Cyanobacteria Toxin Deeper Dive & Some Other Stuff

Barry H. Rosen, Ph. D.
Florida Gulf Coast University
World-Class Scholar and Professor

brosen@fgcu.edu

239-745-4589

THE WATER SCHOOL AT **FGCU**

You may need to wear gloves while listening

phycobiome

Photo Credit: Nara Sousa

Cyanobacteria: Key cyanotoxin-producing organisms: a phylogenetical diverse group

Unicellular forms
Microcystis, Woronichinia

Order Chroococcales

Filamentous (non-N fixers)
Lyngbya, Phormidium,
Microcoleus, Oscillatoria
Planktothrix, Microseira

Order Oscillatoriales

Filamentous (heterocystous)
Dolichospermum
Aphanizomenon
Raphidiopsis
Nodularia
Anabaenopsis
Cylindrospermum
Cuspidothrix, Chrysosporum

Order Nostocales

Some genera are known to make more than one kind of toxin

Other toxin-producing freshwater organisms

Haptophytes

Prymnesins-cytotoxic, neurotoxic and ichthyotoxic

- *Prymnesium parvum*- massive fish kills
- Amphibians
- Crustaceans
- Shellfish
- Toxic blooms in 21 state

Field and laboratory guide to freshwater cyanobacteria harmful algal blooms for Native American and Alaska Native communities Open-File Report 2015-1164
By: Barry H. Rosen and Ann St. Amand USGS

Toxic Algae in Inland Waters of the Conterminous United States—A Review and Synthesis
Water **2023**, *15*(15)

Other toxin-producing freshwater organisms

Euglenophytes

Euglenophycin-ichthyotoxic

- *Euglena sanguinea* (and others)
- Inhibit mammalian tissue and microalgae growth

USGS

Families of cyanotoxins

- **Hepatotoxins**
  - Disrupt proteins that keep the liver functioning, may act slowly (days to weeks)
- **Neurotoxins**
  - Cause rapid paralysis of skeletal and respiratory muscles (minutes)
- **Dermatotoxins**
  - Produce rashes and other skin reactions, usually within a day (hours)
- **b-N-methylamino-L-alanine**
  - Excitotoxin, killing neurons
  - Potentially linked to ALS, Parkinson's, Alzheimer's
- **Cyanopeptides beyond microcystins**
  - Protease inhibitors
  - TBD Anabaenopeptins: What We Know So Far

microcystins (300+ variants)
nodularin
cylindrospermopsin

anatoxin-a
guanitoxin (anatoxin-a (s))
saxitoxin
neosaxitoxin

lyngbyatoxin

BMAA & DABA

anabaenopeptins

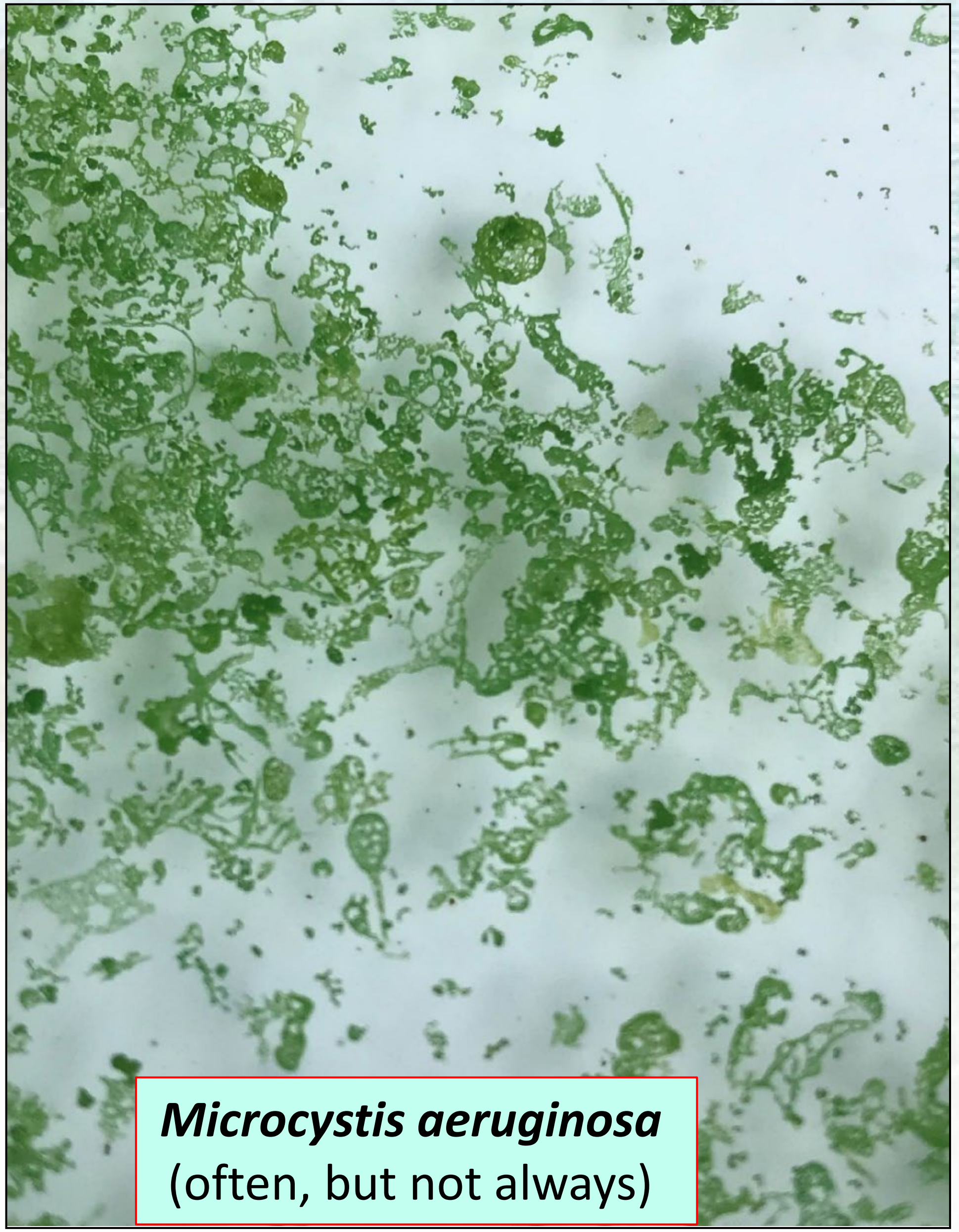
Potent Cyanotoxins: Comparison

*Lethal Dose$_{50}$ (μg/kg of body weight)

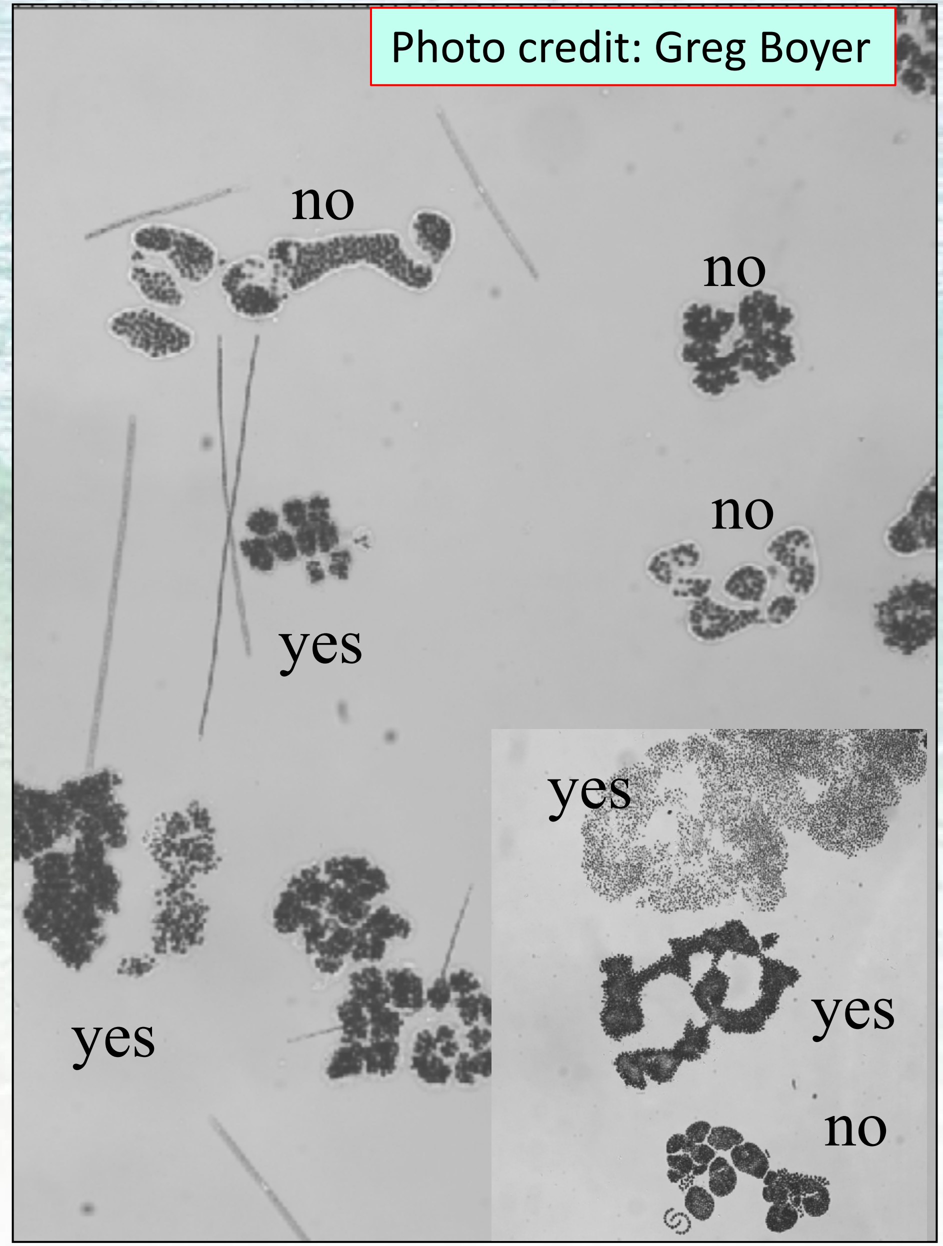
Saxitoxin	9	Ricin	0.02
Guanitoxin Anatoxin-a(s)	20-40	Cobra toxin	20
Microcystin LR	50	Curare	500
Anatoxin-a	200	Strychnine	2000
Nodularin	50		
Cylindrospermopsins	200		

Exposure routes: data is based on ingestion/direct dosing. Little has been done on inhalation effects, but are believed to be 10 times more potent.

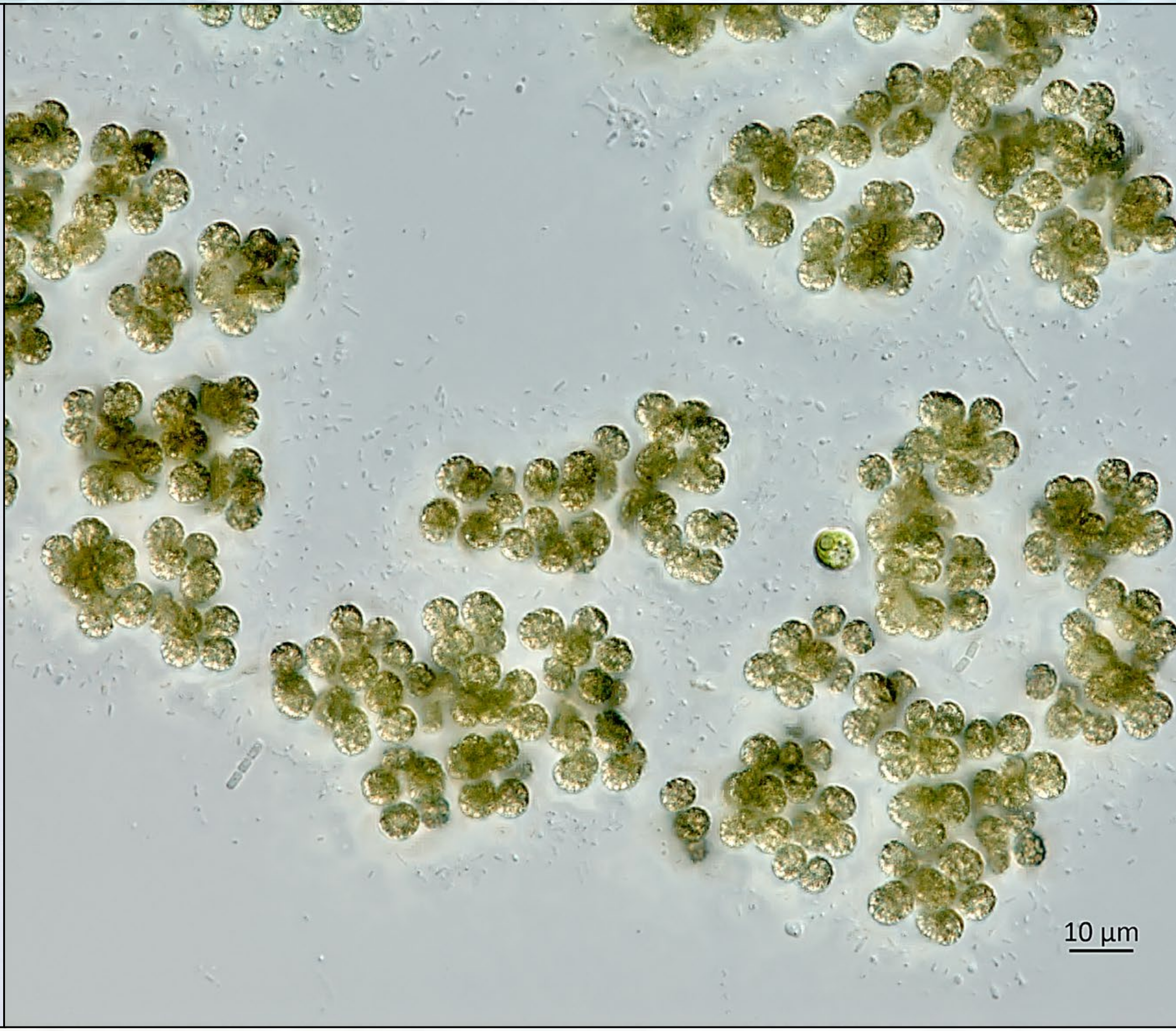
Can not use visual cues to tell toxicity



Microcystis aeruginosa
(often, but not always)



