HAB Monitoring: Test kits, lab data or both? Pro and cons of each

Greg Boyer

Director, Great Lakes Research Consortium

Professor of Biochemisty

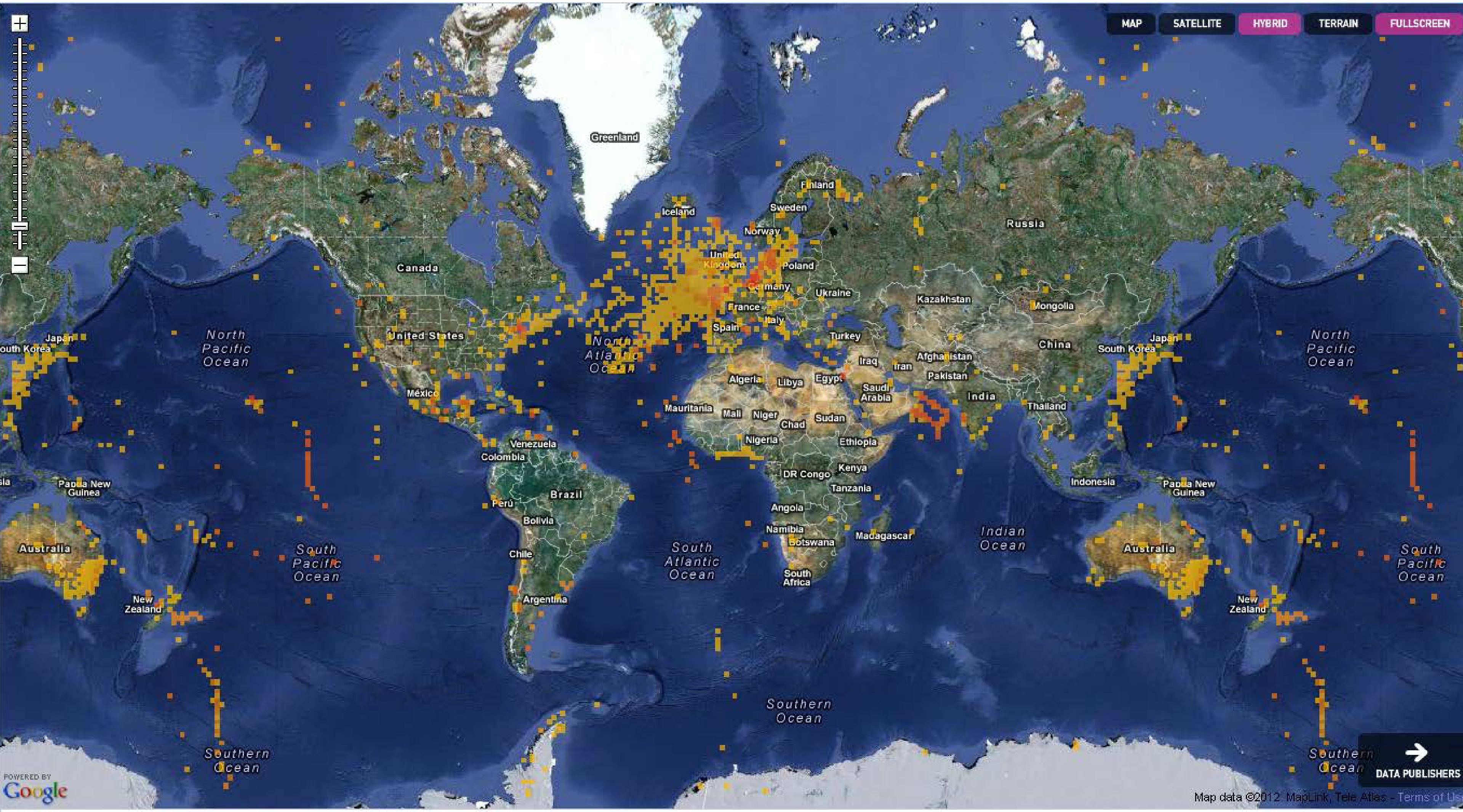
State University of New York

College of Environmental Science

and Forestry, Syracuse, NY

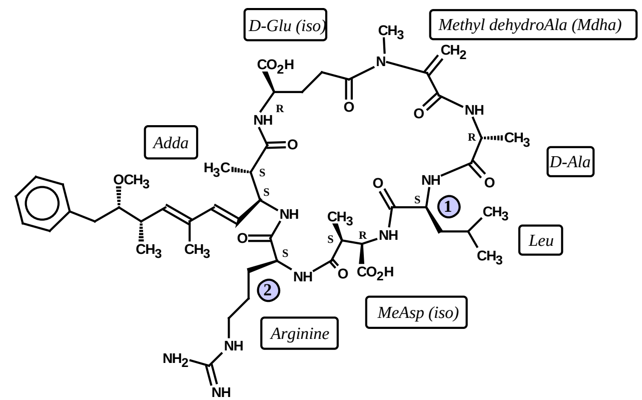
MERHAB-LGL
Harmful Algal Bloom
Monitoring and Event Response in the Lower Great Lakes

What is this picture?



Topics to be covered:

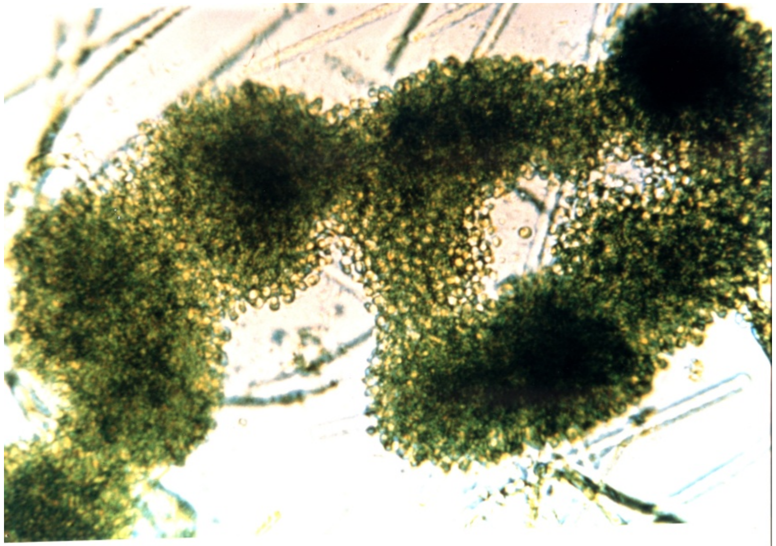
- Very fast review on Cyanobacteria Toxins



- What tools do we have?

- How do we use them in practice?

Microcystins
(and nodularins)



- *Microcystis aeruginosa*
  - Also produced by a number of other species.

