Understanding Cyanobacterial Ecological Strategies

Barry H. Rosen, Ph. D.

Office of the Southeast Regional Director

(CFLWSC) Orlando, FL

brosen@usgs.gov

407-803-5508

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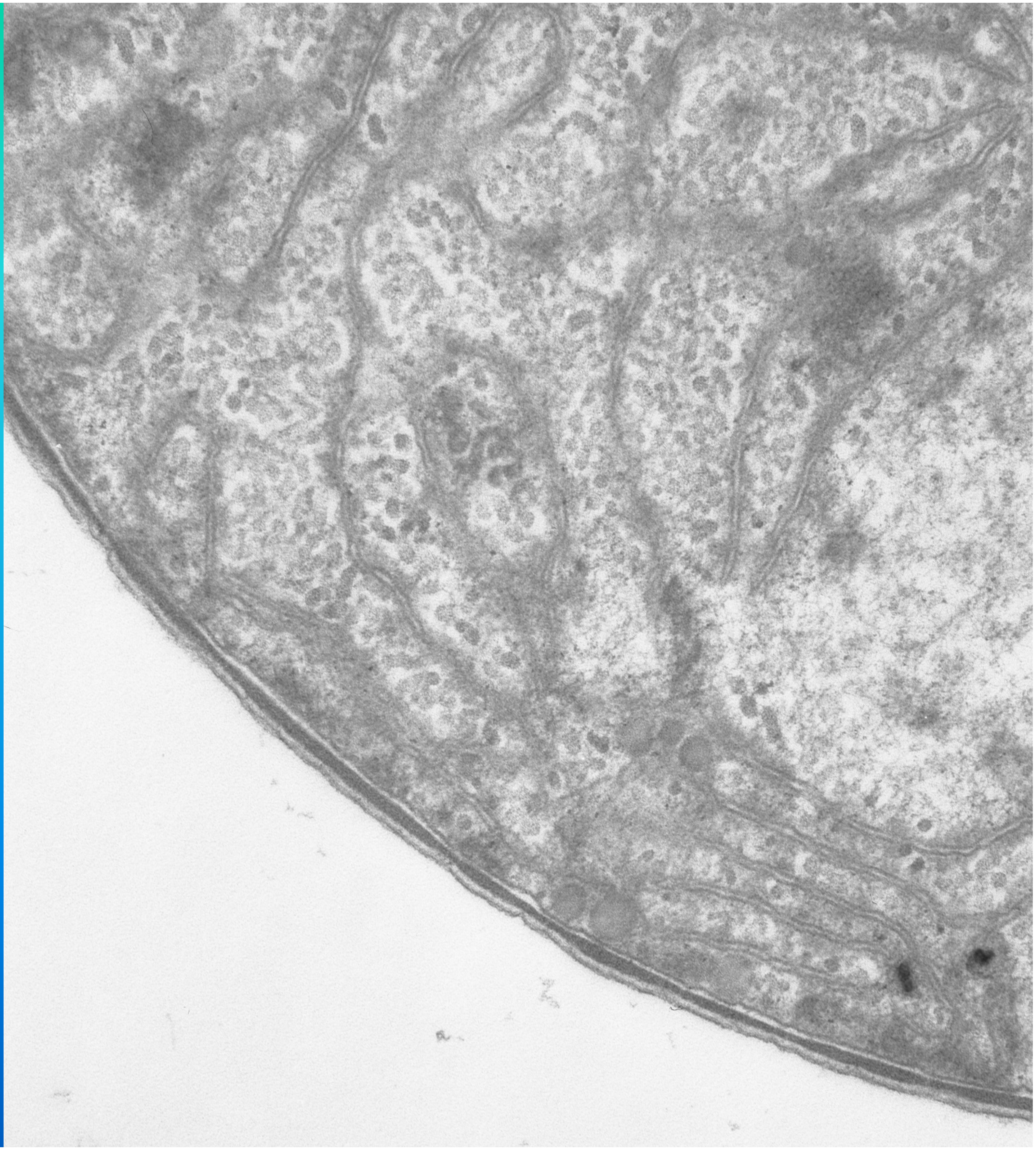
Cyanobacteria

(aka blue-green algae; cyanoHABs)

- gram negative bacteria
- pigments in thylakoids



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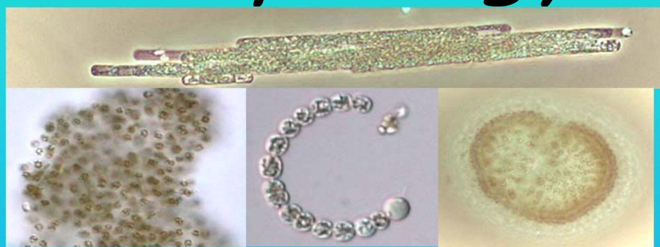
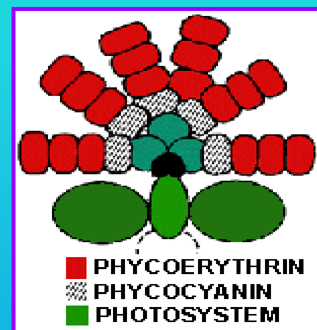
Ecological strategies for cyanobacteria: a sample

