot of peptides!
- Guanitoxin (anatoxin-a(s))
- Rare congeners of commonly measured classes
- Other alkaloids
- Retinoic acids
- Toxic amino acids (BMAA etc)
- Yet to be discovered!

## CyanoMetDB, a comprehensive public database of secondary metabolites from cyanobacteria

Martin R. Jones<sup>a</sup>, Ernani Pinto<sup>b</sup>, Mariana A. Torres<sup>c</sup>, Fabiane Dörr<sup>c</sup>, Hanna Mazur-Marzec<sup>d</sup>, Karolina Szubert<sup>d</sup>, Luciana Tartaglione<sup>e</sup>, Carmela Dell'Aversano<sup>e</sup>, Christopher O. Miles<sup>f</sup>, Daniel G. Beach<sup>f</sup>, Pearse McCarron<sup>f</sup>, Kaarina Sivonen<sup>g</sup>, David P. Fewer<sup>g</sup>, Jouni Jokela<sup>g</sup>, Elisabeth M.-L. Janssen<sup>a</sup>

**Open Access Database**

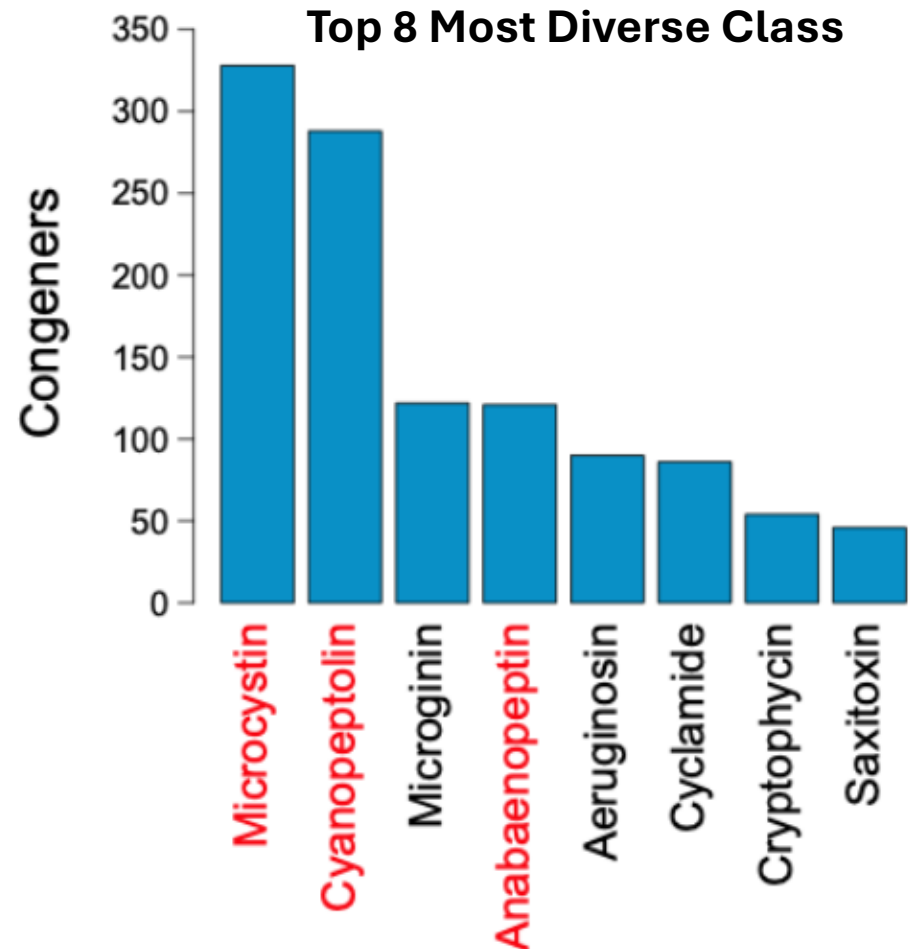
private lists > 850 references >2000 structural codes

to be included in

- normal
- Mel#rag
- PubChem
- npatis
- CompTox

**2010 metabolites in 99 classes**

## CyanoMetDB: Microcystins, Cyanopeptolins and Anabaenopeptins Are Among the Most Diverse



# Toxicity of Anabaenopeptins and Cyanopeptolins

## Anabaenopeptins

- Inhibits protein phosphatase 1 and 2A and carboxypeptidases
  - Spoof et al. (2016) *Marine Drugs* 14
  - Sano et al. (2001) *J. Nat. Prod* 64
- Neurotoxicity in crustaceans
  - Pawlik-Skowrońska et al. (2021) *Toxicon* 198
- Inhibits growth and reproduction in worms (*C. elegans*)
  - Lenz et al. (2019) *Chemosphere*, 214
- Zebrafish – developmental toxicity (Dr. Kurt Svoboda)
- Human cell lines – hyperphosphorylation (Dr. Michael Laiosa)

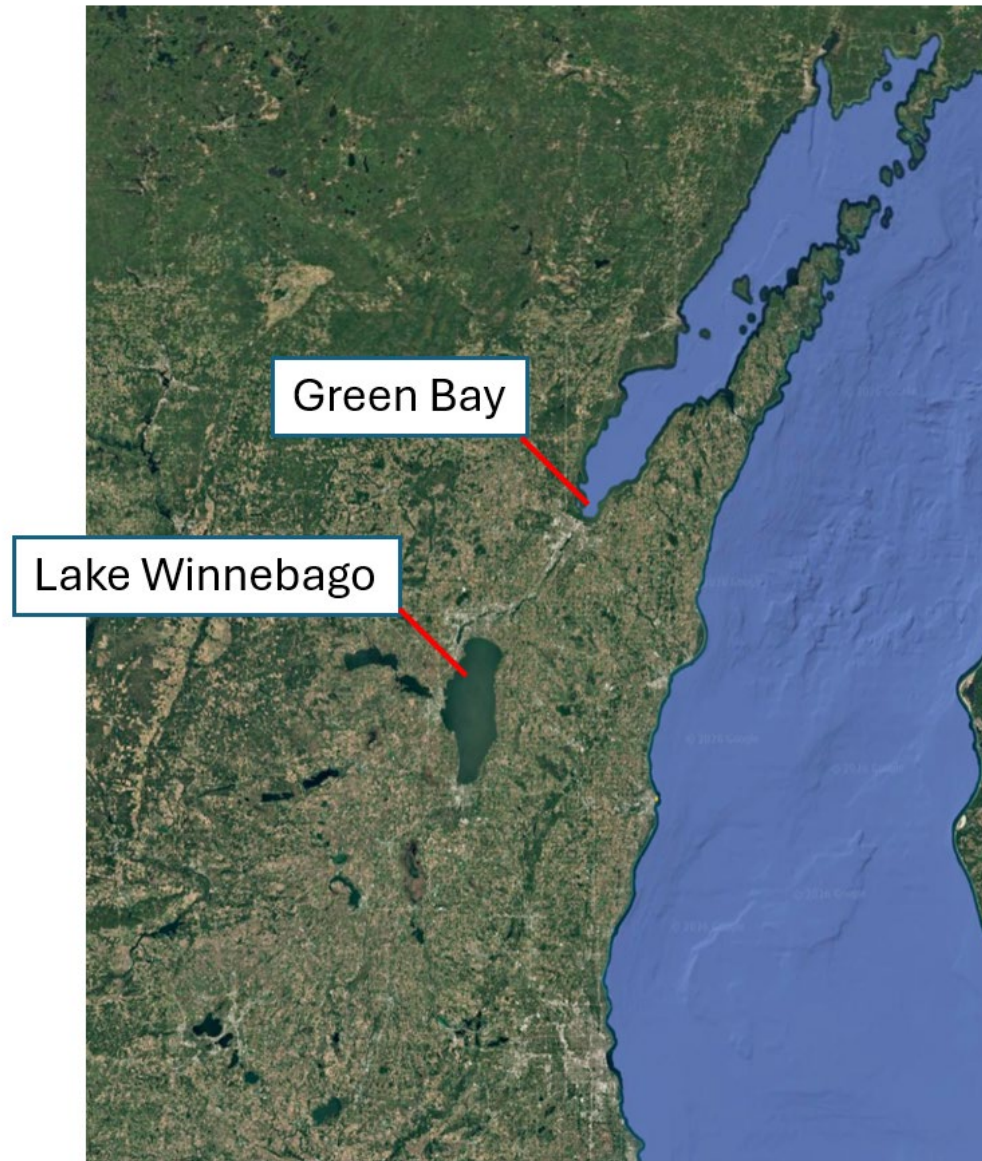
## Cyanopeptolins

- Inhibits various serine proteases and protein phosphatase 1
  - Gademann et al. 2010. *J. Nat. Prod.* 73
  - Okano et al. 1999. *Tetrahedron Letters* 40
  - Kaya et al. 1999. *Tetrahedron Letters* 37
- Neurotoxicity in crustaceans and zebrafish
  - Faltermann et al. 2014. *Aquat Toxicol* 149
  - Gademann et al. 2010. *J. Nat. Prod.* 73
- Inhibits growth and reproduction in worms (*C. elegans*)
  - Lenz et al. (2019) *Chemosphere*, 214