- **Peptide Toxins:**

  90+ different variants

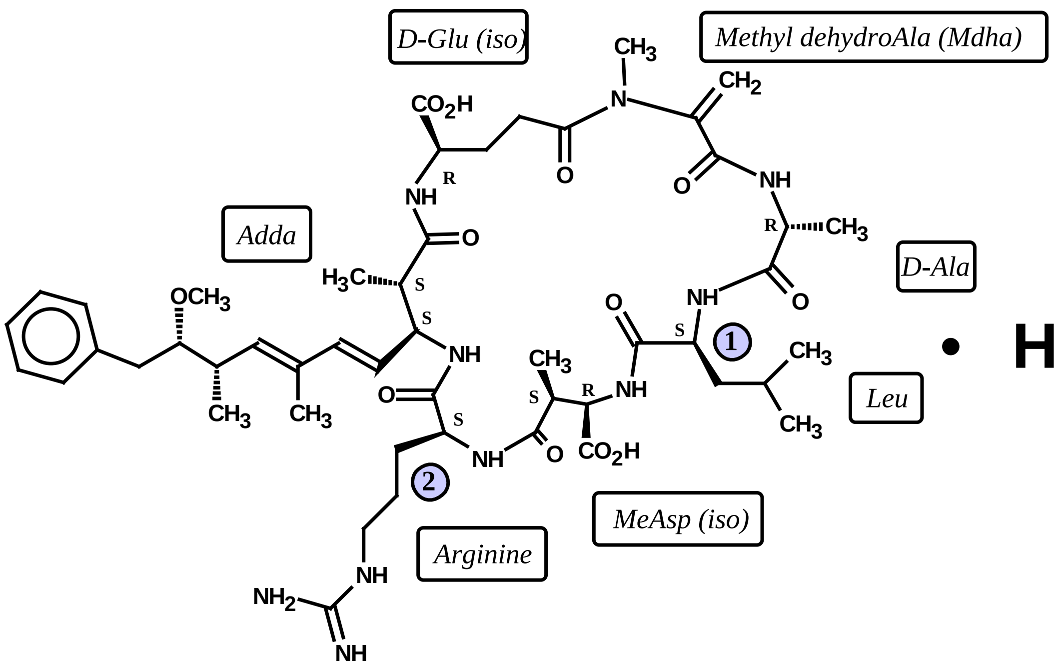
  15 common in NYS

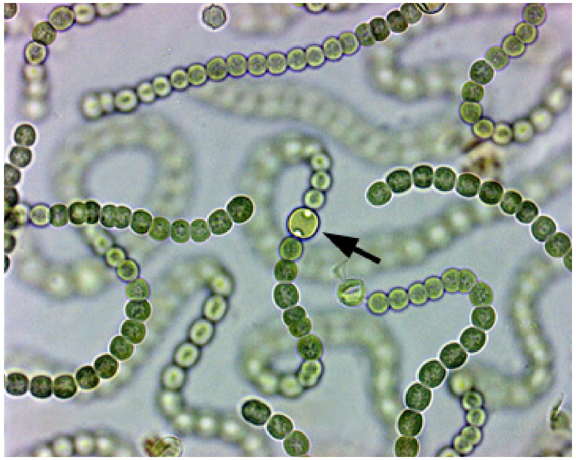
  very stable toxin

- **Hepatotoxins:**

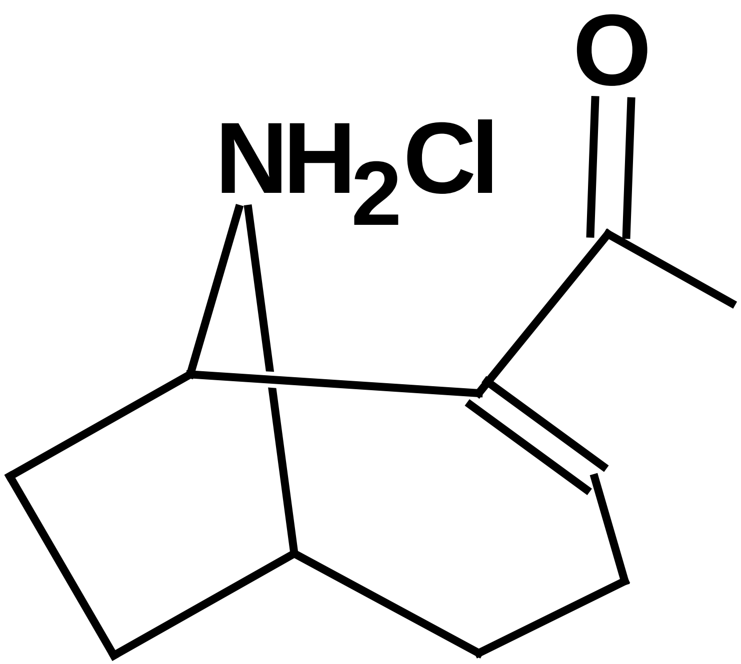
  1 ug/L (ppb) in drinking water

  20 ug/L in recreational water

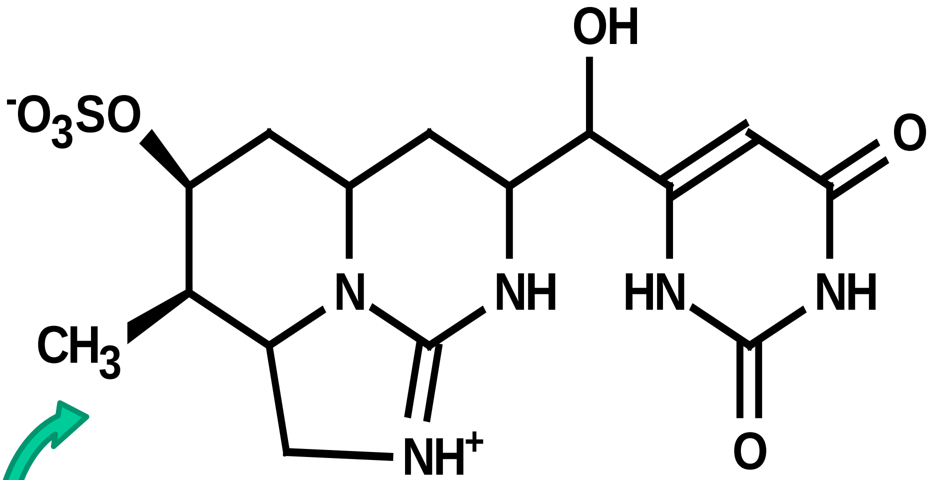




Anatoxin-a



- Potent Neurotoxin.
  - LD-50: 200 μg kg^{-1} (ATX-a)
  - Responsible for a number of animal fatalities.
- Causative organisms include:
  - *Anabaena species (many)*
  - *Oscillatoria sp.*
  - *Aphanizomenon sp.*
  - *Planktothrix sp.*
- ***At least 6 variations on the structure (4 metabolites).***



Cylindrospermopsin

LD-50: 300 μg kg^{-1}

Causative species:

Cylindrospermopsis raciborskii

Aphanizomenon ovalisporum

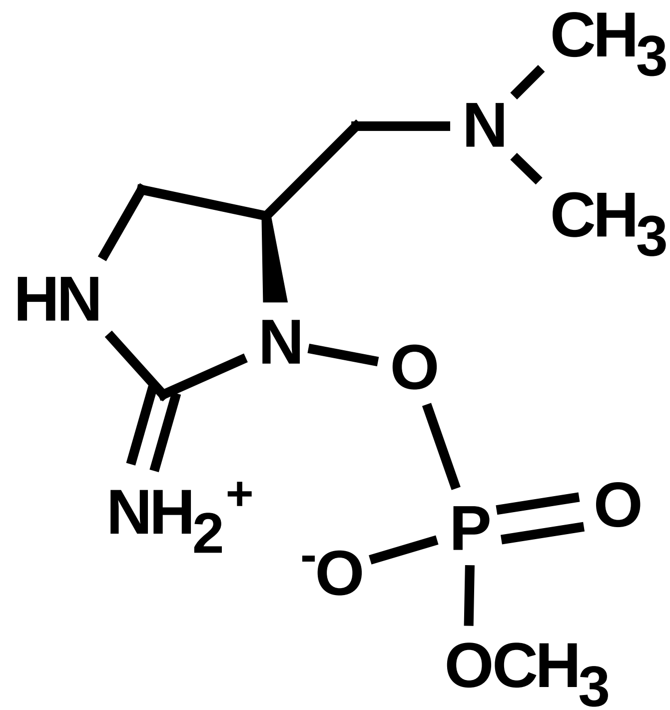
Anatoxin-a(S)

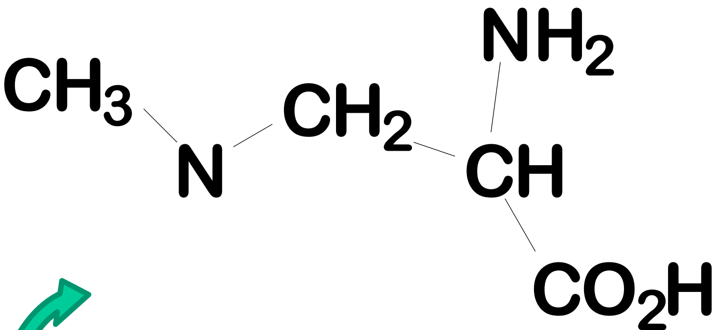
Organophosphate Neurotoxin.

cholinesterase inhibitor

LD-50: 20 μg kg^{-1}

Anabaena species





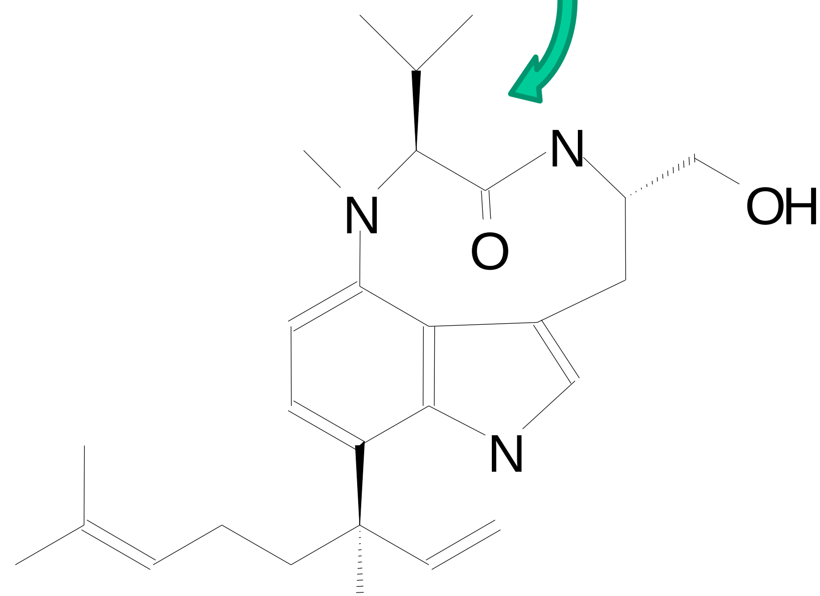
BMAA

β-methyl amino alanine
Produced by many species of cyanobacteria (aquatic and terrestrial) including *Microcystis, Anabaena* and *Aphanizomenon*

Lyngbyatoxin and other dermatoxins

Skin irritants or gastrointinal toxins (swimmers itch).

