US ERA ARCHIVE DOCUMENT

## Cyanobacteria 101 (and why you need to know more)

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algae cyanobacteria

HABs

microcystin

saxitoxin

Goog	e

algae	32 million
cyanobacteria	2.9 million
HABs	372 K
microcystin	314 K
saxitoxin	195 K
paralytic	143 K
amnesic	56 K
cyanotoxins	48 K

\*paralytic shellfish poisoning (PSP)

amnesic shellfish poisoning (ASP)

cyanotoxins



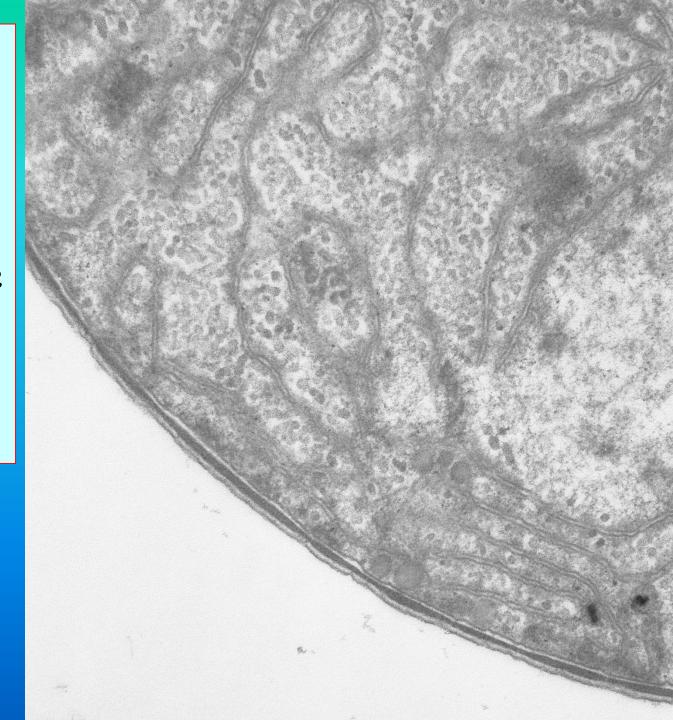


#### Cyanobacteria

(aka bluegreen algae; cyanoHABs)

·gram negative bacteria

pigments in thylakoids





#### Where are cyanobacteria a problem?

Lakes, reservoirs, rivers, streams, wetlands

Estuaries and coastal systems

Marine systems



CyanoHABs







SeaGrant

#### Why are we concerned about cyanoHABs?



Toxicity

Hypoxia

Taste and odors

Aesthetics





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









#### 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 (90+ variants) nodularin cylindrospermopsin

anatoxin -a anatoxin -a (s) saxitoxin neosaxitoxin

lyngbyatoxin

**BMAA** 



#### Cyanotoxins are highly potent

#### Compounds & LD<sub>50</sub> (ug/kg)

Saxitoxin	9	Ricin	0.02
Anatoxin-a(s)	20	Cobra toxin	20
Microcystin LR	50	Curare	500
Anatoxin-a	200-250	Strychnine	2000

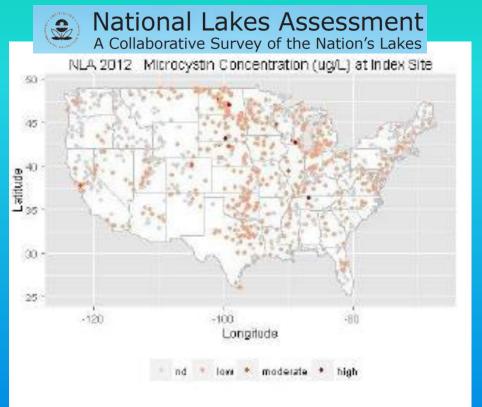
50

Cylindrospermopsins 200



Nodularin

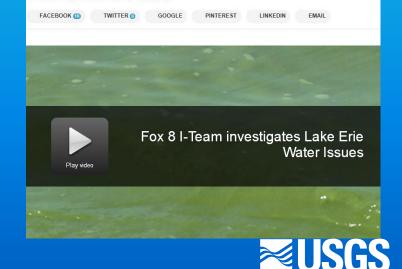
#### How common are toxic blooms?



