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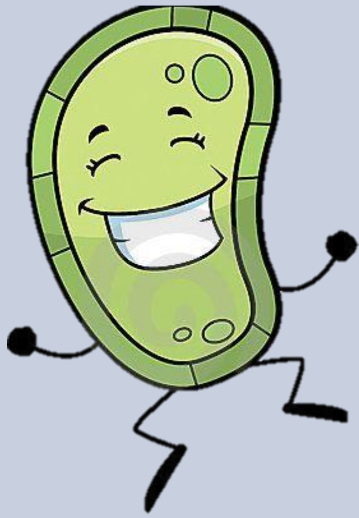
Water and Community: A Public Forum on HABs

Testing for Toxins
Assessing Whether a Cyanobacterial Bloom is Harmful or Not

Co-Sponsored by Cayuga Lake Watershed Network and Cayuga Lake Floating Classroom

Fun facts about “blue-green algae”

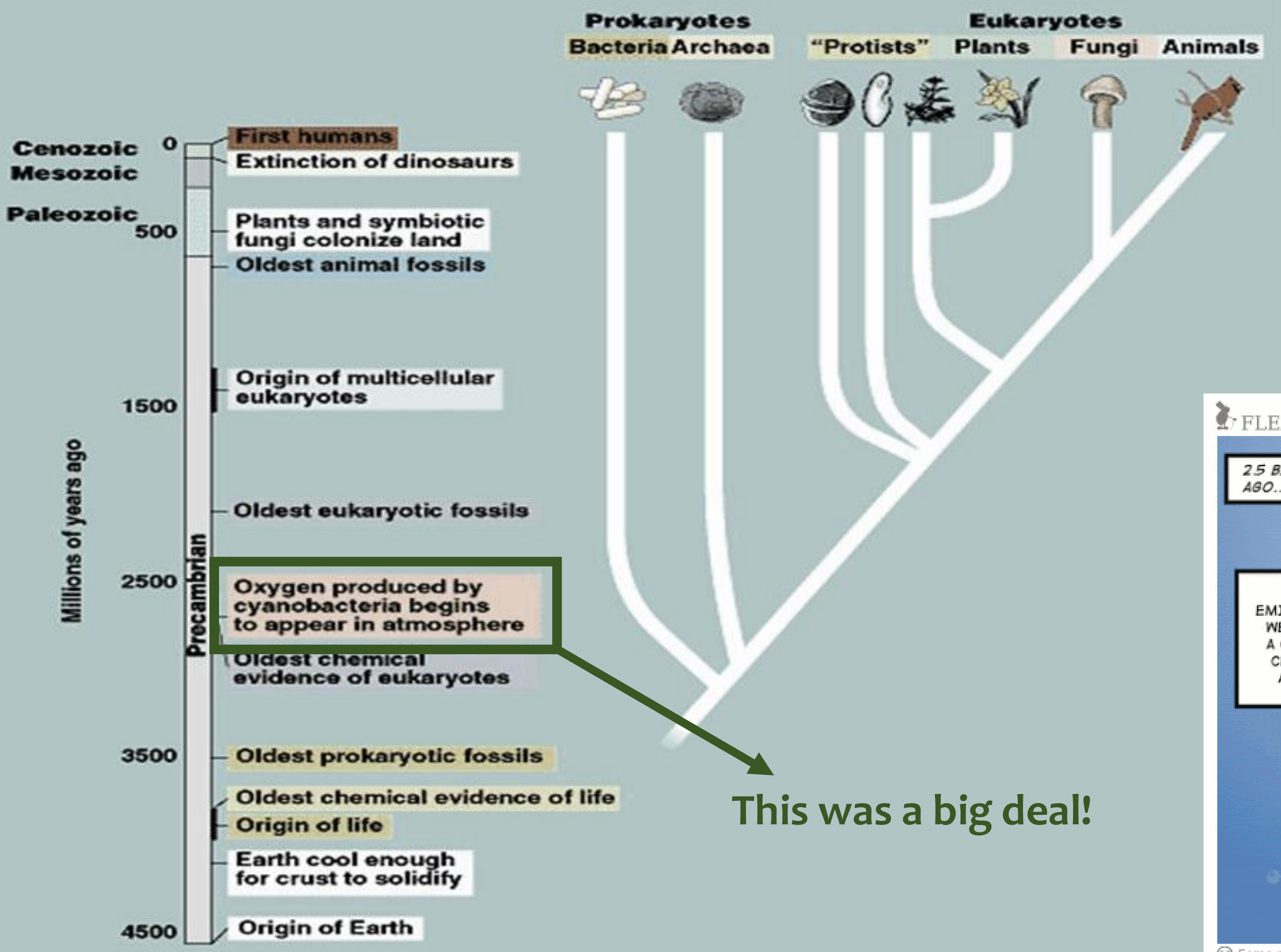


Scientists at first mistook cyanobacteria for algae and called them “blue-green algae” because of their color. The name stuck. But really they are gram-negative bacteria, a large group that includes E. coli.