Morphology



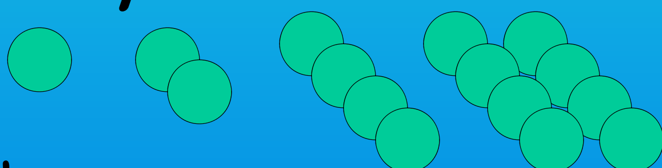
grazing, floating

Pigments



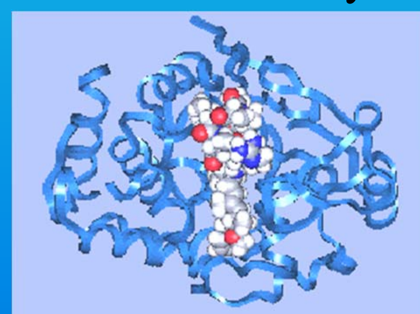
Buoyancy Regulation

Rapid Growth



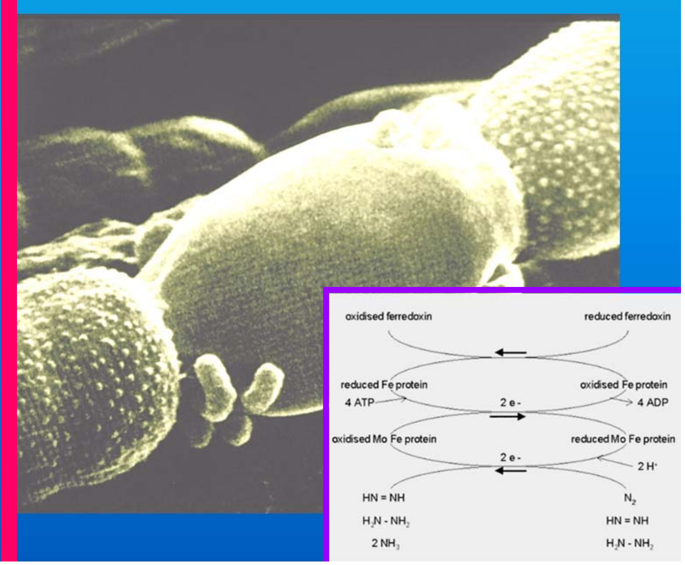
temp

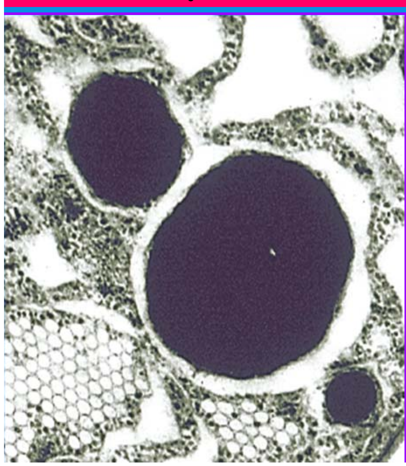
Toxicity



microcystin
LR complex

Nitrogen Fixation





trace, P,
C, N,

Nutrient Storage

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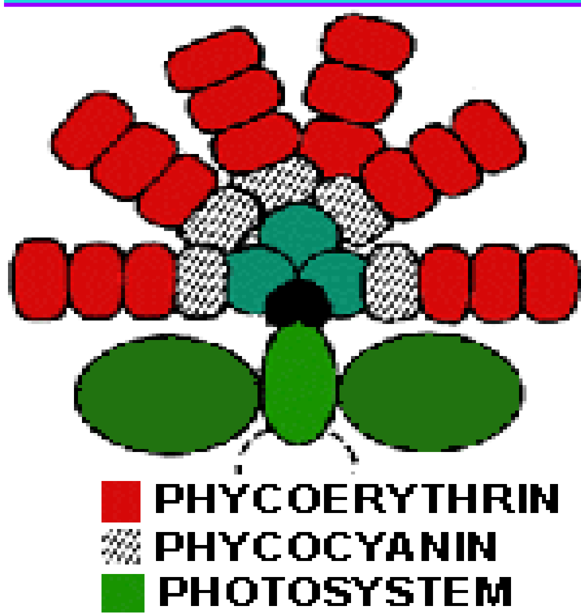
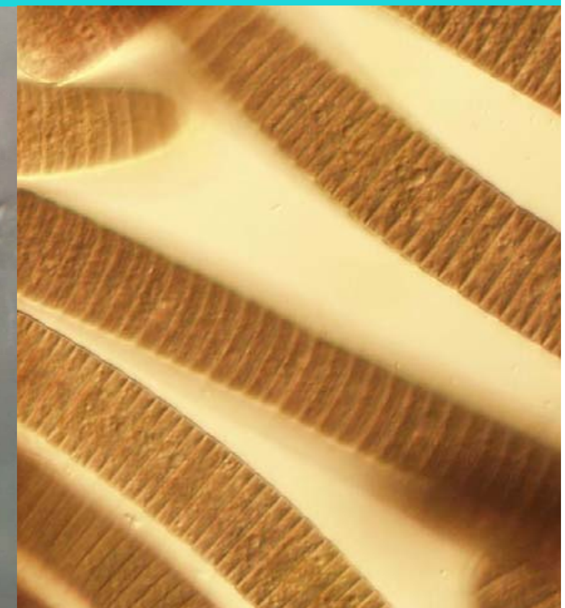
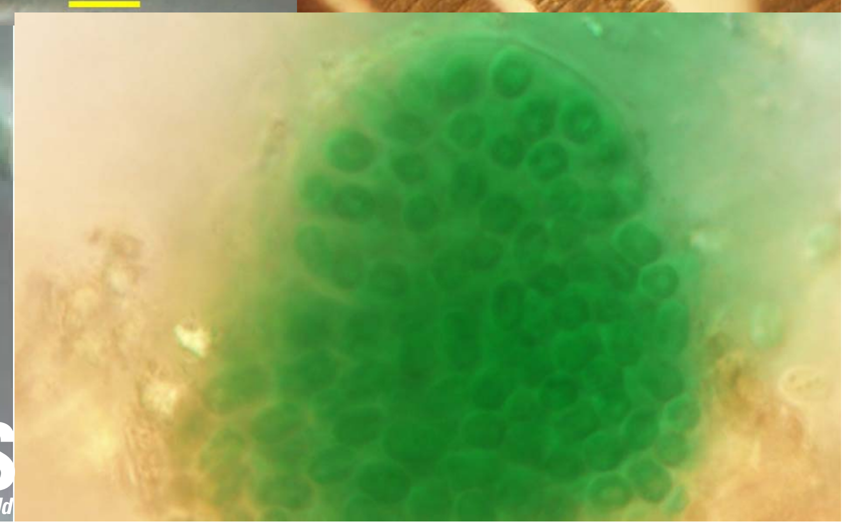
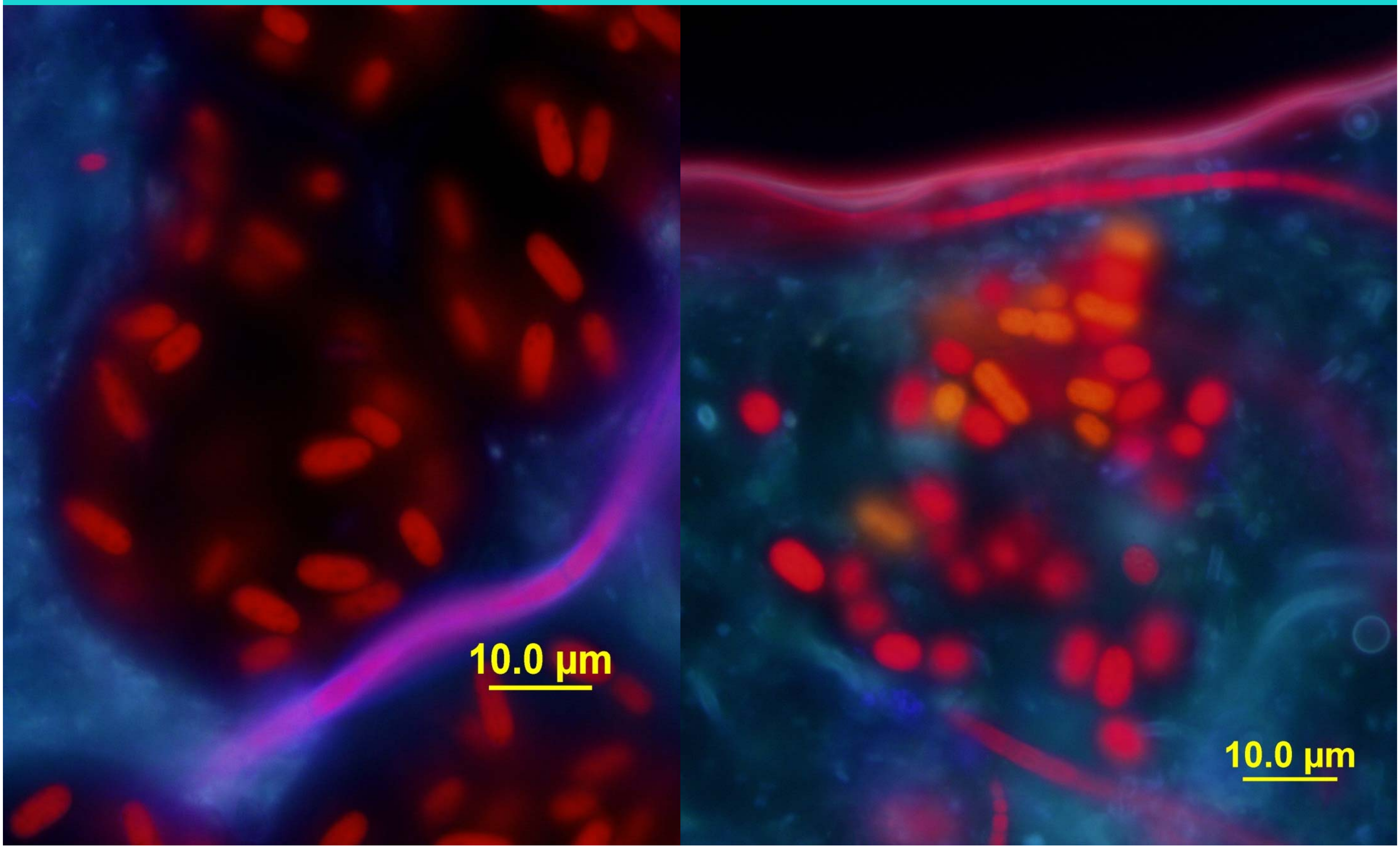
Ecological Strategies: complimentary pigments for maximizing photosynthesis







