Introduction to Cyanobacteria, Toxins, and Taste-and-Odor Compounds

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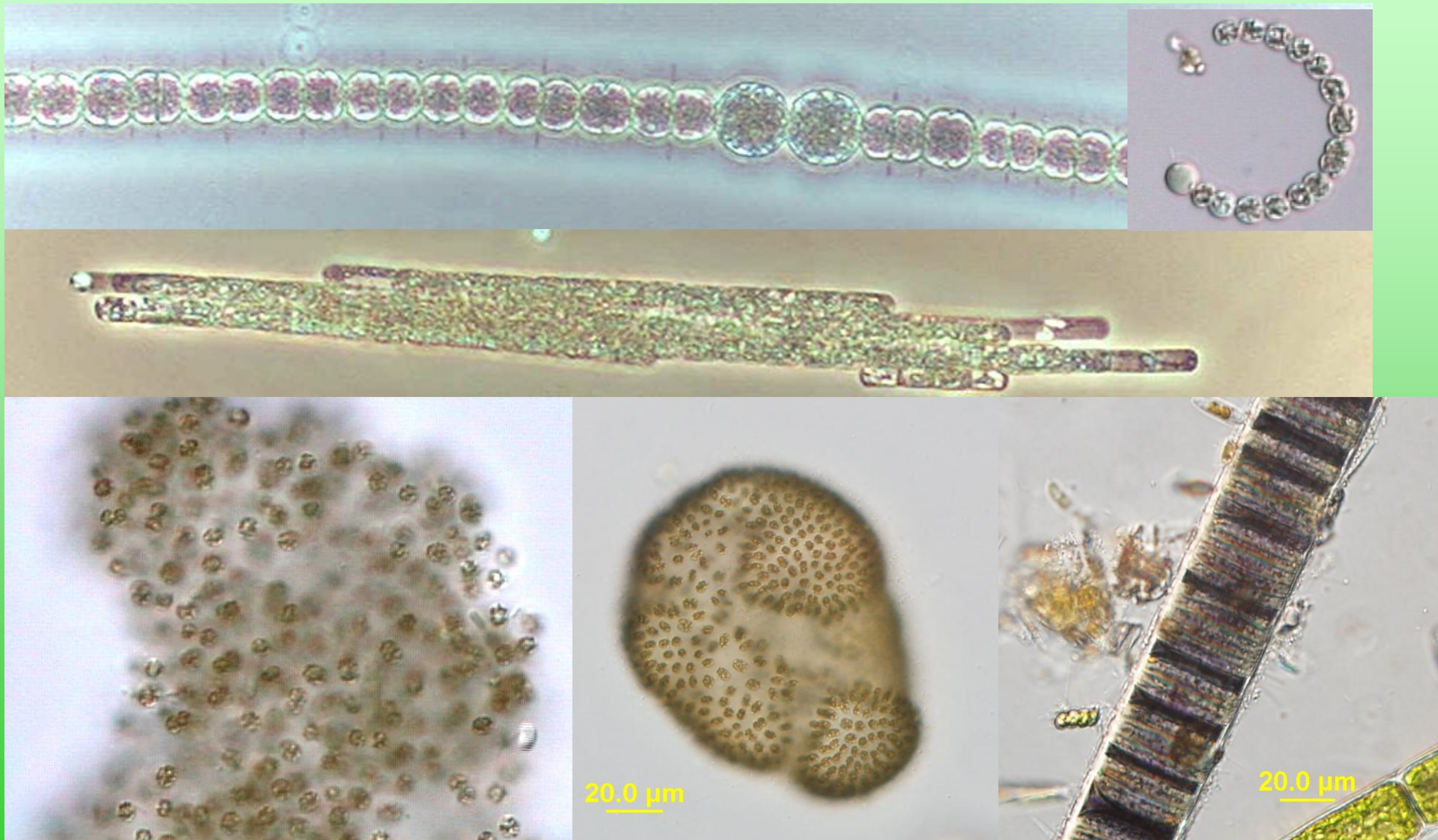
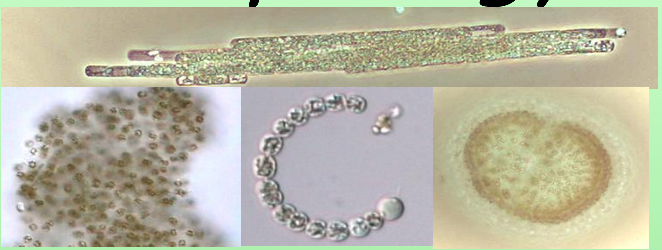
Cyanobacteria – What they look like



