Cyanobacteria Ecological Strategies and Impacts on Human Health

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Director

brosen@usgs.gov 407-738-0669 algae cyanobacteria

HABs

microcystin

saxitoxin

Google

algae	73 million
cyanobacteria	5.7 million
HABs	1.4 million
microcystin	554 K
Microcystis	594 K
saxitoxin	227 K
Cyanotoxins	157 K
amnesic	63 K

*paralytic shellfish poisoning (PSP)

*amnesic shellfish poisoning (ASP)



*coastal & marine

Where are cyanobacteria a problem?

Lakes, reservoirs, rivers, streams, wetlands

Estuaries and coastal systems

Marine systems



CyanoHABs



Why are we concerned about cyanoHABs?



Toxicity

Hypoxia

Taste and odors

Aesthetics





So why do we care about them? Some produce cyanobacteria toxins





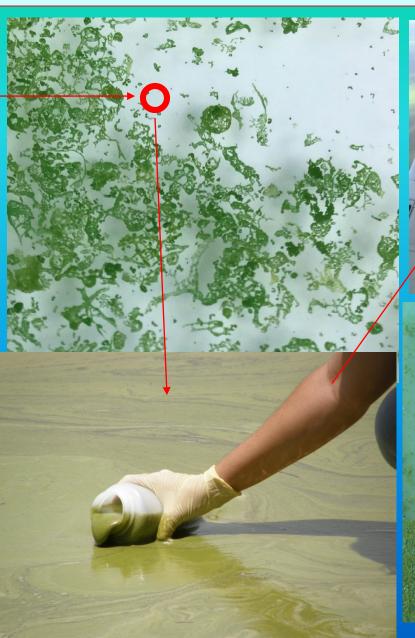


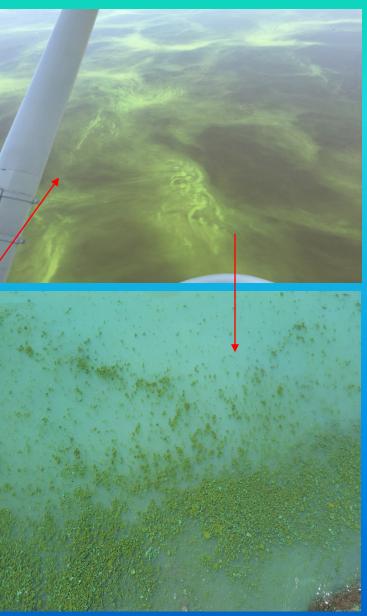


A bloom starts "small"



Note: they are floating







Cyanobacteria

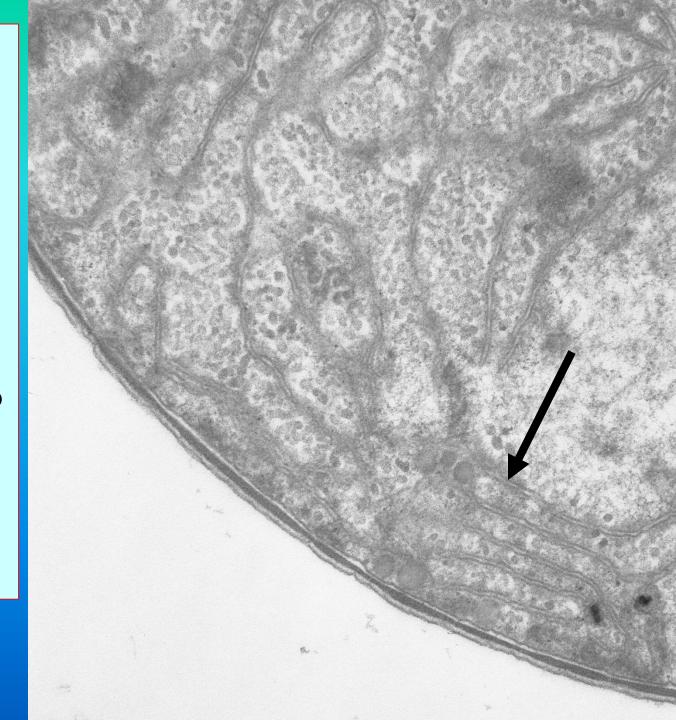
aka

blue-green algae

gram negative bacteria

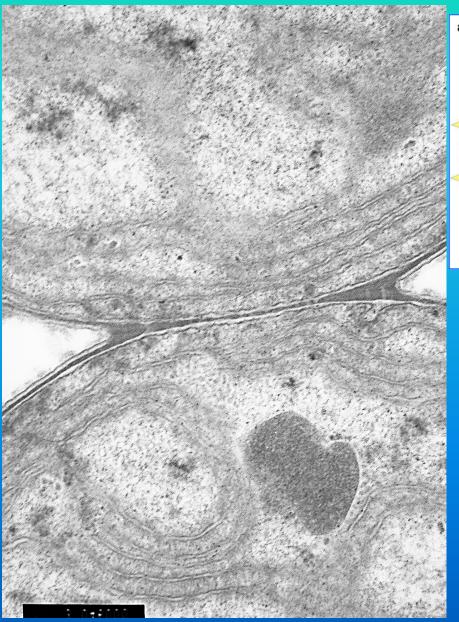
(prokaryotic-i.e. no nucleus or chloroplasts)