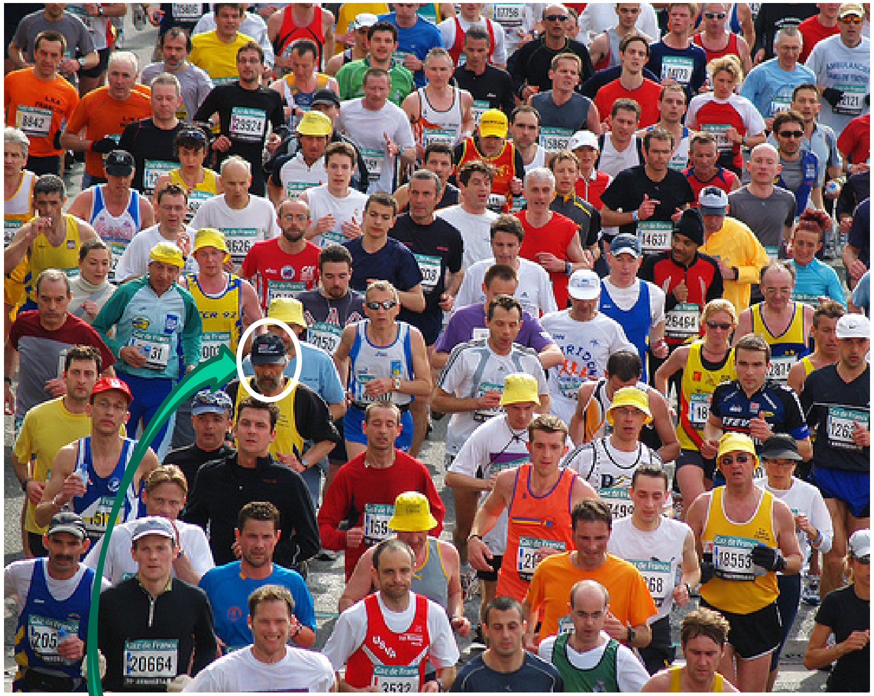
68 different toxins found in marine Lyngbya



Fools rush in where angles fear to tread!:

- Microcystins (90+)
- Anatoxin-a’s (6-7)
- Anatoxin-a(S) (1)
- Cylindrospermopsin (3)
- PSP toxins (56)
- Lyngbyatoxins (68)
- BMAA (1 of 7)
- Protease inhibitors (125+)

» Total: >350



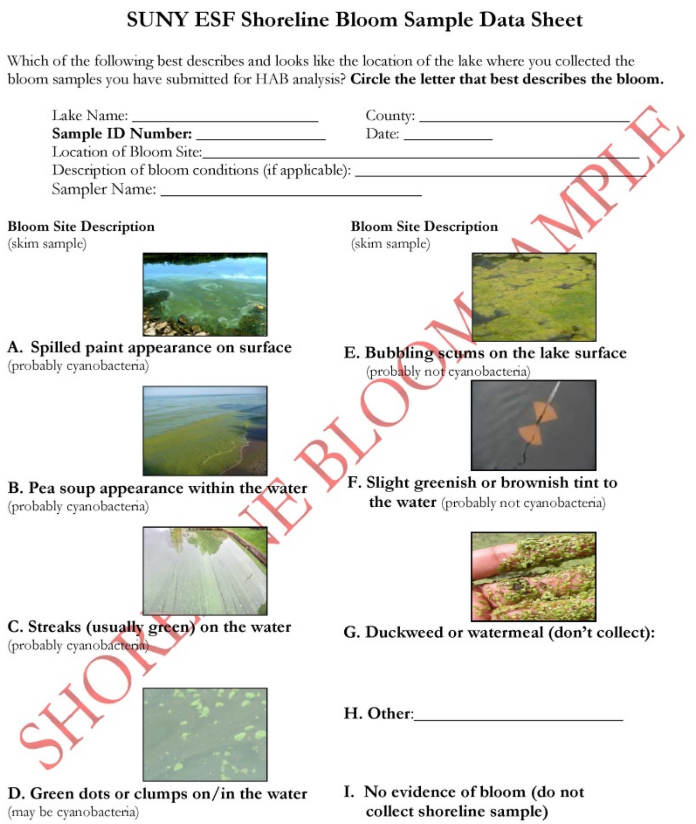
Not all are present in any given sample but finding the one that is responsible for bio-activity is not easy!

What tools are available?

- **Why set up a monitoring program**
  - Do you want to know if blue-green algae are present?
  - Do you want to know if potentially toxic blue-green algae are present?
  - Do you want to know if blue-green algal toxins are present?
  - Do you need to know who and how much?

(A) Are Blue-green algae present in my lake?

- Tools:
  - Visual examination
    - Look for blooms, surface scums and other indications of blooms.
  - Microscope examination
    - Most potentially toxic genera are easy to identify under a microscope.



SUNY ESF Shoreline Bloom Sample Data Sheet

Which of the following best describes and looks like the location of the lake where you collected the bloom samples you have submitted for HAB analysis? **Circle the letter that best describes the bloom.**

Lake Name: ____________ County: ____________
Sample ID Number: ____________ Date: ________
Location of Bloom Site: ____________
Description of bloom conditions (if applicable): ____________
Sampler Name: ____________

Bloom Site Description
(skim sample)

A. Spilled paint appearance on surface
(probably cyanobacteria)

B. Pea soup appearance within the water
(probably cyanobacteria)

C. Streaks (usually green) on the water
(probably cyanobacteria)

D. Green dots or clumps on/in the water
(may be cyanobacteria)

Bloom Site Description
(skim sample)

E. Bubbling scums on the lake surface
(probably not cyanobacteria)

F. Slight greenish or brownish tint to the water (probably not cyanobacteria)

G. Duckweed or watermeal (don't collect):

H. Other: ____________

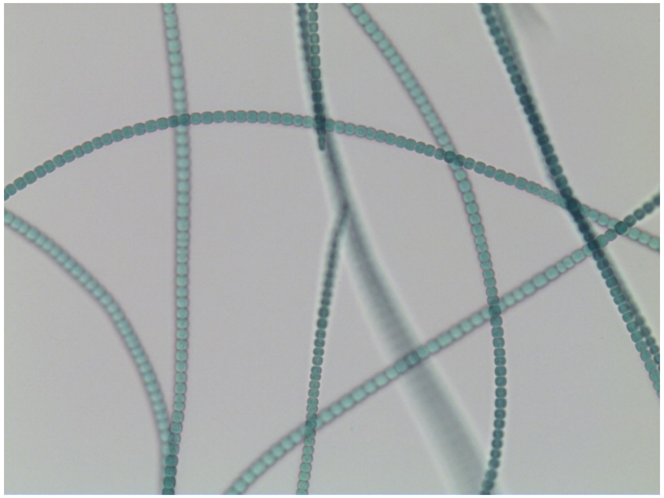
I. No evidence of bloom (do not collect shoreline sample)

Blue-green algal blooms often look like paint on the water

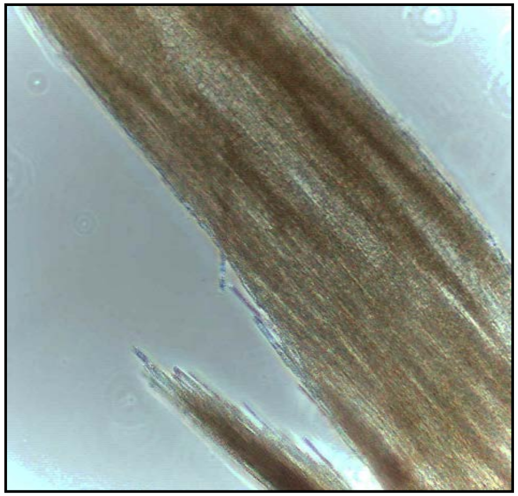
Blue and white crust forming on bloom that is decaying

Cost: $0

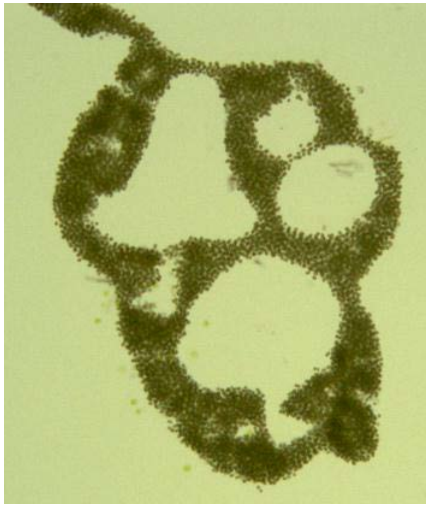
Cyanobacteria are pretty easy to identify under a microscope………



Anabaena



Aphanizomenon



Microcystis

Known to a generation of scientists as Anni, Fanni and Mike
(3 most common bloom forming species)

Cost: $250-$3000

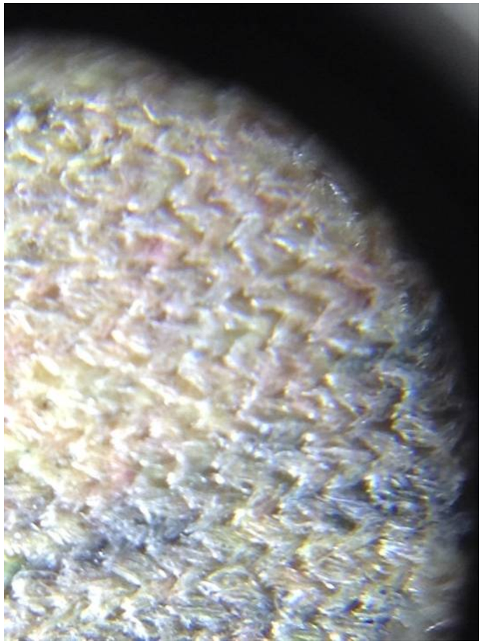
Microscope adaptors are available for smart phones