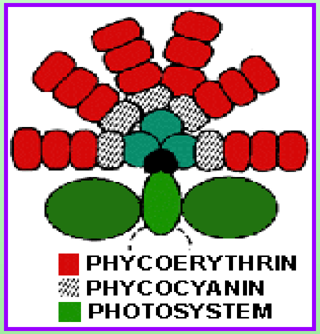
Ecological strategies for cyanobacteria

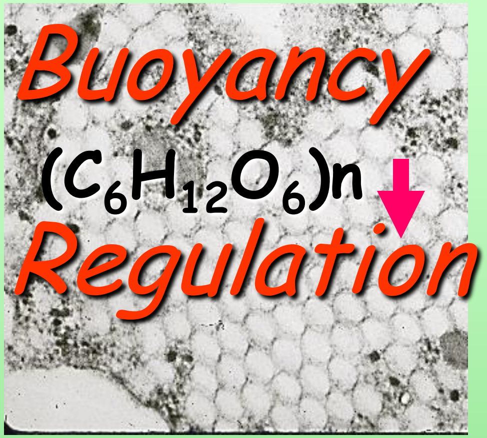
Morphology



grazing, floating

Pigments



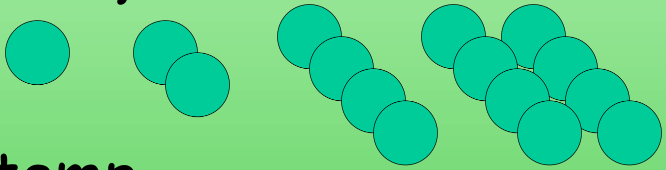


Buoyancy

$(C_6H_{12}O_6)n$ ↓

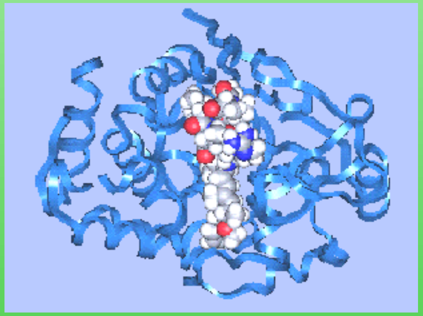
Regulation

Rapid Growth



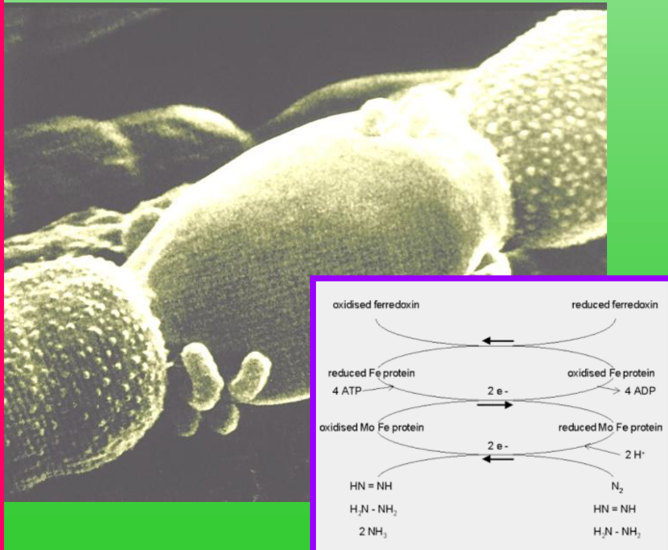
temp

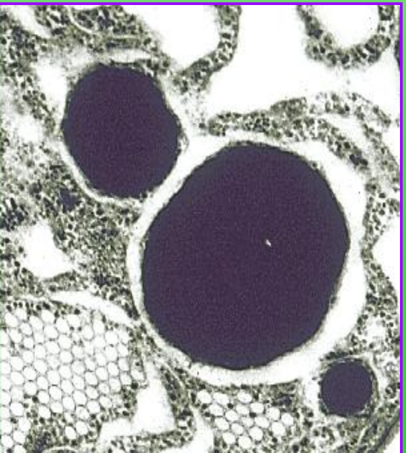
Toxicity



microcystin
LR complex

Nitrogen Fixation





trace, P,
C, N,

Nutrient Storage

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Very dense blooms are symptomatic of eutrophication: cyanoHABs

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Why are we concerned about cyanoHABS?

- **Taste and odor** problems
- **Toxic** to zooplankton, fish, shellfish, domestic animals and humans
- Cause hypoxia and anoxia, leading to fish kills
- Aesthetic problems, loss of recreational and fishing value of affected waters

TUESDAY

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"The entire river is green. I think they're having St. Patrick's Day early. I've lived here 30 years and I've never seen anything like it."

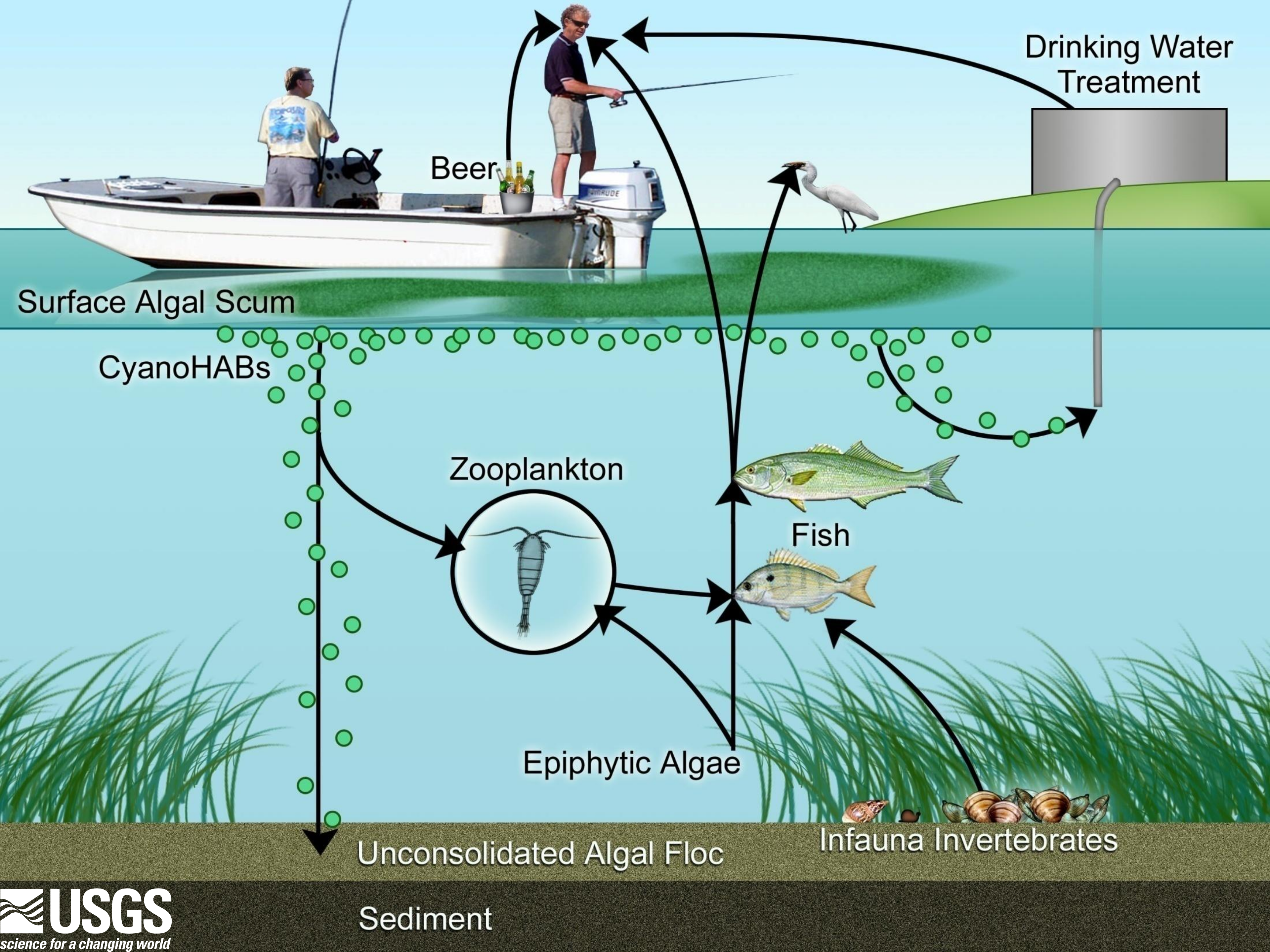
Ewww! River's a 'mess'

Rivers Coalition met with local lawyers to develop a legal strategy to battle deteriorating conditions in the estuary.