Threshold	Level	% (N)
Non detect	<0.1	53% (674)
Low	0.1-10	46% (538)
Medium	10-20	0.5% (8)
High	>20	0.5% (8)

- Toxic blooms are very common and have been reported almost every state of the nation.
- Found worldwide

I-TEAM: Could it happen here? The Untold Story of the Toledo Water Crisis



#### Ecological strategies for cyanobacteria: a sample

#### Morphology

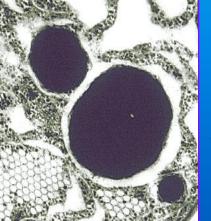


grazing, floating

#### Rapid Growth



temp



trace, P, C, N

Nutrient Storage

#### Pigments.

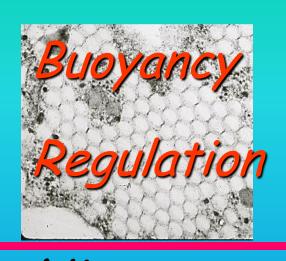


#### Toxicity

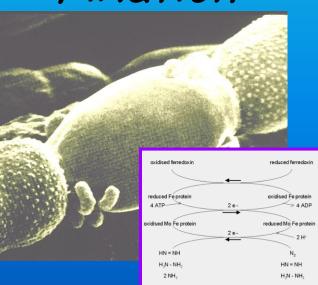


microcystin LR complex

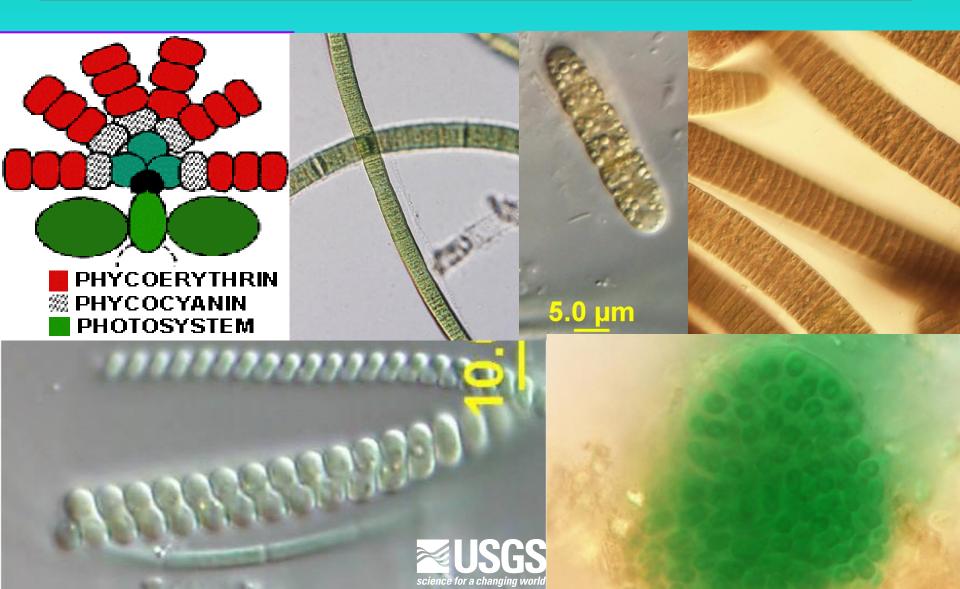




#### Nitrogen **Fixation**

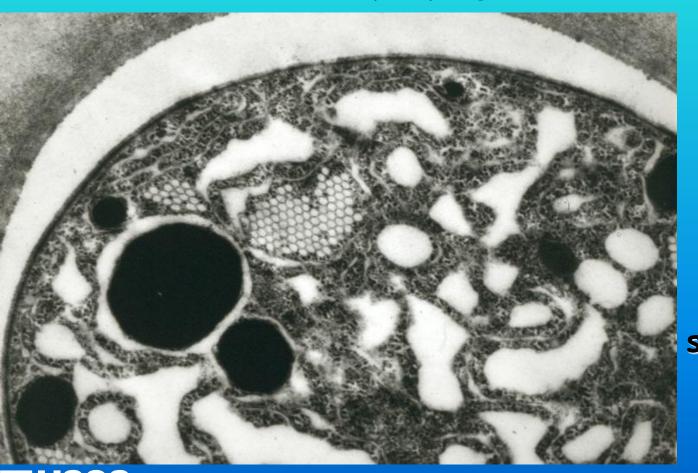


## Ecological Strategies: complimentary pigments for maximizing photosynthesis



## Ecological Strategies: internal structures for optimizing placement in the water column

Gas Vesicles: Buoyancy regulation and vertical migration



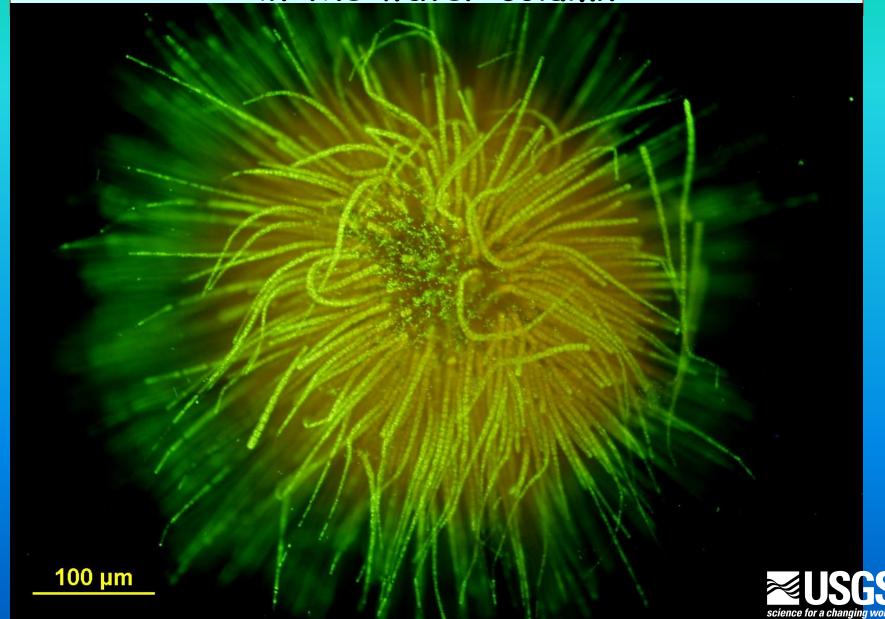
Low light

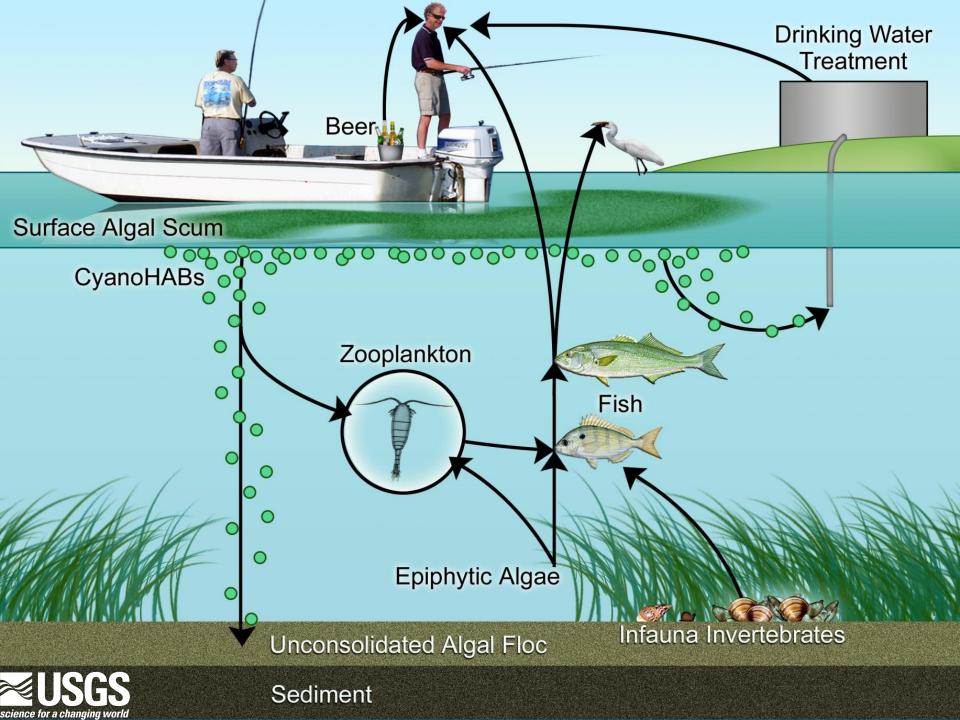
(C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>)n