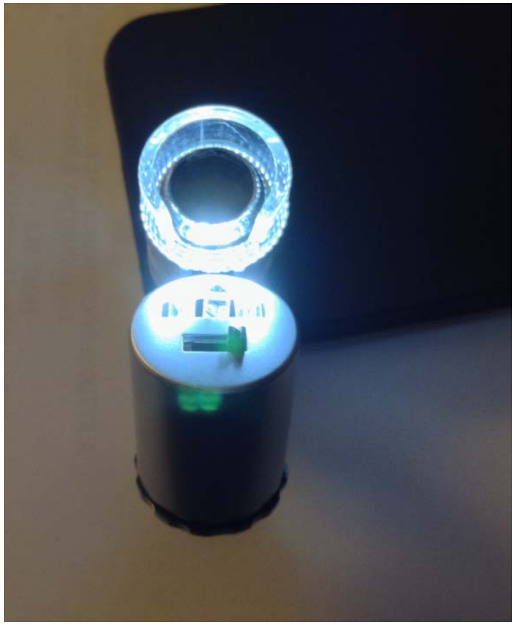
CSLAP@esf.edu
glboyer@esf.edu

I-phone 4s microscope attachment
(60x microscope for i-phone)

Cost: $9-$120

No promises





There are instruments that can replace your eyes

Cost: $12,000

Water quality sondes can be equipped with sensors for chlorophyll (all algae) or cyanobacteria (phycocyanin)

Dock or buoy-mounted sensors exist (Cyano-watch).

Pro: Easy to use
Con: hard to calibrate.

Cost: $6,000

Cost: $600

Fluorometer List

Chlorophyll

- Turner Designs AlgalWatch
- HydroLab DS5
- YSI 6600 sonde

- BBE FluoroProbe
- Turner Designs 10-AU
- Turner Designs Cyclops

Phycocyanin

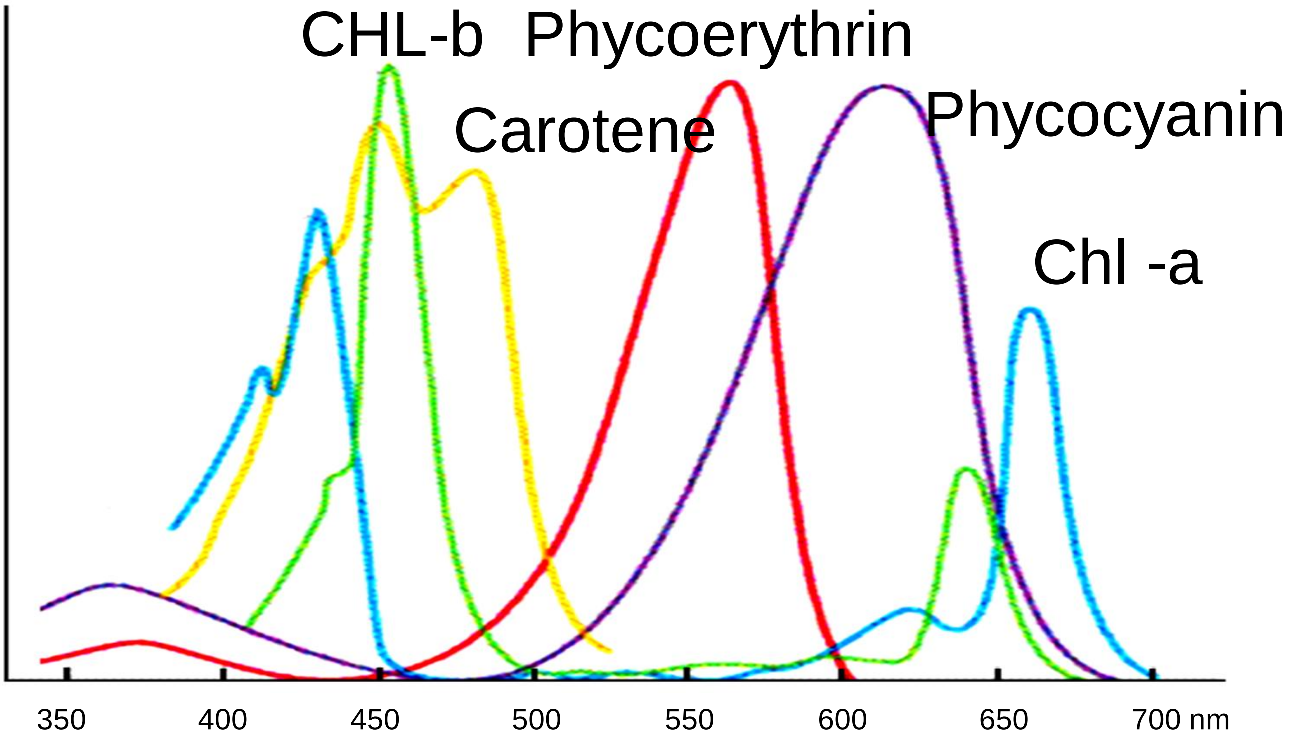
- TD CyanoWatch
- HydroLab DS5
- YSI 6600 Sonde

- BBE (Cyano-specific Chl)
- Turner Designs 10-AU
- Turner Designs Cyclops
  - PC, PE, CDOM, CHL

How do these sondes work?:

All plants collect light which is used for photo-synthesis.

All plants contain chlorophyll



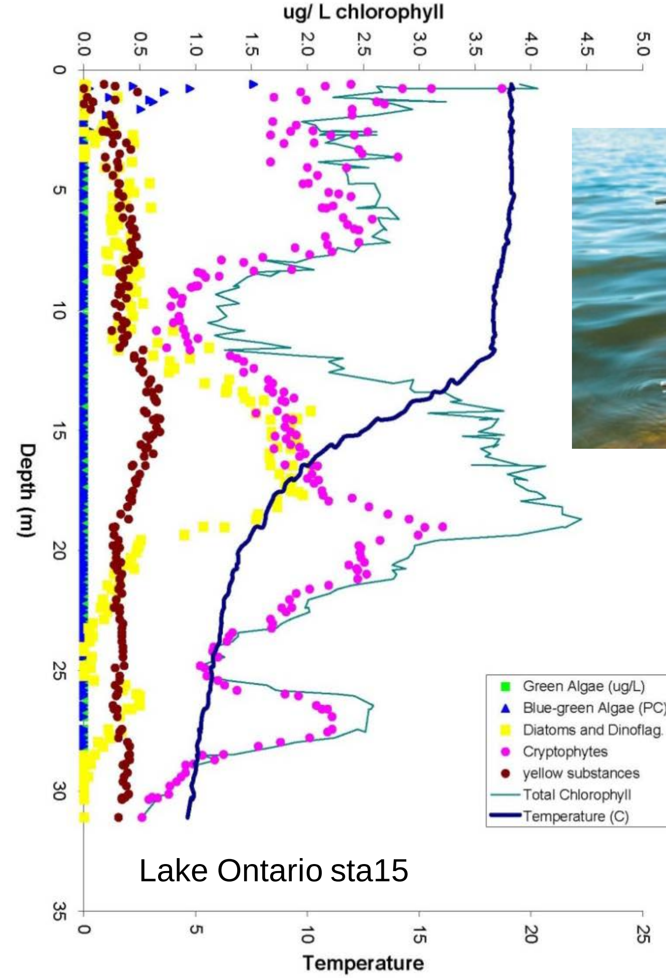
Different algae use different pigments to collect light

- Green Algae
- Yellow algae (dinoflagellates and diatoms)
- Blue-green algae

We can use the pigments to estimate the amount of algae in the water

CSLAP uses such a sonde for its monitoring.

- BBE FluoroProbe rapidly determine five classes of algae
- Your CSLAP report contains % BGA chl



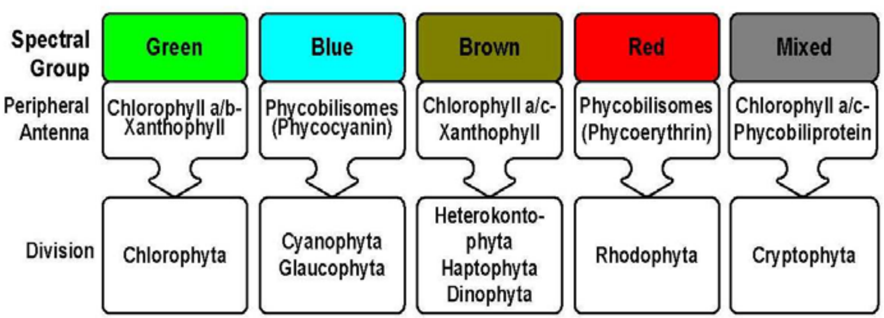
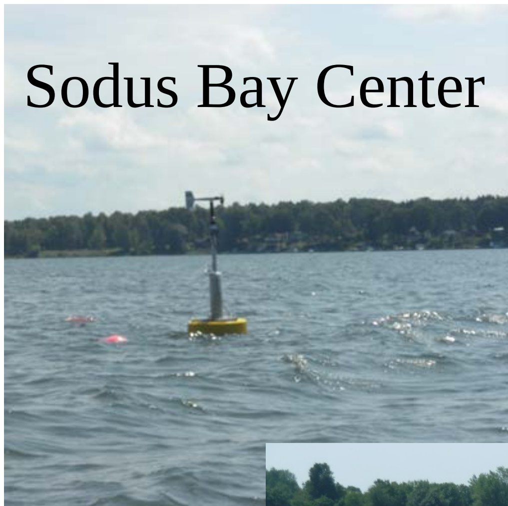