It's back to class for St. Lucie students

With only a few minor hiccups, the school year started with 2,090 more pupils enrolled than last year.

Energy bill not a quick fix, says Bush at signing


Drinking Water
Treatment
Beer
Surface Algal Scum
CyanoHABs
Zooplankton
Fish
Epiphytic Algae
Infauna Invertebrates
Unconsolidated Algal Floc
Sediment
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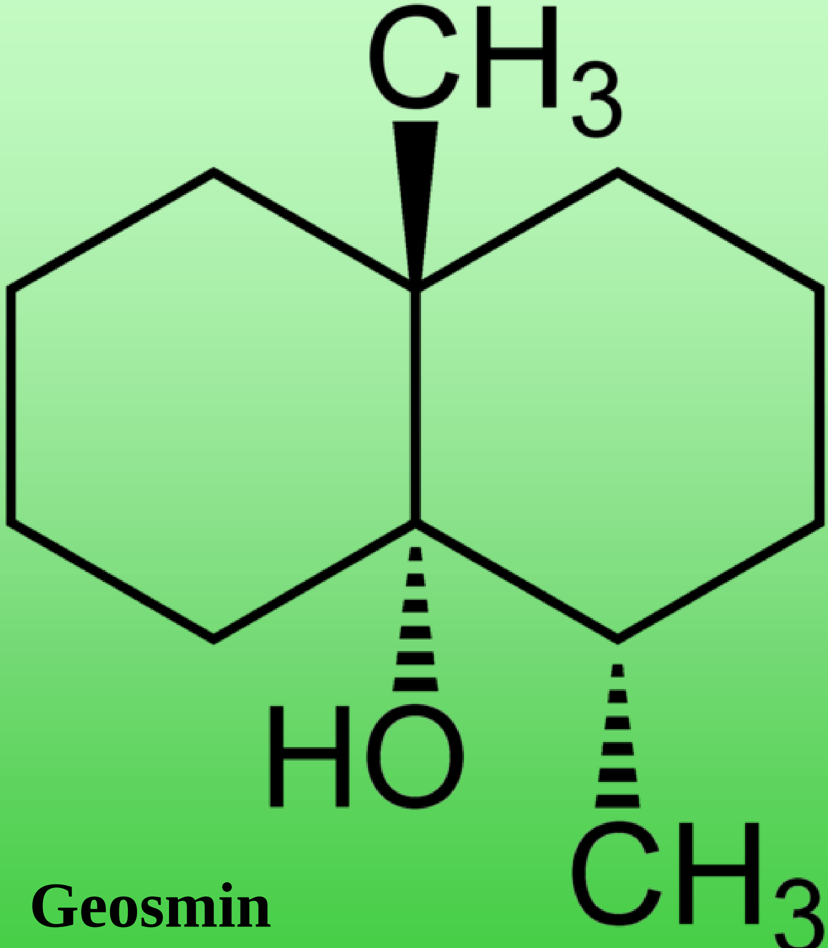
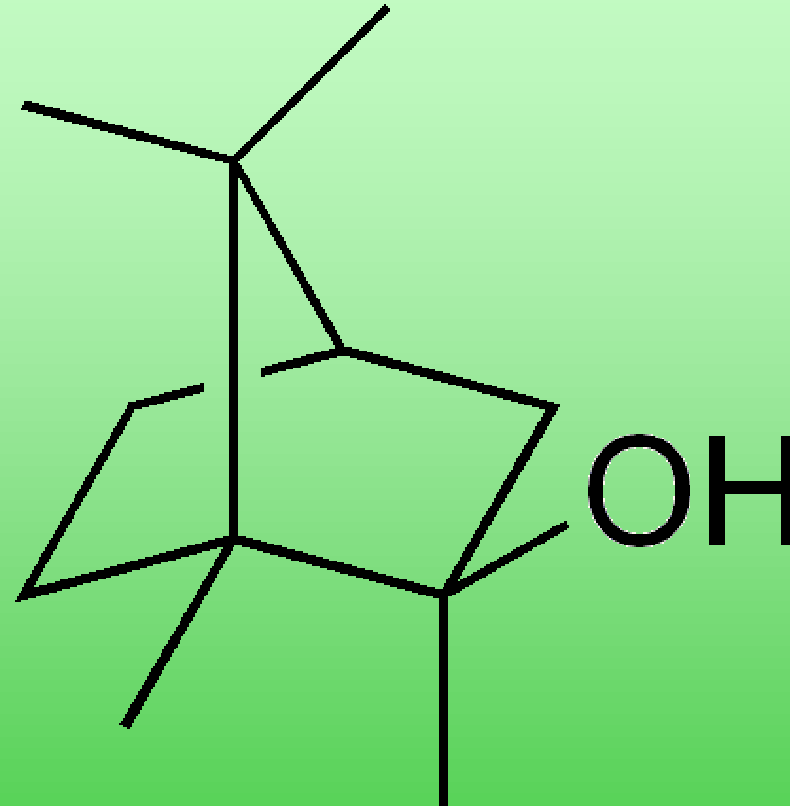
Taste and Odor Compounds



Geosmin

$C_{12}H_{22}O$



2-Methylisoborneol

$C_{11}H_{20}O$

Occurrence and health significance ranking of cyanotoxins

- Microcystins - most common, widespread poisonings
- Anatoxins - common; many animal poisonings
- Cylindrospermopsins - common; poisonings Australia
- Lyngbyatoxins - probably in continental US; poisonings in South & Central Pacific
- Saxitoxins - sporadic; animal deaths
- BMAA - world wide; potential major health significance
- LPS - world wide; health significance unclear