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

$(C_6H_{12}O_6)n$

Nutrients scavenged whilst near lake sediments or thermocline

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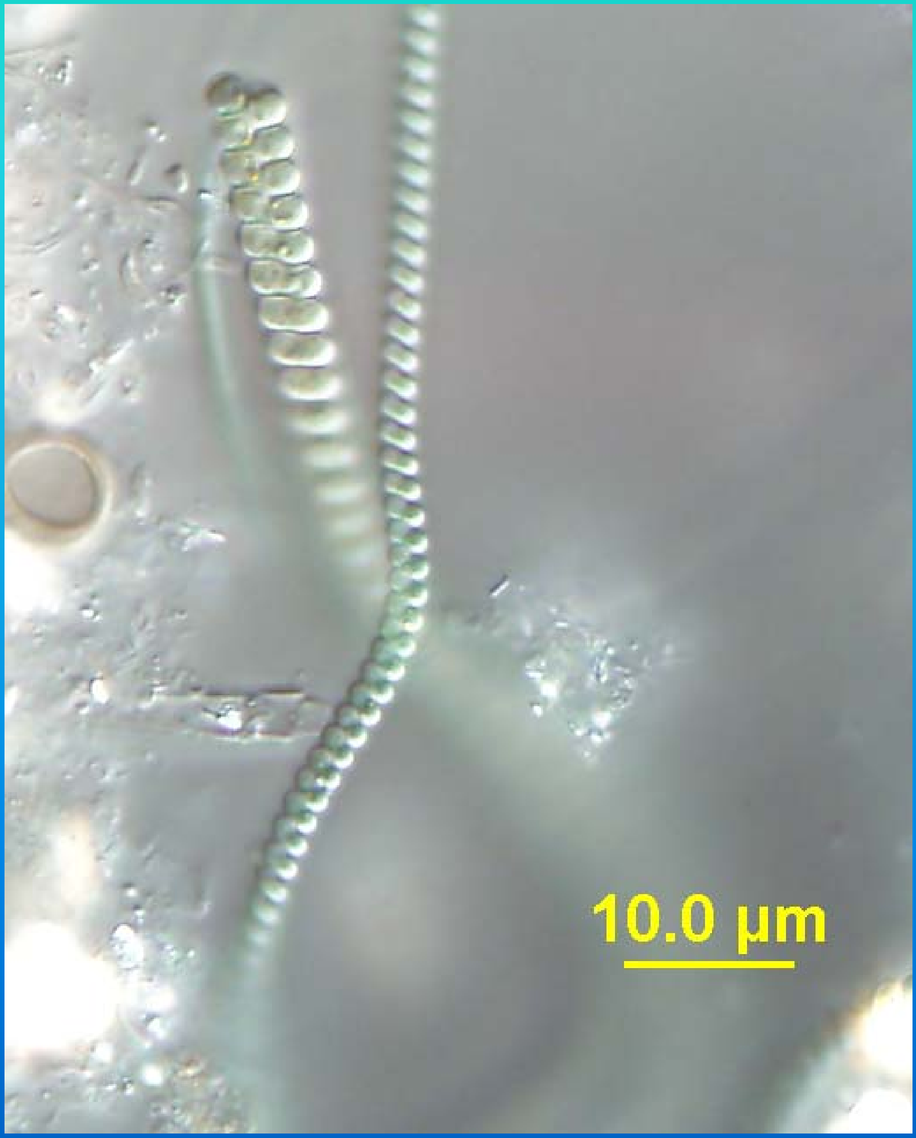
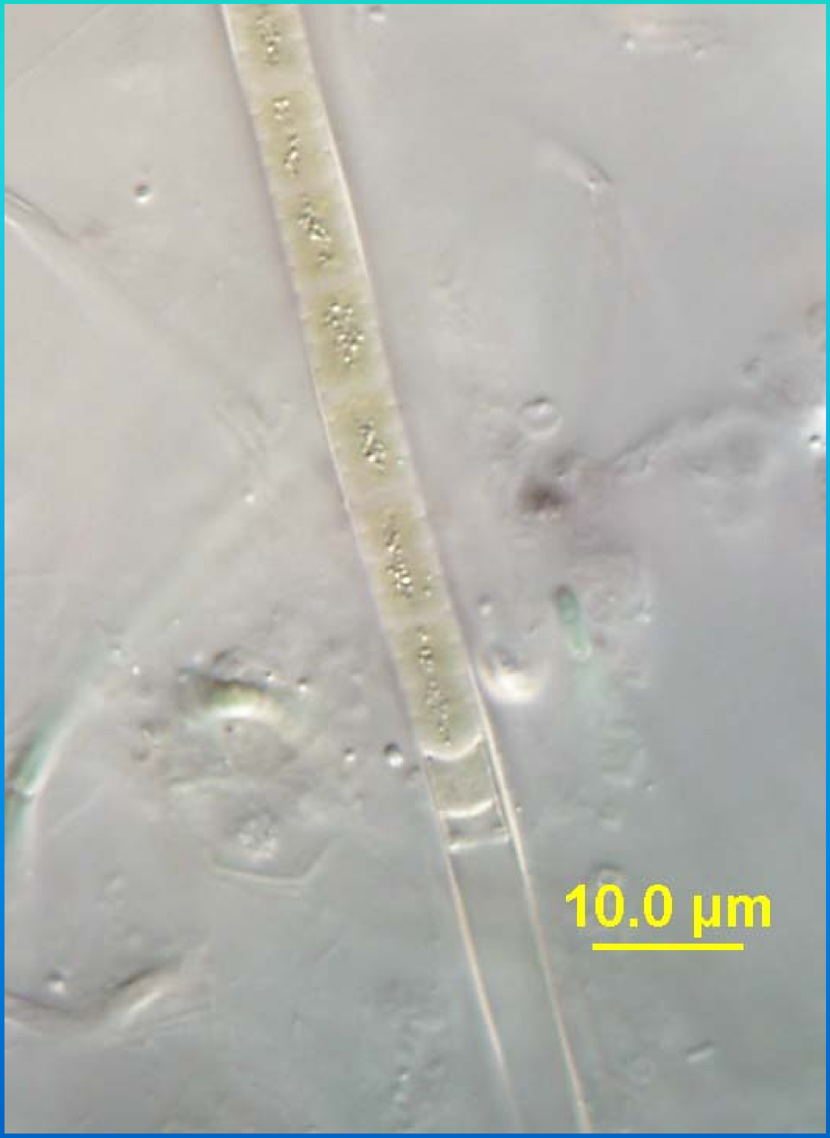
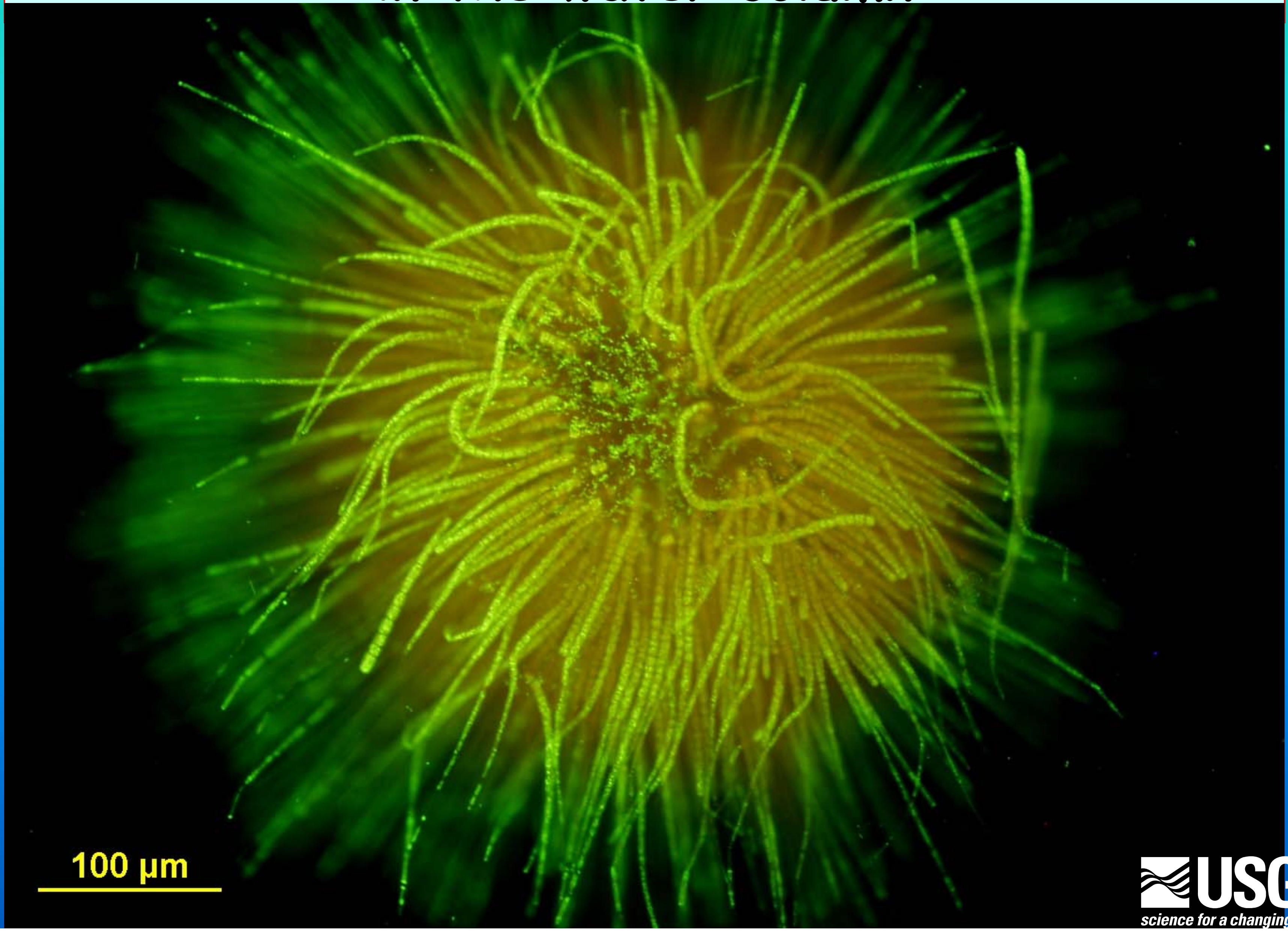
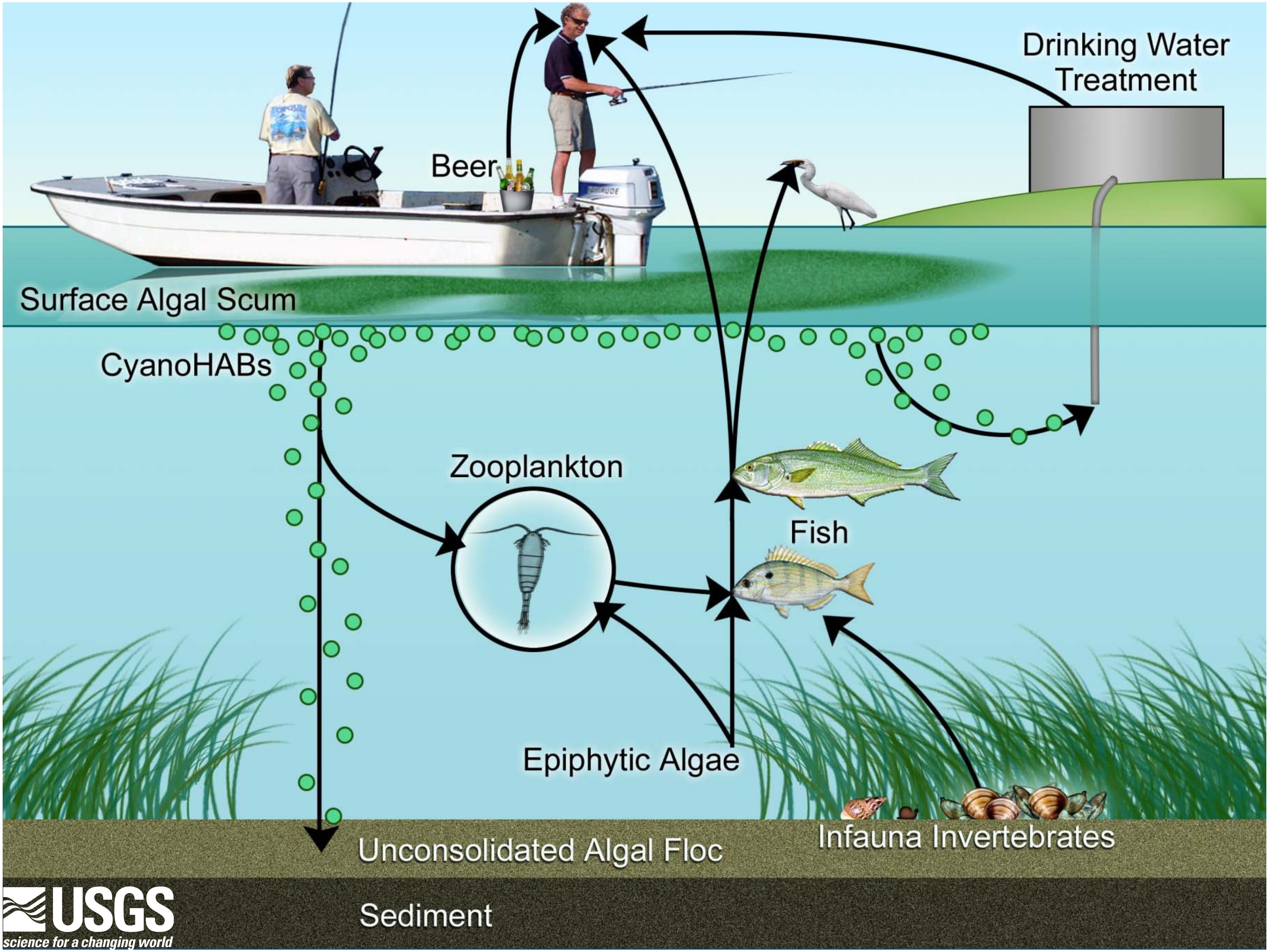
Ecological Strategies: Motility in sediments