Known microcystin producers-planktonic

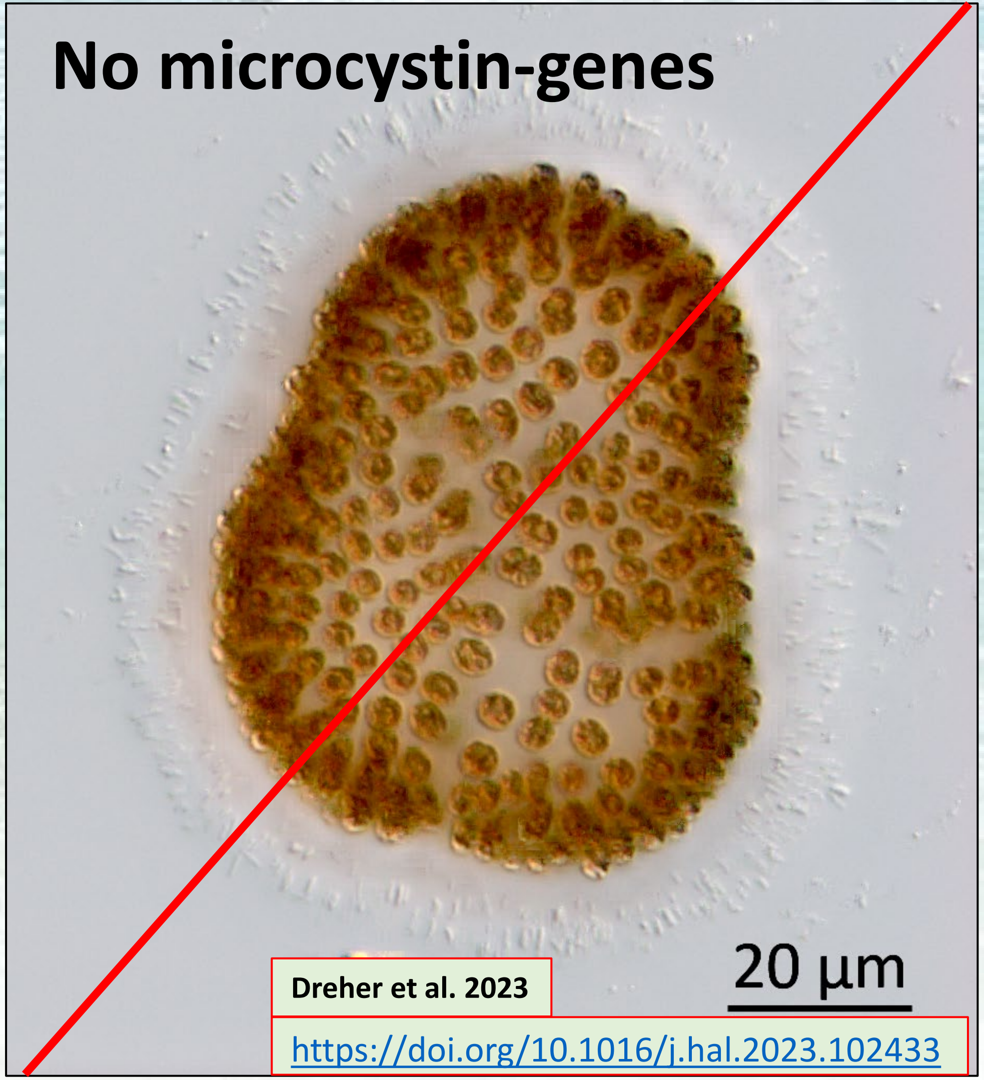


Microcystis aeruginosa

Microcystis viridis

Known microcystin producer...or not

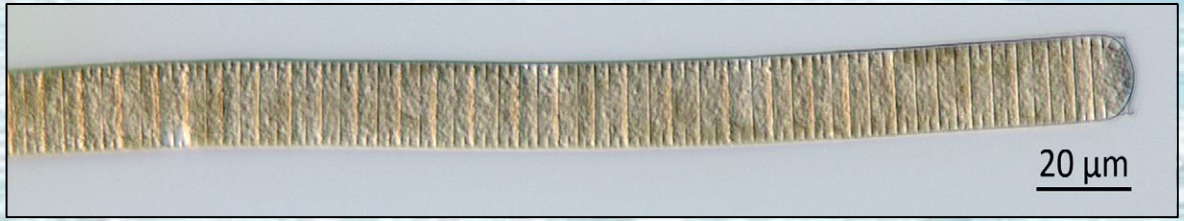
Microcystis wesenbergii



Woronichinia naegeliana

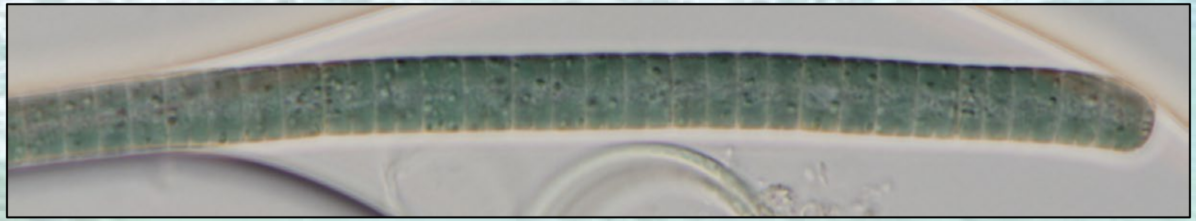
Known filamentous, non-heterocystous producers

Planktothrix

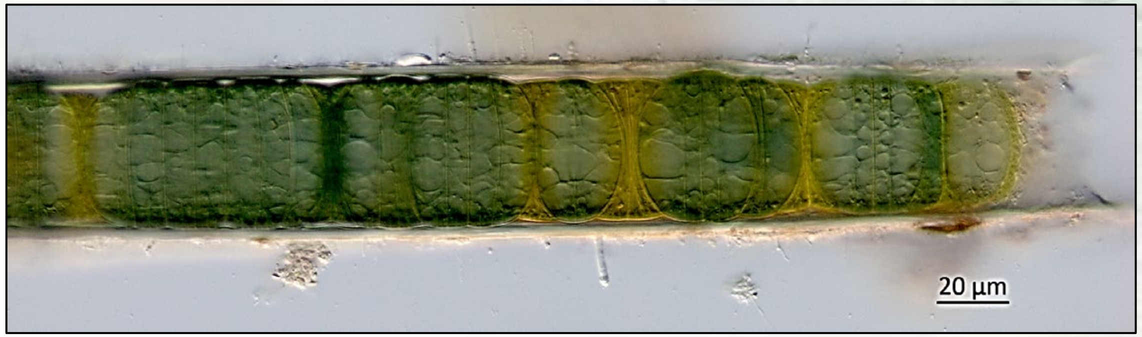


Oscillatoria

Lyngbya



Phormidium



Microseira

Microcoleus

Just one example: Known heterocystous producers

Dolichospermum flos-aquae

Cylindrospermopsin
Saxitoxin

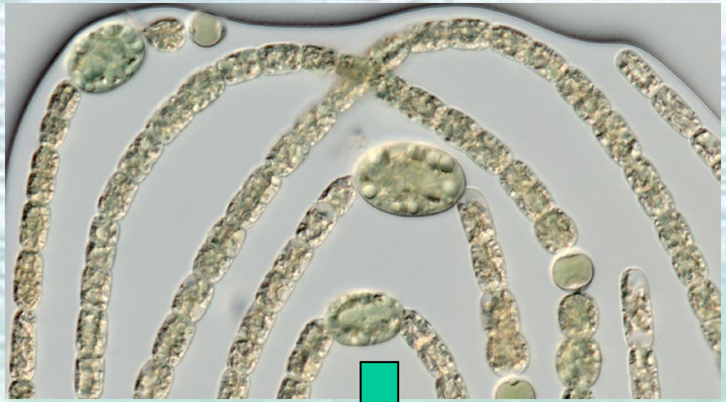
Populations / Strains

Dolichospermum lemmermannii Heater Creek, Detroit Reservoir 5-27-20

Cells must leak toxin to the water (naturally) or be forced to release toxin (for analytical techniques)

- **toxins are important metabolic compounds**
- **“expensive” to synthesis**
- **not just going to “leak” from a healthy cell**

phycocyanin-covered shoreline



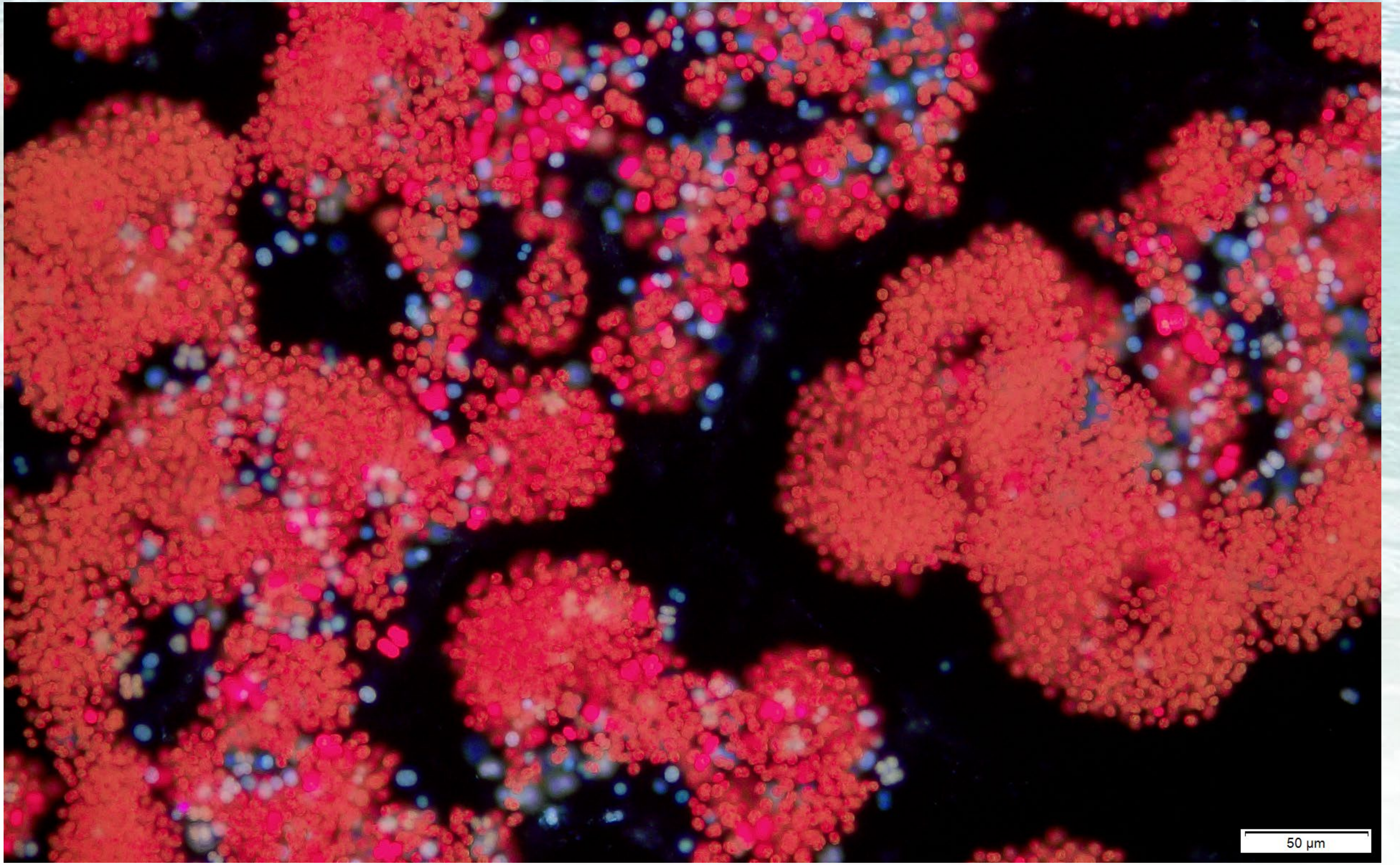
Microphotographs of Cyanobacteria Documenting the Effects of Various Cell-lysis Techniques
https://pubs.usgs.gov/of/2010/1289/

Physical or chemical lysis to release toxins:
Freeze-thaw, sonication, bead beating, etc.

intra-cellular & extra-cellular

How much is in the cells, how much has leaked to the water, total toxin per liter?

Not all cells in a colony are in the same physiological state



Epifluorescence microscopy- *Wide Blue Cube*-with excitation 450-480 nm, emissions above 515 nm)

Microcystins

- **Very common**
  - produced by a number of other species
- **Cyclic Peptides**

  300+ structural variants (congeners)

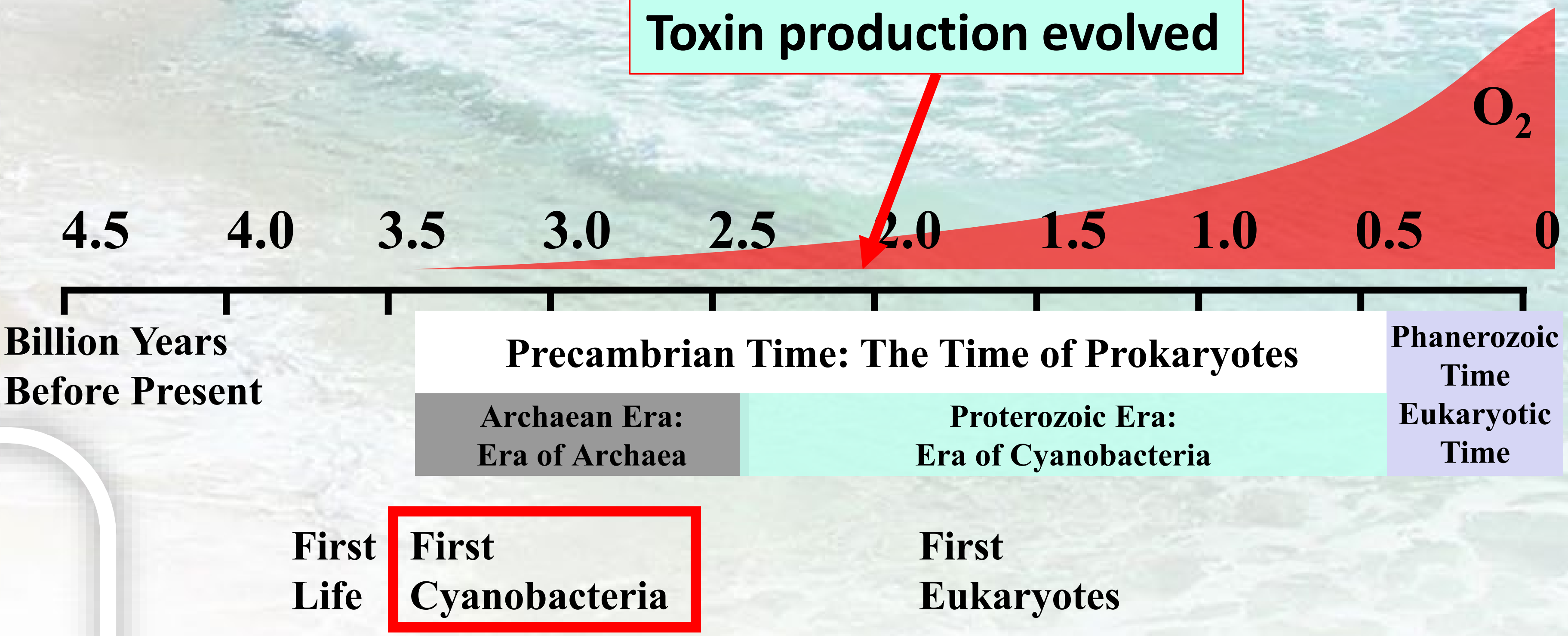
  + 200 others related compounds: nodularins, anabaenapeptins, etc.
- **Microcystins are hepatotoxic** (protein phosphatase inhibitor - PP1a)

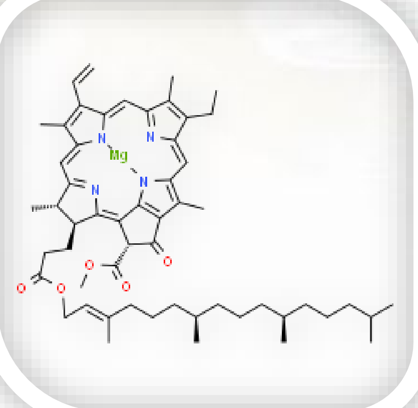
  LD-50: 20-1500 microcystin LR/μg kg^{-1} in fish….Called "fast death factor"
- **Potent tumor promotor**
- **Non-ribosomal peptide synthetase genes, in modules, each adding one amino acid**

All and more!