Exposure Routes - Inhalation, Ingestion, Dermal contact

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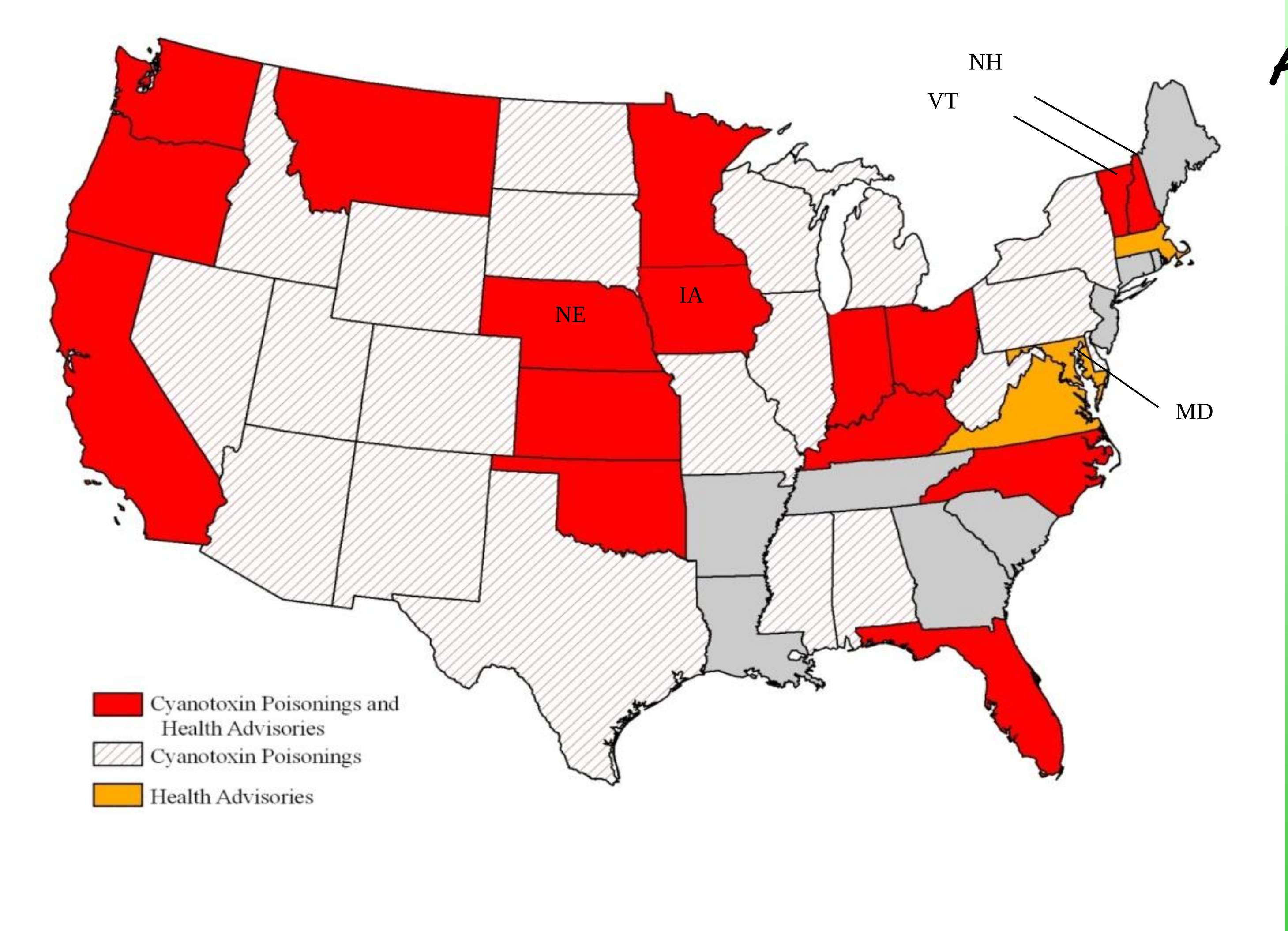
How common are toxic blooms?



At least 36 states have anecdotal reports of human or animal poisonings associated with cyanotoxins

After Graham and others, 2009

Cyanotoxins are highly potent

Compounds & LD_{50} (ug/kg)

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

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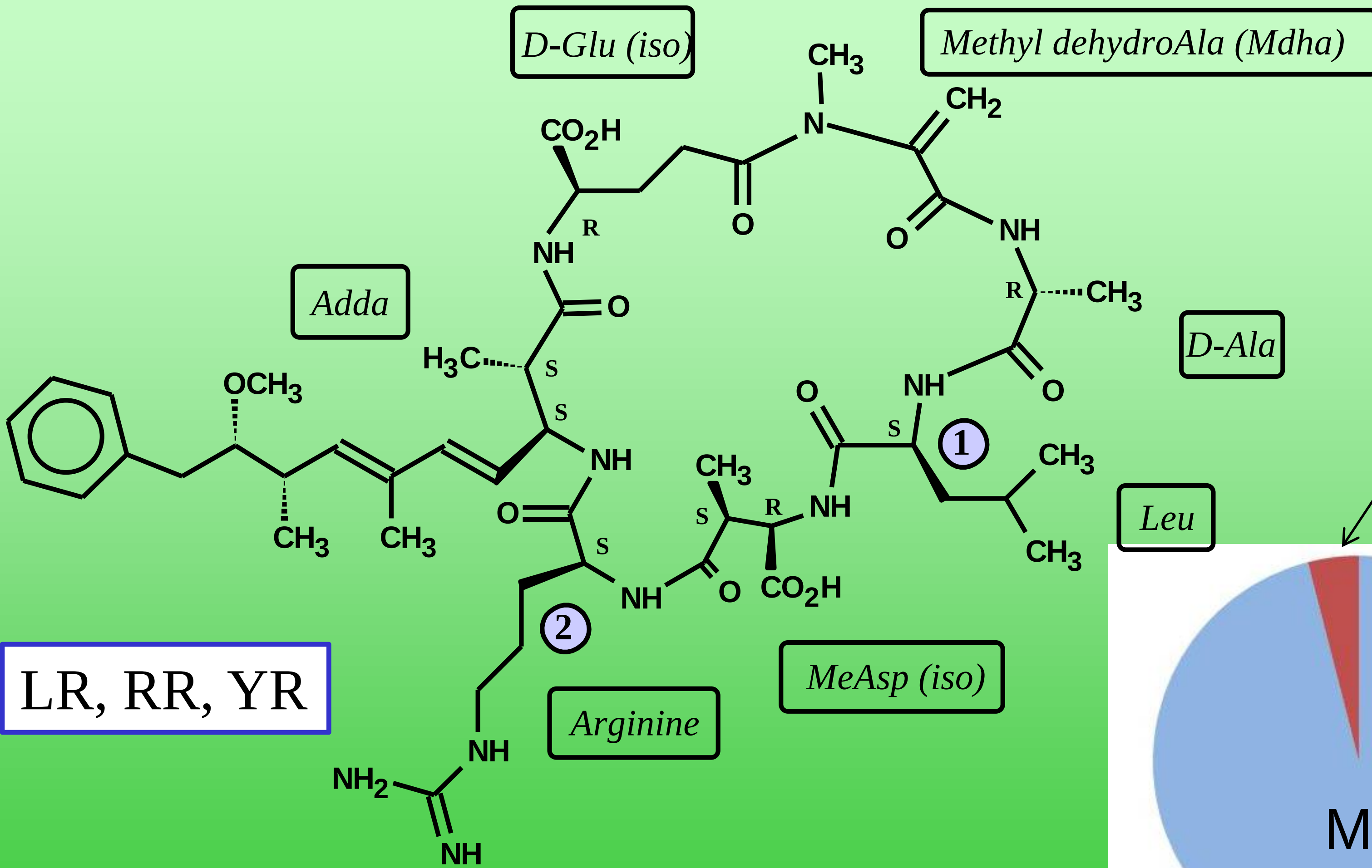
Microcystins