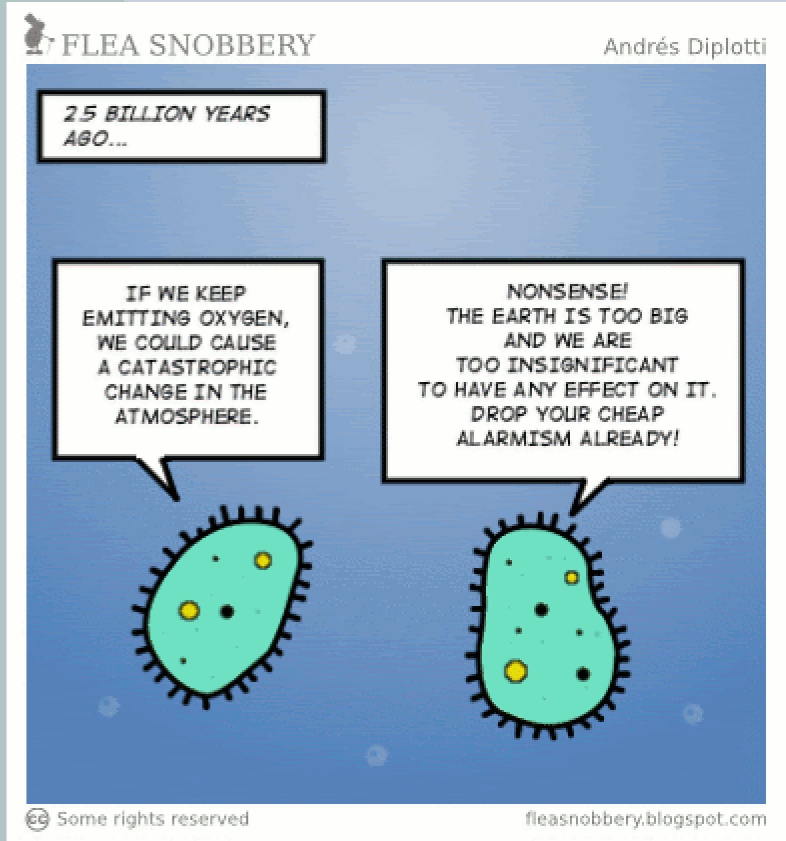
Cyanobacteria have been around for close to 3 billion of the Earth’s 4.5 billion years. They are the only group of bacteria that perform photosynthesis by splitting water and creating oxygen, O_2, as a by-product.

There are hundreds of species of cyanobacteria, and they are everywhere – in the oceans, in freshwater lakes, and in soil.

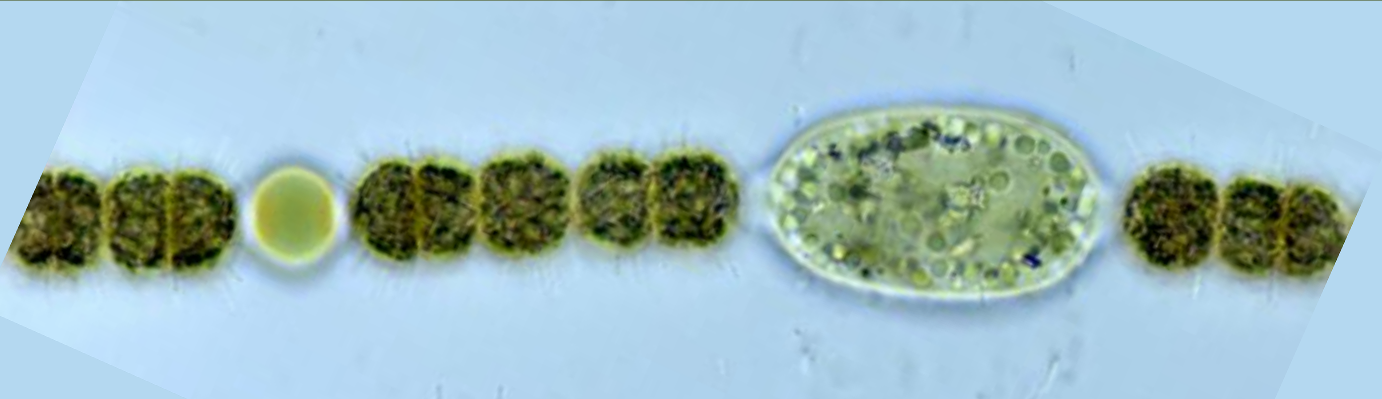
From about 2.5 to 0.5 billion years ago, the oxygen “waste” from the photosynthetic activity of cyanobacteria built up to about 20% of the Earth’s atmosphere, paving the way for higher life forms -- like us.