Great Oxygenation Event-lead by the Cyanobacteria!

(wipe out as many competitors as possible!)





chlorophyll *a*

Why toxins? Scavenger for iron, nitrogen storage, grazing? (pre-date metazoans), oxidative stress, quorum sensing, allelopathy. **Human perspective is inadequate.**

Microcystin Congeners
are identified and named based on variable amino acids

review

https://www.mdpi.com/2072-6651/11/12/714#

85% have the ADDA

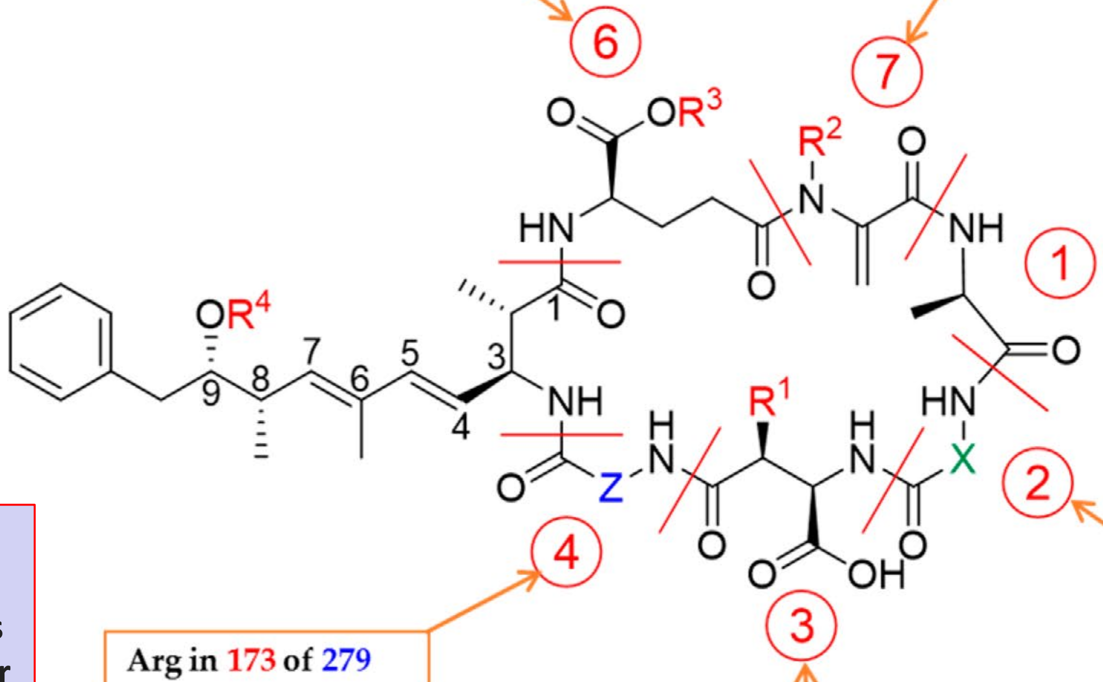
Mdha in 189 of 279
Dha in 27 of 279
Dhb in 15 of 279
E-Dhb in 10 of 279
Z-Dhb in 2 of 279
Mdhb in 1 of 279
Mser in 13 of 279
Ser 11 of 279
Thr in 2 of 279
Cys and Cys(O) adducts to Mdha in 4 of 279
GSH and GS(O) adducts to Mdha in 3 of 279
γ-GluCys and γ-GluCys(O) adducts to Mdha in 2 of 279

D-Glu in 267 of 279
D-Glu(OMe) in 11 of 279
D-Glu(OC_3H_7O) in 1 of 279

D-Ala in 219 of 279
D-Leu in 35 of 279
Gly in 15 of 279
D-Ser in 3 of 279
D-Met in 2 of 279
D-Met(O) in 2 of 279
D-Phe in 1 of 279
D-Val in 1 of 279
D-Hil in 1 of 279

alanine

Adda in 221 of 279
ADMAdda in 36 of 279
DMAdda in 16 of 279
(6*Z*)-Adda in 2 of 279
Adda(OH) in 1 of 279
Adda(O) in 1 of 279
dmAdda in 2 of 279



about 20% (55 of 279) appear to be the result of chemical or biochemical transformations of MCs that can occur in the environment or during sample handling and extraction of cyanobacteria

Arg in 173 of 279

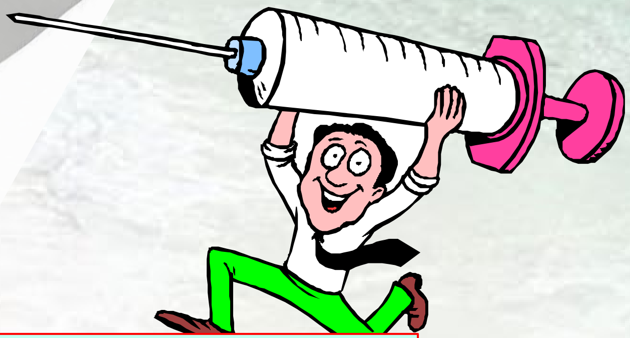
Leu in 95 of 279

D-Masp in 157 of 279
D-Asp in 122 of 279

Mouse Bioassays

Non-selective: most toxins
Not very sensitive (mg concentrations)
Shellfish monitoring

Inject, observe reaction-minutes to overnight; perform necropsy
i.e., bloody liver = hepatotoxin



Intraperitoneal inoculation

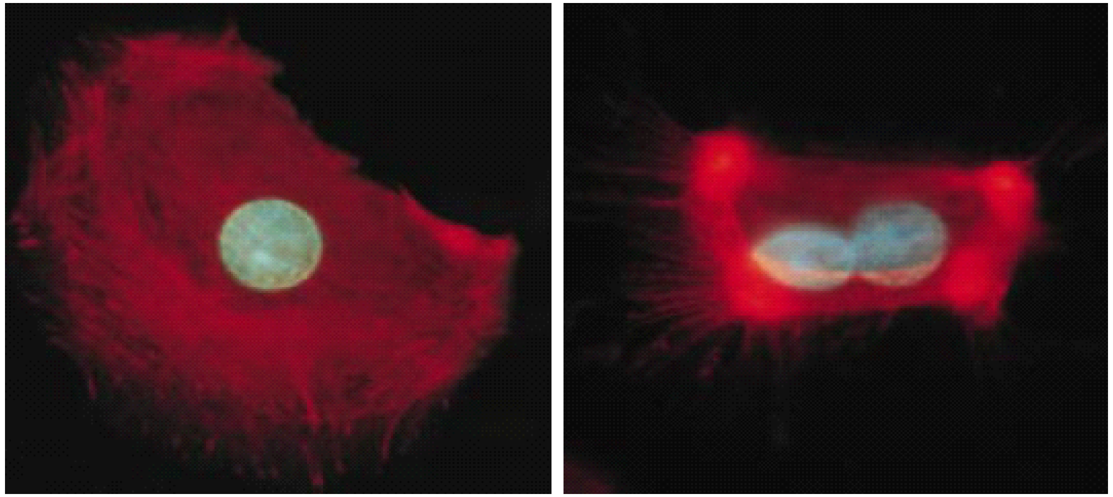
Oral: ingestion experiments

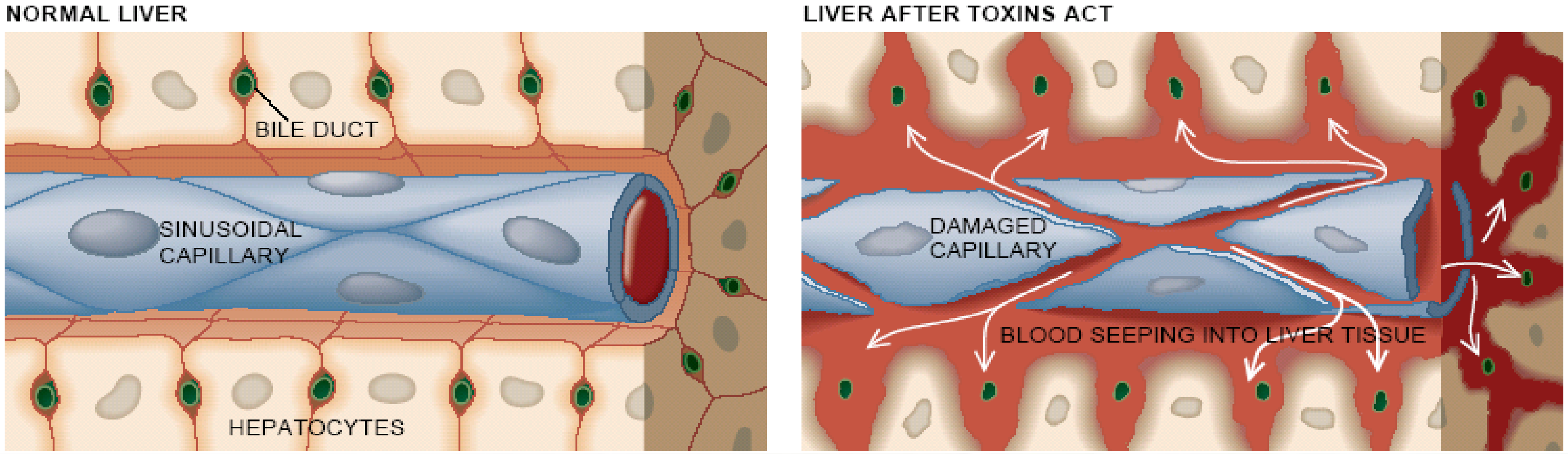
Microcystin exposure: response

- Uptake by bile acid transporter
- Inhibit protein phosphatases 1 and 2A
- Affects cytoskeleton, cell cycle, general metabolism, apoptosis

Hepatotoxicity

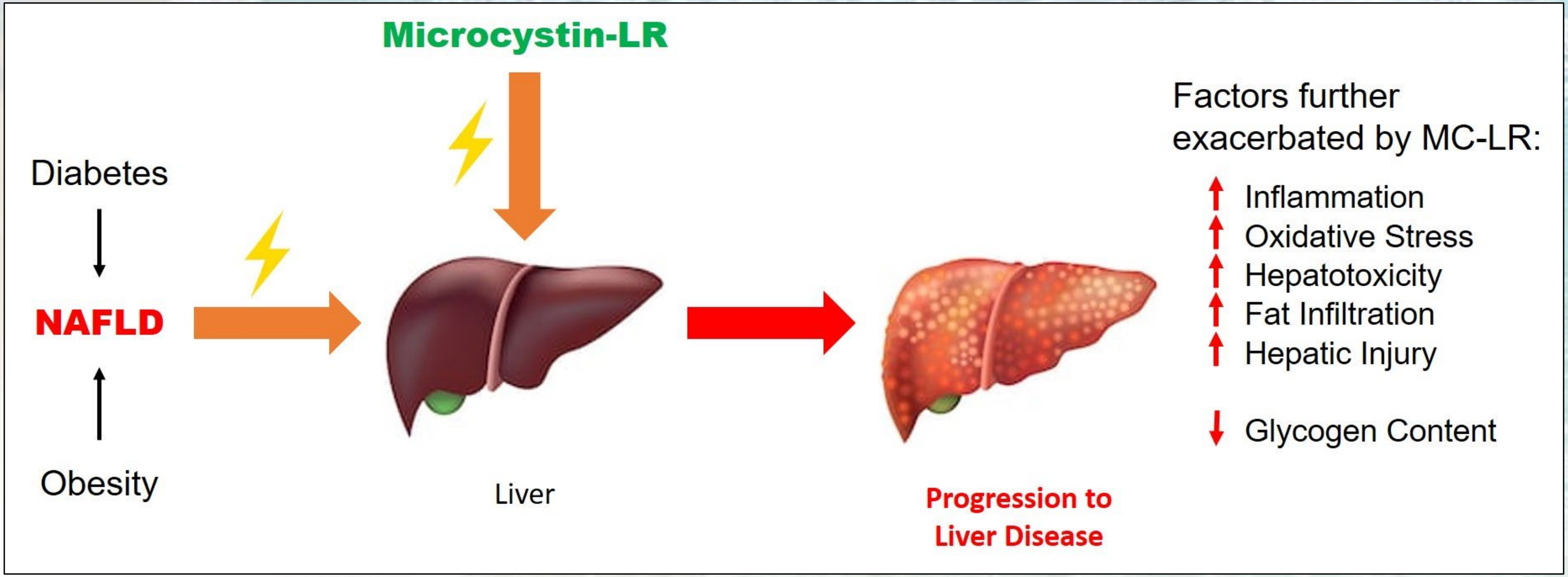
MICROFILAMENTS (*red threads in micrographs*), structural components of cells, are usually quite long, as in the rat hepatocyte at the left. But after exposure to microcystins (*right*), microfilaments collapse toward the nucleus (*blue*). (This cell, like many healthy hepatocytes, happens to have two nuclei.) Such collapse helps to shrink hepatocytes—which normally touch one another and touch sinusoidal capillaries (*left drawing*). Then the shrunken cells separate from one another and from the sinusoids (*right drawing*). The cells of the sinusoids separate as well, causing blood to spill into liver tissue. This bleeding can lead swiftly to death.