Ecological Strategies: morphology for staying in the water column




Drinking Water Treatment
Beer
Surface Algal Scum
CyanoHABs
Zooplankton
Fish
Epiphytic Algae
Unconsolidated Algal Floc
Infauna Invertebrates
Sediment
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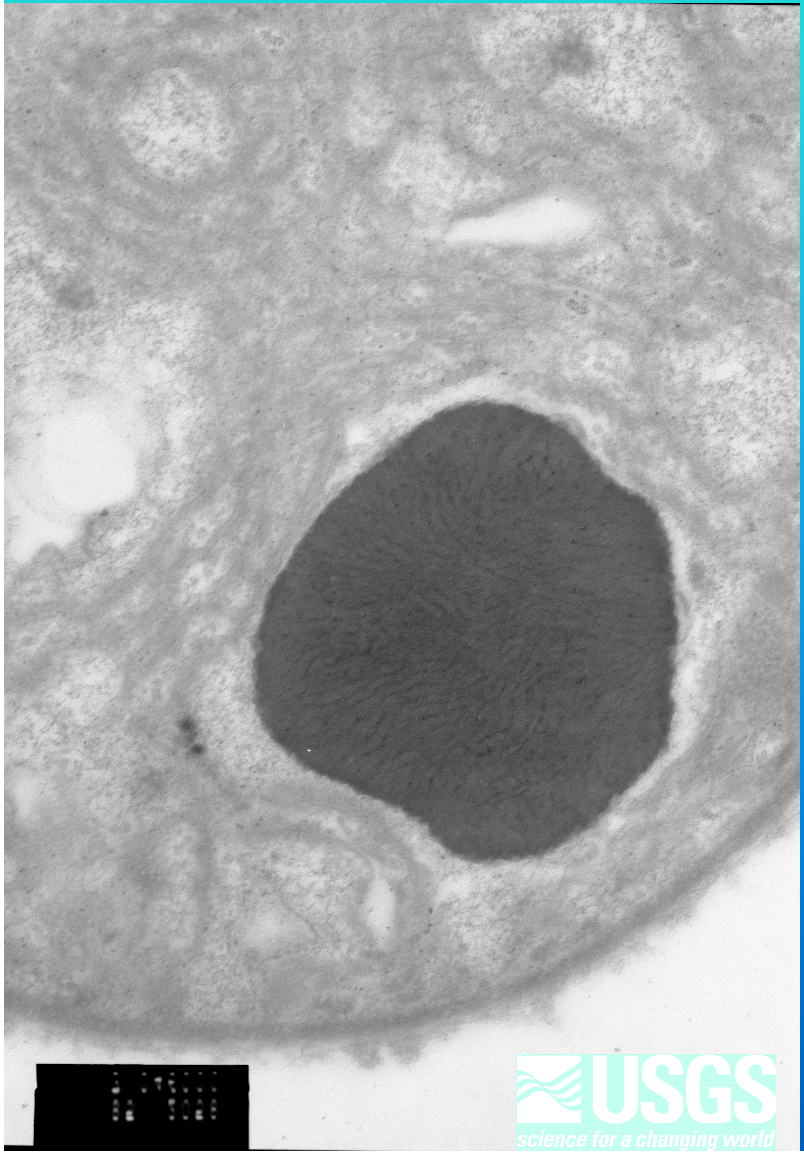
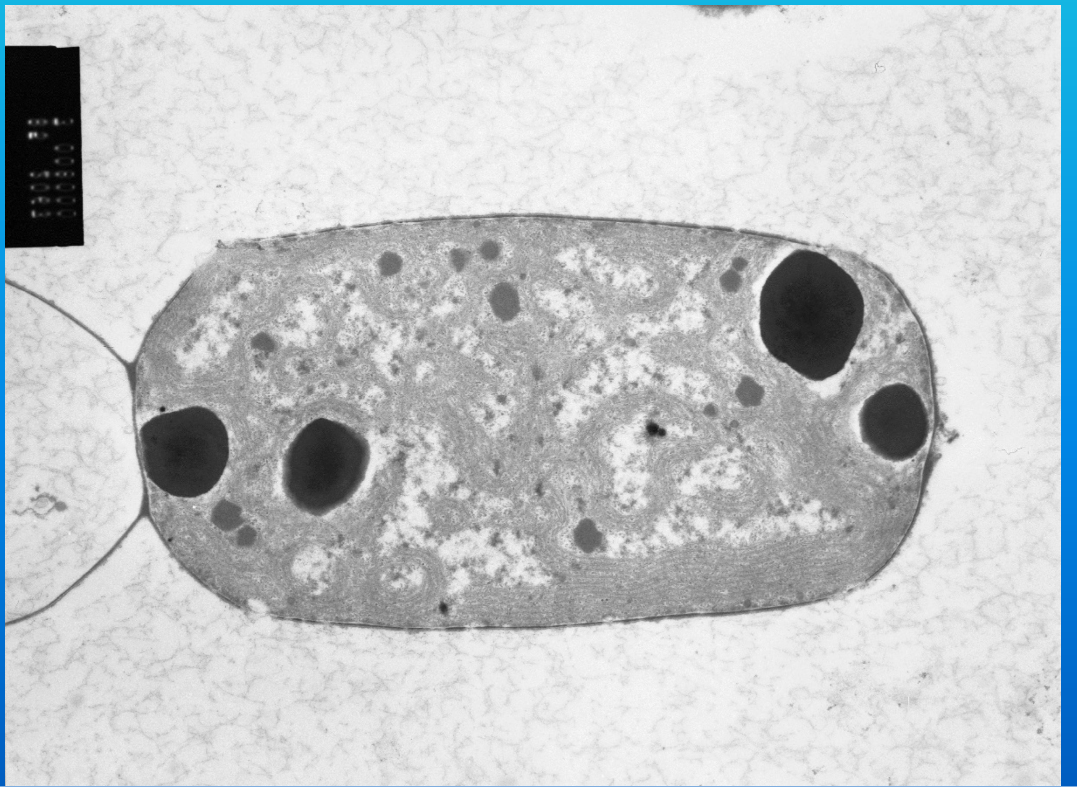
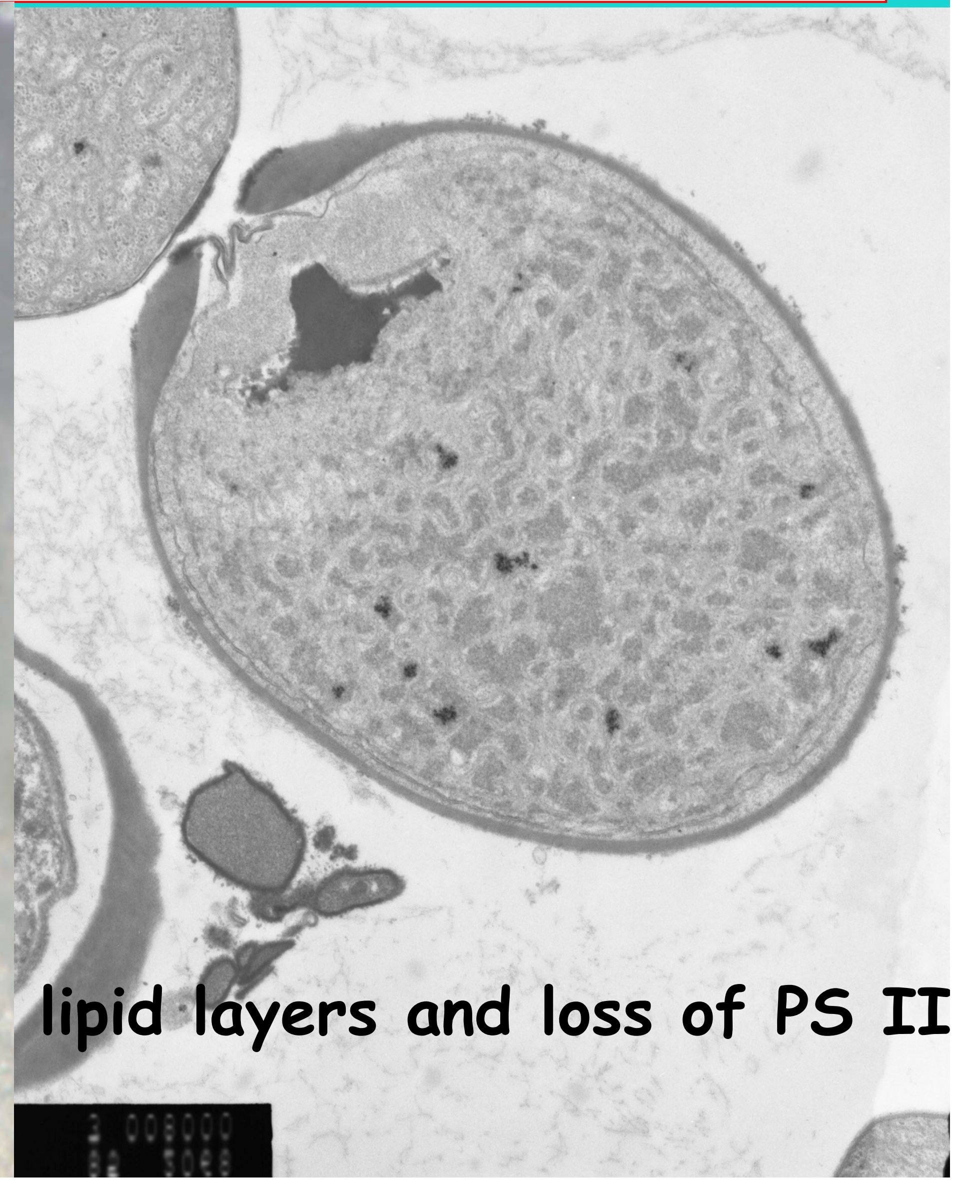
Ecological Strategies: luxuriant nutrient uptake and storage & metal sequestration