Nutrients
scavenged whilst
near lake
sediments or
thermocline



Ecological Strategies: morphology for staying in the water column

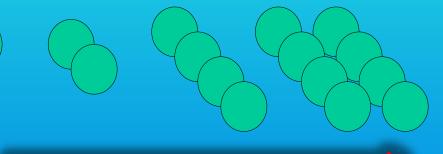




### Ecological Strategies: thermophiles grow fast and will be worse as the climate warms



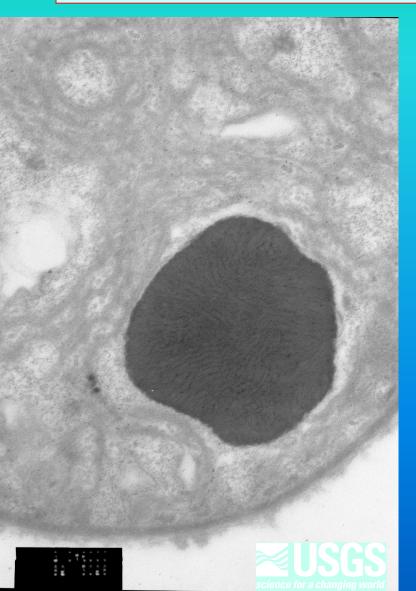
#### Rapid Growth



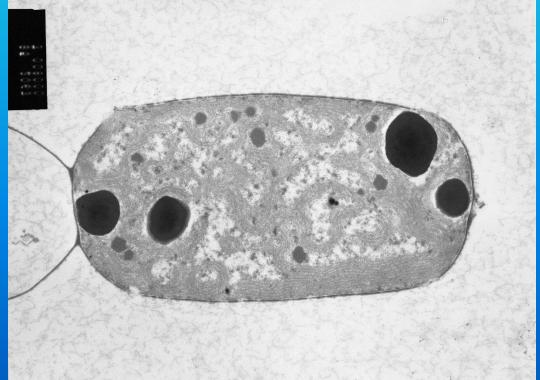
temperature

3 "doublings" or divisions every day

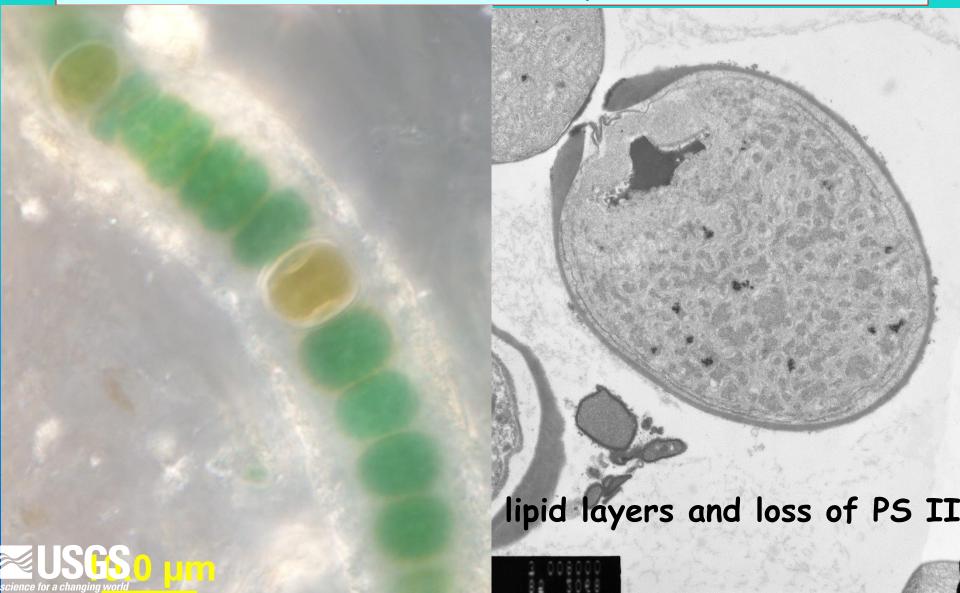
## Ecological Strategies: luxuriant nutrient uptake and storage & metal sequestration



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



## Ecological Strategies: make your own nitrogen from the atmosphere





# D-Glu (iso) CH<sub>3</sub> Methyl dehydroAla (Mdha) CH<sub>2</sub> NH O OCH<sub>3</sub> NH O CH<sub>2</sub> NH O OCH<sub>3</sub> CH<sub>3</sub> D-Ala OCH<sub>3</sub> NH CH<sub>3</sub> CH<sub>3</sub> CH<sub>3</sub> D-Ala OCO<sub>2</sub>H Arginine NH O MeAsp (iso)

#### Microcystins

- Mostly Microcystis
   aeruginosa (very common)