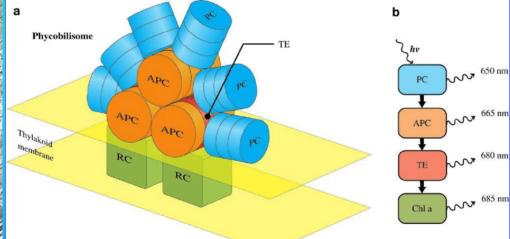
chlorophyll in thylakoids





Ecological Strategies: light harvesting





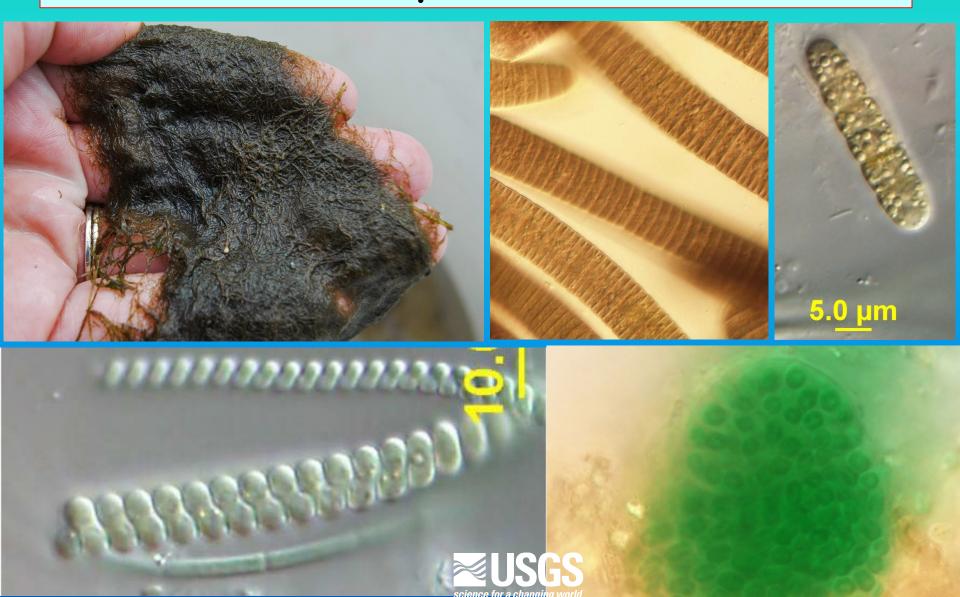
Dartnell et al. 2011

Photosynthesis:

- 1) H₂O is split (oxygen waste product)
- 2) energy captured in biochemical form (ATP, NADPH)



Ecological Strategies: pigments lead to a variety of "colors"



Ecological Strategies: cyanobacterial art







Ecological Strategies: Staying in the light (photic zone), but much more

Gas Vesicles: Buoyancy regulation and vertical migration

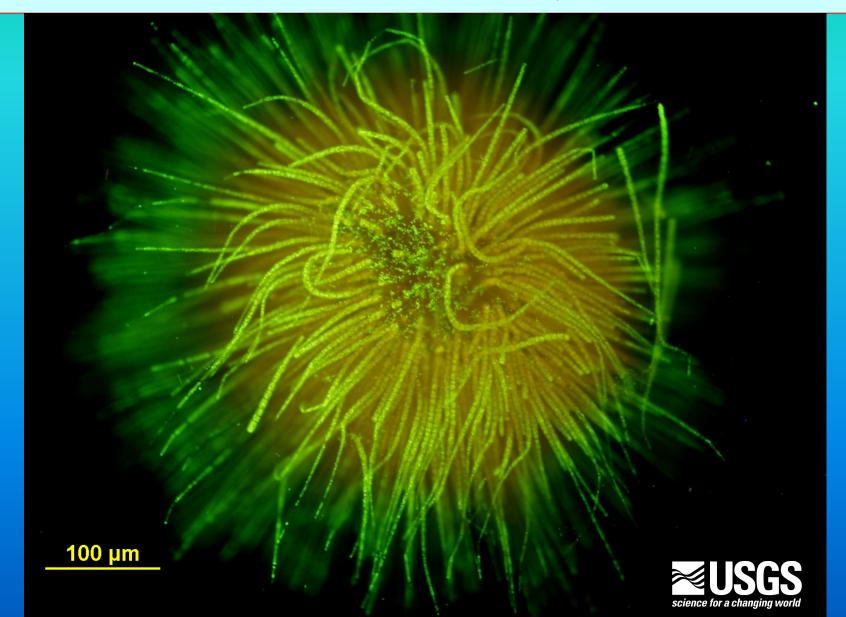


Low light

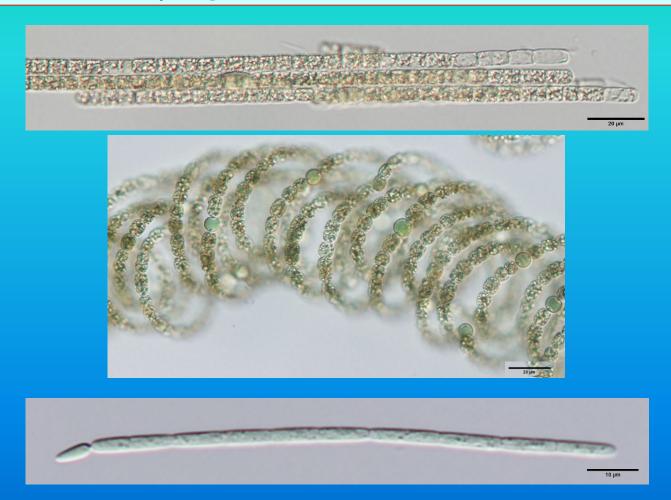
 $(C_6H_{12}O_6)n$

Nutrients
scavenged whilst near
lake sediments or
thermocline

Ecological Strategies: morphology for staying in the water column

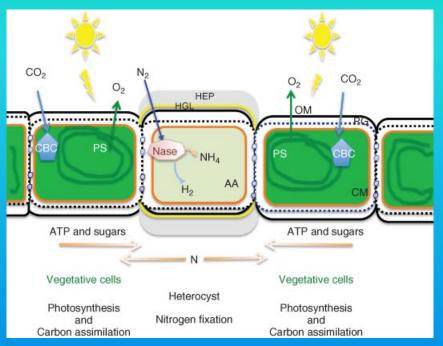


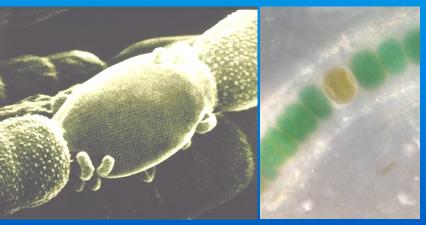
Ecological Strategies: morphologies for staying in the water column