- *Microcystis*
- non-N fixer.
- Very common
  - Also produced by a number of other species.

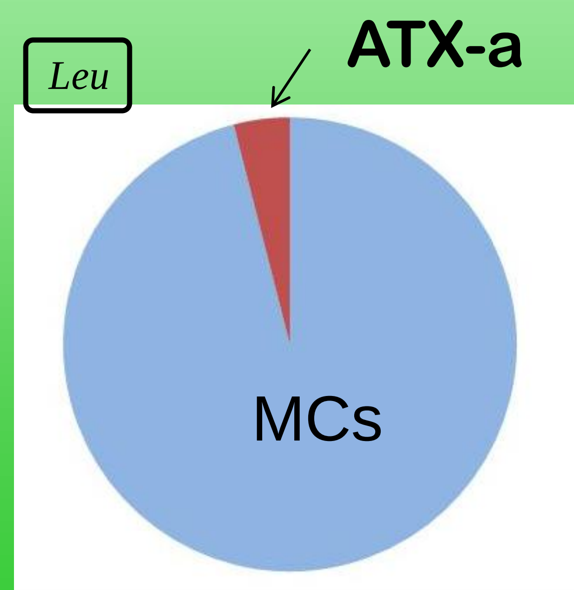
- Peptide Toxins:
  90+ structural variants
  + 200 others related compounds:
  nodularins, anabaenapeptins, etc.

- Microcystins are hepatotoxic
  LD-50: 25-60 μg kg^{-1}
- Called "**fast death factor**"
  Potent tumor promotor

Microcystins: most common in the North East



LR, RR, YR



Bloom events

Microsystin-producing strains include:

- *Microcystis aeruginosa*
- *M. veridis*
- *M. botrys*
- *Oscillatoria limosa*
- *Anabaena flos-aquae*
- *A. lemmermannii*
- *A. circinalis*
- *Planktothrix agardhii*
- *P. mougeotii*
- *Nostoc spumigena*
- *N. species*
- *Anabaenopsis millerii*
- *Haphalosiphon hibermicus*
- *Gleotrichia sp.*

Difficult to use taxonomy to predict toxicity

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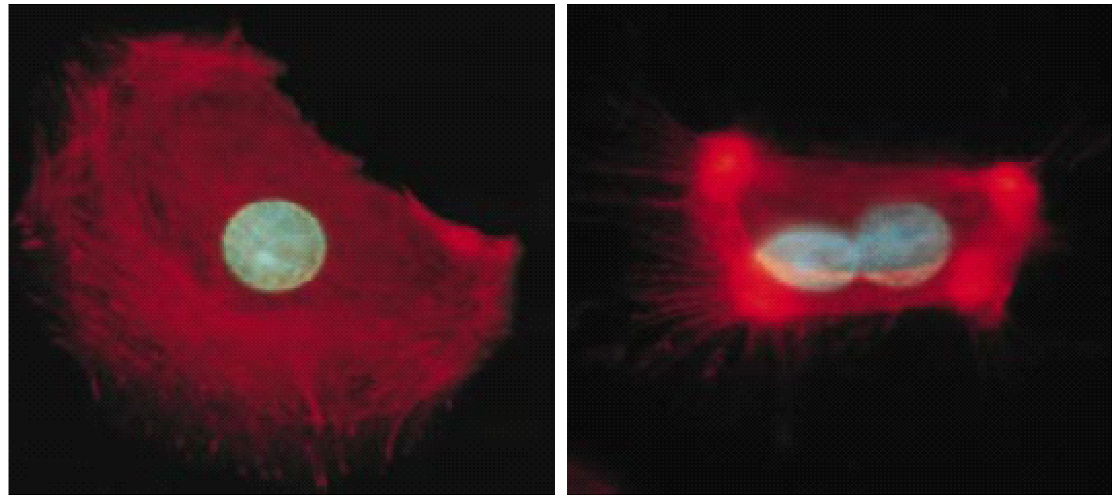
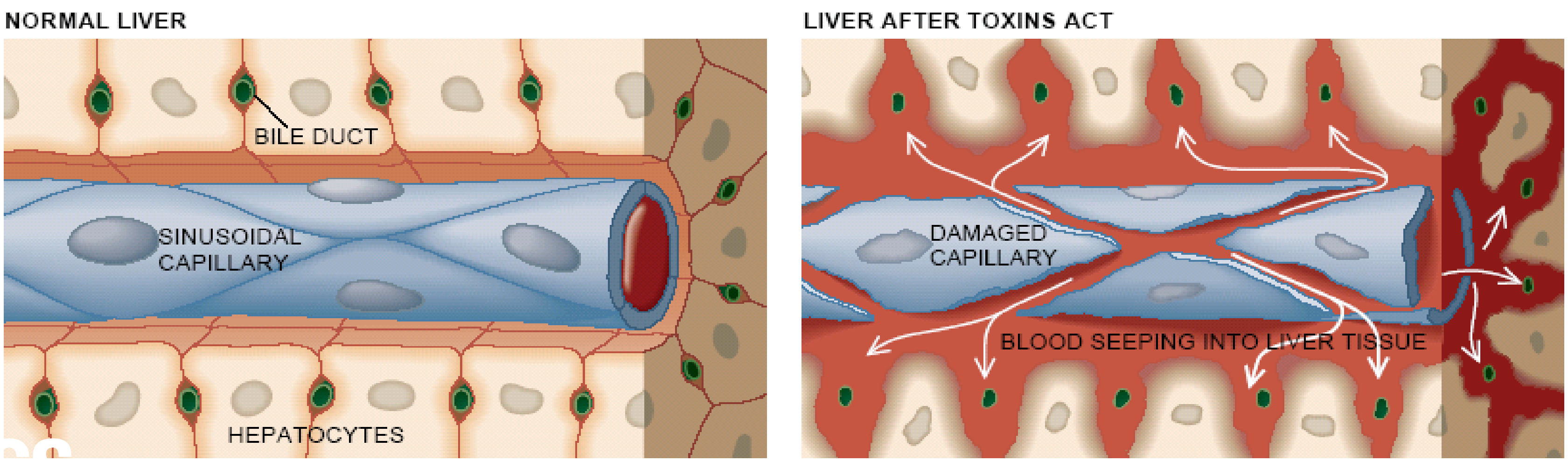
Microcystin exposure: response