  - Also produced by a number of other species.
- Potent hepatotoxin
   LD-50: 25-60 μg kg<sup>-1</sup>
- Called "fast death factor"
- Potent carcinogen
- · Guide line values in water:
  - 1 ppb drinking water
  - 10-20 ppb recreational contact
- Peptide Toxins:
   90+ structural variants



#### 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 as 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.

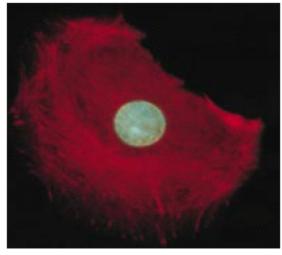
Potential health effects from longer exposure to higher levels of algal toxins in drinking water include gastroenteritis and liver and kidney damage. The health advisory values are based on **exposure for 10 days**.

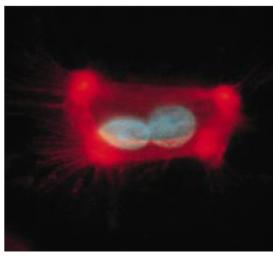


#### Microcystin exposure: response

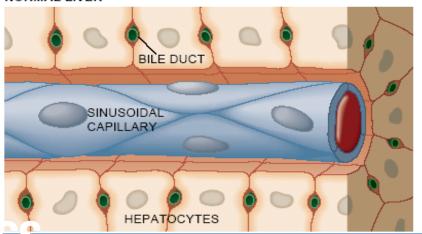
- Uptake by bile acid transporter
- · Inhibit protein phosphatases 1 and 2A
- · Affects cytoskeleton, cell cycle, general metabolism, apoptosis

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.