Ecological Strategies: fix your own nitrogen from the atmosphere







- Lipid layer around cell wall
- Loss of oxygenic photosystem II
- Visible pigment difference

Ecological Strategies: fix your own nitrogen from the atmosphere (the heterocyte)







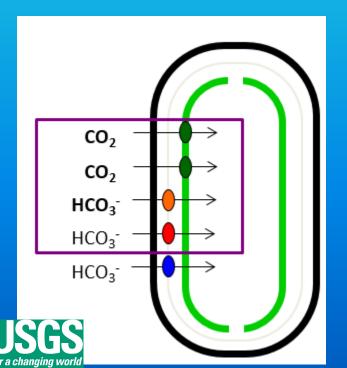


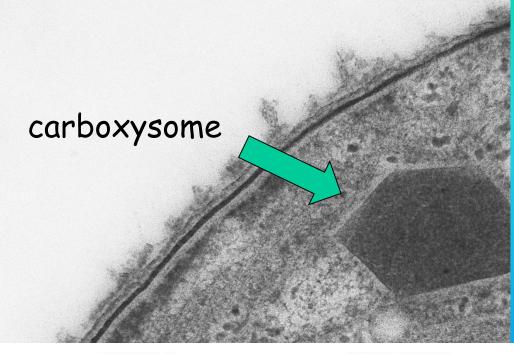


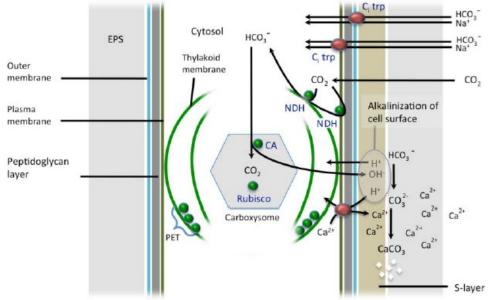
Ecological Strategies: carbon dioxide concentrating mechanism

Take in CO_2 and bicarbonate, up to 1000-fold over that in the surrounding water.

 5 separate transport systems; some require ATP



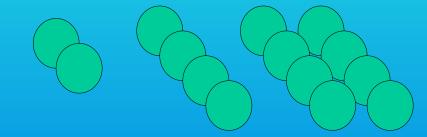




Ecological Strategies: thermophiles-grow fast and will be worse as the climate warms



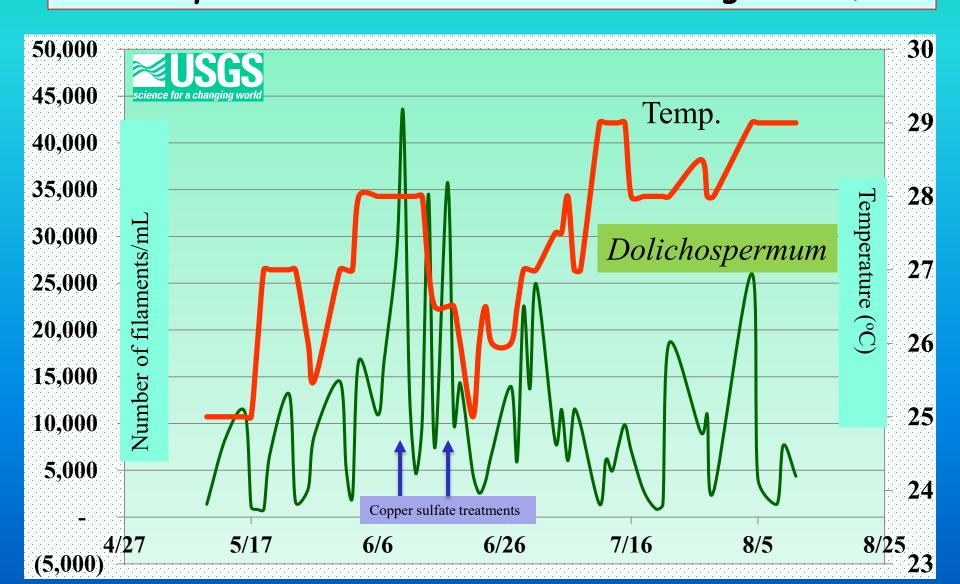
Rapid Growth



temperature

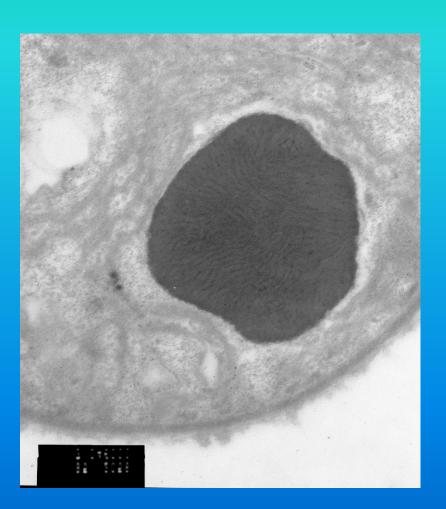
3 "doublings" or divisions every day

Ecological Strategies: Temperature Case Study Dolichospermum circinale in the Hillsborough River, FL

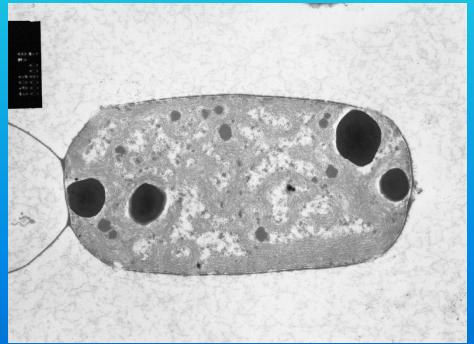




Ecological Strategies: luxuriant nutrient uptake and storage & metal sequestration