- 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.





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

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.

Andrew Humpage ISOC-HAB Ch. 16

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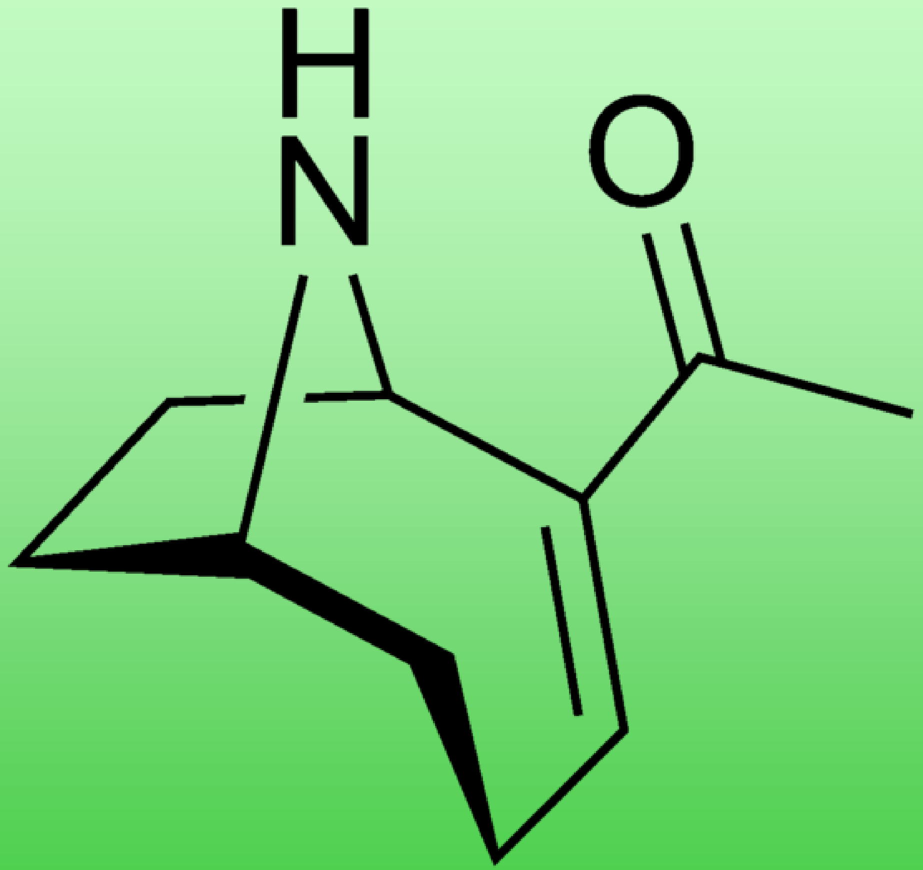
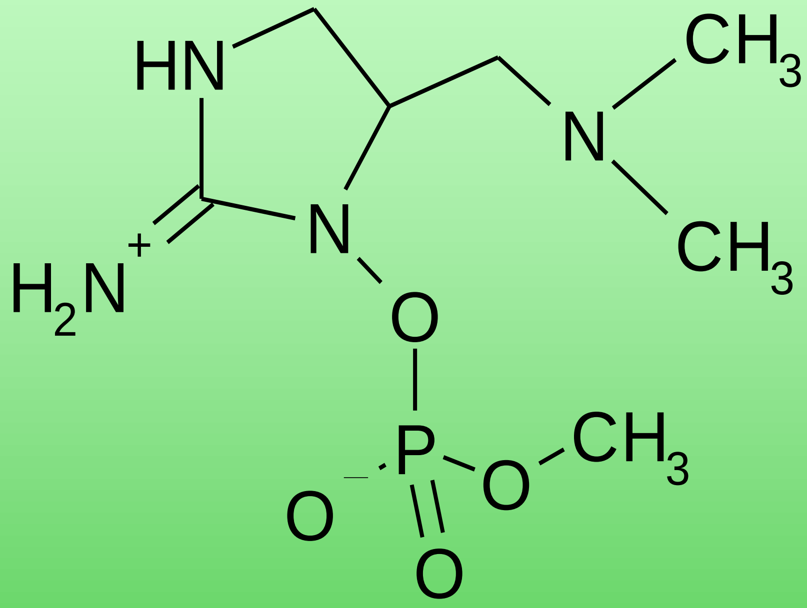
Anatoxin-a and a(s)



amine alkaloid



organophosphate (pesticide-like)

Anabaena lemmermannii

Anatoxin-a strains include:

• Anabaena flos-aquae	Canada, Finland Germany, US
• Anabaena planctonica	Italy
• Aphanizomenon sp.	Finland, Germany
• Cylindrospermum sp.	Finland
• Microcystis sp.	Japan
• Oscillatoria sp. benthic	Scotland, Ireland
• Planktothrix sp.	Finland