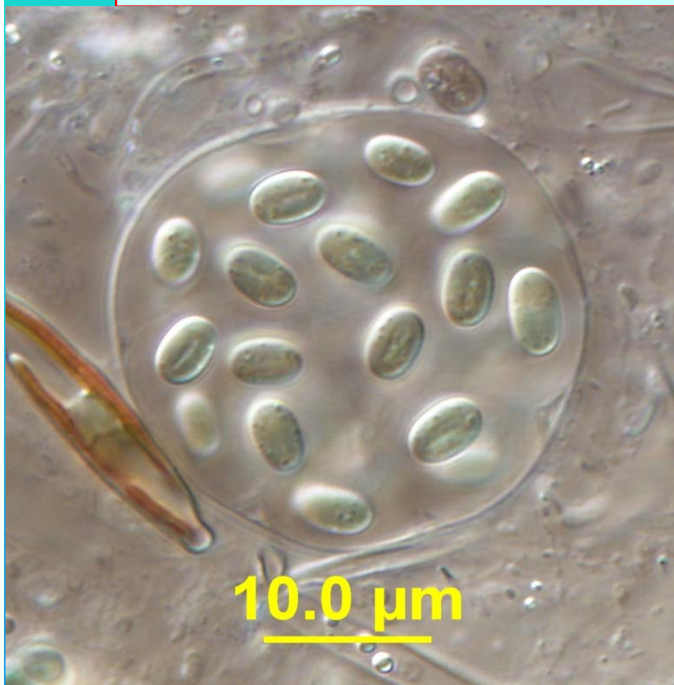
- Contain protein, lipids, polyP
- Na, Mg, Ca, K, Mn, Fe, Cu

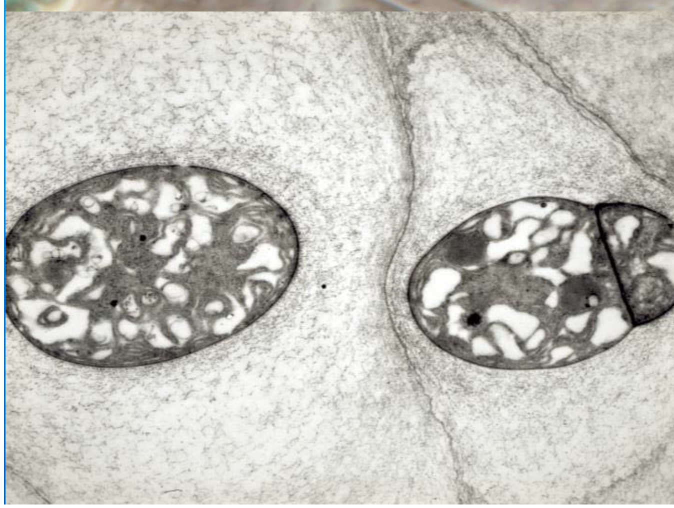


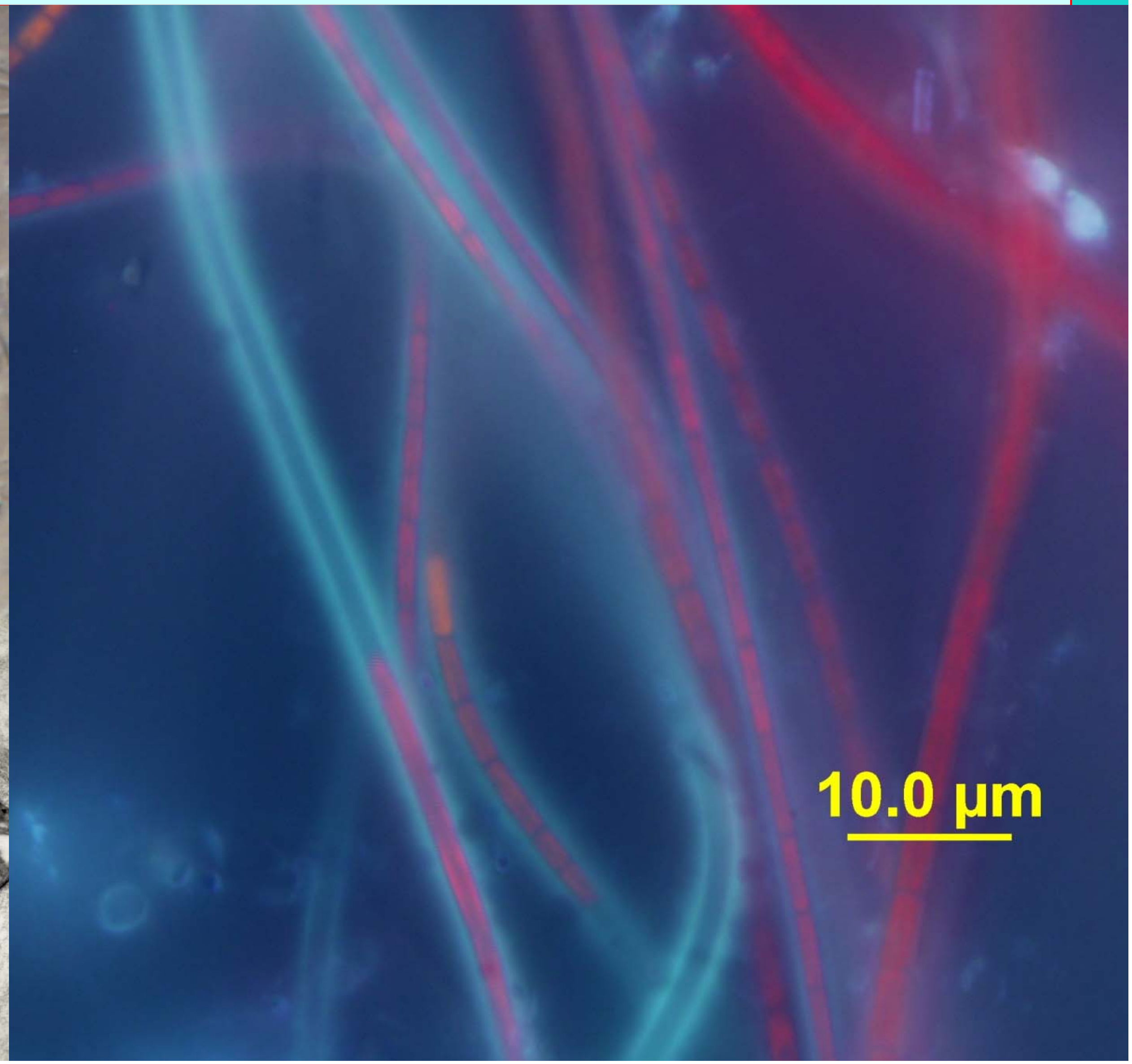
Ecological Strategies: make your own nitrogen from the atmosphere