# Lake Winnebago to Green Bay

(living laboratories for cyanotoxin discovery)

- Frequent, seasonal cyanobacterium blooms
- Impacted by agricultural nutrient runoff (dairy industry)
- Recreational uses: boating, swimming, fishing
- Lake Winnebago used as a drinking water source for four cities
- Lower (southern) Green Bay is an EPA listed Area of Concern

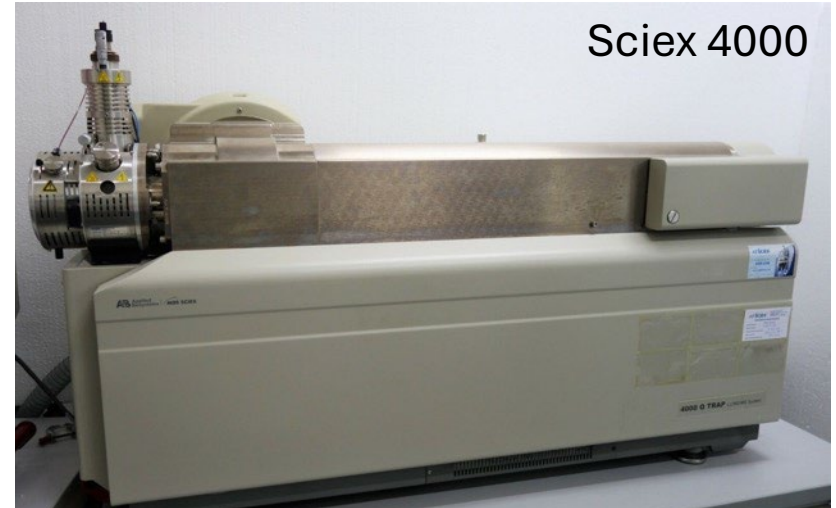


## **Scientific Questions:**

What is the temporal and spatial distribution of microcystins, anabaenopeptins and cyanopeptolins?

# Methods

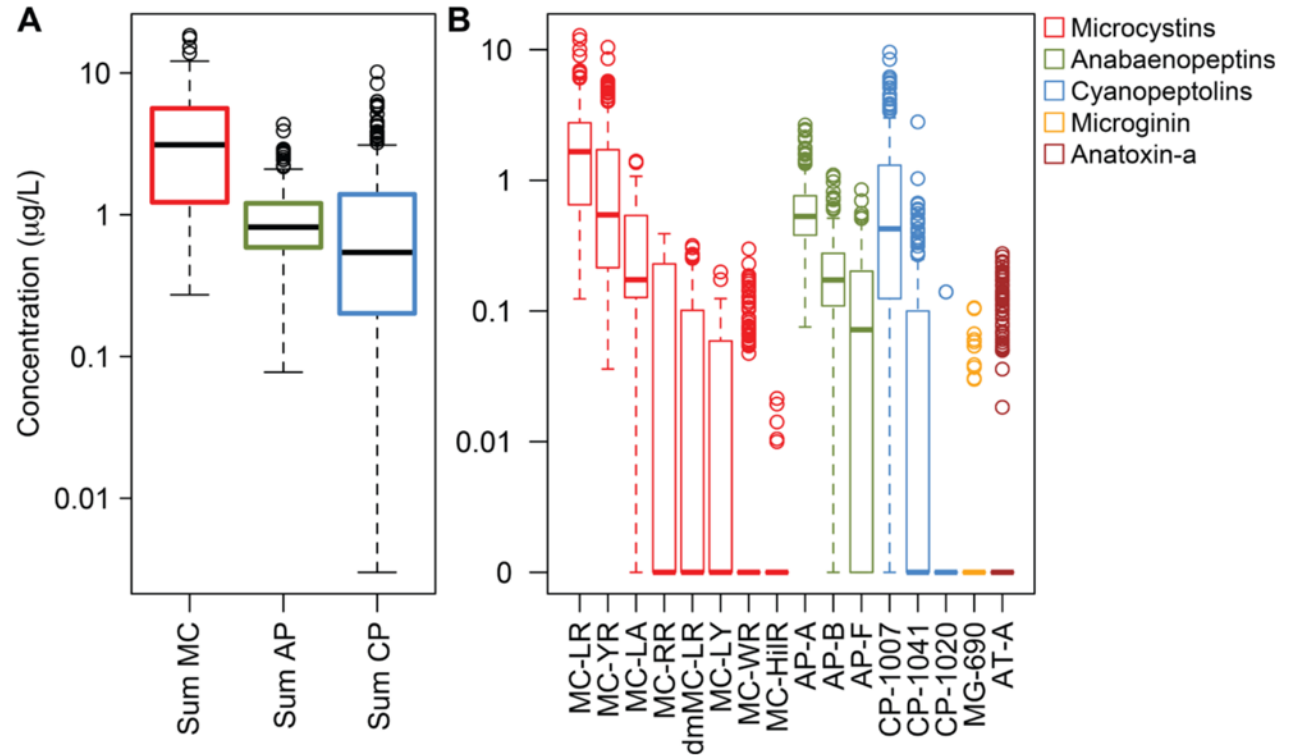
- Extraction from Water Samples
  - Lyophilization
  - Freeze/Thaws
  - 70% methanol/0.1% formic acid
- LC-MS/MS Analysis
- Reverse Phase HPLC separation
- Scheduled multiple reaction monitoring in positive mode
- Targeted, Quantitative Analysis
  - 4 to 11 microcystins
  - 3 anabenopeptins
  - 3 cyanopeptolins
  - 1 microginin
  - Anatoxin-a
  - Nodularin



# Anabaenopeptins and Cyanopeptolins Co-Occur with Microcystins

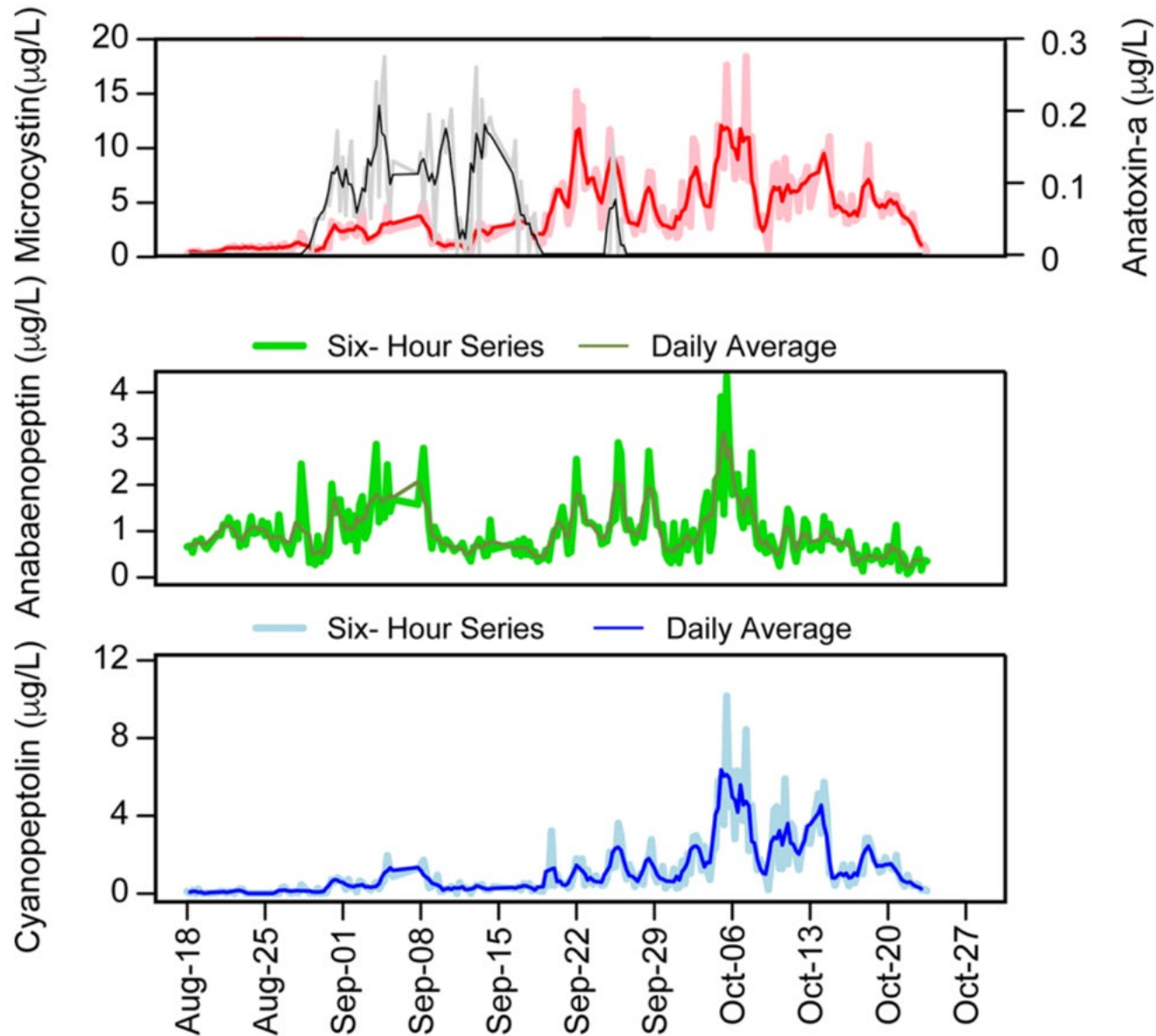
- Blooms contain a complex mixture of different toxins (mainly peptides)
- This is likely only a fraction of the toxin diversity
- Anabaenopeptins occur at a similar range of concentrations at microcystins
- Risk analyses should consider the effect of toxin mixtures