Wayne Carmichael ISOC-HAB Ch. 4, Scientific American, January, 1994

Microcystin exposure: tumor promotion



Microcystin Toxicokinetics, Molecular Toxicology, and Pathophysiology in Preclinical Rodent Models and Humans

source

Drinking Water Guidelines

0.3 micrograms per liter for **microcystin**

0.7 micrograms per liter for **cylindrospermopsin**

- children younger than school age
- **exposure for 10 days**.

Link

1.6 micrograms per liter for microcystin

3.0 micrograms per liter for cylindrospermopsin

- all other ages

EPA Issued Health Advisories for Algal Toxins in Drinking Water *Release Date: 05/06/2015*

Recreational Guidelines

8 micrograms per liter for **microcystins**
15 micrograms per liter for **cylindrospermopsin**

- All age groups

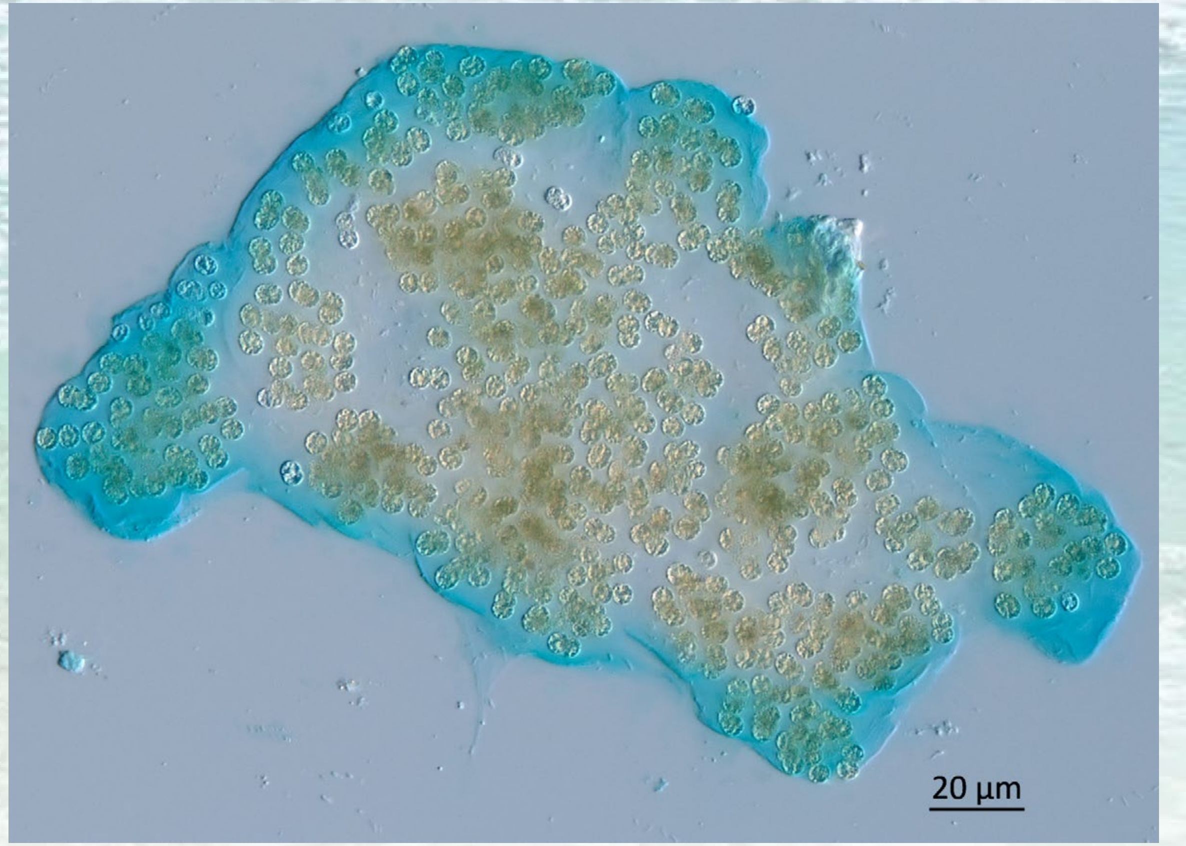
EPA Issues Recommendations for Recreational Water Quality Criteria and Swimming Advisories for Cyanotoxins

Release Date: 05/22/2019

https://www.epa.gov/newsreleases/epa-issues-recommendations-recreational-water-quality-criteria-and-swimming-advisories

Observation: filaments are more delicate than colonial forms

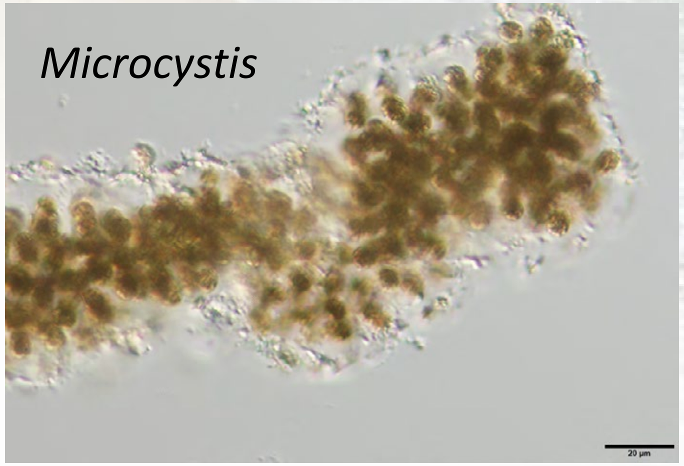
Dolichospermum mucosum



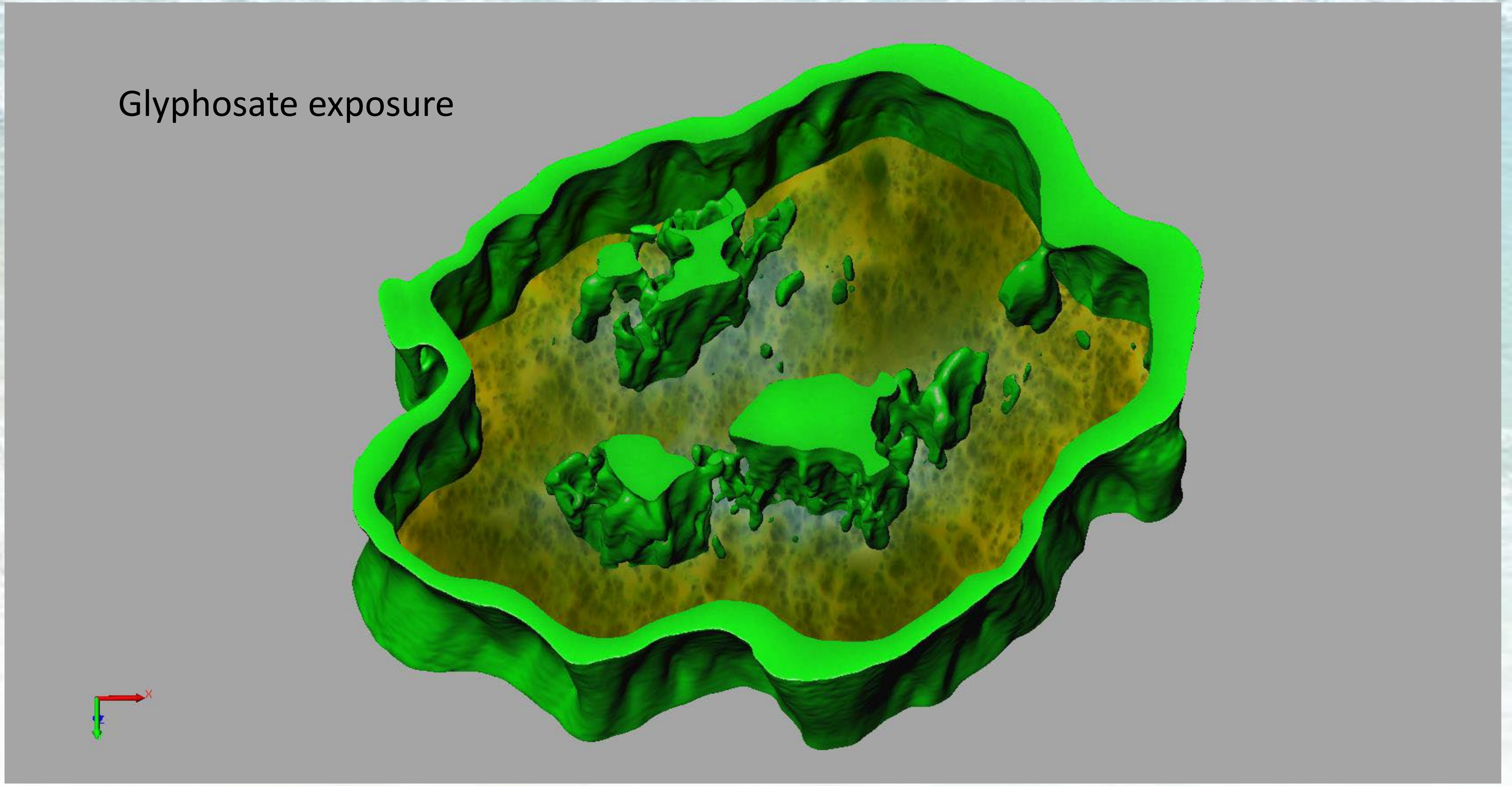
Alcian Blue Stain-acid mucosubstances

Environmental variable in mucilage production: does this leaking of toxins?

Exposure to stress: Salinity, desiccation, glyphosate stimulate mucilage production



https://doi.org/10.3133/sir20185092



Photosynthate shunted into "protection". How does that change the allocation for toxin production in the cells?

Microsystin-producing strains include:

- *Microcystis aeruginosa*
- *M. wesenbergii*
- *M. viridis*
- *Oscillatoria limosa*
- *Dolichospermum flos-aquae*
- *D. lemmermannii*
- *D. circinale*
- *Planktothrix agardhii*
- *Nostoc spumigena*
- *Anabaenopsis*
- *Haphalosiphon hibermicus*
- *Gloeotrichia sp.*

And more

Another route of exposure: Aerosolization

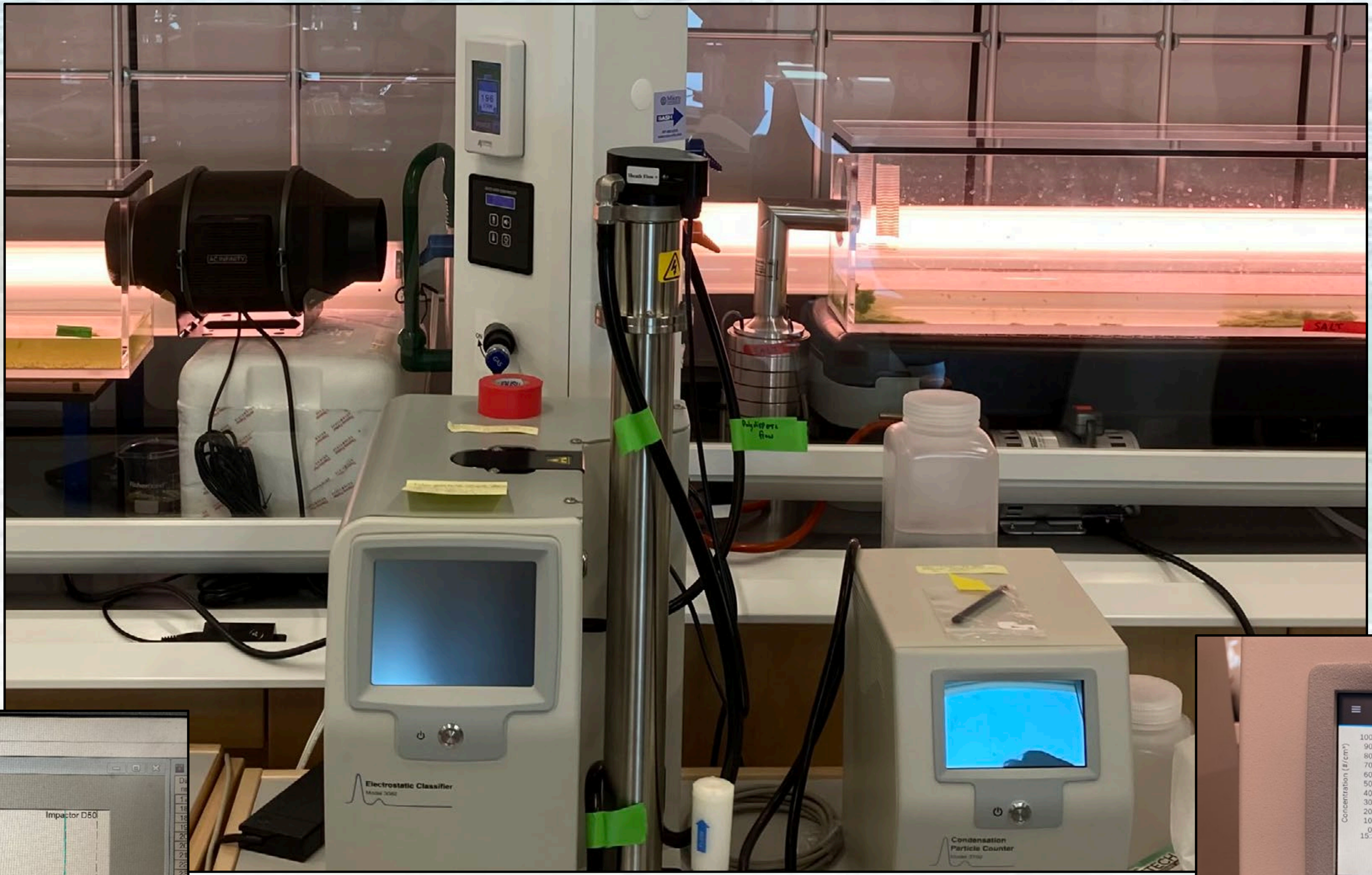
cascade air impactor
(Tisch Environmental)
simulate the lung

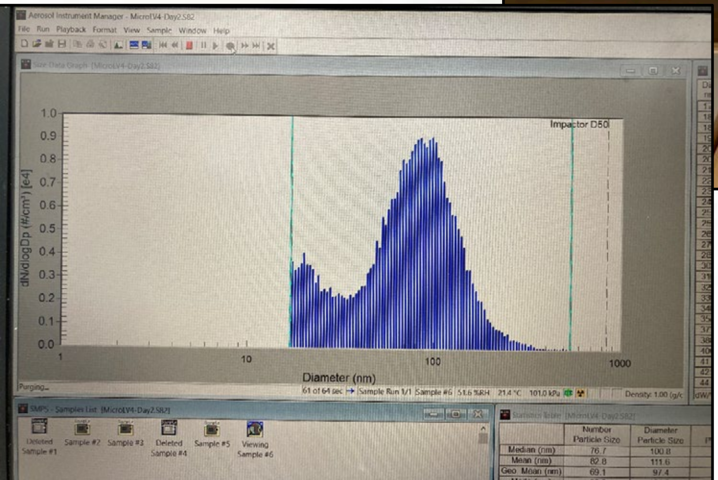
Note: whole, viable organisms reach the lowest level of the air impactor

Exposure?