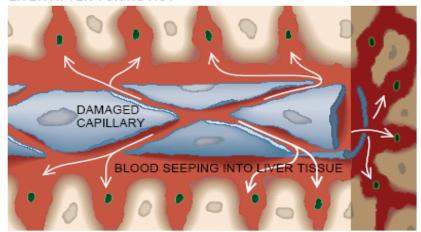




NORMAL LIVER



LIVER AFTER TOXINS ACT



#### Microcystin exposure: tumor promotion



- Epidemiology in China:
  - Contaminated drinking water ↔ primary liver and colon cancer.
- Injection of toxin ± initiator:
  - Increased size/number of liver cancer precursors.
- Oral M. aeruginosa. extract:
  - Skin papillomas larger/heavier
  - No effect on duodenal

tumours or lymphoma.

**Colon cancer precursors** 

larger





#### **Anatoxins**

#### **Anatoxin-a**

actylcholine agonist

#### Anatoxin-a(S)

acetylcholinesterase inhibitor





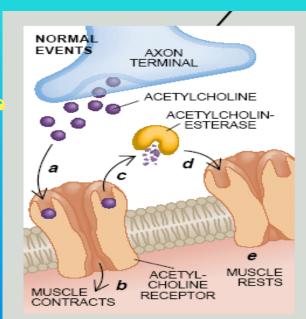
Anabaena flos-aquae & lemmermannii

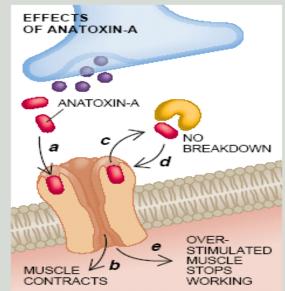


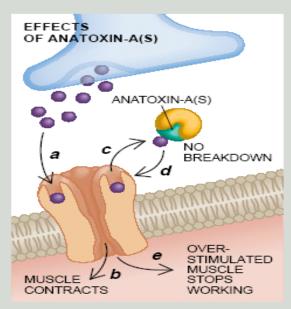
#### Anatoxin-a and a(s)

Anabaena

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).



#### Has anyone ever died from these toxins?

#### Not in the US. Most affects are with animals:

.....associated with the *Anabaena flos-aquae* bloom were estimated deaths of 5000-7000 gulls, 560 ducks, 400 coots, 200 pheasants, 50 squirrels, 18 muskrats, 15 dogs, 4 cats, 2 hogs, 2 hawks, 1 skunk, 1 mink, plus numerous song birds."

Storm Lake, Iowa, 1952





Toxins 2015, 7, 1048-1064; doi:10.3390/toxins7041048

ISSN 2072-6651

www.mdpi.com/journal/toxins

Article

#### Cyanobacteria and Algae Blooms: Review of Health and Environmental Data from the Harmful Algal Bloom-Related Illness Surveillance System (HABISS) 2007–2011

Lorraine C. Backer 1,\*, Deana Manassaram-Baptiste 2, Rebecca LePrell 3 and Birgit Bolton 4

**Table 1.** The number of reports recorded in Harmful Algal Bloom-related Illness Surveillance System (HABISS) from 2007 to 2011, by year, and the reason why the data were collected.

	Reason	Reason for Bloom-Related Data Collection (Percent by Year)				
Year	Routine	Bloom Report	<b>Health Event</b>	Fishkill	Total	
	Monitoring	Response	Response	Response	Reports	
2007	167 (96)	1 (<1)	5 (3)	0	173	
2008	509 (90)	7(1)	41 (7)	8(1)	565	
2009	1344 (93)	55 (4)	28 (19)	23 (2)	1450	
2010	977 (94)	25 (2)	19 (2)	16(2)	1037	
2011	1248 (95)	31 (2)	20 (52)	10(1)	1309	
Total Reports	4245	119	113	57	4534	