## Lake Winnebago



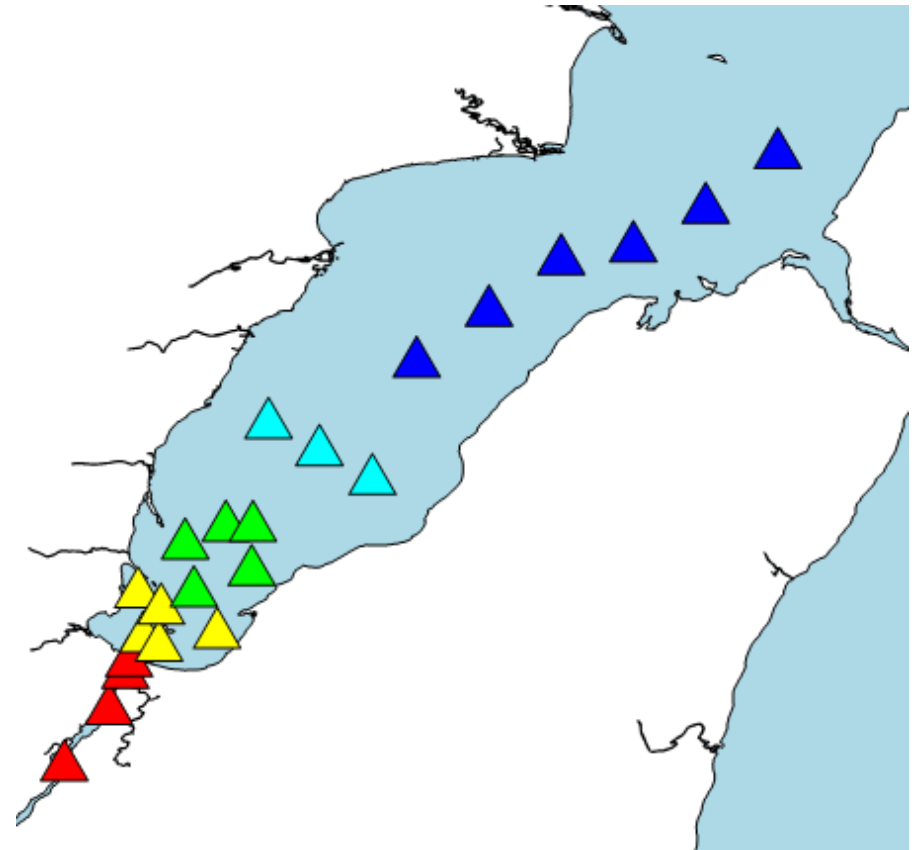
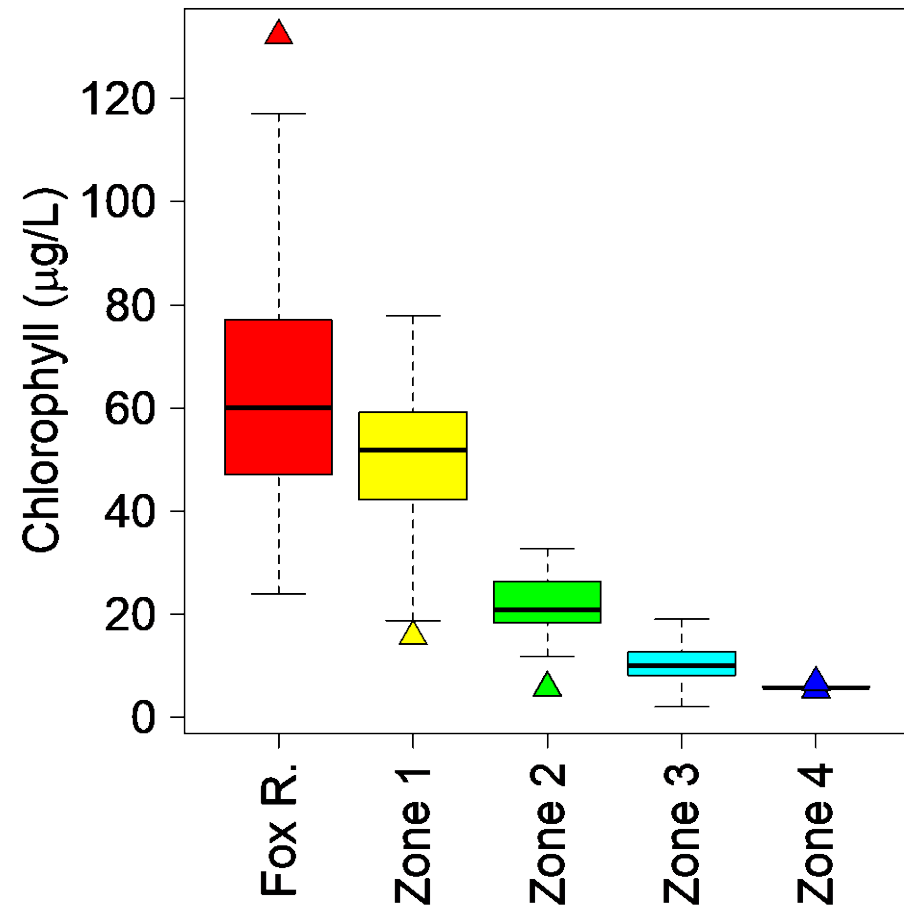
# Temporal Trends of Cyanopeptides and anatoxin-a in Lake Winnebago

- Anabaenopeptins follow a different trend (less variable) compared to microcystins and cyanopeptolins



Miller et al. 2019.. Environmental Science & Technology. 53(10):5661-5670.