Quantifying Aerosols

X-ray particle **sizer** and **counter**





Experimental setup: Control vs Salinity

Control vs. Salinity

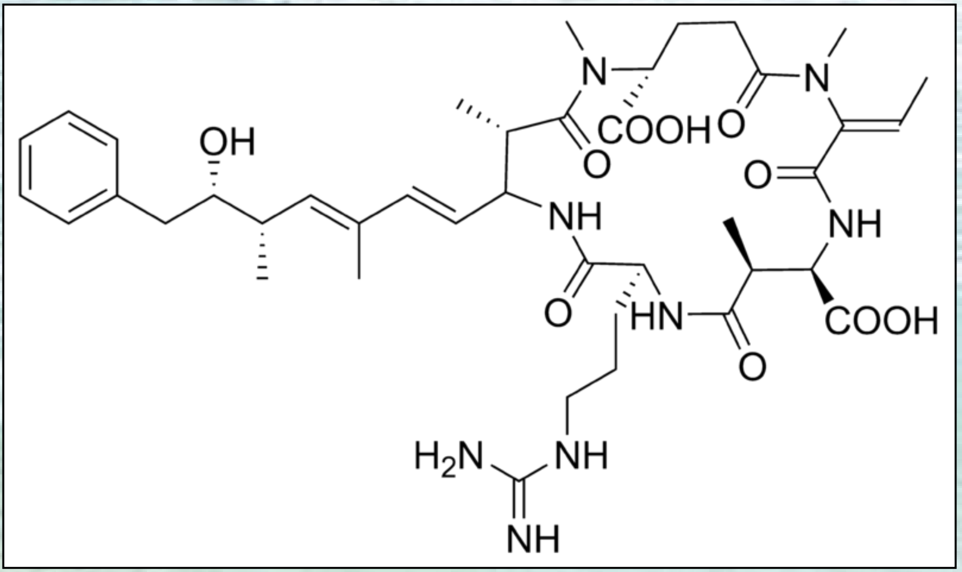
Sample	Run	Control/Salt	Location	Time	MC-LR (ppt)	D-Asp3MC-LR (ppt)	MC-HilR (ppt)
MLV1-C 72 FF	1	C	FF	Day 3	4.58		
MLV3-C 72 F1	3	C	F1	Day 3	6.37		
MLV3-C 72 FF	3	C	FF	Day 3	2.7		
MLV6-S 96 FF	6	S	FF	Day 4	3.69		
MLV1-C 0hr Tank	1	C	Tank	Day 0	46530	2400	1860
MLV1-C 96hr Tank	1	C	Tank	Day 4	67200	5970	1930
MLV6-C 0hr Tank	6	C	Tank	Day 0	940		
MLV6-C 96hr Tank	6	C	Tank	Day 4	1530		
MLV6-S 96hr Tank	6	S	Tank	Day 4	3010		

Plenty in the source material

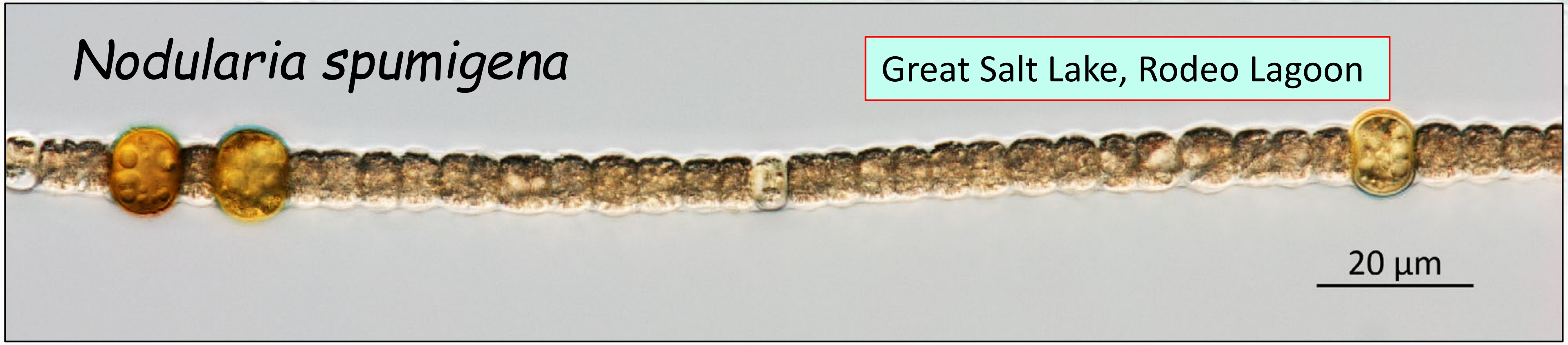
Judy Westrick, Lumigen Instrument Center at Wayne State

Nodularin-R

- Cyclic nonribosomal peptide
- Brackish; world-wide
- Freshwater
- Hepatotoxin (same as microcystin LR)
- Significant homology of structure and function with microcystins



Microcystins/Nodularins (ADDA)
SAES, ELISA, 96 tests



Nodularia spumigena

Anatoxins

LD$_{50}$
200 µg/kg

Very Fast
Death Factor

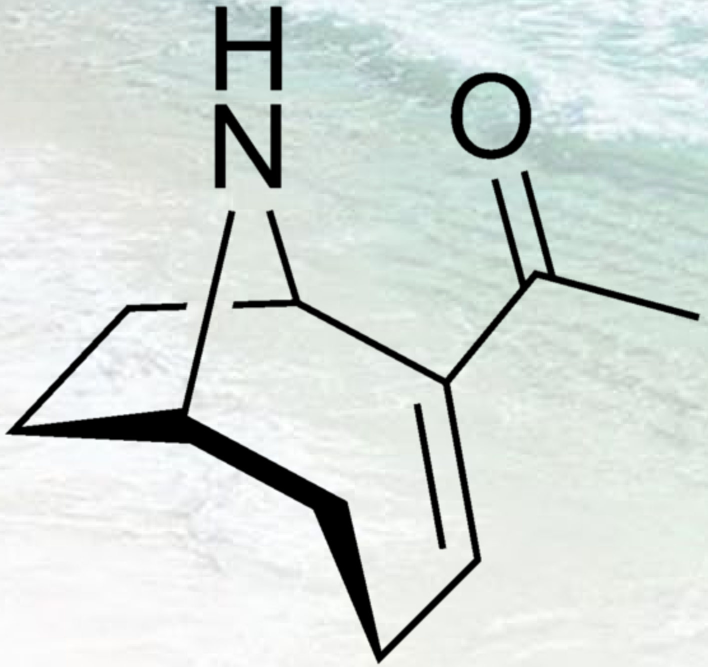
Mouse Bioassay:

- Paralysis
- Tremors
- Mild convulsions
- Salivation
- Respiratory arrest

2-7 mins... death

Anatoxin-a

actylcholine agonist

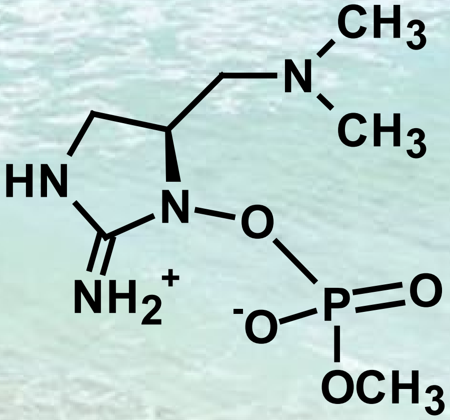


https://doi.org/10.1016/j.hal.2019.101737

~~Anatoxin-a(S)~~

Guanitoxin (2020)

acetylcholinesterase inhibitor

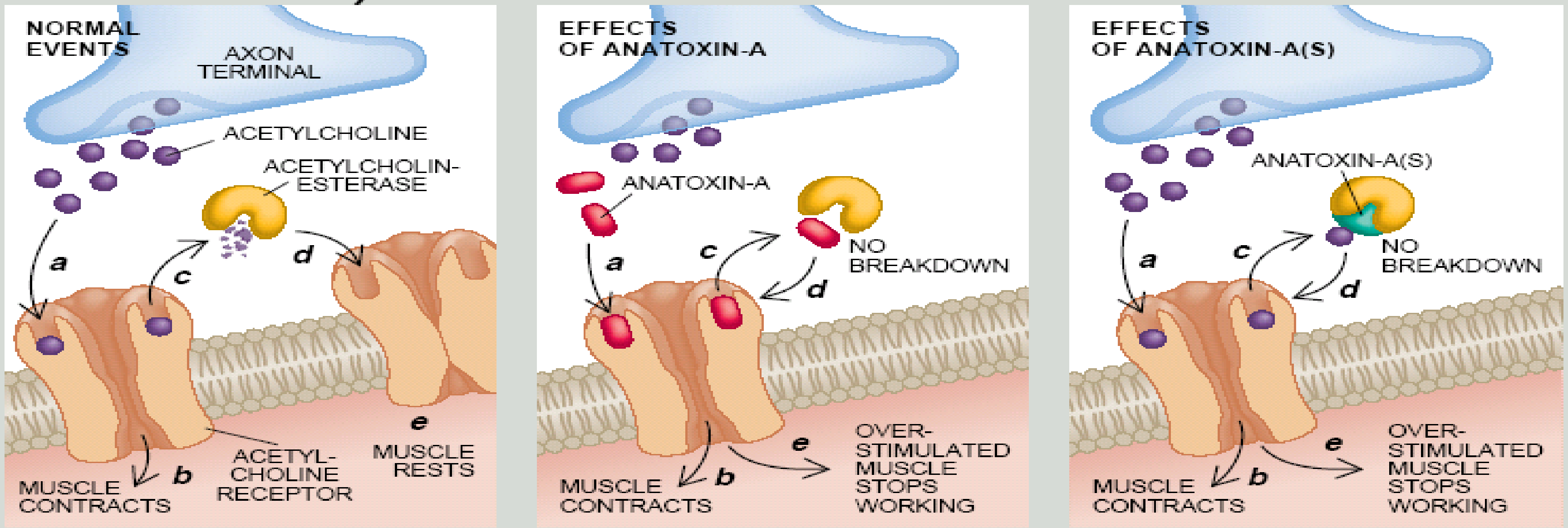


Dolichospermum flos-aquae &
D. lemmermannii

Anatoxin-a and Guanitoxin

Anatoxin-a: nicotinic acetylcholine receptor agonist
Guanitoxin: Acetylcholinesterase irreversible inhibitor

Neurotoxicity

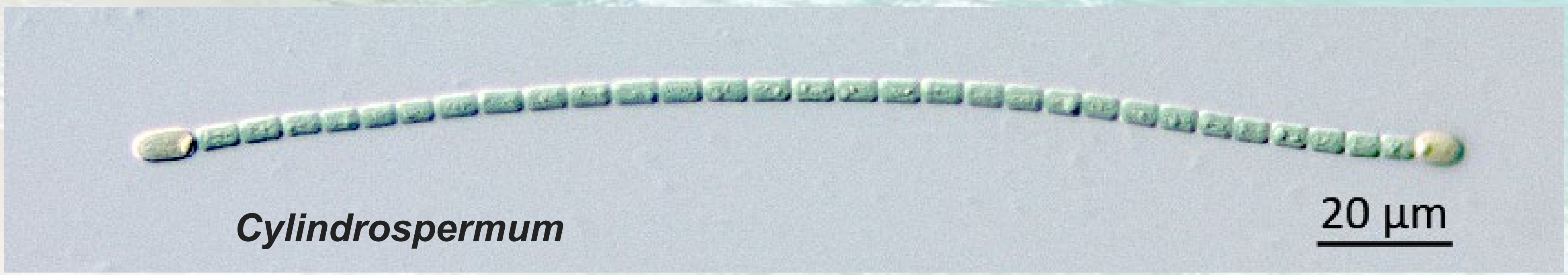


Anatoxin-a and anatoxin-a(s) (*center and right panels*) overexcite muscle cells by disrupting the functioning of the neurotransmitter acetylcholine. Normally, acetylcholine molecules (*purple*) bind to acetylcholine receptors on muscle cells (*a in left panel*), thereby inducing the cells to contract (*b*). Then the enzyme acetylcholinesterase (*yellow*) degrades acetylcholine (*c*), allowing its receptors and hence the muscle cells to return to their resting state (*d* and *e*). Anatoxin-a (*red in center panel*) is a mimic of acetylcholine. It, too, binds to acetylcholine receptors (*a*), triggering contraction (*b*), but it cannot be degraded by acetylcholinesterase (*c*). Consequently, it continues to act on muscle cells (*d*). The cells then become so exhausted from contracting that they stop operating (*e*). Anatoxin-a(s) (*green in right panel*) acts more indirectly. It allows acetylcholine to bind to its receptors and induce contraction as usual (*a* and *b*), but it blocks acetylcholinesterase from degrading acetylcholine (*c*). As a result, the neurotransmitter persists and overstimulates respiratory muscles (*d*), which once again eventually become too fatigued to operate (*e*).