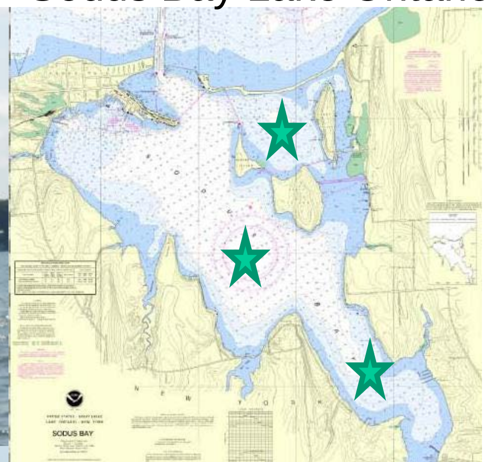
Fig. 1: Assignment of several algal divisions in spectral groups

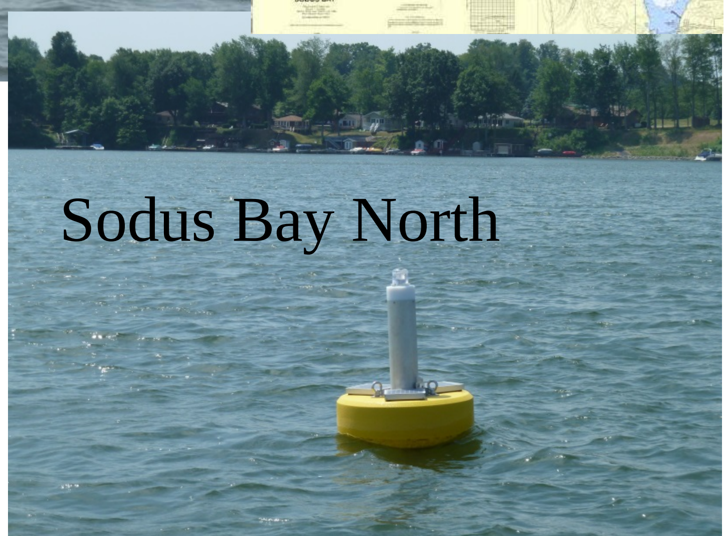
Cost: $30,000

Sondes are commonly used on buoys.

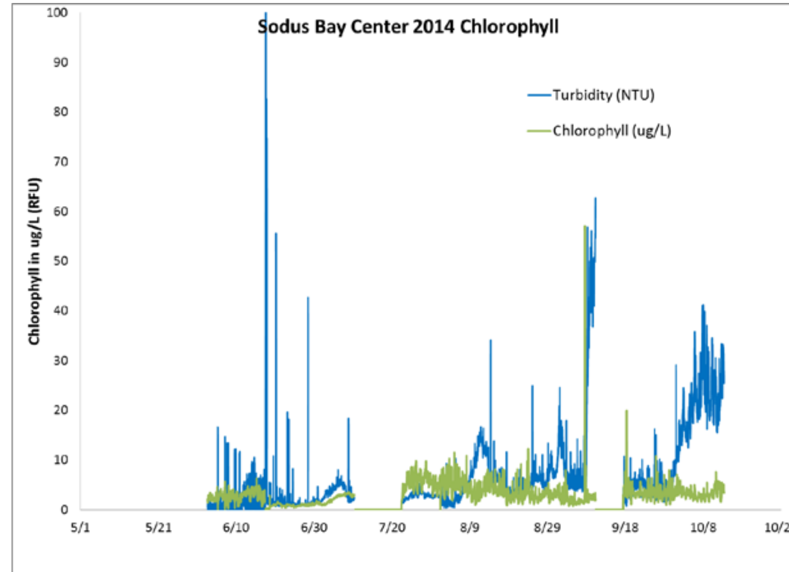
Sodus Bay Lake Ontario

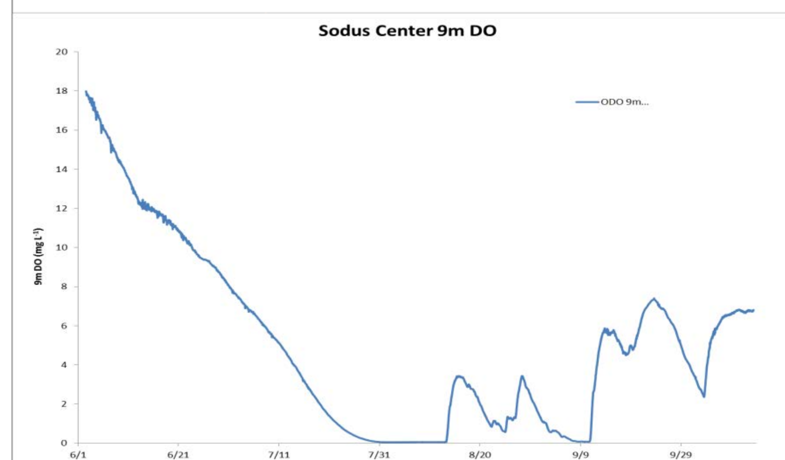






Docks work also!





Cost: $40,000-$75,000

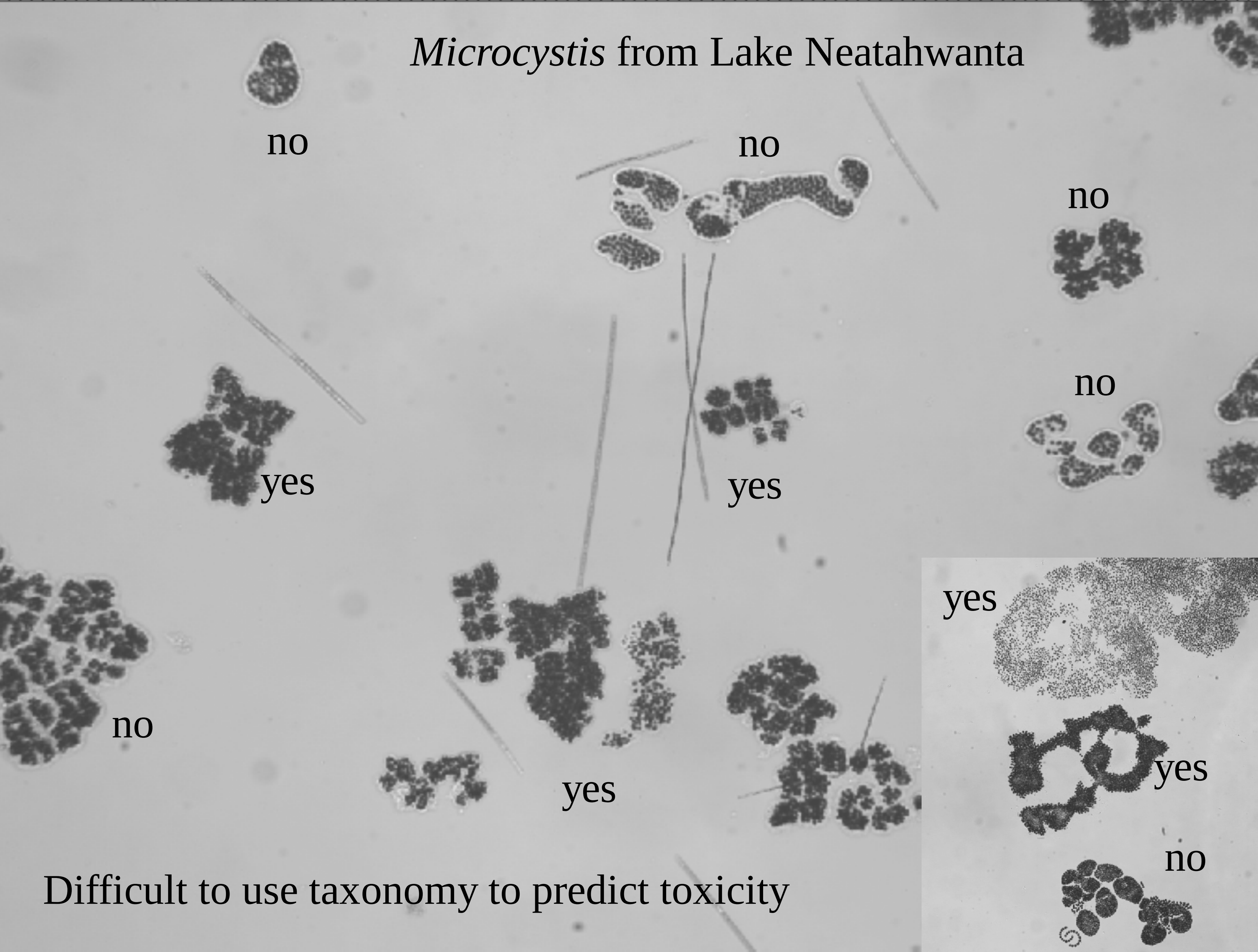
(B) Are the Blue-green algae in my lake potentially toxic?