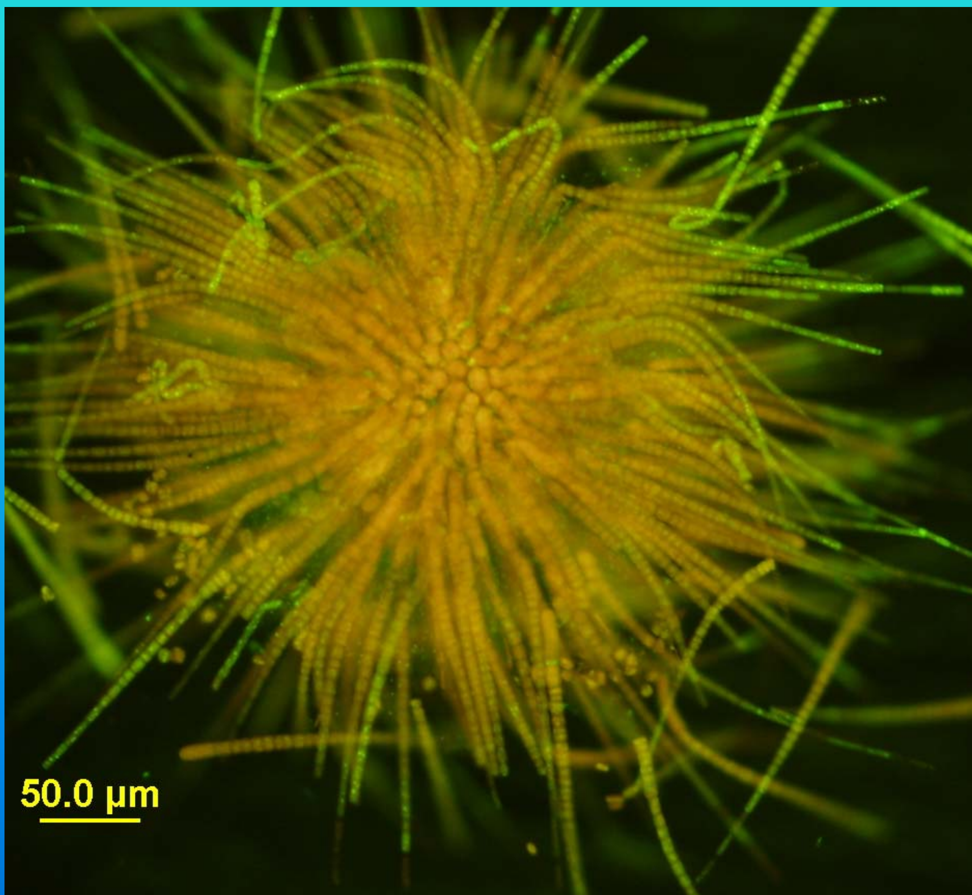
Ecological Strategies: desiccation tolerant (exopolymeric substances-often pigmented)