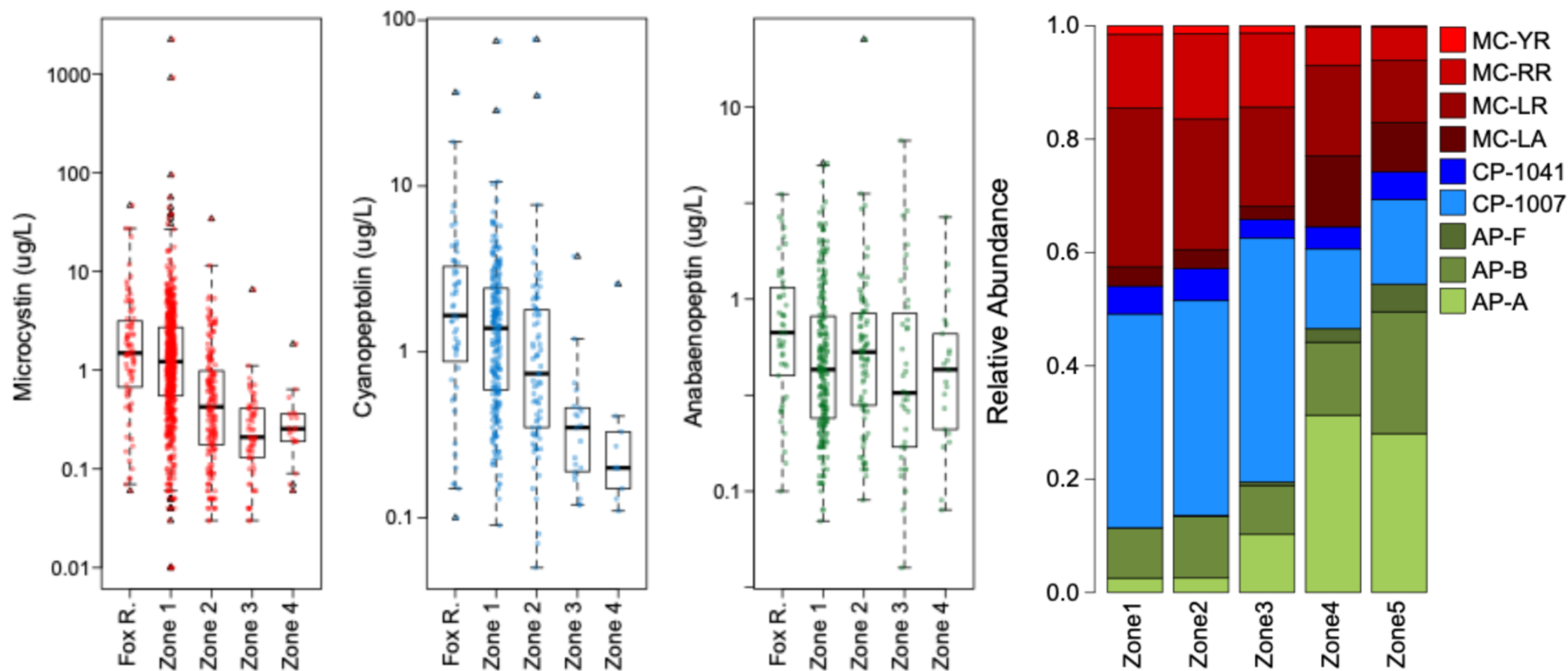
# Green Bay Trophic Gradient



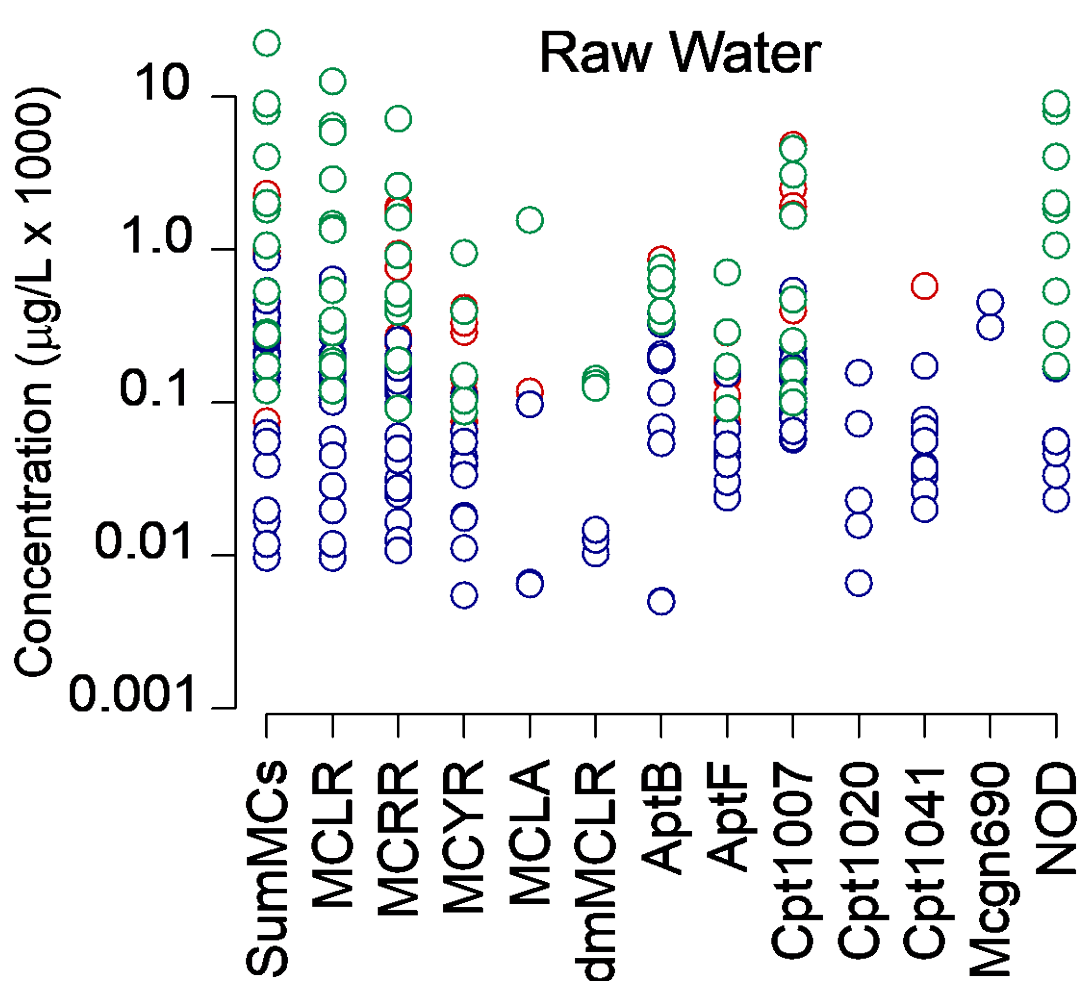
# Spatial Distribution of Cyanopeptides in Green Bay Along the Trophic Gradient (2016 – 2020)

Absolute Abundance (Log Scale)