This was a big deal!



"Blue-green algae" have learned a thing or two about thriving on planet Earth in the 3 billion or so years they've been around:



- They are **autotrophs** (like plants) that make their organic carbon from carbon dioxide (CO2) in the air using photosynthesis.
- Many species "**fix**" nitrogen (N_2) in the air into chemical forms that they can use as nutrients such as nitrate (NO_3) and amino acids.
- They have **gas vacuoles** that they use like swim bladders to move up and down in the water column

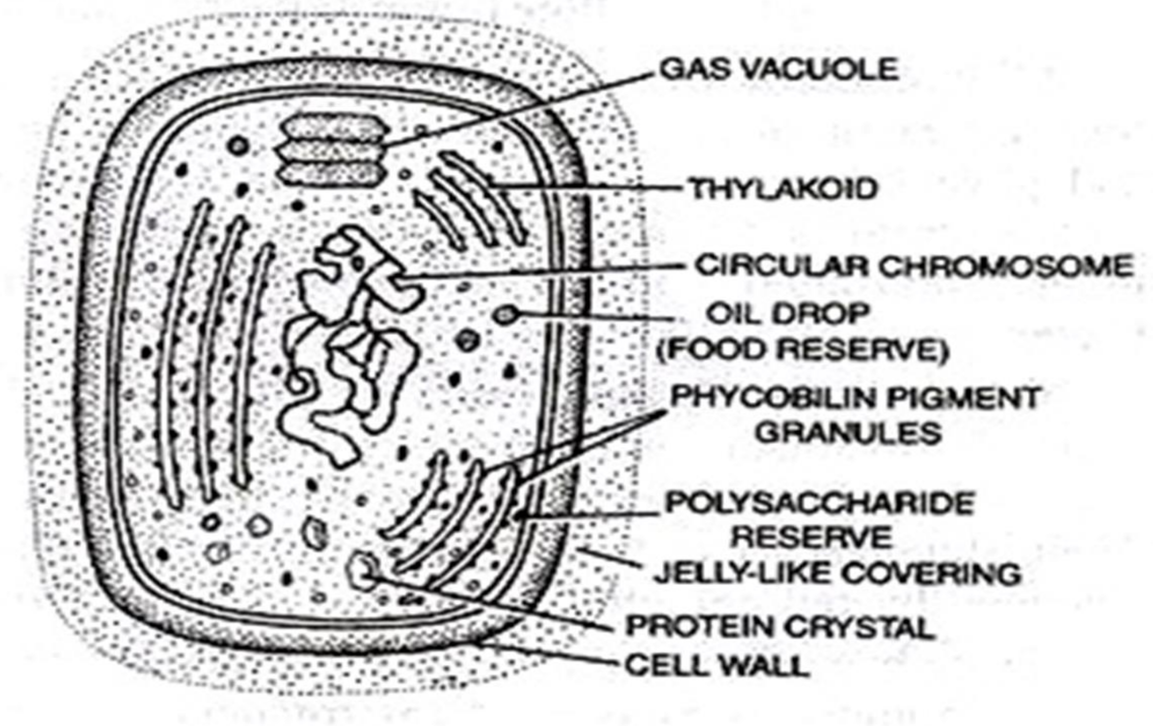
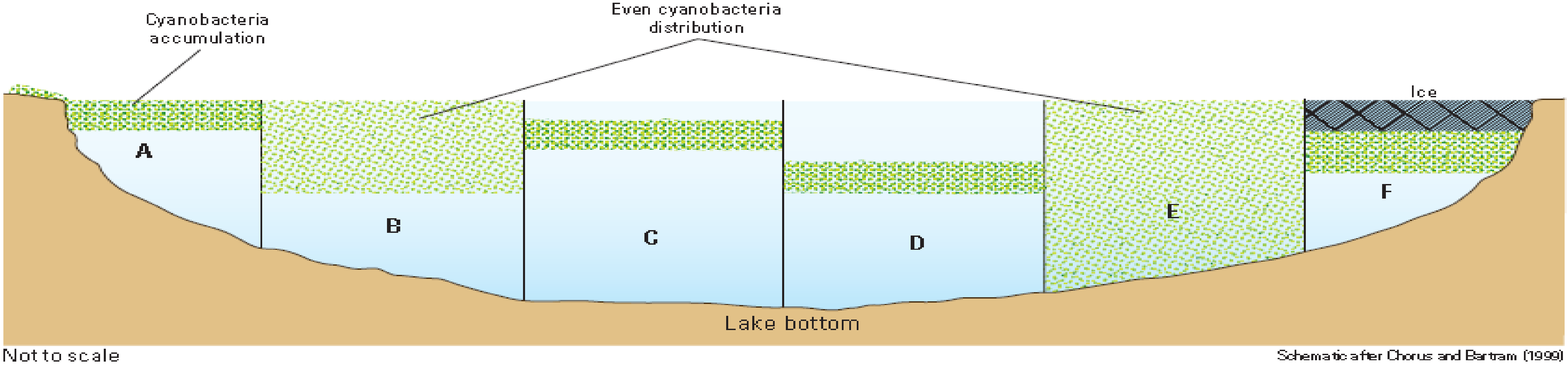


Fig. 2.18. Ultrastructure of a cyanophycean cell.