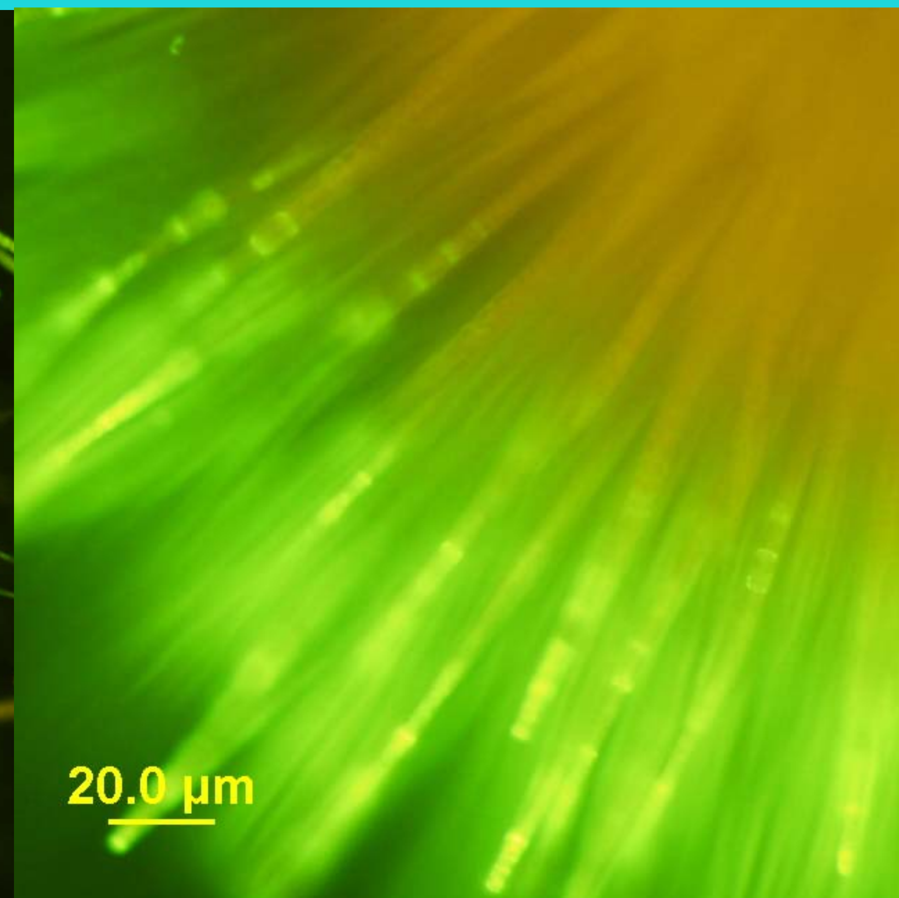




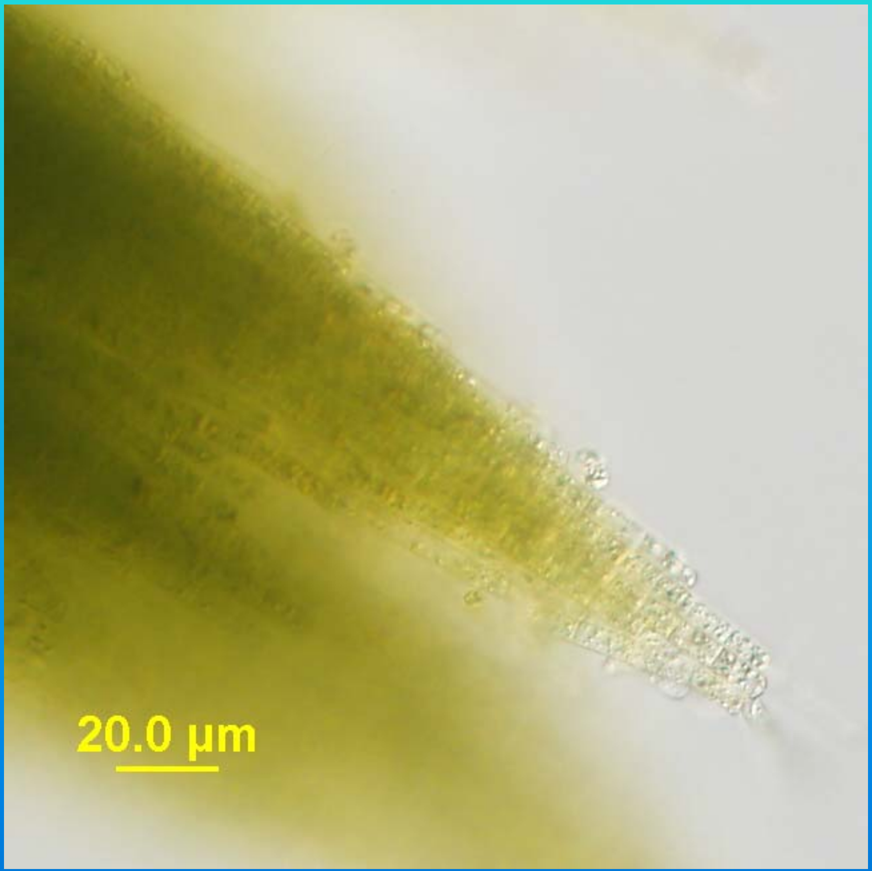


Ecological Strategies: morphology to prevent grazing



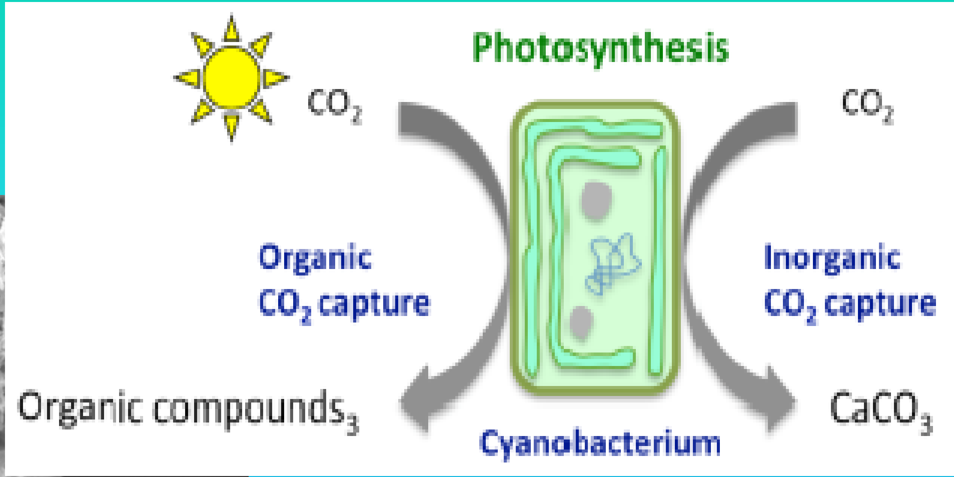


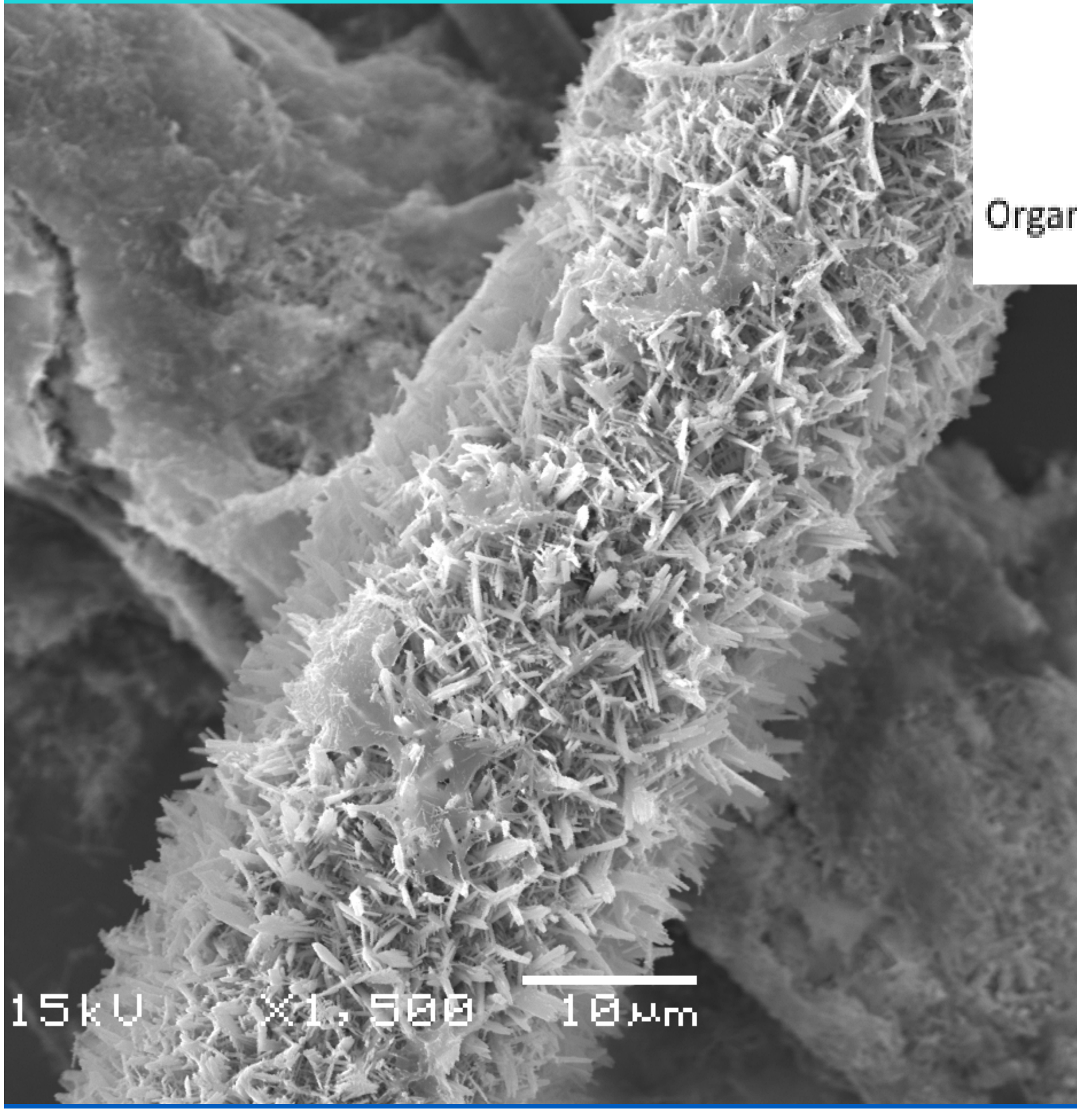
Ecological Strategies: morphology to prevent grazing

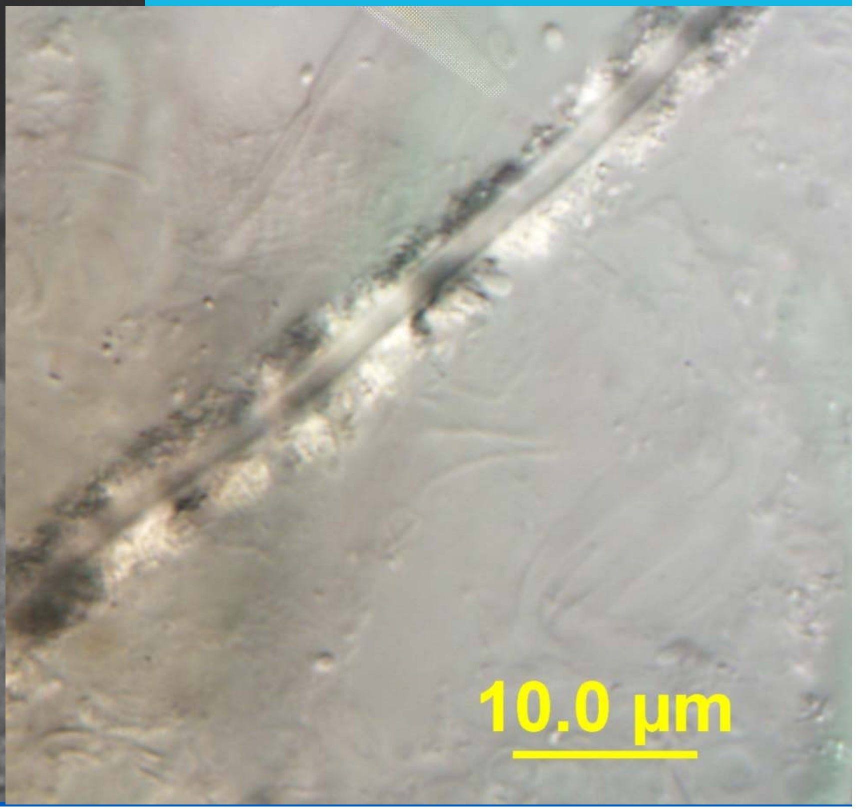


Ecological Strategies: armor to prevent grazing?