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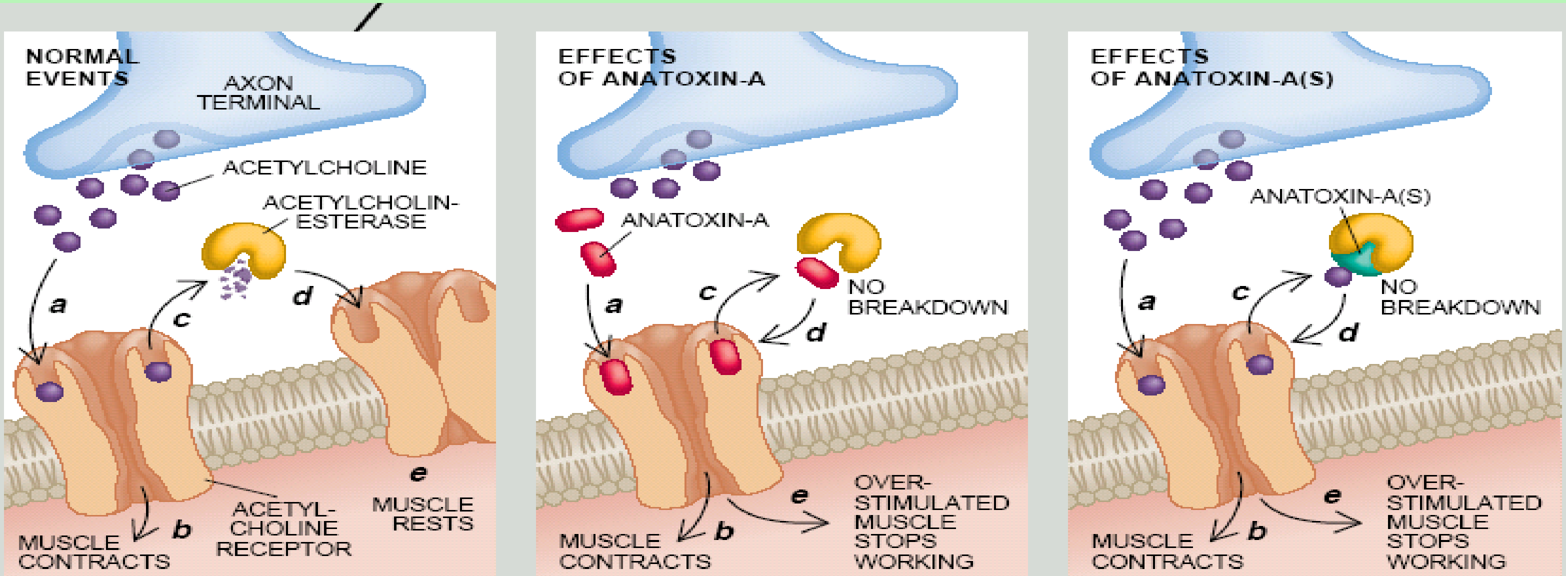
Anatoxin-a and a(s)

Anatoxin-a:
Acetylcholine receptor agonist
Anatoxin-a(s):
Cholinesterase 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*).

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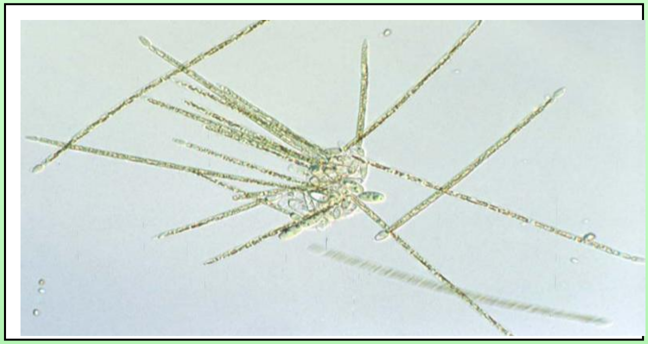
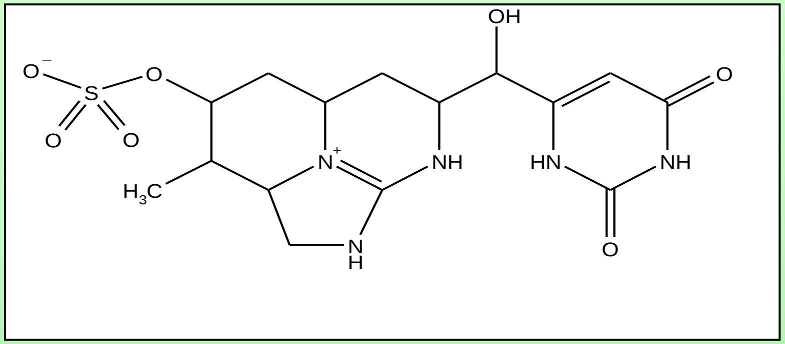
Wayne Carmichael ISOC-HAB Ch. 4, Scientific American, January, 1994

Cylindrospermopsin



Cylindrospermopsis



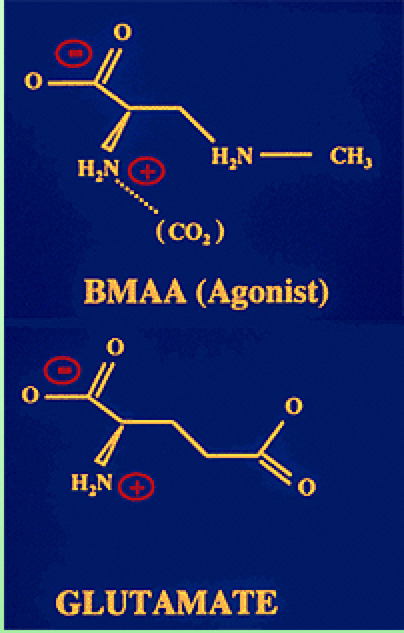
Alkaloid Toxin

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

Alkaloid: a complex heterocyclic multi-ring system. Includes a pyridine and pyrimidine ring and a sulfate substituent

β-Methyl Amino Alanine (BMAA) & Neurodegenerative Disease

- Made by almost all cyanobacteria
- BMAA, cycad plant, flying foxes, indigenous Chamorro people of Guam