Cyanobacteria can use their gas vacuoles to distribute themselves in the water column



EXPLANATION

Potential water column distributions of cyanobacteria

- **A** Shoreline, near-shore, and open water accumulations and scums
- **B** Even distribution throughout the photic zone or epilimnion
- **C** Specific depth in the photic zone
- **D** Metalimnetic bloom (special case of **C**)
- **E** Even distribution throughout the water column
- **F** Under ice bloom

Figure 6. Potential water column distributions of cyanobacteria.

New research indicates cyanobacteria produce many chemical compounds with potential uses in medicine

Cyanobacteria as a group produce a vast array of chemicals as part of their normal physiological processes and metabolism.

Natural products from cyanobacteria are under active investigation in the lab (tissue culture and animal models) as starting points for developing new drugs.

A review published in 2011 described:

- Ten (10) chemicals with **anti-cancer** activity
- Five (5) chemicals with **antiviral** activity
- Eight (8) chemicals with **antibacterial** activity
- Five (5) chemicals with **antiprotozoal** activity
- Two (2) chemicals with **immunomodulatory** activity

A few of these chemical compounds have advanced to clinical trials so far.

Cyanobacteria also produce chemicals that unfortunately happen to target fundamental molecular biological processes

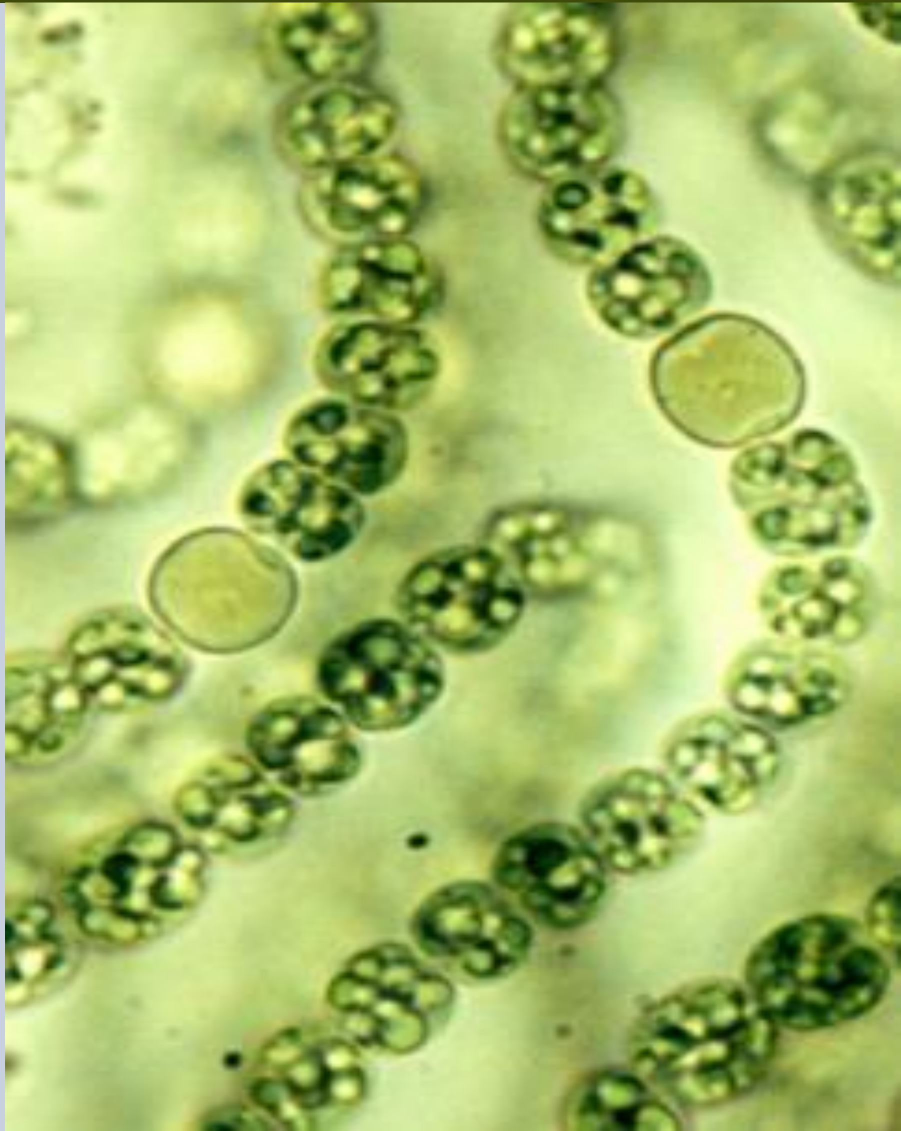
Liver Toxins	Microcystis Cylindrospermopsins Nodularins	**Acute toxicity:** Internal bleeding, shock, death **Mechanism:** Interfere with protein dephosphorylation **Chronic toxicity:** Potential carcinogens, tumor promotors
Nerve Toxins	Anatoxins Saxitoxins (Paralytic Shell Fish Poisins) Neosaxitoxins BMAA	**Acute toxicity:** Seizure, paralysis, respiratory failure, death **Mechanism:** Block nerves from transmitting signals; BMAA under investigation **Chronic toxicity:** BMAA only – Possible link to neurodegenerative diseases (ALS, Parkinson's, Alzheimer's)
Skin Irritants	Lipopolysaccharides Lyngbyatoxin-a aplysiatoxins	**Acute toxicity:** Skin rashes, intestinal upset **Mechanism:** : Research suggests less toxicity with cyanobacteria than other gram-negative bacteria **Chronic toxicity:** Tumor Promotors
Taste and Odor Compounds	Geosmin 2-methylisoborneol	**Smells and tastes bad**