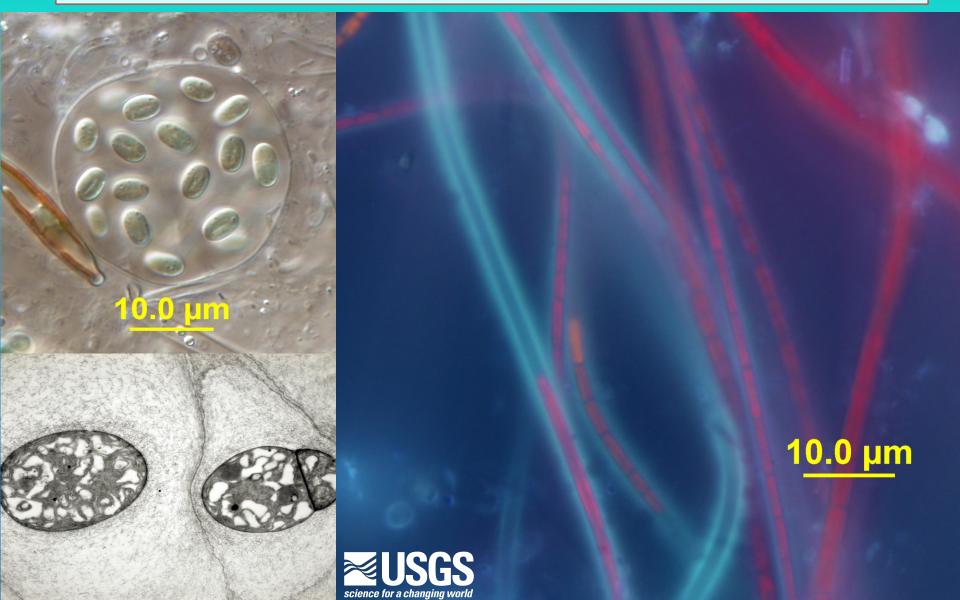


- Contain protein, lipids, polyP
- Na, Mg, Ca, K, Mn, Fe, Cu



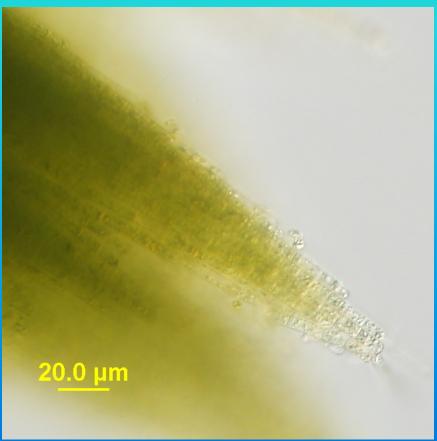


Ecological Strategies: desiccation tolerant (polysaccharide sheath-often pigmented)



Ecological Strategies: morphology to prevent grazing







Ecological Strategies: 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
 - Neurological: linked to ALS

microcystin (240+ variants) nodularin cylindrospermopsin

anatoxin -a anatoxin -a (s) saxitoxin neosaxitoxin

lyngbyatoxin

BMAA



Cyanotoxins are highly potent

Compounds & LD_{50} (µg/kg)

Saxitoxin 9 Ricin 0.02

Anatoxin-a(s) 20 Cobra toxin 20

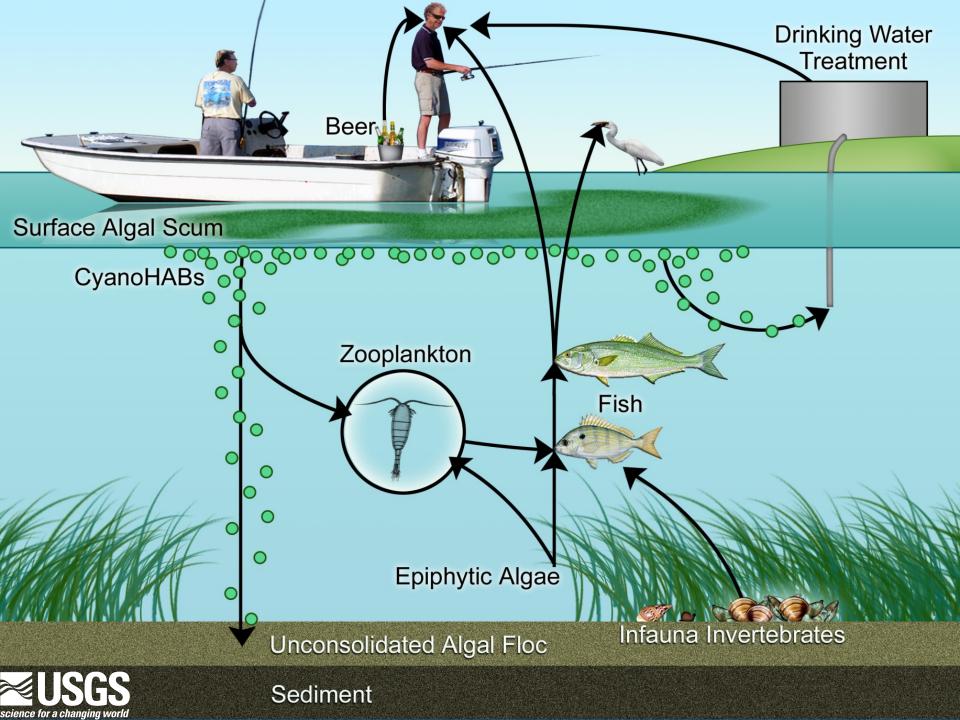
Microcystin LR 50 Curare 500

Anatoxin-a 200-250 Strychnine 2000

Nodularin 50

Cylindrospermopsins 200







Planktothrix

D-Glu⁶ Mdha⁷

Adda⁵

$$H_3C$$
 OMe
 H_3C
 OMe
 H_3C
 OMe
 H_3C
 OMe
 OMe

Microcystins

- Microcystis aeruginosa
- non-N fixer
- Very common
 - Also produced by a number of other species.
 - Peptide Toxins:
 190+ structural variants
 + 200 others related
 compounds: nodularins,
- Microcystins are hepatotoxic
 LD-50: 25-60 μg kg⁻¹

anabaenapeptins, etc.