- Tools:
  - Visual examination cannot tell toxic from non-toxic
  - Molecular approaches can tell toxic from non-toxic strains.
  - These techniques best belong in a CSI-lab.
    - Answer specific questions

<u>Algae known to make MC's</u>

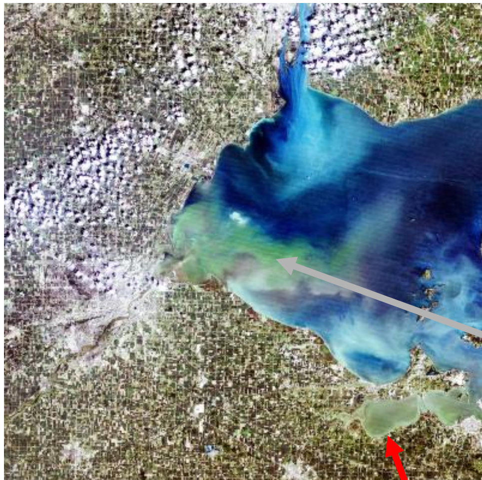
Microcystis aeruginosa
M. veridis
M. botrys
Oscillatoria limosa
Anabaena flos-aquae
A. lemmermannii
A. circinalis
Planktothrix agardhii
P. mougeotii
Nostoc spumigena
N. species
Anabaenopsis millerii
Haphalosiphon hibermicus
Gleotrichia sp.

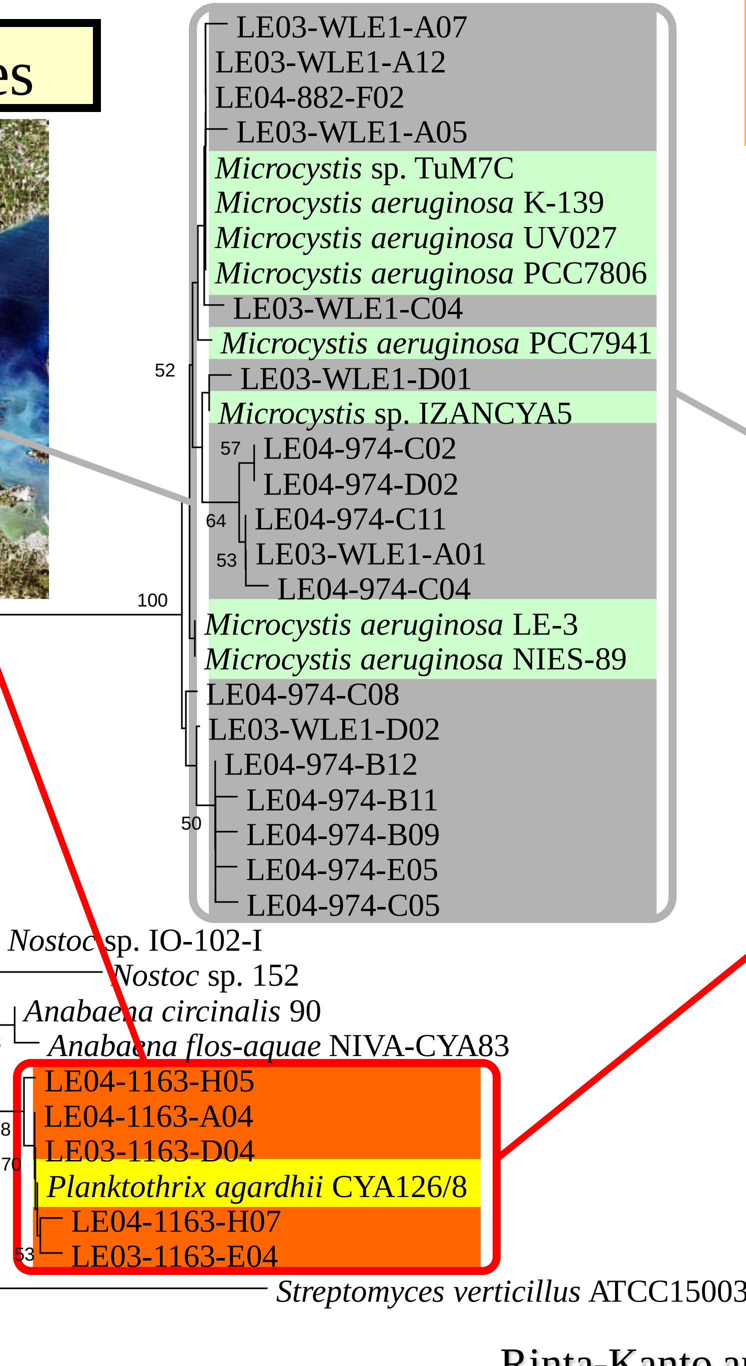
Microcystis from Lake Neatahwanta



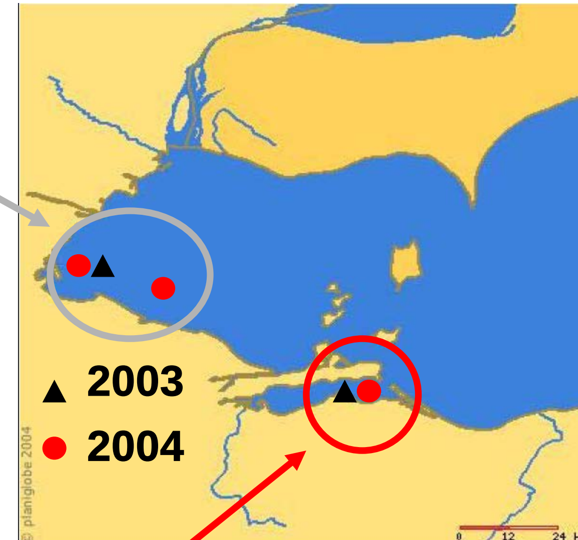
Difficult to use taxonomy to predict toxicity

McyA sequences





Cost: $thousands to set up; $50 per sample



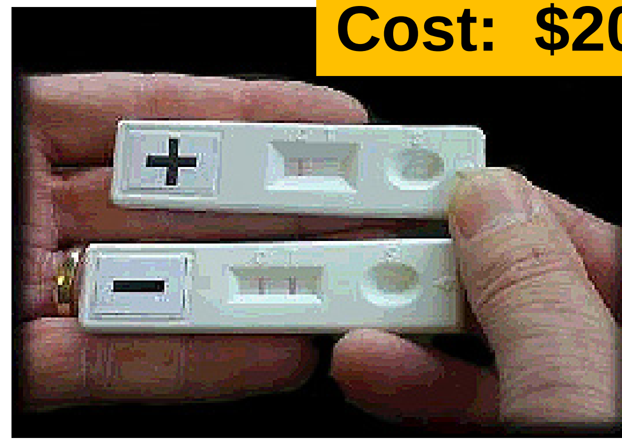
2 different populations producing the same toxin!!

Rinta-Kanto and Wilhelm, 2006, AEM 72:5083

(C) Are the Blue-green algal toxins in my lake?

- Tools:
  - Need to actually measure the toxins in the water.
    - Approach 1: Assay
    - Approach 2: Analysis
  - Both approaches can be field or laboratory-based.
  - Some assays are easy to do in the field.