Wayne Carmichael ISOC-HAB Ch. 4, Scientific American, January, 1994

Anatoxin-a Producers

Anatoxin-a and homoanatoxin-a produced by benthic and planktonic cyanobacteria worldwide:
Dolichospermum*, *Cuspidothrix*, *Phormidium*, *Oscillatoria*, *Tychonema* and *Cylindrospermum



Guanitoxin Producers

LD_{50}

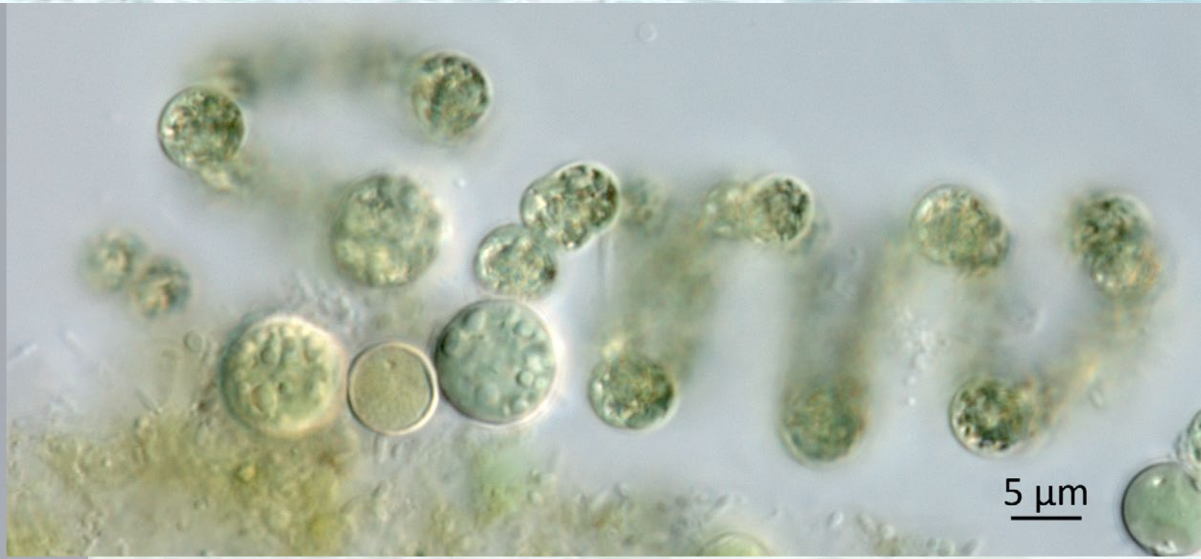
Guanitoxin Anatoxin-a(s) **20-40**

natural organophosphate neurotoxin

(at least in Brazil)

Dolichospermum crassum

Sphaerospermopsis torques-reginae



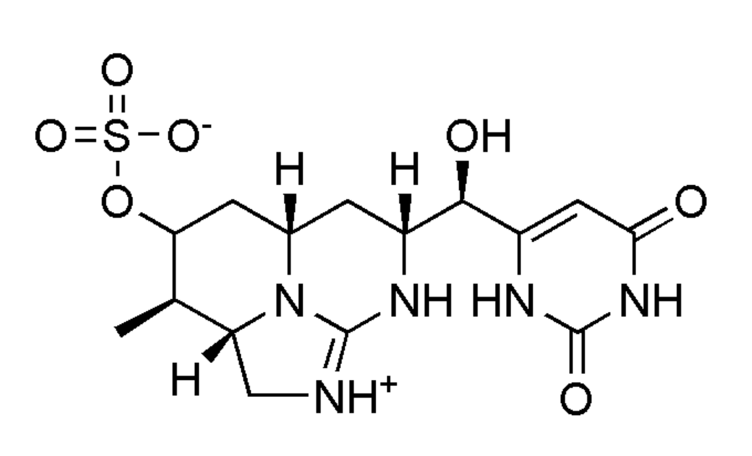
Biosynthesis of Guanitoxin Enables Global Environmental Detection in Freshwater Cyanobacteria

J. Am. Chem. Soc. 2022

Cylindrospermopsin

Raphidiopsis*, formerly *Cylindrospermopsis

- Gastrointestinal effects
- Hepatotoxicity
- Liver necrosis
- Kidney effects
- Inhibition of protein synthesis



Alkaloid Toxin

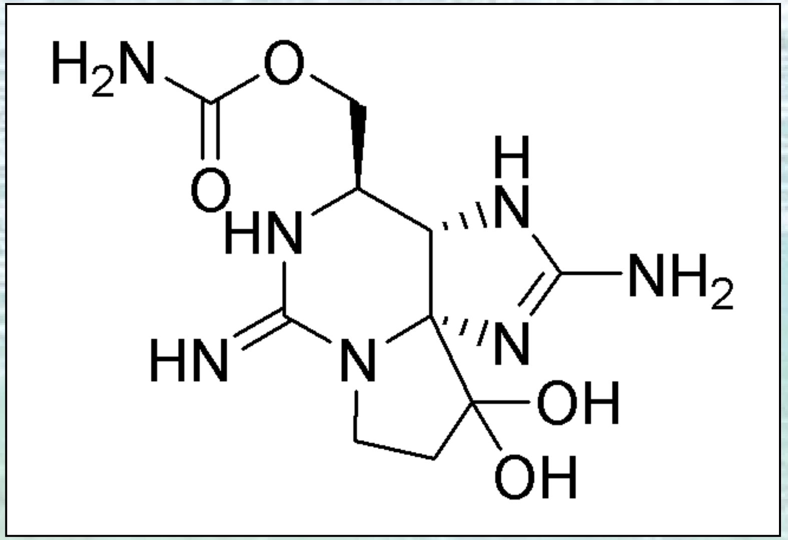
- Covalently modify DNA and/or RNA
- Resistant to degradation by pH and temp-persistent (boiling water won't help)

deoxycylindrospermopsin, a non-toxic metabolite of *R. raciborskii*

Saxitoxin

LD_{50}
Saxitoxin 9

Dolichospermum circinale
Aphanizomenon gracile & *AFA*
Raphidiopsis raciborskii
Microseira wollei
Planktothrix sp.

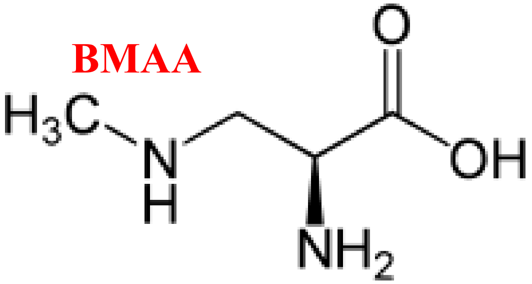


- **Neurotoxin**
  (voltage-gated sodium channels); respiratory failure
- **Aka-Paralytic Shellfish Poisoning (PSP)**
- **Numerous marine dinoflagellates; acquired genes from the cyanobacteria?**

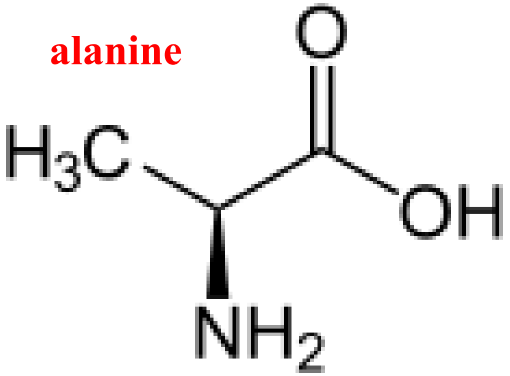
β-methyl amino alanine (BMAA) & 2,4-diaminobutyric acid (DABA)

- **Non-protein amino acid** (900 exist)
- **Made by almost all cyanobacteria?**
- **DABA is a metabolite of BMAA**

BMAA



alanine



Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 2013; Early Online: 1–9
informa healthcare

REVIEW ARTICLE

Is exposure to cyanobacteria an environmental risk factor for amyotrophic lateral sclerosis and other neurodegenerative diseases?

WALTER G. BRADLEY[1], AMY R. BORENSTEIN[2], LORENE M. NELSON[3], GEOFFREY A. CODD[4], BARRY H. ROSEN[5], ELIJAH W. STOMMEL[6] & PAUL ALAN COX[7]

After 100 samples tested for free BMAA, I have **not** found it.

100⁺ samples tested for free *BMAA & DABA*

Analytical Techniques

Liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS)
BMAA
A SeQuant ZIC-HILIC 3.5µm 2.1 x 150 mm HILIC column was used in separation of BMAA, BAMA, 2,4-DAB, AEG, and 3,4-DAB with mobile phases acetonitrile and water containing formic acid per Foss et al. (2018). The $[M+H]^+$ ion for BMAA and its isomers (*m/z* 119) was fragmented and the product ions (*m/z* 73, 76, 88, 101, 102) were monitored. The IS (d_3-BMAA; *m/z* 122) was fragmented and product ion (*m/z* 105) monitored. Differentiation of isomers was made by retention time, LFSMs, and relative abundance of product ions. The internal standard method was used to determine LFSM returns. The method detection limits were determined to be 5 ng/mL (ppb) for BAMA and BMAA and 10 ng/mL for AEG, 3,4-DAB; and 2,4-DAB.

Foss, A.J., Chernoff, N., Aubel, M.T., 2018. The analysis of underivatized β-Methylamino-L-alanine (BMAA), BAMA, AEG & 2,4-DAB in Pteropus mariannus mariannus specimens using HILIC-LC-MS/MS. Toxicon 152, 150–159.

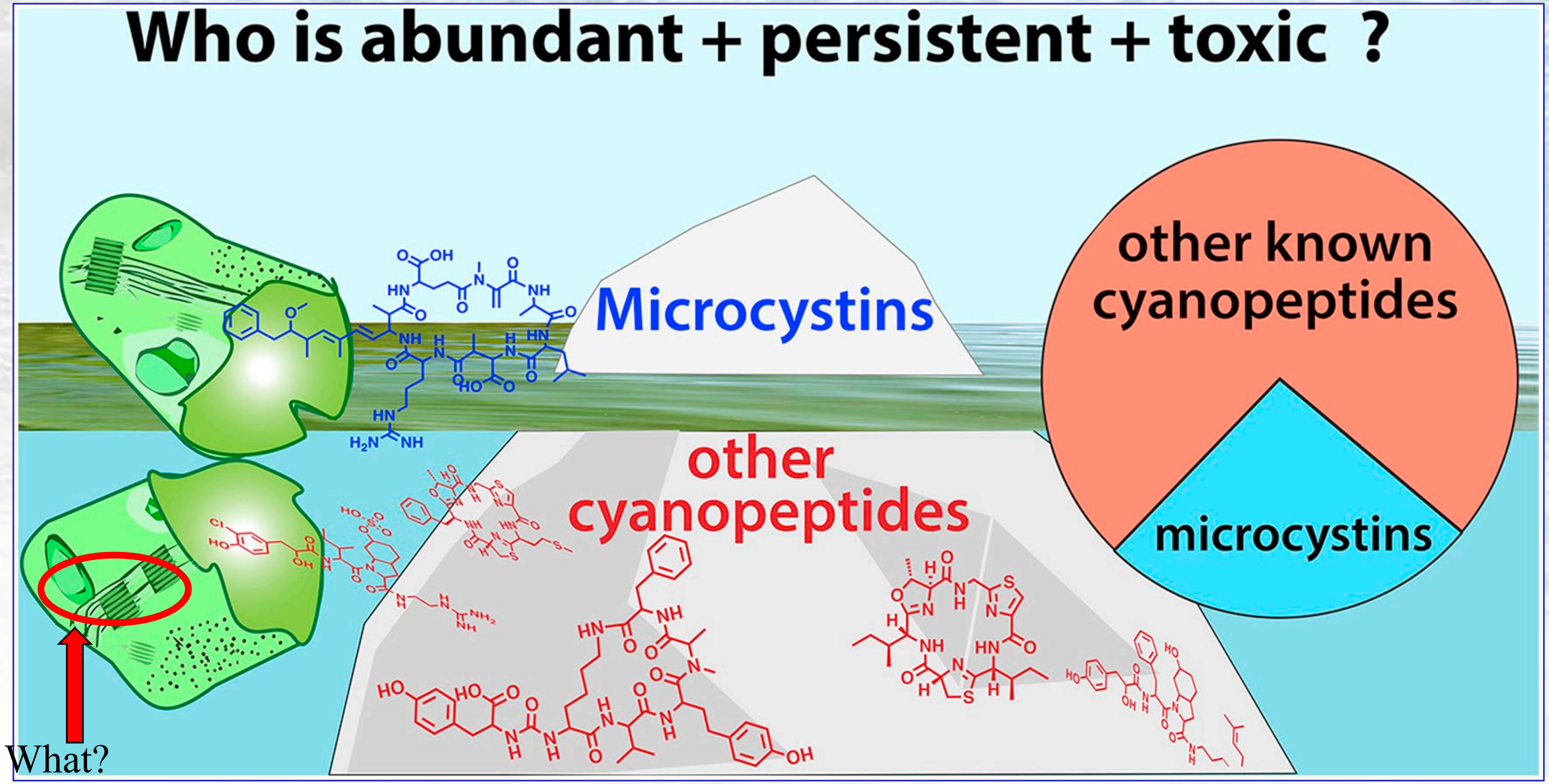
Sample ID	BMAA
Microcystis RL	ND
Microcystis LV	ND
Planktothrix RL	ND
Dolichospermum	ND
Phormidium tinctorium	ND
Microseira	ND
Cape Coral 9/12/21	ND
Pahokee Bloom 9/29/21	ND
Pahokee Bloom 3/30/22	ND
Microcystis sp.	ND
Blowout Creek	ND
Cougar Reservoir	ND
Franklin Lock	ND
Trafford 6/15/21	ND
Trafford 2/22/21	ND
Roland Martin Marina	ND
MDL (ng/mL):	*5*

RA, D., SA, B., SL, B. *et al.* Is Exposure to BMAA a Risk Factor for Neurodegenerative Diseases? A Response to a Critical Review of the BMAA Hypothesis. *Neurotox Res* **39**, 81–106 (2021). https://doi.org/10.1007/s12640-020-00302-0

Cyanopeptides such as anabaenopeptides (96+)

"cyclic hexapeptides demonstrated inhibitory activity towards phosphatases and proteases, which could be related to their toxicity and adaptiveness against zooplankters and crustaceans"