Most Suspicious Blooms Are Not HABs

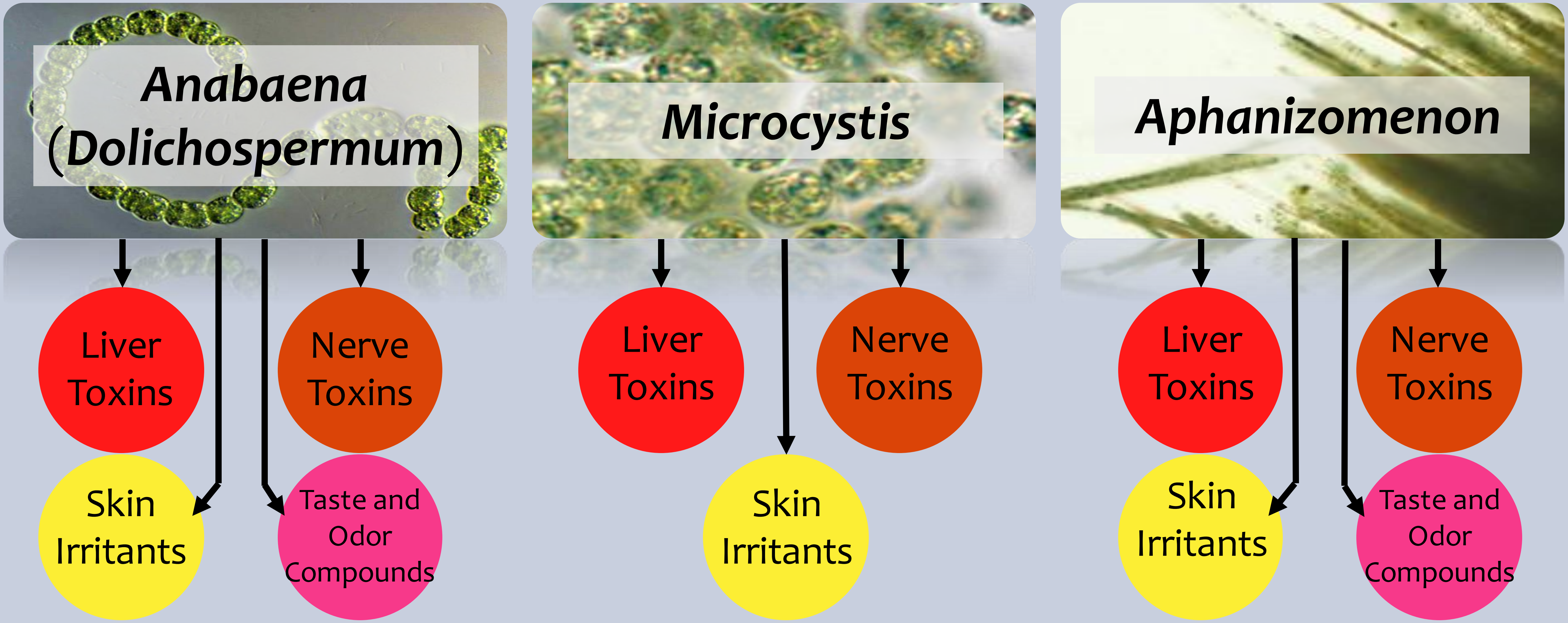
Managing public health risks from Harmful Algal Blooms (HABs) is complicated due to several factors:

- Cyanobacterial blooms may be confused with blooms of regular algae
- In many cases, cyanobacterial blooms do not produce harmful toxins
- A cyanobacterial bloom may be non-toxic initially, then start producing toxin, presumably as the result of an (unknown) environmental “trigger”

The challenge for public health officials is identifying which blooms are cyanobacteria and which of those produce toxins



Each genus of cyanobacteria is capable of producing more than one class of toxin



Specific toxins can’t be predicted on the basis of genus!