Relative Abundance



# Cyanobacterial Peptides in Raw Drinking Waters, Lake Winnebago (2011 – 2014)

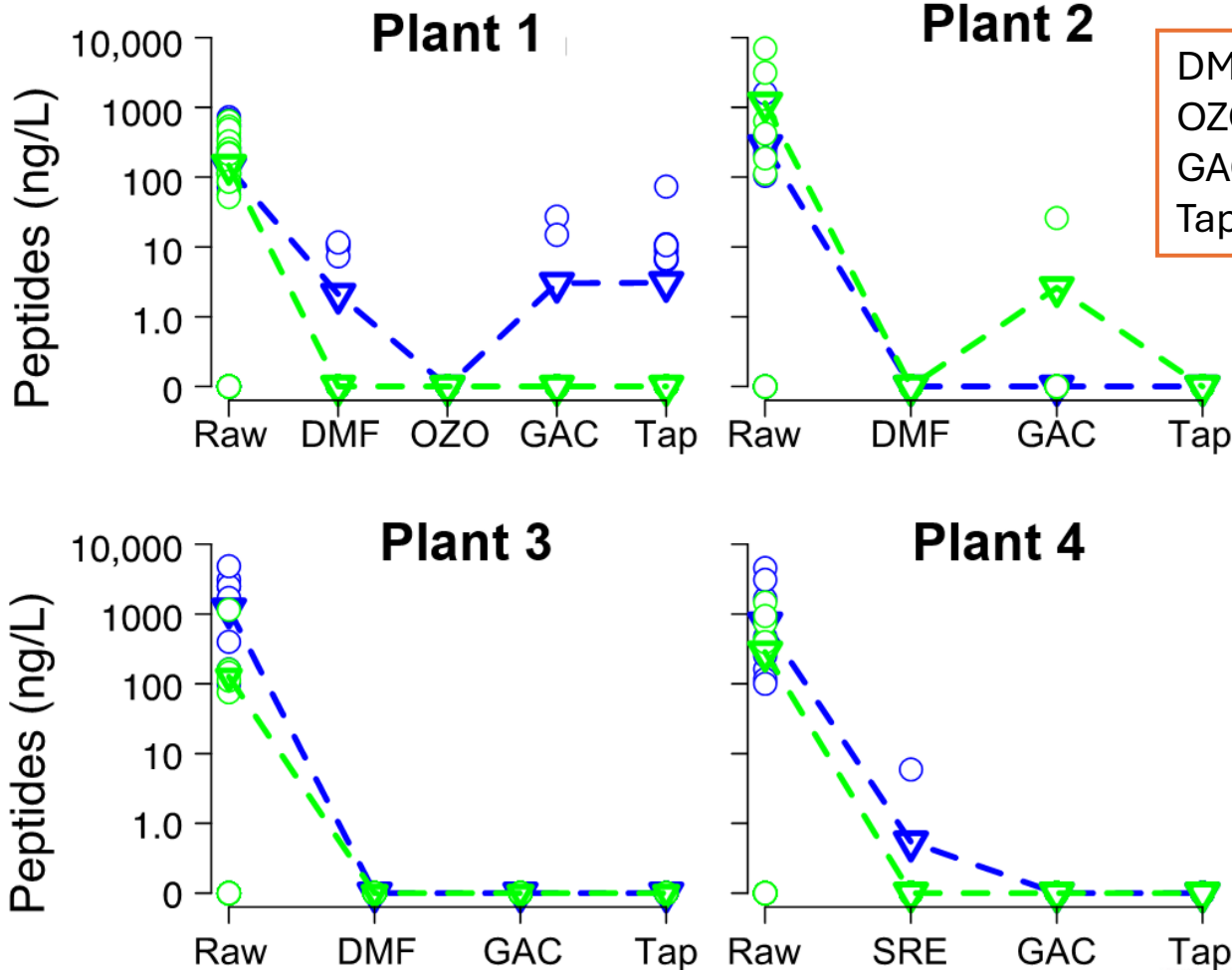


● Drinking Water Intakes



# Removal of Anabaenopeptins and Cyanopeptolins in Four Drinking Water Plants Drawing from Lake Winnebago

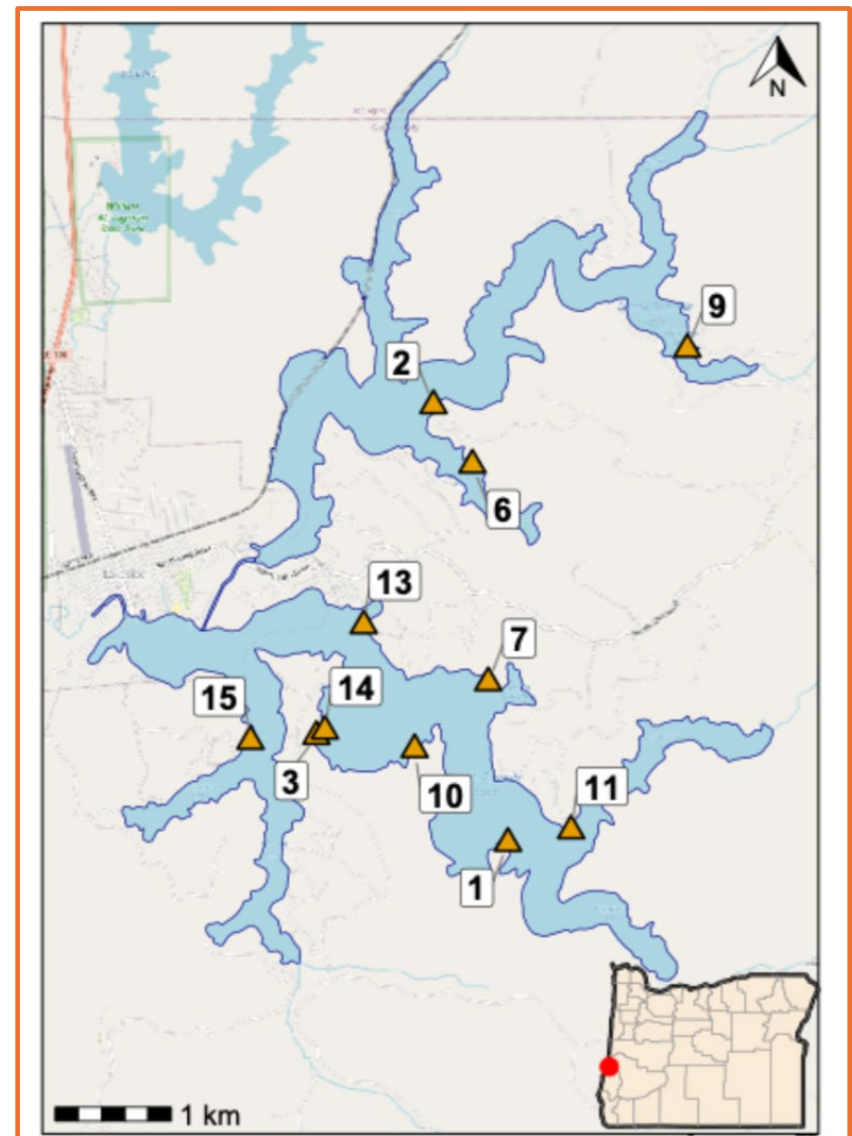
-△- Cyanopeptolin Trend    -▽- Anabaenopeptin Trend



DMF = dual media filtration  
 OZO = ozonation  
 GAC = granulated activated carbon  
 Tap = finished water

# Tennmile Lake, Oregon

- Coastal lake community
- Residential homes draw water directly from the lake.
- Water is treated using home filtration systems of varying types.
- Volunteers were recruited to sample water before and after filtration for a year.
- Microcystins, anabaenopeptins and cyanopeptolins were quantified in samples using LC-MS/MS.



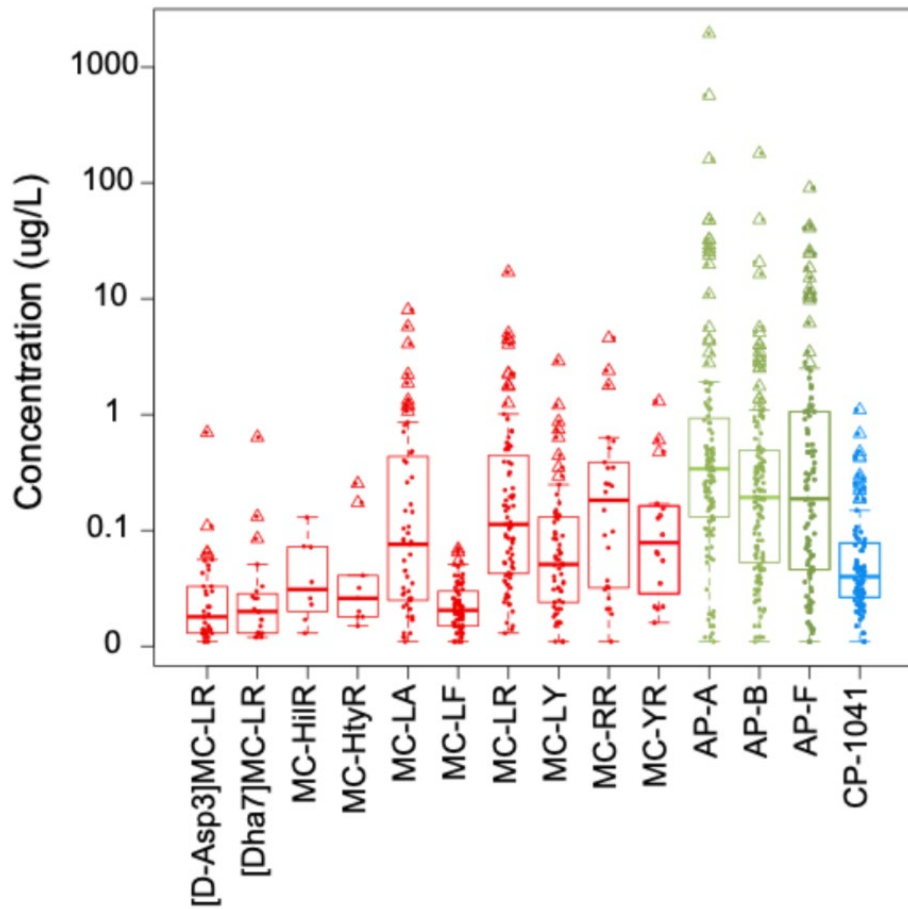
Project funded by the Natural Hazards Center and the NSF Civic Innovation Challenge program, **Amber Roegner**, Project Lead, University of Oregon Center for Global Health

# Tenmile Treatment Systems

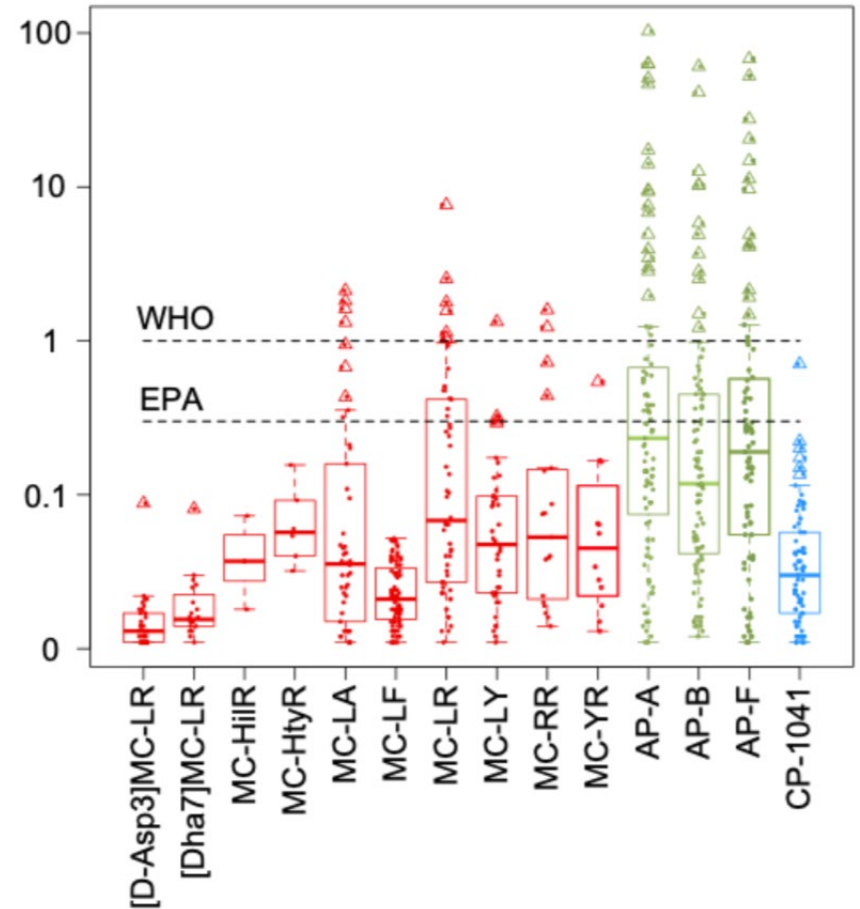
Site	Holding Tank	Chlorine Feed Injector	Sand Filter	Sediment Filter	Carbon Filter (1st)	Particulate Filter	UV Treatment	Carbon Filter (2nd)	Oxidation Filter	Well
1			X		X		X	X		
2										X
3				X						
6		X	X		X	X				
7						X	X			
9				X	X		X			
10	X	X	X		X	X				
11	X		X		X	X	X			
13										
14	X				X	X	X		X	
15	X					X	X			