Cost: $20



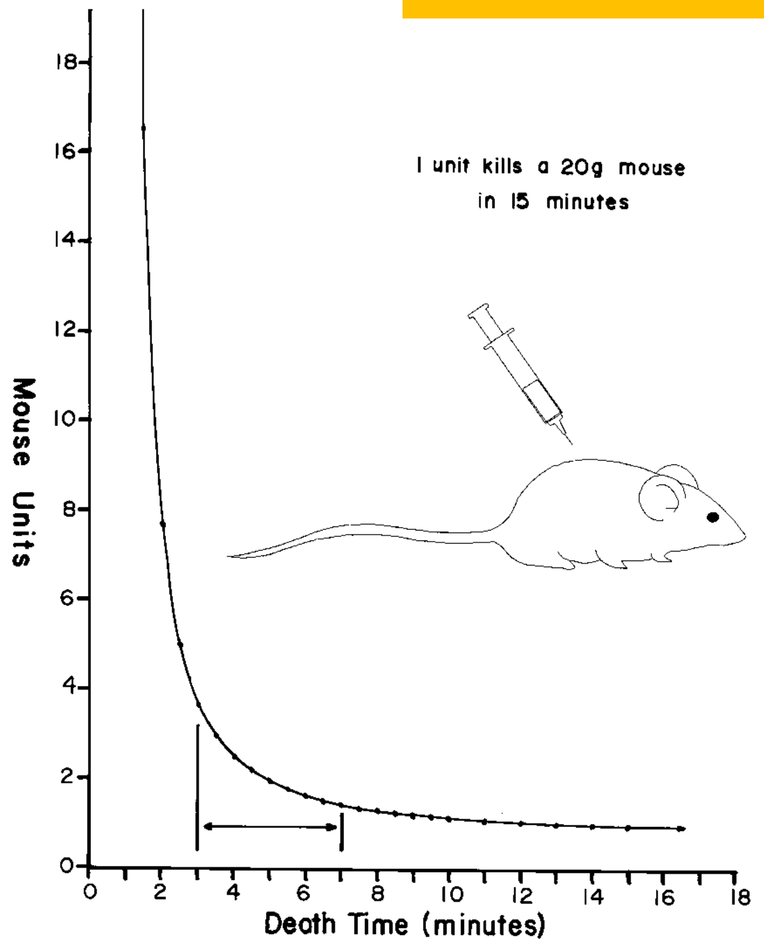
Cost: too much

Boyer lab at SUNY-ESF

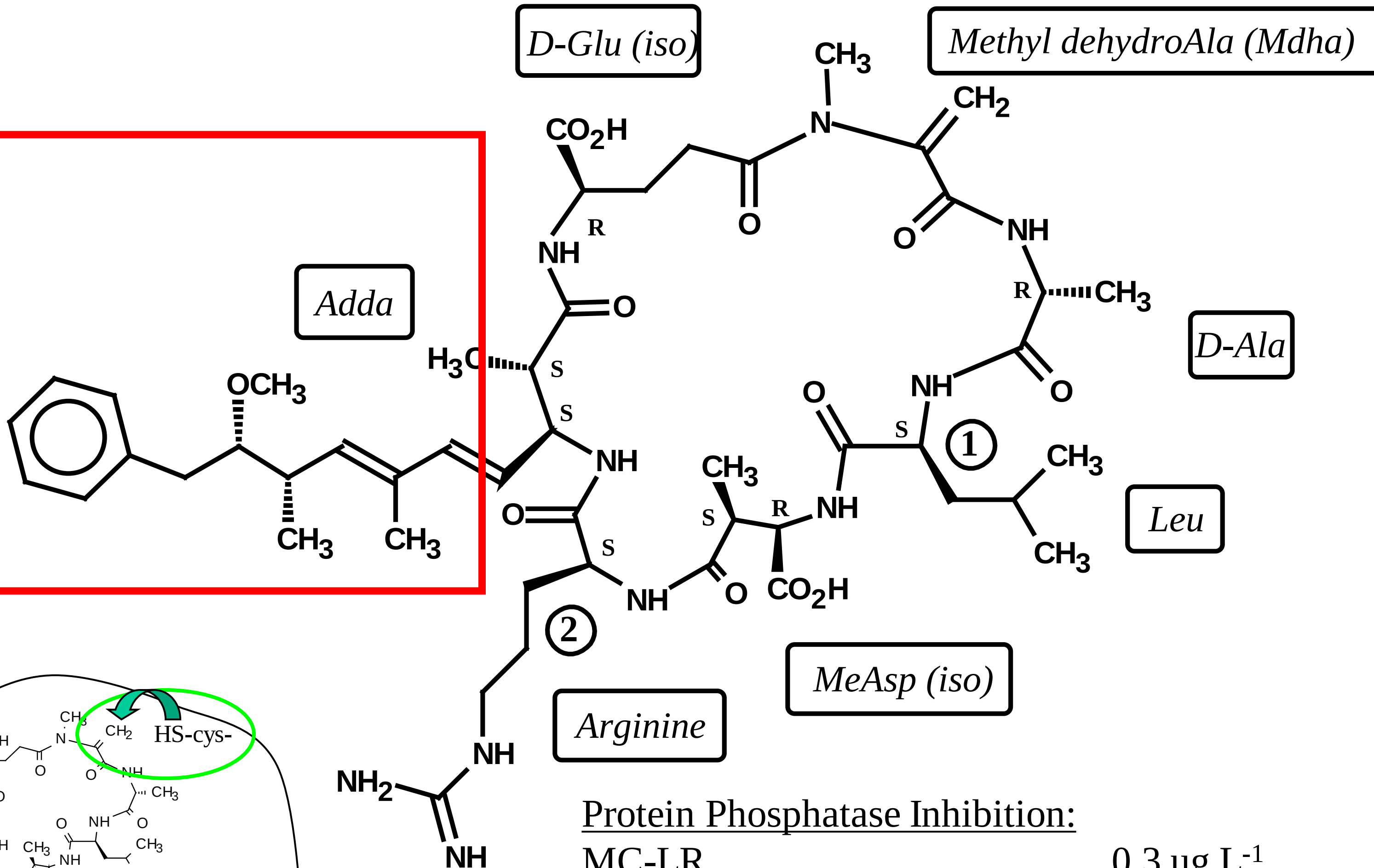
An assay detects a class of compounds

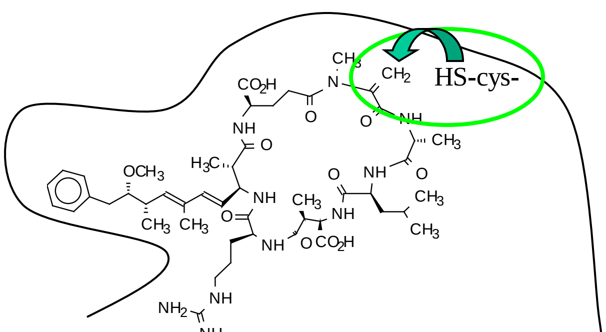
Cost: $120

- It can be bioactivity based (e.g. mouse bioassay, protein phosphatase inhibition assay for microcystins, Acetylcholinesterase activity for anatoxins)
- It can be structure based (e.g. antibody based kits)



Not all compounds behave the same in an assay



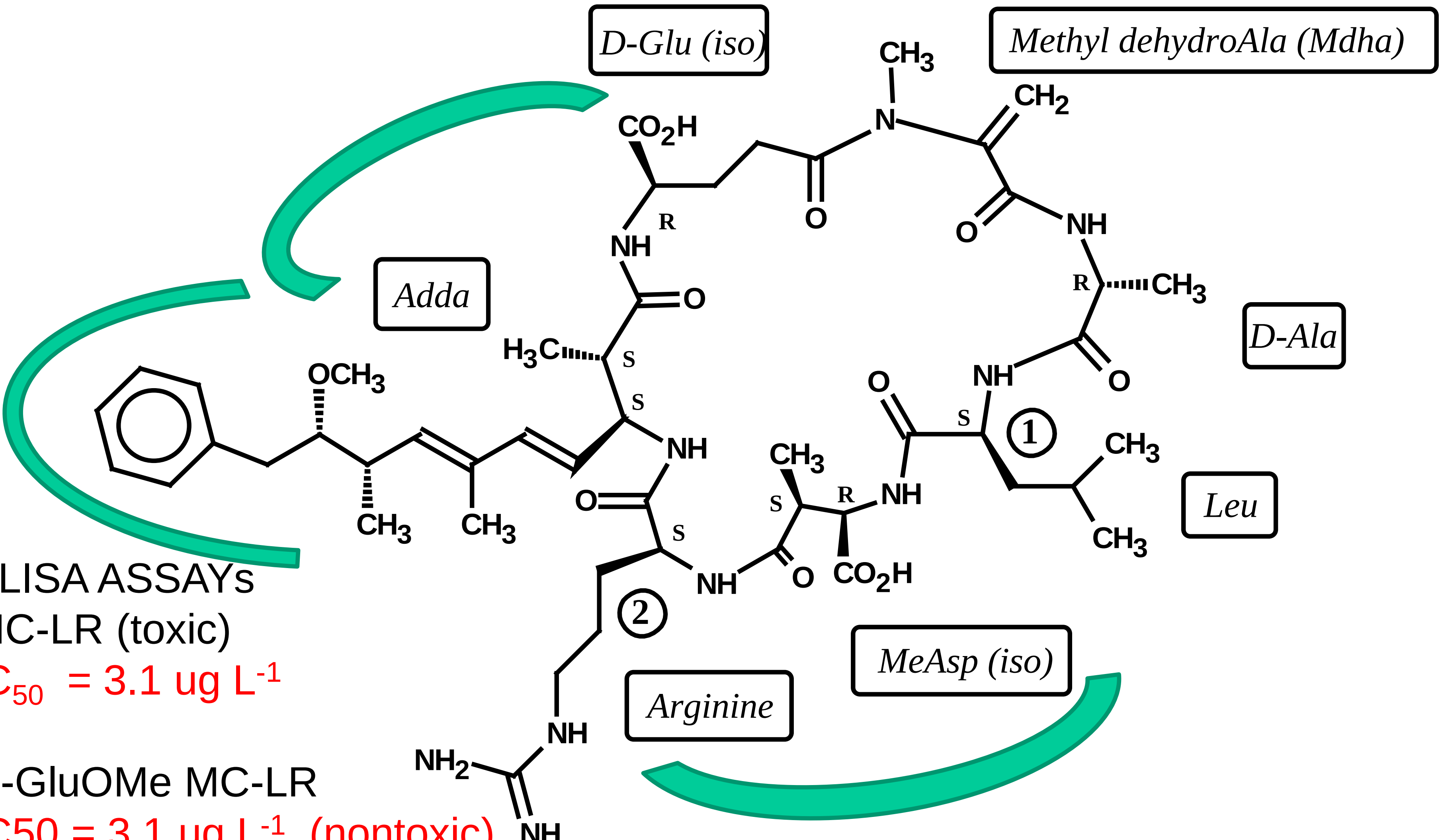


Smith et al 2010

<u>Protein Phosphatase Inhibition:</u>

MC-LR		0.3 ug L^{-1}
Dha7-MC-LR		5.0 ug L^{-1}
6(z) ADDA MC-LR	>	100 ug L^{-1}

Different antibodies give different results
Antibody response is not toxicity



ELISA ASSAYs

MC-LR (toxic)

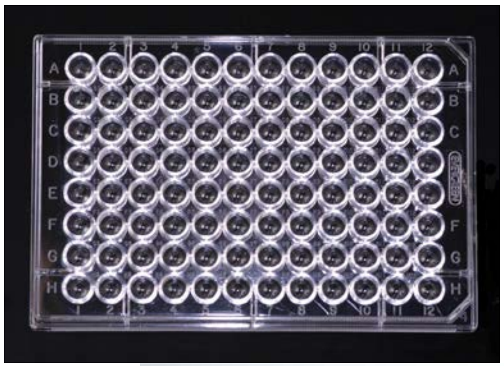
IC_{50} = 3.1 ug L^{-1}

D-GluOMe MC-LR

IC50 = 3.1 ug L^{-1} (nontoxic)

Antibody kits can be simple or complex

- Lab uses Microtiter plates to run “96” at a time (fast).