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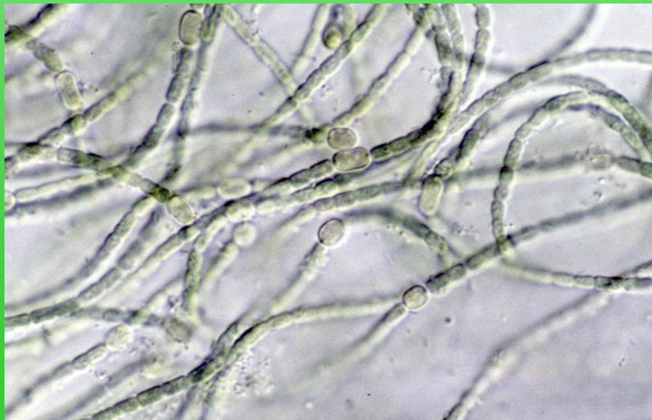

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Toxicity: Key Issues

- Acute and chronic toxicity levels - how much can be tolerated?
- Synergistic effects - those with liver or nerve disorders at higher risk
- Exposure routes – ingestion, skin, inhalation
- Water treatment options - avoid cell lysis, remove or neutralize toxins (i.e., chlorine works on microcystin, but not cylindrospermopsin)

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?

Regulations and Guideline

- No regulations or guidelines in the US

- EPA's OW placed cyanobacteria & cyanotoxins on the Contaminant Candidate List (CCL)
  - Evaluate and make regulatory determinations

- EPA's National Center for Environmental Assessment is producing draft toxicological reviews for US priority cyanotoxins
  - Anatoxin-a
  - Cylindrospermopsin
  - Microcystins LR, YR, RR, LA

Purpose is to derive reference doses for CCL compounds

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Drinking Water Guidelines

		Microcystins
• WHO	1998	1 µg / L (LR)
• Brazil*	2000	1 µg / L (All, Reg)
• France	2001	1 µg / L (LR)
• Australia **	2001	1.3 µg / L (LR tox eq)
• Canada	2002	1.5 µg / L (LR tox eq)
• New Zealand	2005	1 µg / L (LR tox eq)
• Oregon		1 µg / g (Health Food)

* Or 6,500 cells/ml

** Draft guidelines for cylindrospermopsin

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Thanks to:

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& TOXICOLOGIST
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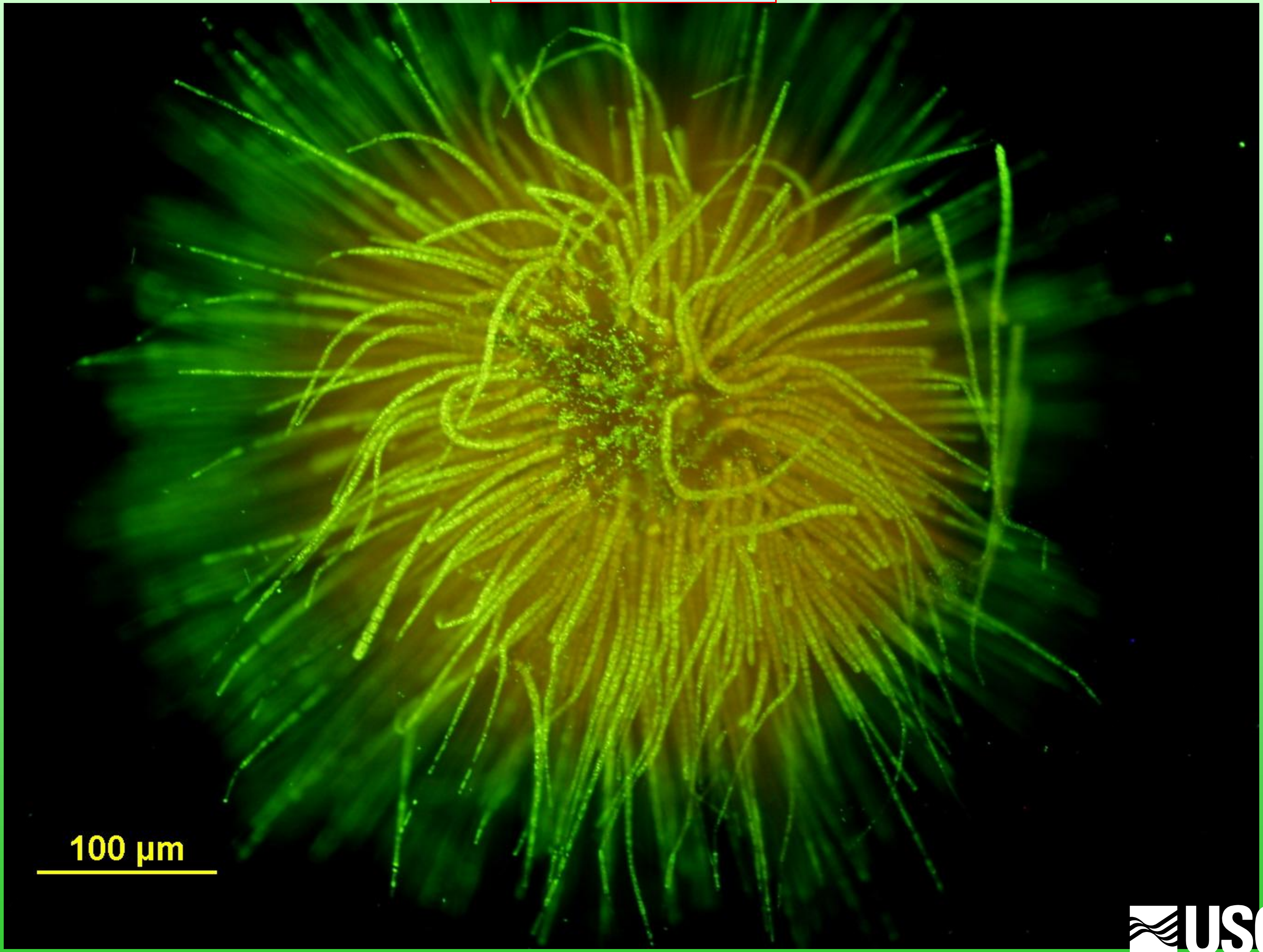
SWIM AT YOUR
OWN RISK

Thank You!



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