Toxins can persist in the water after a bloom has disappeared

Toxins are produced inside cyanobacterial cells, released into the water as cells die, and degraded in the environment within hours or days, depending on the particular toxin and local environment. Some toxin may also be released by living cells.

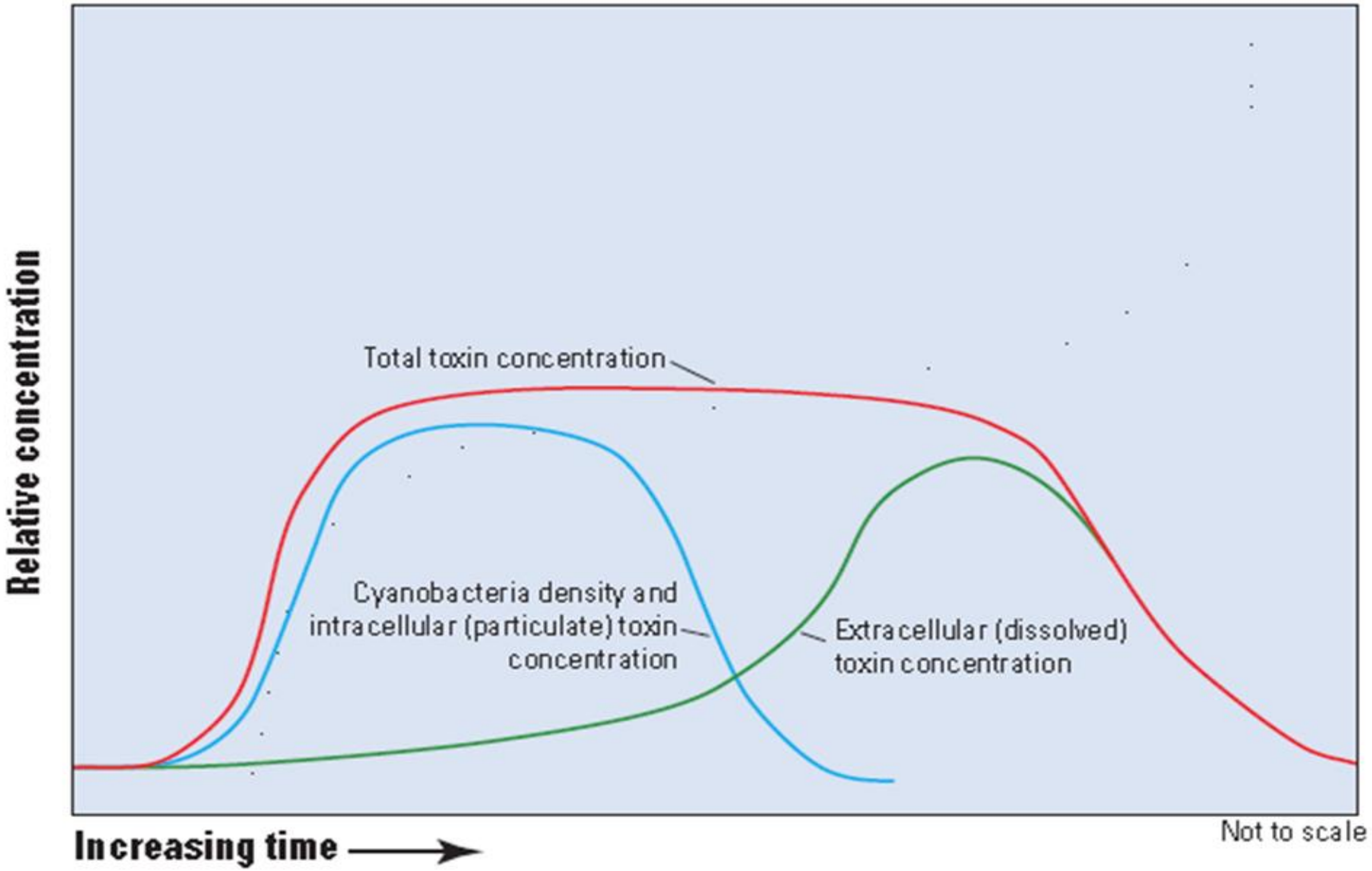
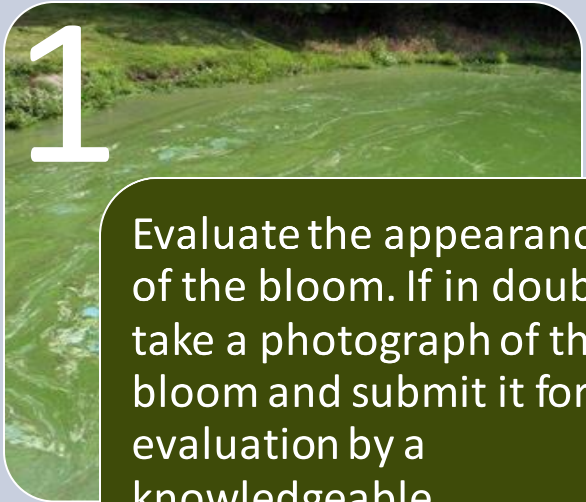