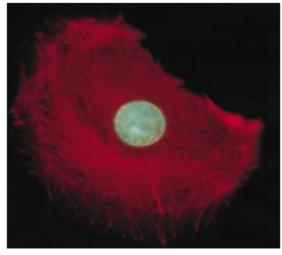
Called "fast death factor"
 Potent tumor promotor

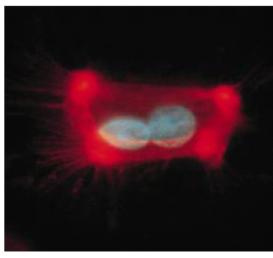


Microcystin exposure: response

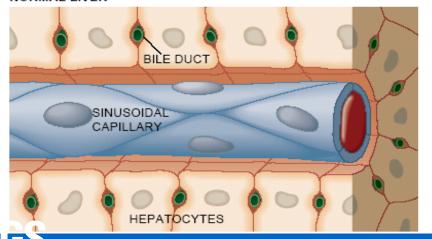
- Uptake by bile acid transporter
- · Inhibit protein phosphatases 1 and 2A
- · Affects cytoskeleton, cell cycle, general metabolism, apoptosis

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.

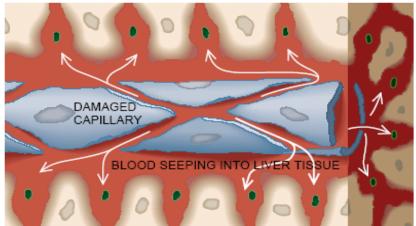




NORMAL LIVER



LIVER AFTER TOXINS ACT



Drinking Water Guidelines

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

The health advisory values for algal toxins recommend 0.3 micrograms per liter for microcystin and 0.7 micrograms per liter for cylindrospermopsin at levels not to be exceeded in drinking water for children younger than school age.

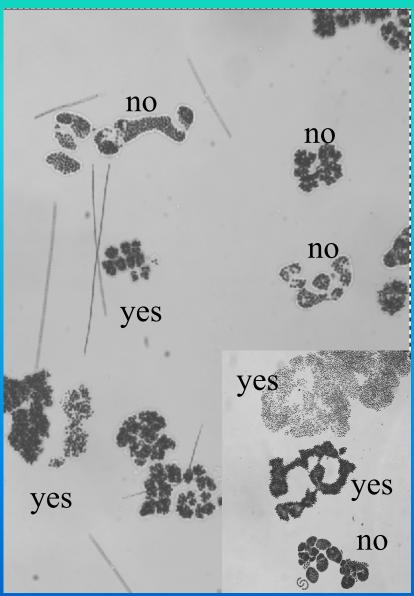
For all other ages, the health advisory values for drinking water are 1.6 micrograms per liter for microcystin and 3.0 micrograms per liter for cylindrospermopsin.

Potential health effects from longer exposure to higher levels of algal toxins in drinking water include gastroenteritis and liver and kidney damage. The health advisory values are based on **exposure for 10 days**.



Can not use taxonomy to predict toxicity







Microcystin "Ecology"

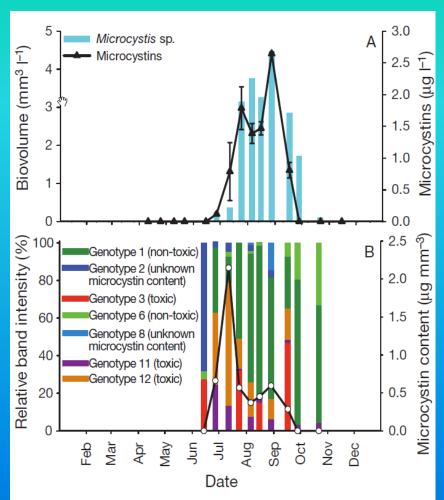


Fig. 2. Seasonal dynamics of Microcystis and microcystin concentrations in Lake 't Joppe. (A) Microcystin concentrations and total biovolume of Microcystis. Error bars show $\pm SD$ of the microcystin concentrations (N = 3). (B) Microcystin contents (solid line) and genotype dynamics of Microcystis based on relative band intensities. Microcystin concentrations are expressed as microcystin-LR equivalents

Microcystis genotype succession in relation to microcystin concentrations in freshwater lakes

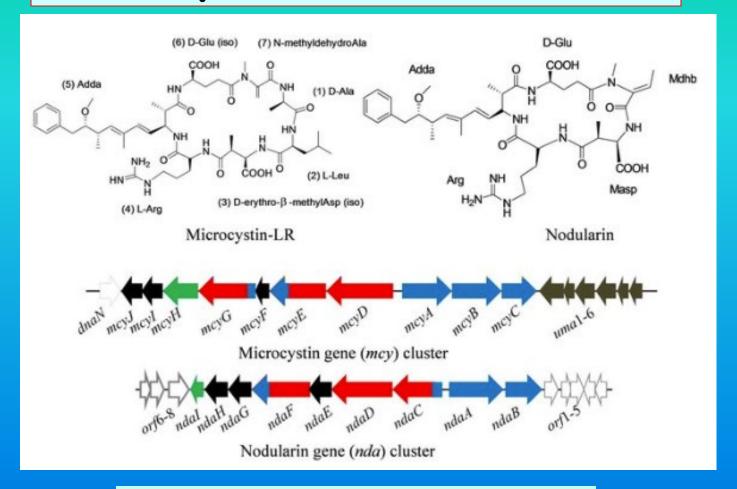
W. Edwin A. Kardinaal^{1,3,*}, Ingmar Janse^{2,4,*}, Miranda Kamst-van Agterveld², Marion Meima², Josje Snoek¹, Luuc R. Mur¹, Jef Huisman¹, Gabriel Zwart^{1,2,5}, Petra M. Visser^{1,**}