# Cyanopeptides in Tenmile Lake and Tap Water (2023 – 2024)

## Lake Water



## Tap Water

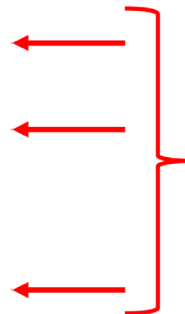


# Tenmile Treatment Systems and Toxin Removal

Site	Holding Tank	Chlorine Feed Injector	Sand Filter	Sediment Filter	Carbon Filter (1st)	Particulate Filter	UV Treatment	Carbon Filter (2nd)	Oxidation Filter	Well
1			X		X		X	X		
2										X
3				X						
6		X	X		X	X				
7						X	X			
9				X	X		X			
10	X	X	X		X	X				
11	X		X		X	X	X			
13										
14	X				X	X	X		X	
15	X					X	X			

Site	Removal Average $\pm$ SD		
	MC	AP	CP
1	0.09 $\pm$ 0.47	1.31 $\pm$ 8.23	0.00 $\pm$ 0.05
2	0.04 $\pm$ 0.32	-0.56 $\pm$ 2.83	0 $\pm$ 0.03
3	1.57 $\pm$ 4.45	16.89 $\pm$ 43.46	0.08 $\pm$ 0.21
6	0.00 $\pm$ 0.07	-0.15 $\pm$ 0.58	0.00 $\pm$ 0.01
7	-0.03 $\pm$ 0.21	0.06 $\pm$ 0.88	0.00 $\pm$ 0.04
9	0.02 $\pm$ 0.02	0.30 $\pm$ 0.45	0.00 $\pm$ 0.02
10	0.06 $\pm$ 0.08	0.33 $\pm$ 0.53	0.00 $\pm$ 0.01
11	-1.30 $\pm$ 1.53	-29.01 $\pm$ 41.56	-0.08 $\pm$ 0.10
13	0.11 $\pm$ 0.28	1.85 $\pm$ 7.22	0.02 $\pm$ 0.04
14	-0.06 $\pm$ 0.96	0.35 $\pm$ 33.32	0.03 $\pm$ 0.10
15	0.63 $\pm$ 2.69	146.04 $\pm$ 540.68	0.06 $\pm$ 0.17

Removal = Lake - Tap


 Negative removal of anabaenopeptins

# Rare Microcystins and Anabaenopeptins in Dietary Supplements

FOOD SAFETY AND TOXICOLOGY | June 29, 2020

## Microcystin Toxins at Potentially Hazardous Levels in Algal Dietary Supplements Revealed by a Combination of Bioassay, Immunoassay, and Mass Spectrometric Methods

Todd R. Miller\*, Ame Xiong, Jonathan R. Deeds, Whitney L. Stutts, Ingunn A. Samdal, Kjersti E. Løvberg, and Christopher O. Miles

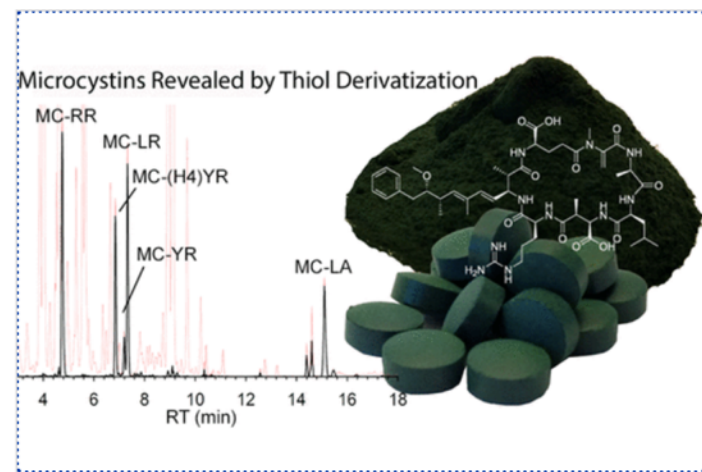
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Supporting Information (1)

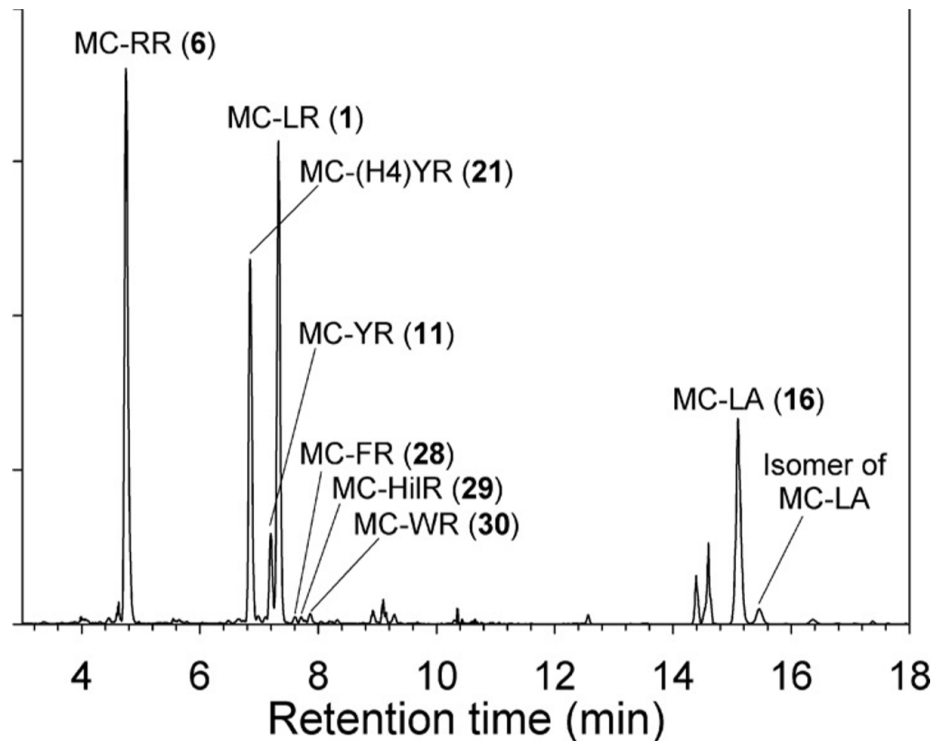
GET IT!

### Abstract

Microcystins (MCs) are hepatotoxic heptapeptides produced by cyanobacteria and are potent inhibitors of protein phosphatases in eukaryotic cells. Algae for dietary supplements are harvested from outdoor environments and can be contaminated with MCs. Monitoring of MCs in these products is necessary but is complicated by their structural diversity (>250 congeners). We used a combination of protein phosphatase inhibition assay (PPIA), ELISA, LC-MS/MS, and nontargeted LC-high-resolution MS (LC-HRMS) with thiol derivatization to characterize the total MCs in 18 algal dietary supplements. LC-MS/MS revealed that some products contained >40 times the maximum acceptable concentration (MAC) of 1 µg/g MCs, but ELISA and PPIA showed up to 50–60 times the MAC. LC-HRMS identified all congeners targeted by LC-MS/MS plus MC-(H4)YR contributing up to 18% of total MCs, along with numerous minor MCs