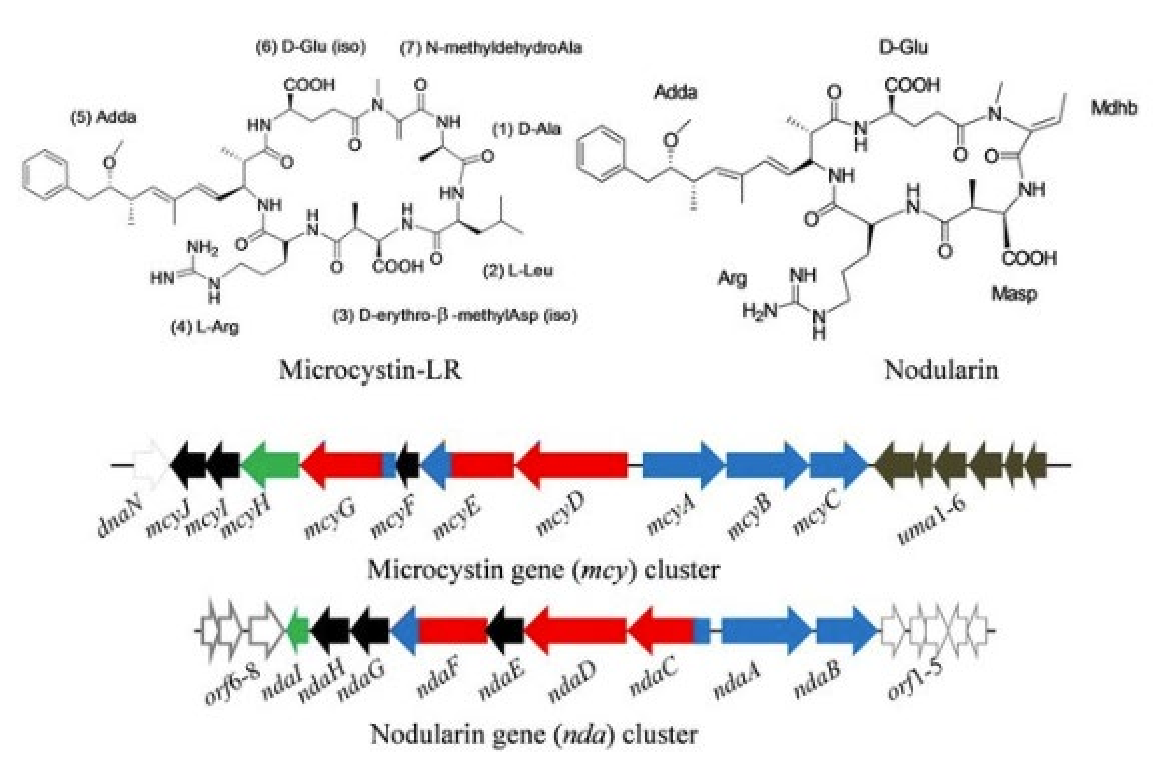
[*Toxins:* 2021: Anabaenopeptins: What We Know So Far]

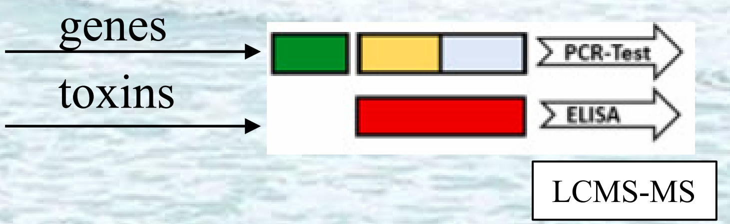


the important functions of microcystins may be substituted with the other Cyanopeptides if no genes present for the microcystins

https://www.sciencedirect.com/science/article/pii/S0043135418310662#!

Toxins vs. Genetics





- Expressing toxin genes
- Non-expressing genes
- Up regulation and down regulation of toxin genes
- **Field and laboratory**

Chemical structure of microcystin (MC-LR) and nodularin (NOD), and their biosynthetic gene clusters, *mcy* and *nda* in the cyanobacteria *Microcystis aeruginosa* PCC7806 and *Nodularia spumigena* NSOR10, respectively. Black – tailoring enzymes, red – polyketide synthases, blue – non-ribosomal peptide synthetases, light black – non-microcystin synthetase, green – ABC transporter (adapted from Tillett et al., 2000; Moffitt and Neilan, 2004; Gehringer et al., 2012; Gehringer and Wannicke, 2014; Gene cluster not drawn to scale).

Potential Toxin Producers… not toxins

Metagenomics

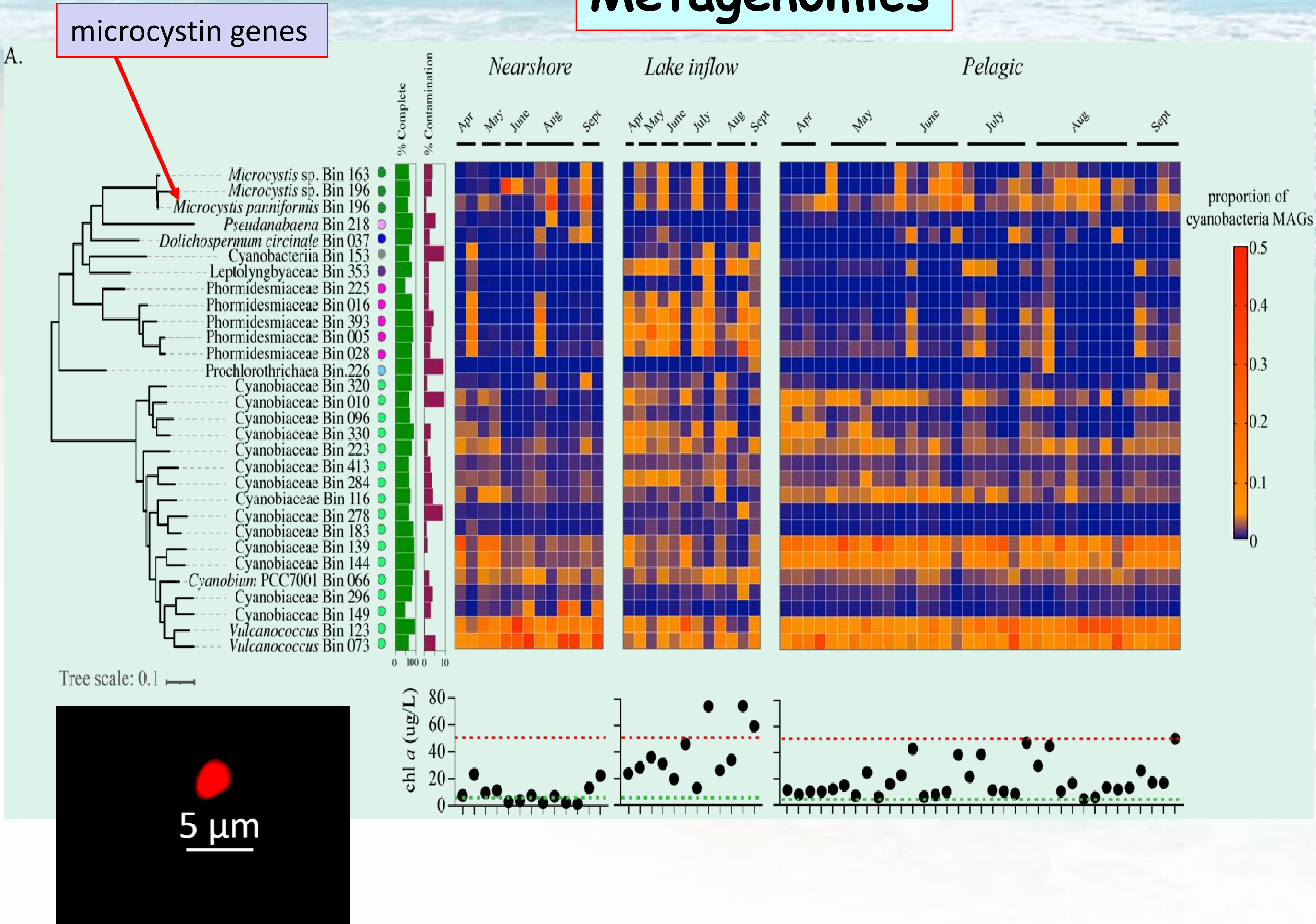
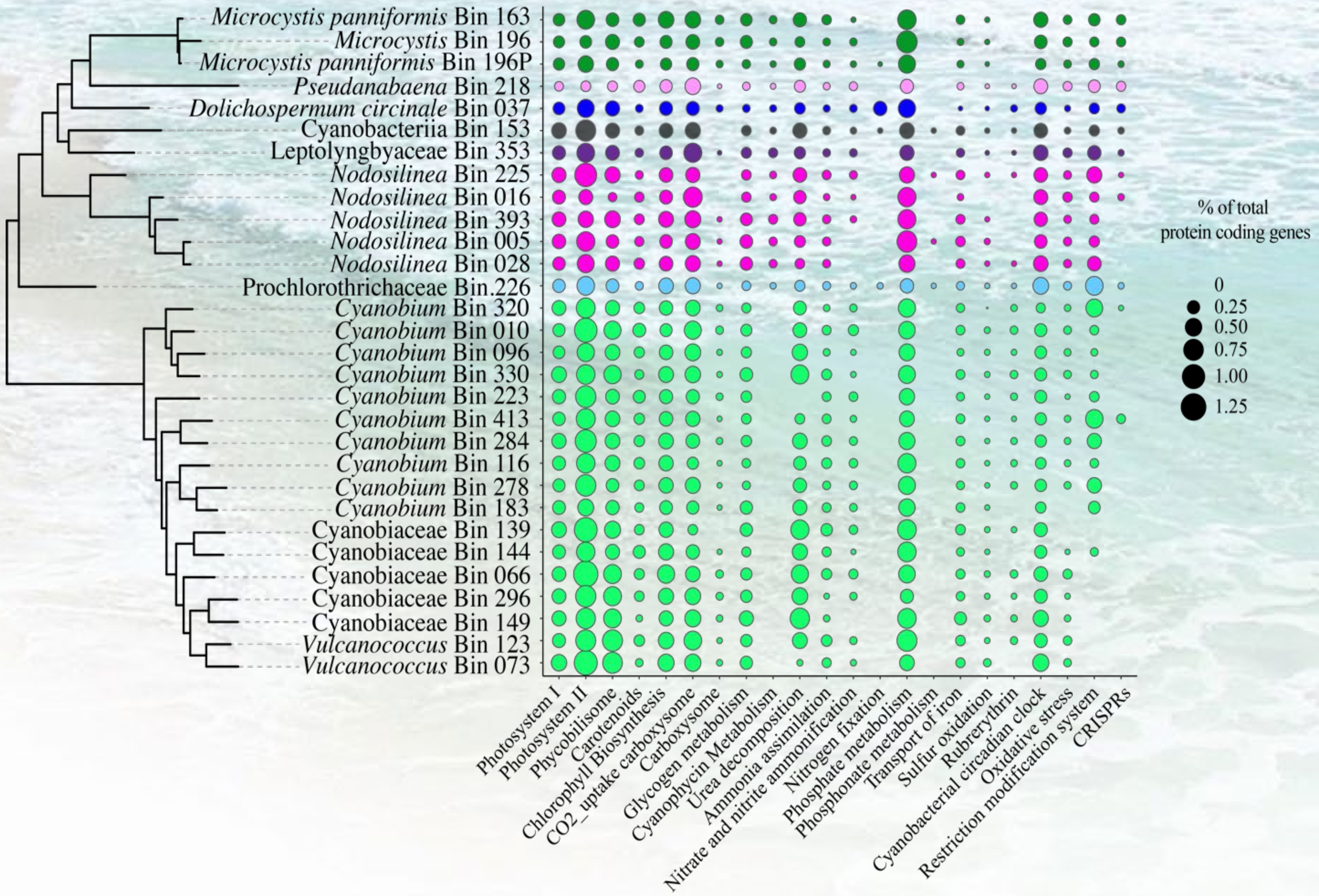


Figure 7. Cyanobacterial diversity in Lake O. Spatiotemporal trends in the abundances of non-redundant cyanobacterial MAGs (>50% complete, <10% contamination) recovered from Lake O metagenome assemblies generated from samples representing a typical bloom season (April-September). MAGs are ordered based on phylogenetic distribution. Taxonomic classification associated with each MAG was determined from GTDB-taxonomy and different color circles represent phylogenetic groups. Chl a concentrations displayed on bottle panel are associated with each sample.