## Documented occurrences of toxic cyanobacteria blooms in the US

- 1925: Farmer lost 125 hogs and 4 cows at Big Stone Lake in South Dakota. (first report in the US)
- 1930: *Microcystis* bloom on Ohio and Potomac Rivers caused intestinal illness in 5,000-8,000 people.
- 1975: Cyanobacterial bloom ied to endotoxic shock in Washington DC.
- 1980: Several cases of illness in Pennsylvania following a bloom.
- 1996-1998: 24 Public water supply companies were surveyed for microcystins. 80% of the samples tested positive. Several examples where treatment of algae with copper sulfate in a drinking water reservoir led to gastroenteritis within 5 days.
- 2004: Approximately 50 people reported illness following exposure to toxic cyanobacterial blooms in Nebraska lakes and reservoirs.
- 2010: 7 people hospitalized after cyanotoxin exposure in Grand Lake St. Mary, Ohio



## Documented occurrences of toxic harmful algal blooms in the US

**Table 3.** Toxins identified in the first water sample collected (2007–2011) by type of water sample.

Torin	Water Type				
Toxin	Fresh	Brackish	Marine	Unknown	Total (%)
Anatoxin	243	2	0	1	246 (7)
Azaspiracid	0	0	1	0	1 (<1)
Brevetoxoins	0	3	0	0	3 (<1)
Cylindrospermopsin	4	0	0	0	4 (<1)
Domoic Acid	0	0	31	0	31(1)
Karlotoxins	0	3	1	0	4 (<1)
Microcytins Total	2629	35	2	10	2676 (81)
Microcytsin LR	21	0	0	0	21(1)
Okadaic Acid	1	2	0	0	3 (<1)
Saxitoxins	296	1	11	3	311 (9)
Unidentified Toxin	0	1	0	0	1 (<1)
Total	3194	47	46	14	3301



## 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



#### 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<sup>-1</sup>



#### Paul S. Sarbanes Environmental Restoration Site at Poplar Island Project



"Unfortunately the same environmental conditions required for the development of HABs also supports the development of wildlife diseases such as <u>avian botulism</u>. Unlike HABs, avian botulism is a bacterial borne disease associated with the bacterium *Clostridium botulinum*.

During the summer of 2012, avian botulism and the presence of a toxin forming HAB resulted in the death or debilitation of more than 700 birds at The Paul S. Sarbanes Environmental Restoration Site at Poplar Island (hereafter referred as Poplar Island) located in the Maryland portion of the Chesapeake Bay."



#### Paul S. Sarbanes Environmental Restoration Site at Poplar Island Project



#### Sea Otters in CA and Chesapeake Bay

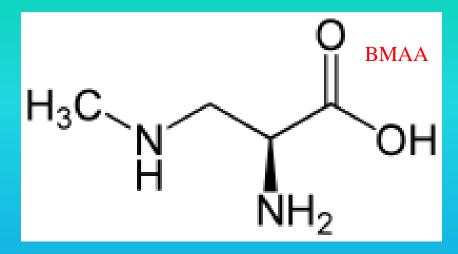
"Twenty one sea otter (*Enhydra lutris kenyoni*) deaths reported in California during the period 1999-2008, showed that cause of death was due to exposure to microcystins (Miller et al. 2010)"

"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)"

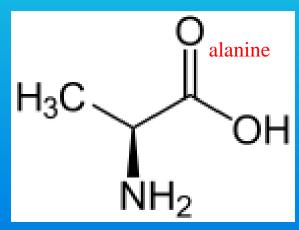


#### $\beta$ -methyl amino alanine (BMAA)

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



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





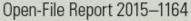


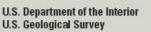
#### Field and Laboratory Guide to Freshwater Cyanobacteria Harmful Algal Blooms for Native American and Alaska **Native Communities**















brosen@usgs.gov

#### Common Filamentous Cyanobacteria

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

Cylindrospermopsis raciborskii (CYN)

20 µr

Chrysosporum ovalisporum (CYN)



Planktolyngbya contorta (MYC)











#### Thank You!