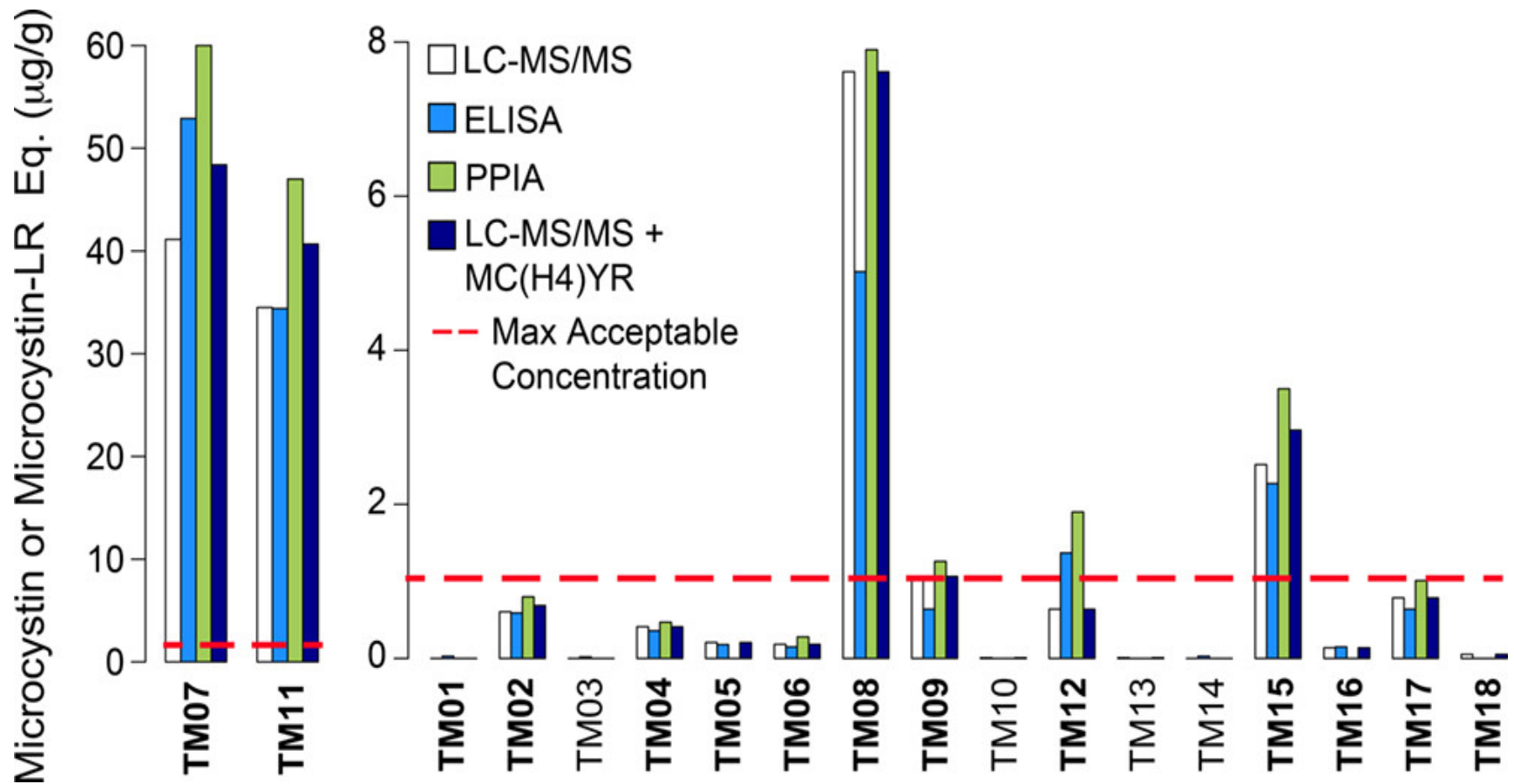


# Microcystin Containing Tetrahydrotyrosine (H4)MC-YR in Dietary Supplements

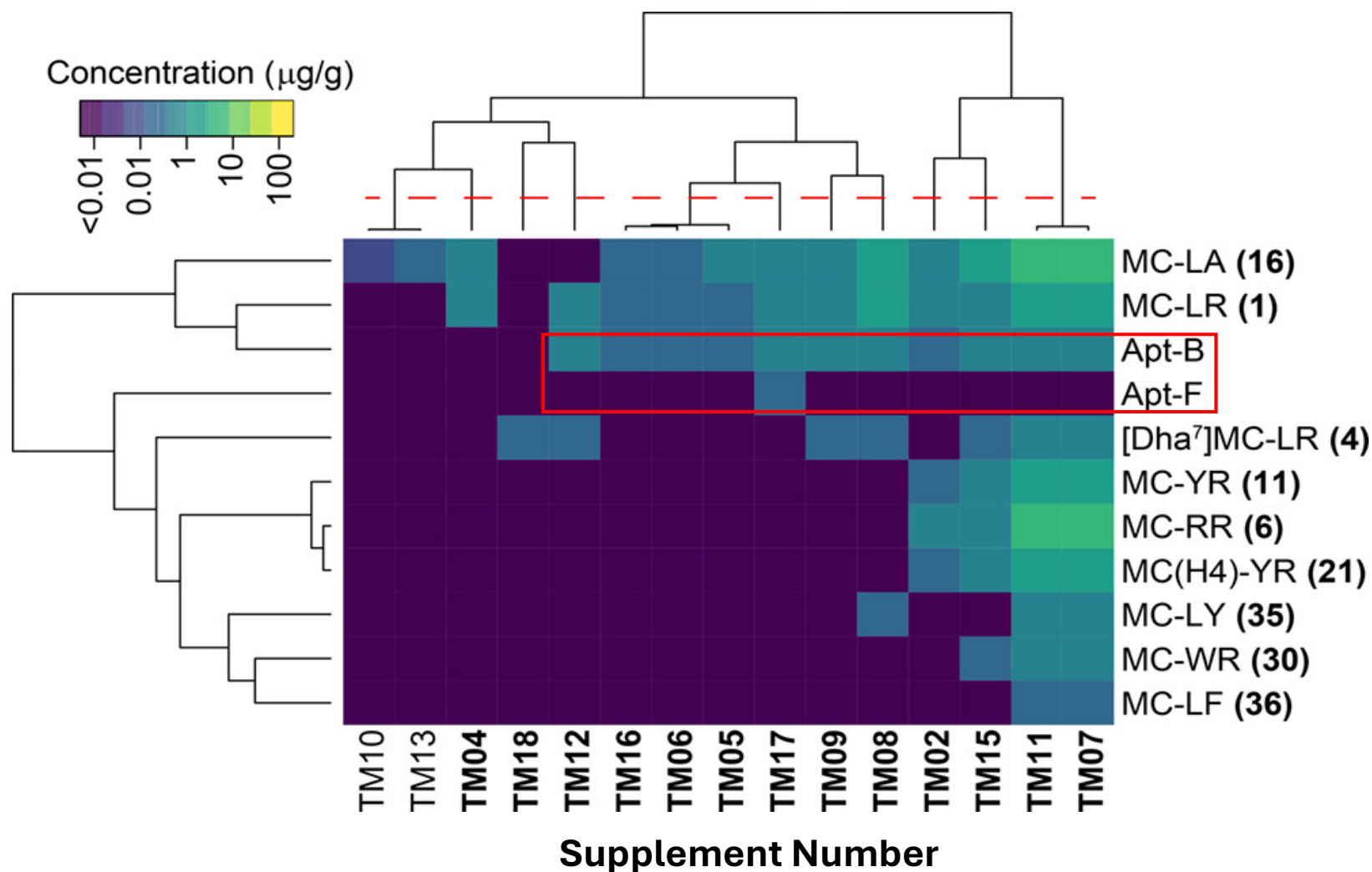


- Microcystin - (H4)YR was discovered in two dietary supplements.
- Based on ionization, (H4)MC-YR contributed significantly to the overall total microcystin.

# Microcystins Over Health Limits in Six Supplements

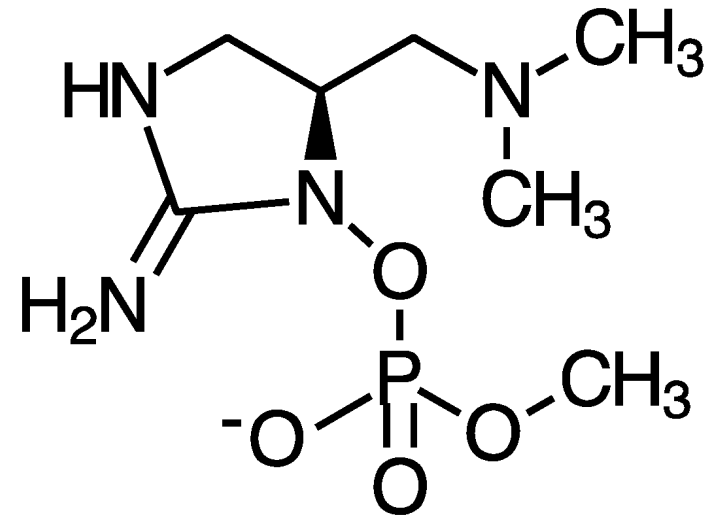


# Anabaenopeptins Present in Nearly All Supplements Tested



# Guanitoxin (formerly anatoxin-a(s)), Potent Freshwater Neurotoxin

- Naturally occurring organophosphate neurotoxin
- Binds irreversibly to acetylcholinesterase
- Originally discovered in *Anabaena flos-aquae* NRC 525-17, from Buffalo Pound Lake, Saskatchewan, Canada.
  - Mahmood and Carmichael, 1987, Toxicon
  - Structure provided by Richard Moore, University of Hawaii, Department of Chemistry
- LD<sub>50</sub> in mice via intraperitoneal injection is 40 ug/kg
  - Saxitoxin = 10 ug/kg
  - MC-LR = 460 ug/kg
  - Cylindrospermopsin = 2100 ug/kg



NRC 525-17 was lost  
Few or no other strains have been available  
No commercial standards available  
Very little is known about its distribution in U.S.