Figure 4. Theoretical temporal distribution of total, particulate, and dissolved cyanobacterial toxin concentrations with respect to cyanobacterial population density.

NYSDEC identifies a toxin-producing bloom in three steps

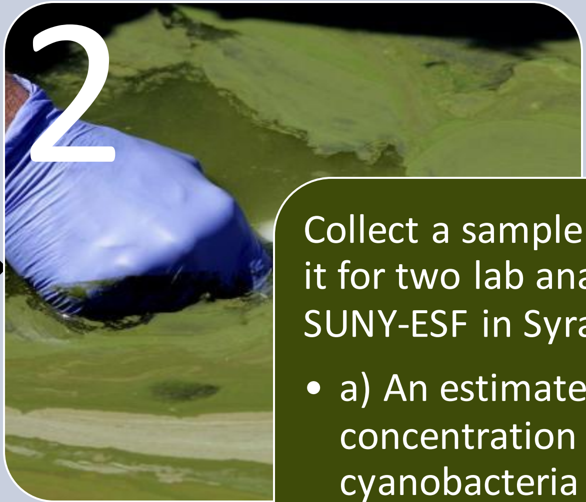
1



Evaluate the appearance of the bloom. If in doubt, take a photograph of the bloom and submit it for evaluation by a knowledgeable professional at the DEC.

If bloom is judged "suspicious," i.e., likely to be cyanobacteria, then:

2



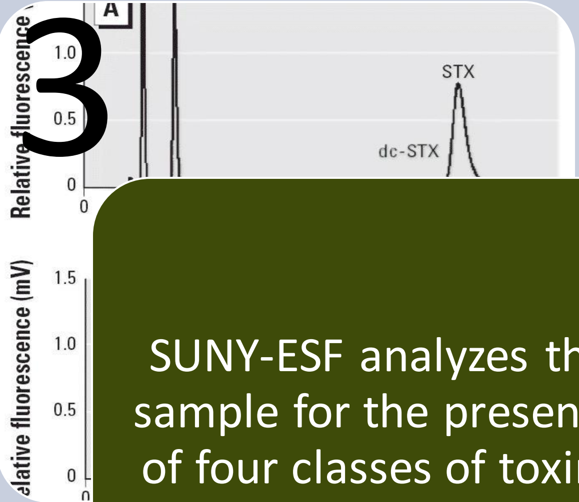
Collect a sample and submit it for two lab analyses at SUNY-ESF in Syracuse:

- a) An estimate of the concentration of cyanobacteria in the sample based on the fluorescence of blue-green (BG) chlorophyll a; and
- b) An examination of the sample under the microscope (~200x magnification) to identify specific cyanobacteria taxa.

If BG chlorophyll a <25 ug/L and/or cyanobacteria taxa do not dominate in the sample, then "**No Bloom.**"

If BG chlorophyll a >25 ug/L and/or cyanobacteria taxa dominate, then:

3



SUNY-ESF analyzes the sample for the presence of four classes of toxins that could pose risks to public health: **Microcystins, anatoxins, cylindrospermopsins, BMAA**

NYSDEC identifies a toxin-producing bloom in three steps (cont'd)

Step 3 (cont'd):

- SUNY-ESF uses a combination of liquid chromatography and mass spectrometry (LC-MS/MS) to analyze confirmed cyanobacterial bloom samples for:

  - Fourteen (14) structural variants of microcystins (most common toxin found in blooms)

  - Several structural variants of anatoxin-a (found only rarely)

  - Cylindrospermopsin (not yet found in New York)

  - BMAA (not yet found in New York)

NYSDEC Classification of confirmed blooms is based on *microcystin* levels

- Classification "Confirmed Bloom:"
  - Combined microcystins <10 ug/L in open water, <20 ug/L near shoreline