Add reagents and measure a change in color.

Compare to a known curve.

Cost: $5000+ 450 per plate

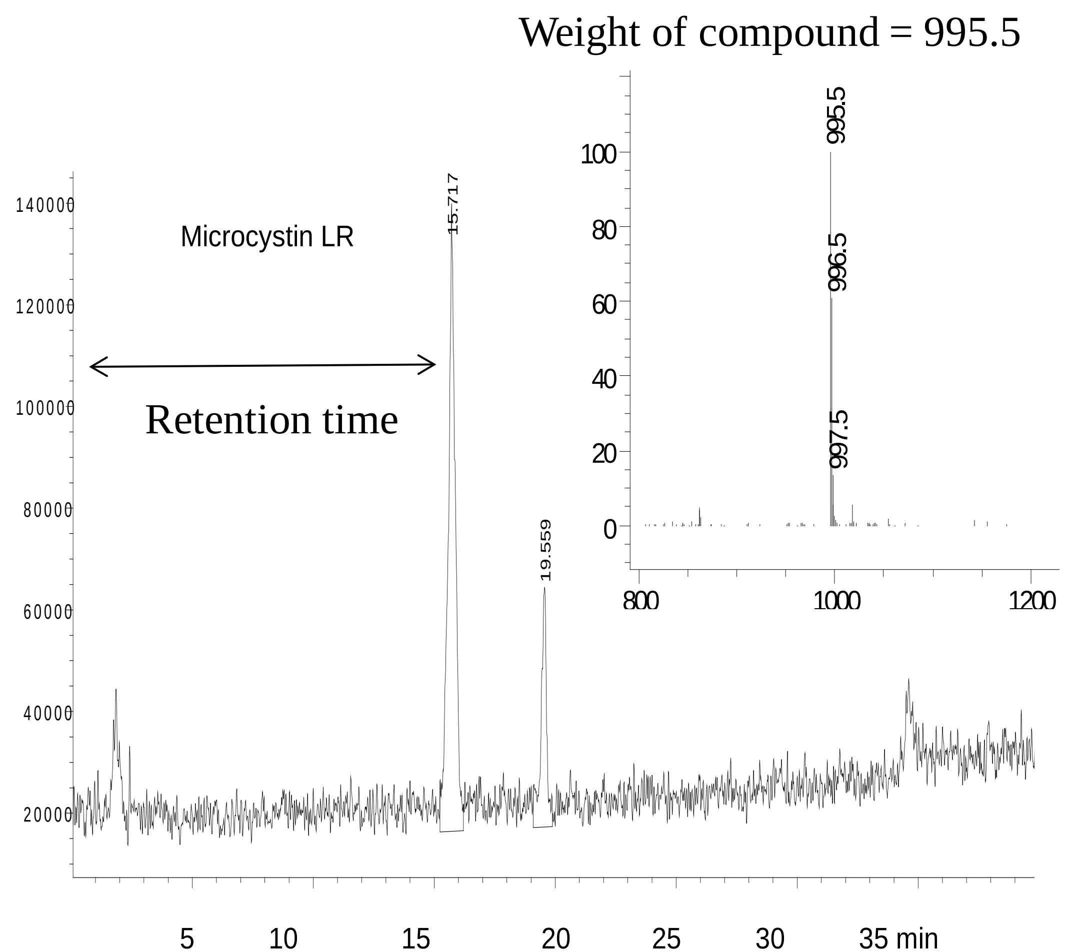
- Field kits use test strips to see if the antibody reacted with “something”.
- Lyse cell and look for an indicator line.

Envirologix
Abraxis

Cost: $20 per sample

In contrast: An analysis tests for 1 or more specific compounds

- It is almost always structure-based.
- Requires the use of standards for each compound.
- Needs expensive instrumentation
  - HPLC
  - LC-MS
  - LC-MS/MS
- These are lab-based tests



Cost: $150,000-$500,000
ESF: $150 per sample

Pros and Cons

Antibody Assays

- Less expensive to start
- Can be done in the field
- Give you an integrative value of all toxins.
- Don’t necessarily know what the antibody measured
- Many false pos/neg.
- Cannot be used for regulatory work

Instrumental Analysis

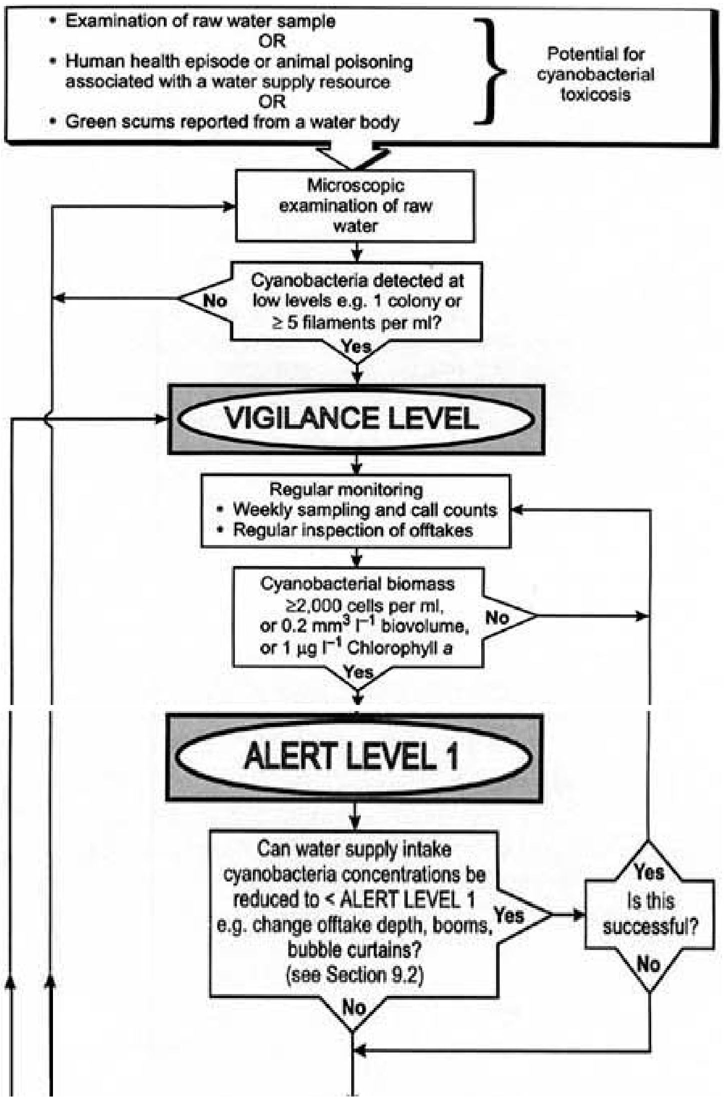
- More expensive to set up
- Requires standards so you only test for what you know
- Harder to do in the field
- Suitable for regulatory use
- Gives you exact compounds
- Can test for multiple toxins

My personal experience with field test strips is that the results are often inconclusive. New ones may be better.

Summary of chemical techniques (and cost):

Toxin	Assays	Analysis
Microcystins	mouse bioassay ELISA ($) Dipsticks ($) PPIA ($)	MALDI-TOF ($$$) HPLC-PDA ($) LC-MS ($$) LC-MS/MS ($$$)
Anatoxin-a	mouse bioassay (ELISA) (?) ACE assay ($)	HPLC-FD ($) LC-MS (/MS) ($$)
Cylindrosperm.	mouse bioassay ELISA ($)	HPLC-PDA ($) LC-MS ($$) LC-MS/MS ($$$)
Anatoxin-a(S)	mouse bioassay ACE assay ($)	LC-MS ($$)
PSP toxins	mouse bioassay ELISA ($) Receptor-binding ($$)	HPLC-FD ($) LC-MS ($$) LC-MS/MS ($$$)
BMAA	none	HPLC-FD ($) LC-MS (/MS) ($$)

Tier-based monitoring strategies



World Health Organization decision tree:

- Initial examination
- Vigilance level
- Alert level 1
- Alert level 2
- Public response

From WHO blue book
Chorus and Bartram, 1999

Summary

- Cyanobacteria produce a number of toxins but not all species are toxic.
- These toxins can be produced by a number of different species making visual monitoring difficult.
  – BUT: NO CYANOBACTERIA NO TOXINS
- There are many tools available to monitor for toxins
  – Visual exam to identify potentially toxic genera or species
  – Molecular techniques to look for toxin biosynthetic genes
  – Chemical assays to detect individual classes of toxins
  – Chemical analysis to detect specific toxins
- You need to choose the correct tool for the job.
  – Different tests look at different things => may not agree.
- Lake Associations need to decide why they want a monitoring program to choose the correct approach.