# Identification of Biosynthetic Genes and Metabolic Synthesis of Guanitoxin



pubs.acs.org/JACS

Article

## Biosynthesis of Guanitoxin Enables Global Environmental Detection in Freshwater Cyanobacteria

Stella T. Lima, Timothy R. Fallon, Jennifer L. Cordoza, Jonathan R. Chekan, Endrews Delbaje, Austin R. Hopiavuori, Danillo O. Alvarenga, Steffaney M. Wood, Hanna Luhavaya, Jackson T. Baumgartner, Felipe A. Dörr, Augusto Etchegaray, Ernani Pinto, Shaun M. K. McKinnie,\* Marli F. Fiore,\* and Bradley S. Moore\*



Cite This: *J. Am. Chem. Soc.* 2022, 144, 9372–9379



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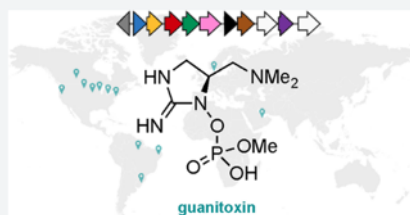


Article Recommendations

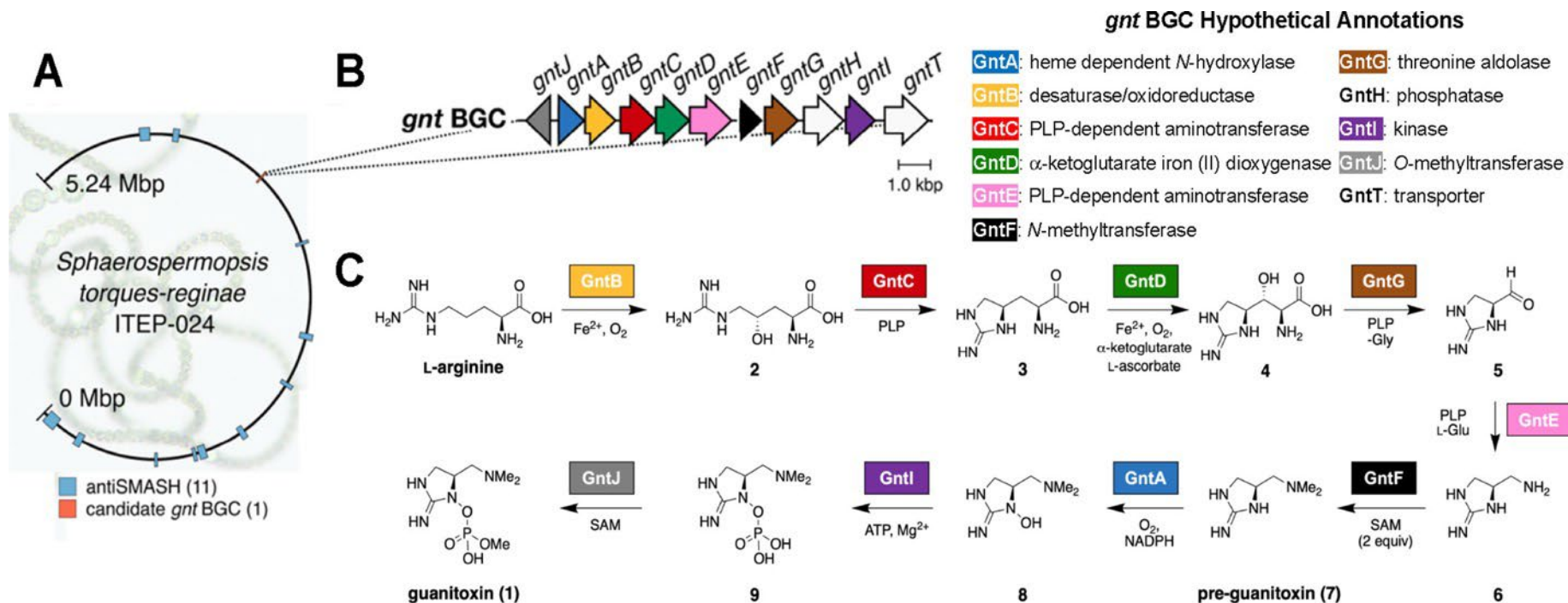


Supporting Information

**ABSTRACT:** Harmful cyanobacterial blooms (cyanoHABs) cause recurrent toxic events in global watersheds. Although public health agencies monitor the causal toxins of most cyanoHABs and scientists in the field continue developing precise detection and prediction tools, the potent anticholinesterase neurotoxin, guanitoxin, is not presently environmentally monitored. This is largely due to its incompatibility with widely employed analytical methods and instability in the environment, despite guanitoxin being among the most lethal cyanotoxins. Here, we describe the guanitoxin biosynthesis gene cluster and its rigorously characterized nine-step metabolic pathway from *L*-arginine in the cyanobacterium *Sphaerospermopsis torques-reginae* ITEP-024. Through environmental sequencing data sets, guanitoxin (*gnt*) biosynthetic genes are repeatedly detected and expressed in municipal freshwater bodies that have undergone past toxic events. Knowledge of the genetic basis of guanitoxin biosynthesis now allows for environmental, biosynthetic gene monitoring to establish the global scope of this neurotoxic organophosphate.



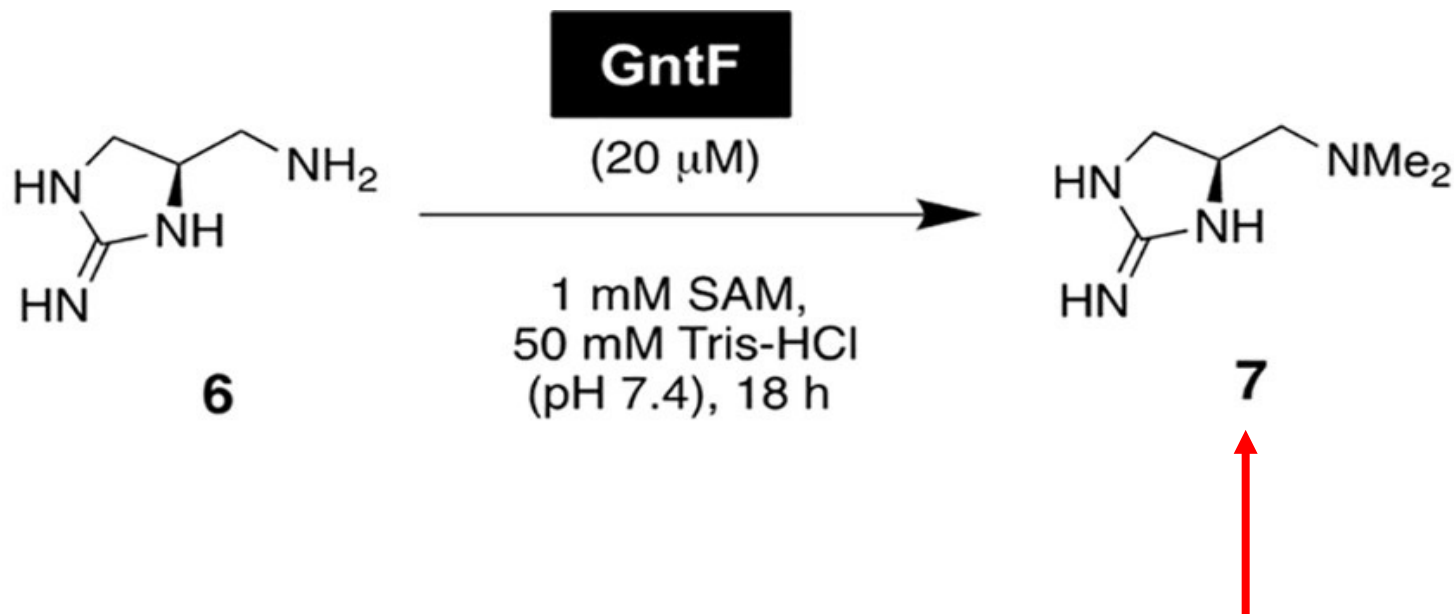
# Biosynthetic Pathway in *Sphaerospermopsis* from Brazil



Lima et al. *Journal of the American Chemical Society* **2022**, 144

Strain ITEP-024 also produces anabaenopeptins  
Lima et al. et al. *ACS Chemical Biology* **2017**.

# Pre-Guanitoxin is a Diagnostic Marker for Guanitoxin Synthesis



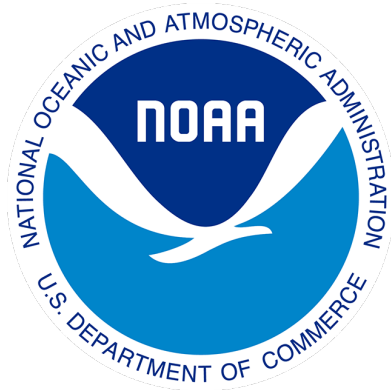
We have a limited supply of pre-guanitoxin from the Moore Lab to share with those interested in using it as an analytical standard.

Pre-Guanitoxin  
-A novel molecule not observed in other characterized biosynthetic pathways

# Acknowledgements



- **Anjana Adhikari**, Doctoral Student, Zilber College of Public Health
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- **Cheslea Weirich**, Former Doctoral Student, Zilber College of Public Health
- **Sarah Bartlett**, Former Doctoral Student, School of Freshwater Sciences
- **Ame Xiong**, Former Technician, Zilber College of Public Health
- **Lucas Beversdorf**, Former Post-Doctoral Fellow



great lakes  
observing system