Metagenomics



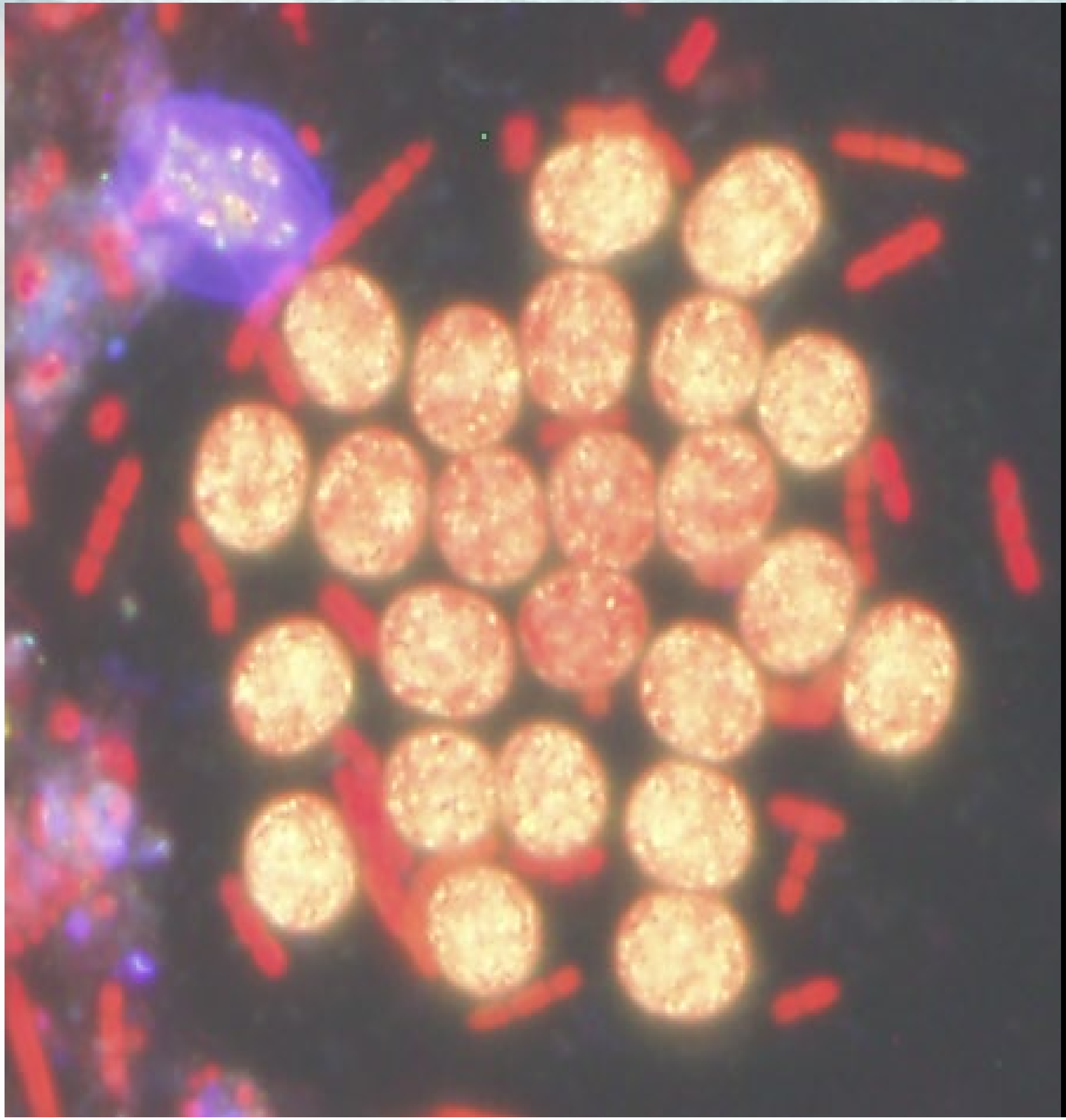
qPCR detection of toxin genes

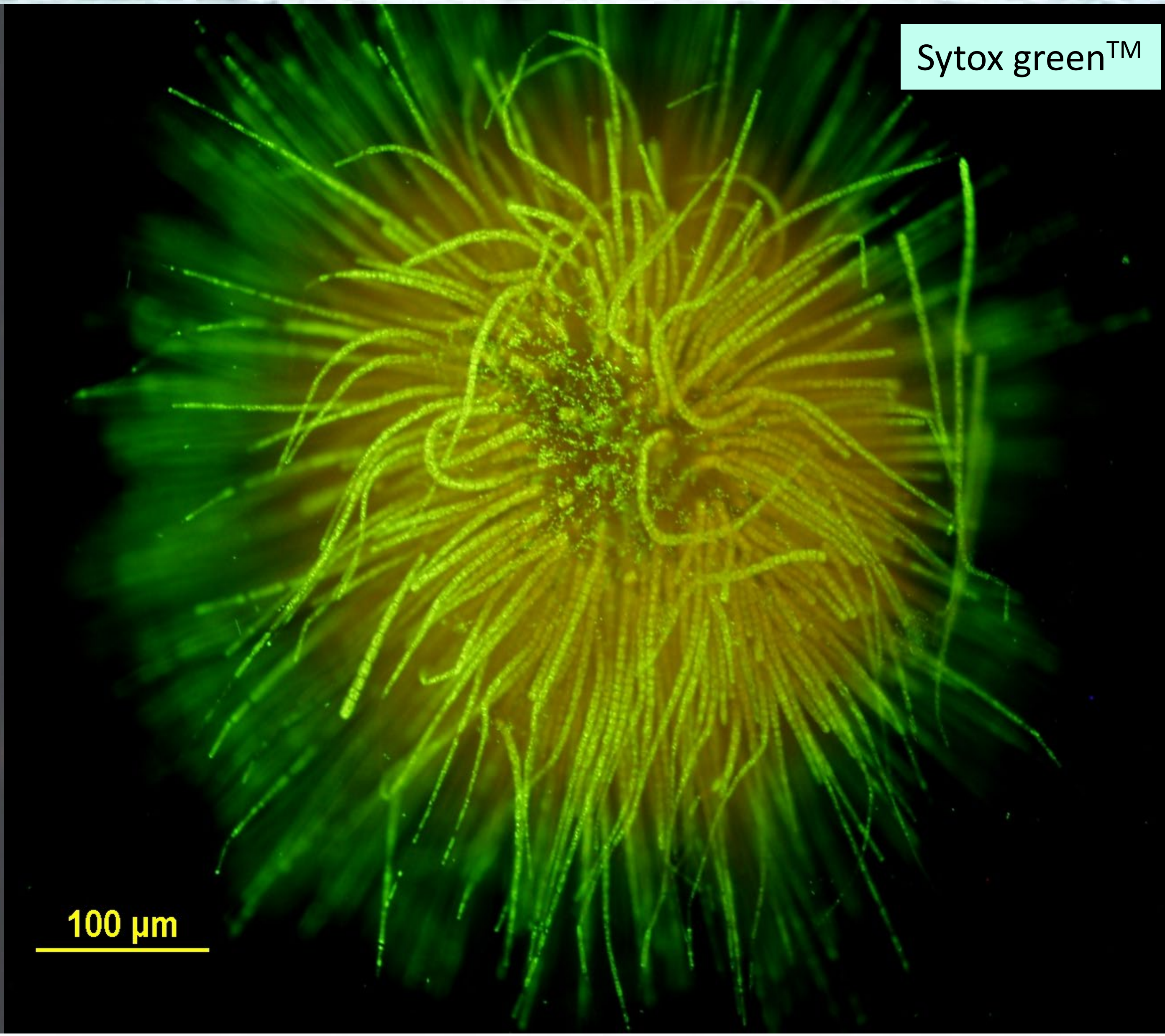
Table 2. Primers used to amplify and/ or sequence cyanotoxins biosynthesis genes and 16S rRNA.

Gene	Primer	Fragment length (bp)	Sequence (5′–3′)	References
*mcy*C	PSCF1	674	GCAACATCCCAAGAGCAAAG	Ouahid et al. [61]
	PSCR1		CCGACAACATCACAAAGGC	
*mcy*D	PKDF1	647	GACGCTCAAATGATGAAAC	
	PKDR1		GCAACCGATAAAAACTCCC	
*mcy*E	PKEF1	755	CGCAAACCCGATTTACAG	
	PKER1		CCCCTACCATCTTCATCTTC	
*mcy*G	PKGF1	425	ACTCTCAAGTTATCCTCCCTC	
	PKGR1		AATCGCTAAAACGCCACC	
*sxt*A	sxtA F	602	AGGTCTTTGACTTGCATCCAA	Ledreux et al. [10]
	sxtA R		AACCGGCGACATAGATGATA	
*sxt*G	sxtGf	893	AGGAATTCCCTATCCACCGGAG	Casero et al. [25]
	sxtGr		CGGCGAACATCTAACGTTGCAC	
*sxt*H	sxtHf	812	AAGACCACTGTCCCCACCGAGG	
	sxtHr		CTGTGCAGCGATCTGATGGCAC	
*sxt*I	sxtIf	910	AGCGCTGCCGCTATGGTTGTCG	
	sxtIr		ACGCAATTGAGGGCGACACCAC	
cyrB	M13	597	GGCAAATTGTGATAGCCACGAGC	Schembri et al. [62]
	M14		GATGGAACATCGCTCACTGGTG	
*cyr*C	M4	650	GAAGCTCTGGAATCCGGTAA	
	M5		AATCCTTACGGGATCCGGTGC	
16S rRNA	27F		AGAGTTTGATCCTGGCTCAG	Neilan et al. [59]
	CYA359F		GGGGAATYTTCCGCAATG GG	Nubel et al. [60]
	1494R		TACGGCTACCTTGTTACGAC	Neilan et al. [59]
	CYA781R		GACTACTGGGGTATCTAATCCCATT	Nubel et al. [60]
	CYA781F		AATGGGATTAGATACCCCAGTAGTC	This study

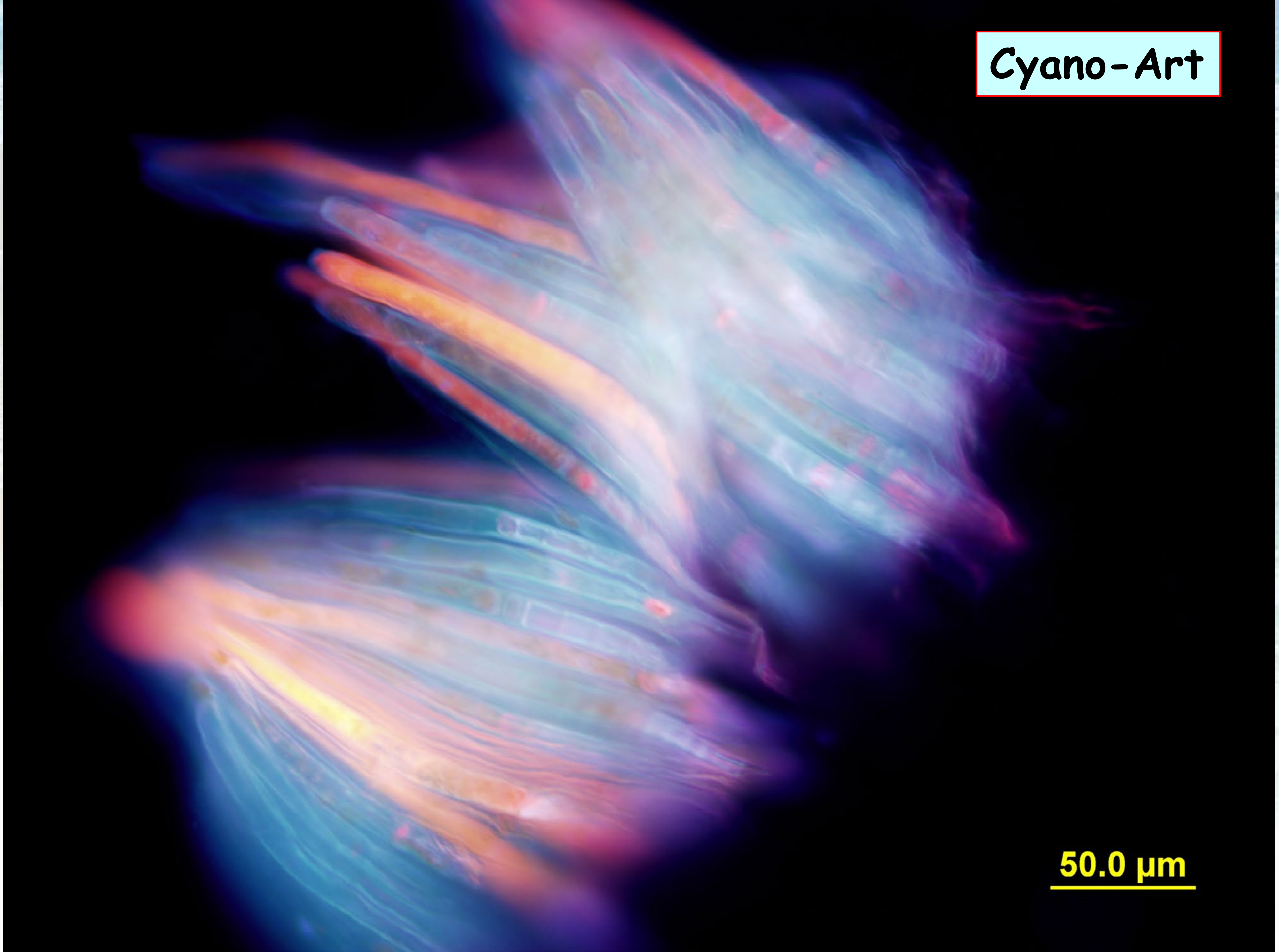
https://doi.org/10.3390/toxins13040258

Phycobiome

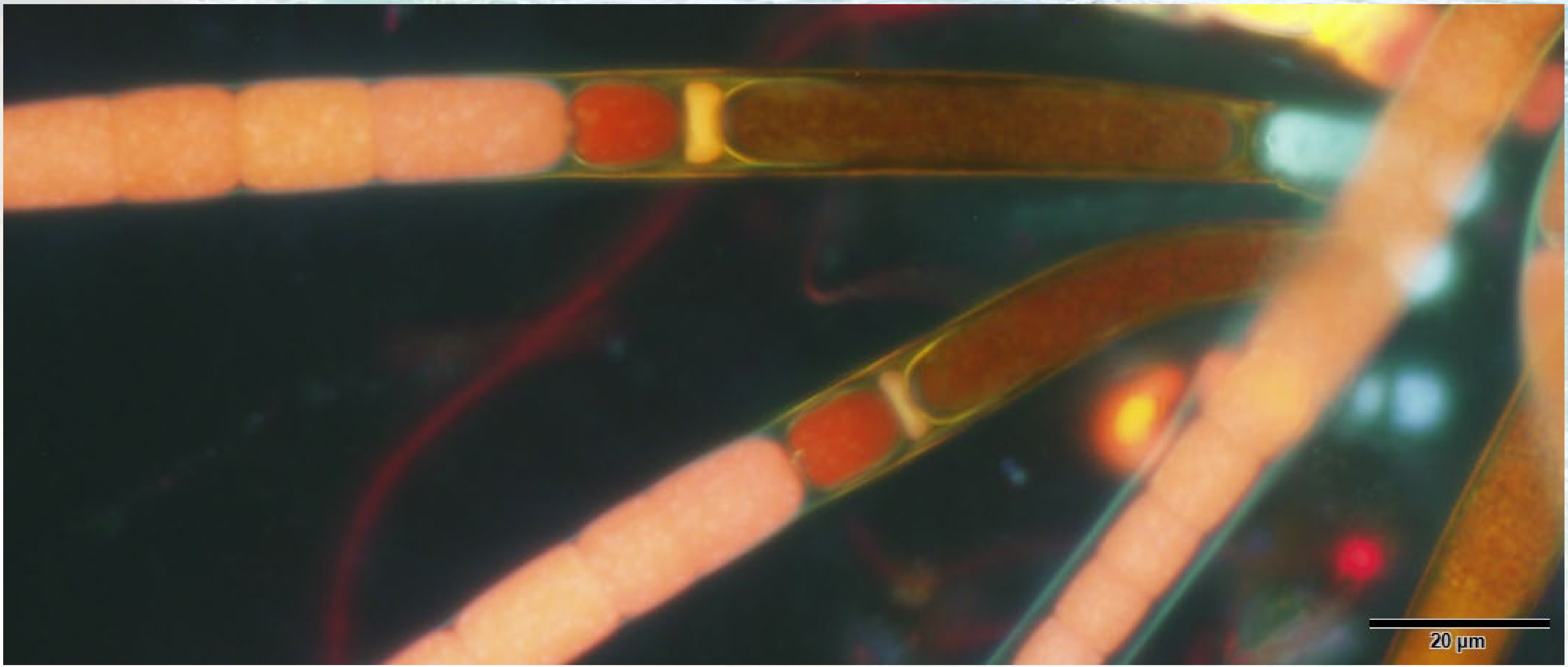




Joe Heller
WARNING
LIFEGUARD,
BACTERIOLOGIST,
MICROBIOLOGIST
PHYCOLOGIST
NOT ON DUTY
SWIM AT YOUR OWN RISK


Cyano-Art
50.0 µm

Thank You!



Live Sample Hunt!

Barry H. Rosen, Ph. D.
Florida Gulf Coast University
USGS Emeritus

brosen@fgcu.edu

239-745-4589

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