Vol. 48: 1-12, 2007

AQUATIC MICROBIAL ECOLOGY Aquat Microb Ecol



Microcystin(s) & Nodularin

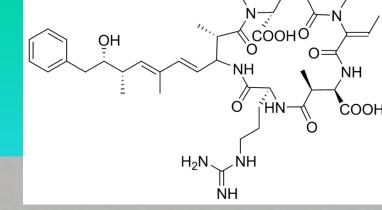


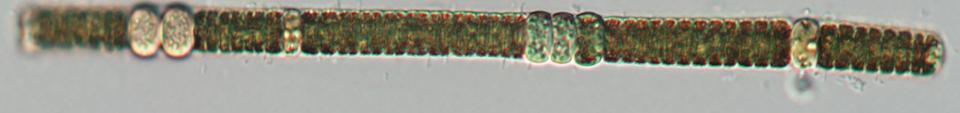
Chemical structure of microcystin (MC-LR) and nodularin (NOD), and their biosynthetic gene clusters, *mcy* and *nda* in the cyanobacteria *Microcystis aeruginosa* PCC7806 and *Nodularia spumigena* NSOR10, respectively. Black – tailoring enzymes, red – polyketide synthases, blue – non-ribosomal peptide 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).



Nodularin-R

Nodularia spumigena





- Cyclic nonribosomal peptide
- > Brackish; world-wide
- Hepatoxin



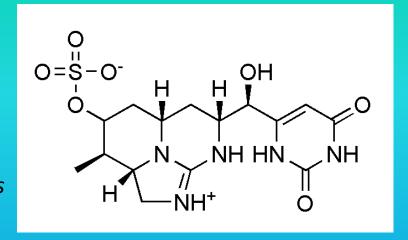




Cylindrospermopsin



Aphanizomenon,
Lyngbya
Dolichospermum
Cylindrospermopsis
Raphidiopsis



- 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 temppersistent



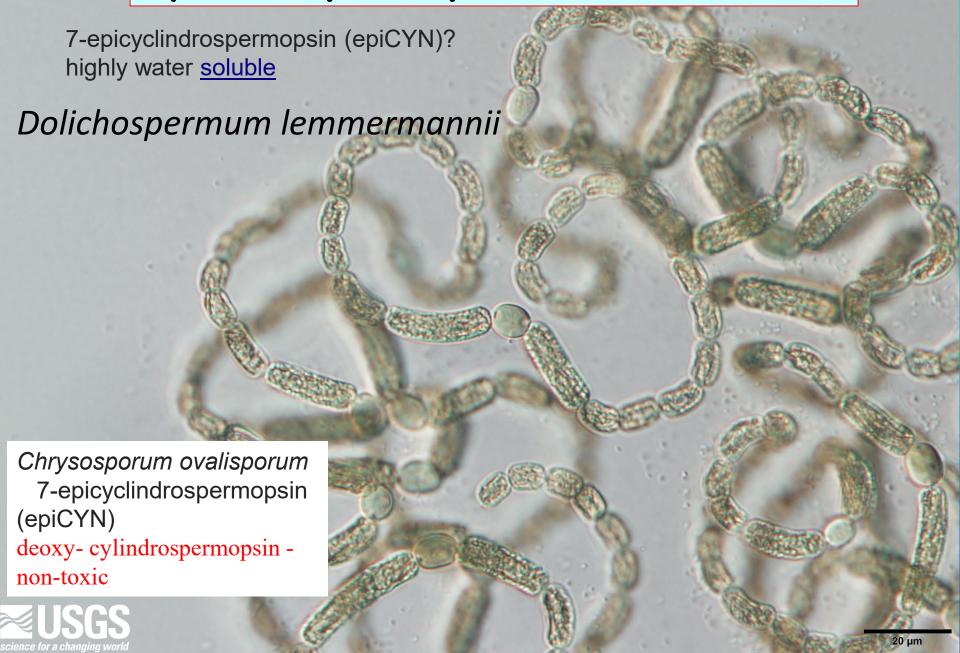
Cylindrospermopsin: Detroit Lake







Cylindrospermopsin: Detroit Lake





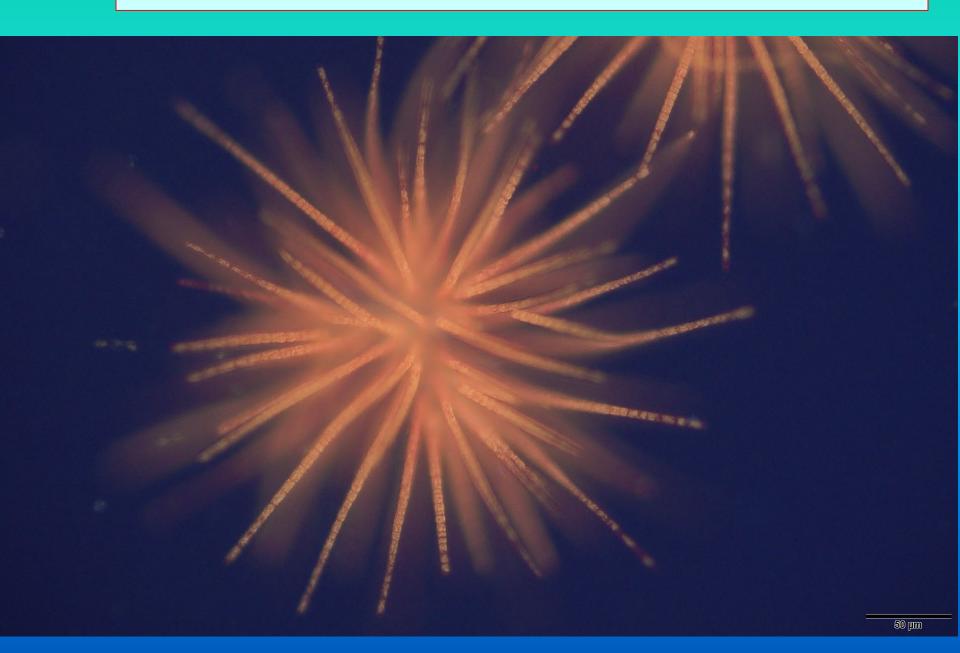
Cylindrospermopsin: Detroit Lake



7-27-18 Detroit Lake: Gloeotrichia



7-27-18 Detroit Lake: Gloeotrichia





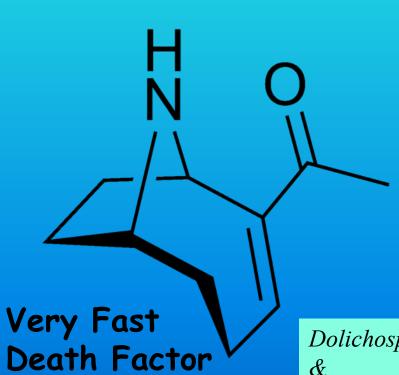
Anatoxins

Anatoxin-a

actylcholine agonist

Anatoxin-a(S)