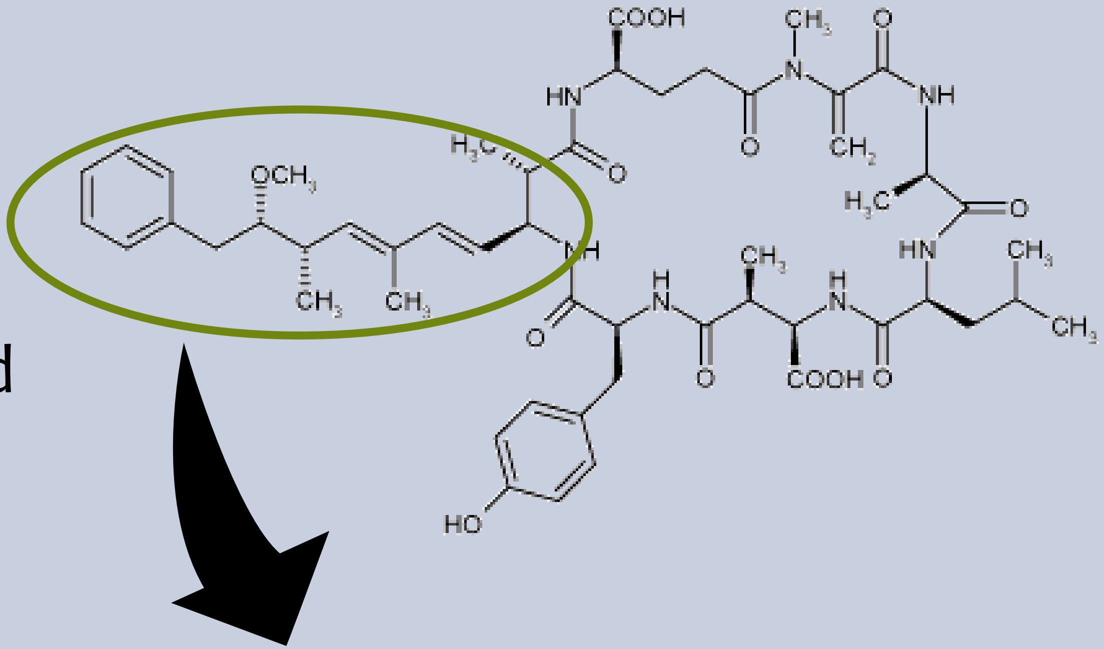
- Classification "Confirmed With High Toxins Bloom:"
  - Combined microcystins >10 ug/L in open water, >20 ug/L near shoreline

- NYSDEC classification is **advisory** and is based on World Health Organization guidelines.

- NYSDEC classification is **non-regulatory**, meaning it is not used to regulate microcystin levels in drinking water or regulated swimming beaches

A Closer Look at Microcystins and How To Test For Them

- Microcystin is a cyclic peptide molecule, i.e., a series of seven (7) amino acids arranged in a circular structure
- There are over 80 structural variants of microcystin
- Most microcystins contain a unique amino acid referred to as "ADDA"
  - One test for microcystins, called an ELISA, is based on an antibody that reacts specifically with the unique ADDA structure
- Microcystins exert their toxicity by blocking liver enzymes from dephosphorylating proteins, thereby poisoning liver function
  - A second test for microcystins, called PPI (protein phosphatase inhibition) measures the inhibition of enzymes that remove phosphate from proteins



ADDA binds to phosphate containing enzymes

NYSDOH uses the ELISA test to regulate microcystin levels in drinking water and at swimming beaches

- NYSDOH approaches HABs from a strictly **regulatory** perspective
- NYSDOH uses microcystin levels to protect the public from HABs
- The NYSDOH's Wadsworth Lab in Albany uses the antibody-based ELISA test, approved by EPA in 2016 and referred to as Method 546, to measure microcystin levels
  - The Wadsworth Lab is the only lab in New York that performs this test
  - NYSDOH does not currently offer Method 546 to commercial labs for certification
- **The Maximum Contaminant Level (MCL) for drinking water is 0.3 ug/L**
- **The allowed level for swimming areas may soon be set at 4 ug/L based on recent EPA guidance**

In Conclusion

Cyanobacteria are an ancient and incredibly diverse group of organisms

Cyanobacteria produce a variety of chemical compounds that happen to be harmful to humans; the most common in New York is microcystin

By inventing water-based photosynthesis that generates oxygen as a by-product, cyanobateria are indirectly responsible for higher forms of life on earth

NYSDOH uses microcystin testing to protect the public from exposure to HABs toxins in drinking water and at swimming beaches

NYSDEC uses testing for microcystins, anatoxins, cylindrospermopsins and BMAA to monitor cyanobacterial blooms

Acknowledgments

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