acetylcholinesterase inhibitor



CH₃

CH₃

CH₃

CH₃

CH₃

CH₃

Dolichospermum flosaquae &

D. lemmermannii



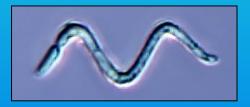


Anatoxin-producing strains include:

- Dolichospermum circinale
- · D. lemmermannii
- · D. planctonicum
- Planktothrix sp.
- · Aphanizomenon flos-aquae
- · Cylindrospermopsis raciborskii
- · Chrysosporum ovalisporum





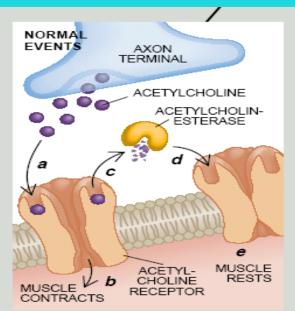


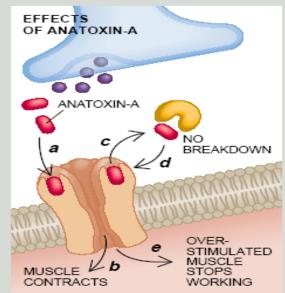


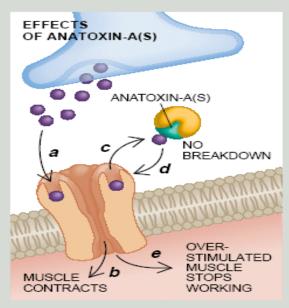


Anatoxin-a and a(s)

Anatoxin-a: Acetylcholine receptor agonist Anatoxin-a(s): Acetylcholinesterase inhibitor







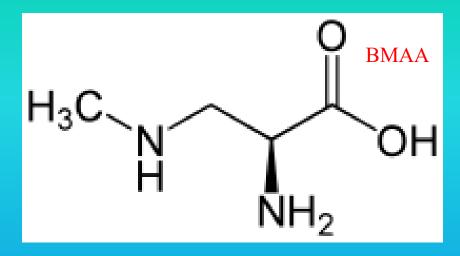
Anatoxin-a and anatoxin-a(s) (center and right panels) overexcite muscle cells by disrupting the functioning of the neurotransmitter acetylcholine. Normally, acetylcholine molecules (purple) bind to acetylcholine receptors on muscle cells (a in left panel), thereby inducing the cells to contract (b). 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 con-

traction (b), but it cannot be degraded by acetyl-cholinesterase (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).

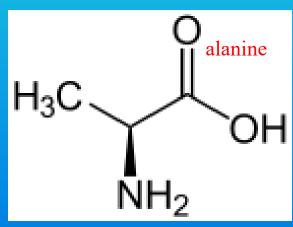


β -methyl amino alanine (BMAA)

- Non-proteinogenic amino acid
- Made by almost all cyanobacteria

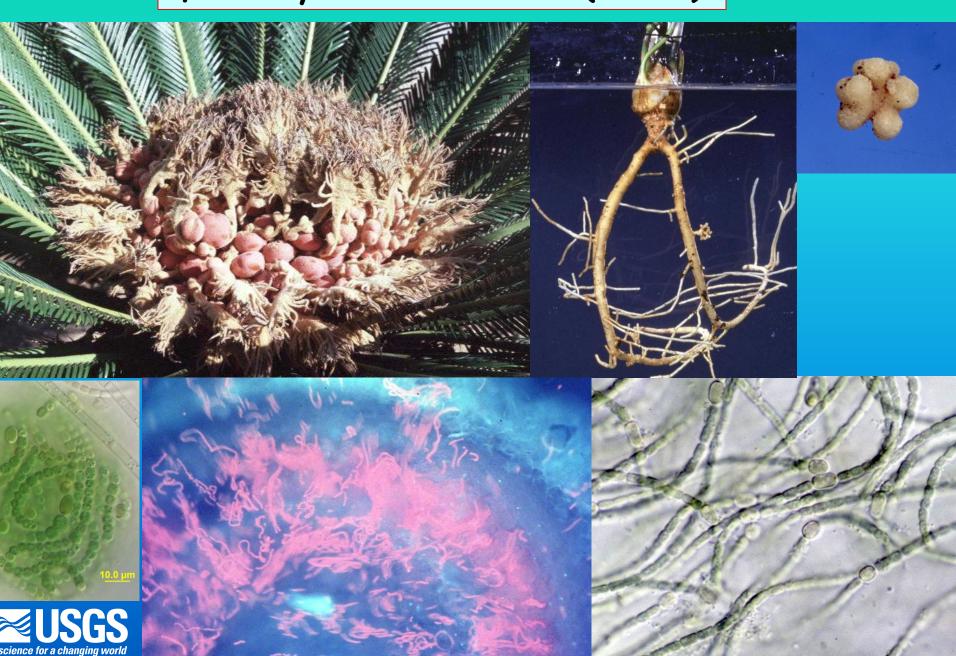


(Cox, Banack, Murch, Rasmussen, Tien, Bidigare, Metcalf, Morrison, Codd, and Bergman. PNAS 2005)



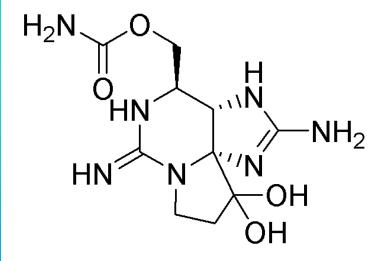


β-methylamino-L-alanine (BMAA)



Saxitoxin

Dolichospermum
Aphanizomenon
Cylindrospermopsis
Lyngbya
Planktothrix



- Neurotoxin (voltage-gated sodium channels); respiratory failure
- Paralytic Shellfish Poisoning (PSP)
- > Numerous marine dinoflagellates



Some things we don't know yet

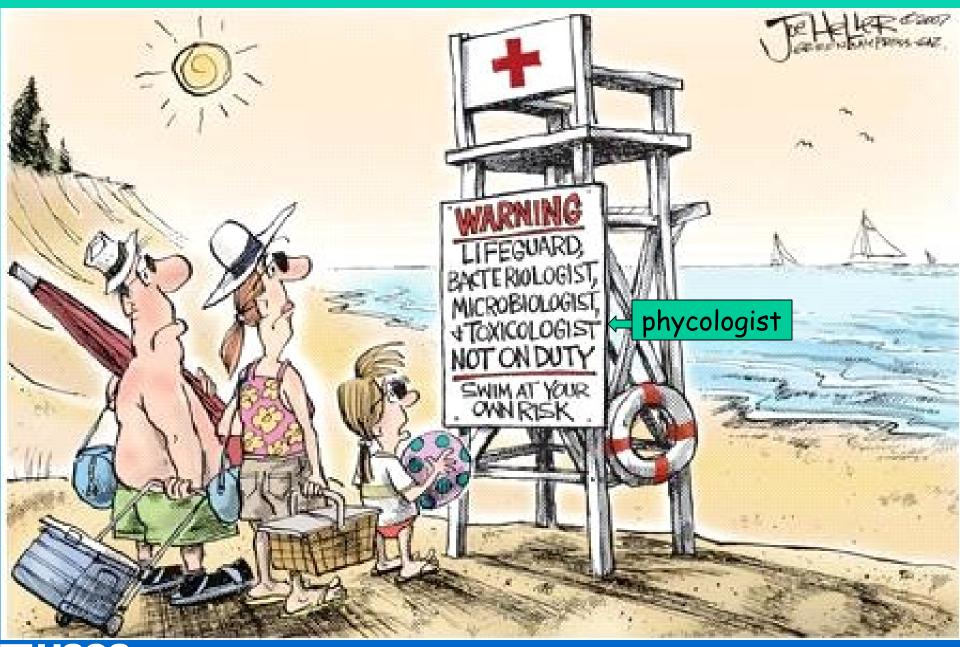
- Environmental triggers for toxin production
- Reasons for high variability of impact on fish and invertebrates
- · Actual degree of impact on humans
- Are more algae producing toxins, or are we just now detecting it?



Current Avenues of My Research

- Environmental triggers for toxin production
- Isolation and culturing of bloom-forming organisms nation-wide: Polyphasic approach, morphology, genetics and toxins
- Phylogenetics: adding to the genetic database for Cyanobacteria







Documented human mortality

1996: Caruaru Brazil

- 1996 outbreak in water supply for a hemodialysis center in Caruaru, Brazil.
- 117 of 136 patients (86%) had symptoms.
- 75 of 136 died (55%) over several month period.
- Microcystins identified in blood and liver tissues.
- Pretreatment (sand, carbon, resin and microfiltration) of the incoming water.
- WHO set limit of 1 μg L⁻¹



Sea Otters in CA and Chesapeake Bay

"Twenty one sea otter (*Enhydra lutris kenyoni*) deaths reported in California during the period 1999-2008, showed that cause of death was due to exposure to microcystins (Miller et al. 2010)"

"During the period 2001-2004 Great Blue Heron (*Ardea herodias*) mortalities in the Chesapeake Bay region were associated with microcystin exposure at a small island freshwater pond (Driscoll et al. 2002)"



How we are exposed

Poison	Causative organism	Vector	Onset
Anatoxin-a	Anabaena spp. Aphanizomenon spp. Planktothrix spp.	Contaminated fresh water	minutes to hours
Anatoxin-a(s)	Anabaena flos-aquae	Contaminated fresh water	minutes to hours
Azaspiracid	Protoperidinium	Shellfish: clams, scallops, mussels, oysters	<24 hours
Brevetoxin	Dinoflagellates Karenia brevis Other Karenia spp.	Contaminated marine waters and shellfish	<24 hours
Ciguatoxins	Dinoflagellates Gambierdiscus toxicus Gambierdiscus spp	Many fish species: eel, grouper, mackerel, snapper	<24 hours
Cylindrospermopsin	Cylindrospermopsis raciborskii, Aphanizomenon ovalisporum	Contaminated fresh water and possibly fish	hours to days
Domoic acid	Pseudo-nitzschia spp. Nitzschia pungens	Shellfish: crab, clams, scallops, mussels, oysters	<24 hours
Lyngbyatoxin	Lyngbya sp.	Contaminated fresh or marine waters	<24 hours
Microcystins	M. Aeruginosa Anabaena spp. Planktothrix spp.	Contaminated fresh water	hours to days
Okadaic acid	Dinophysis sp.	Shellfish: crab, clams, scallops, mussels, crabs	minutes to hours
Saxitoxins	Dinoflagellates and Cyanobacteria (Aphanizomenon sp. Anabaena circinalis)	Shellfish (clams, cockles, mussels, oysters, whelks) or puffer fish	<24 hours
		Contaminated fresh water	Unknown



Taxonomy: Initial ID and some of the major players



Field and Laboratory Guide to Freshwater Cyanobacteria Harmful Algal Blooms for Native American and Alaska Native Communities









U.S. Department of the Interior U.S. Geological Survey







Common Filamentous Cyanobacteria

Lake Mattamuskeet, NC (East and West) July 22, 2015

Cylindrospermopsis raciborskii (CYN)

20 µm

Chrysosporum ovalisporum (CYN)



Planktolyngbya contorta (MYC)





Thank You!