cyanobacteria nucleate $CaCO_3$ precipitation

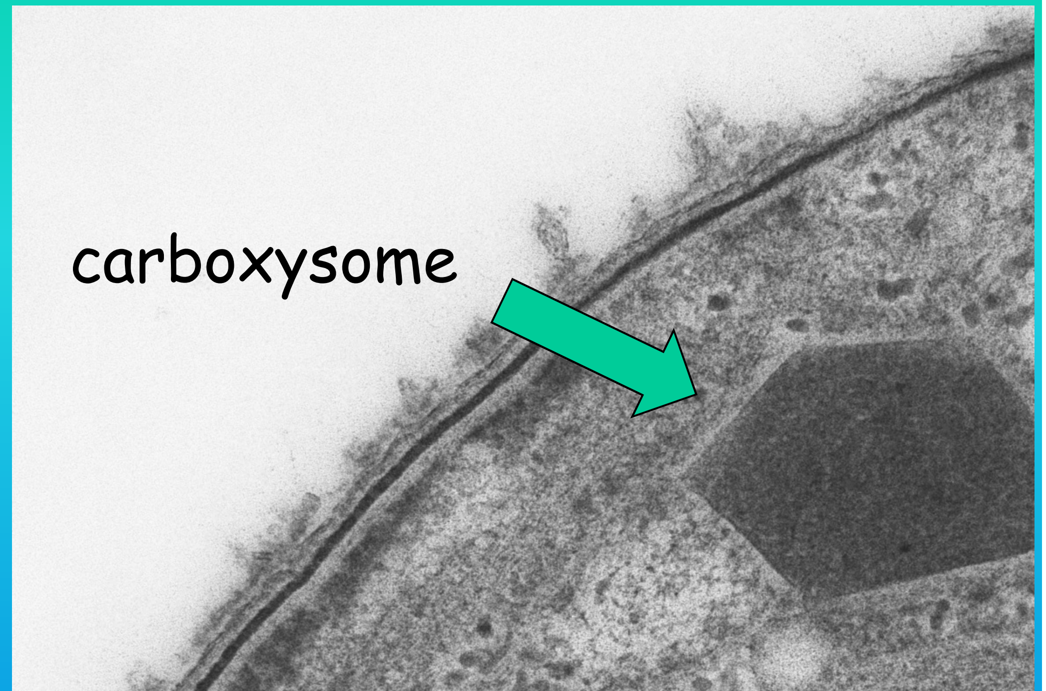


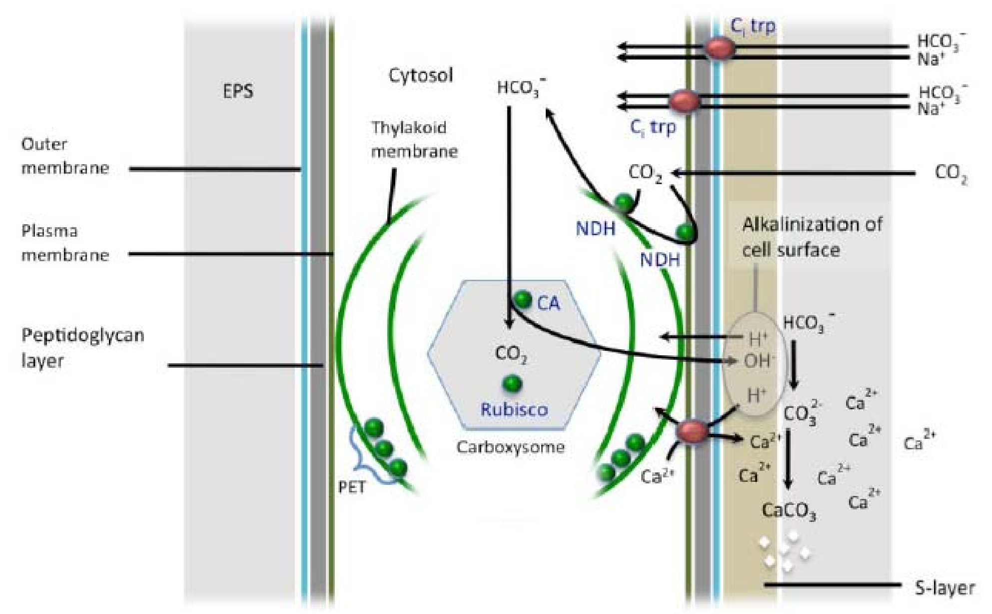




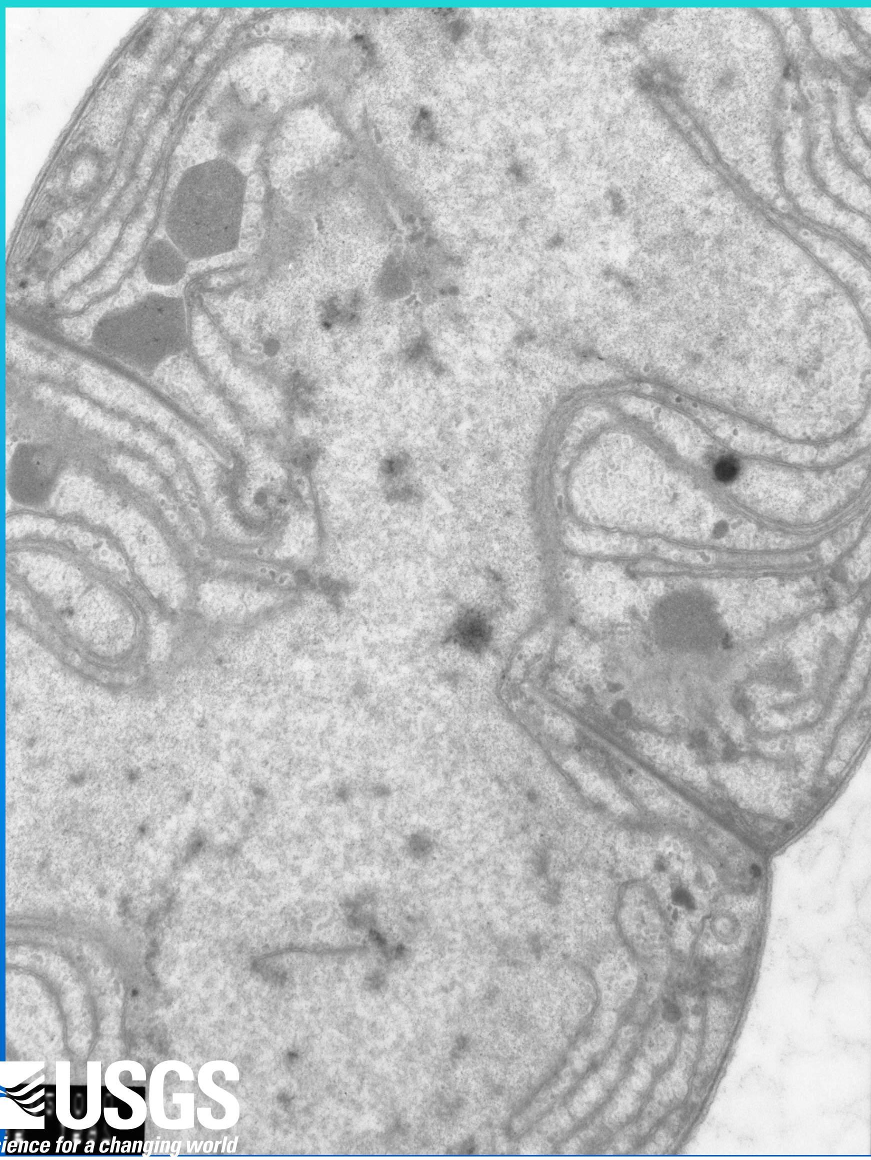
Ecological Strategies: carbon dioxide concentrating mechanism

When bicarboante is limiting, raises the CO_2 using a bio-chemical system that allows the cells to raise the concen-tration at the site of the Rubisco up to **1000-fold** over that in the surrounding medium.

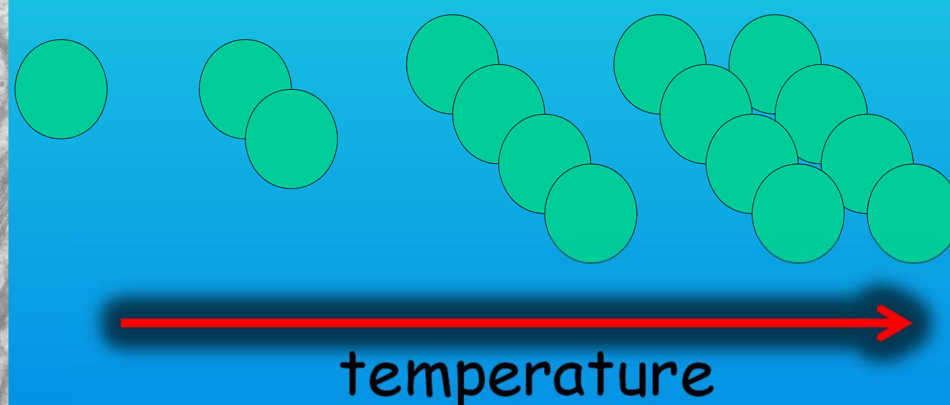




Ecological Strategies: thermophiles grow fast and will be worse as the climate warms



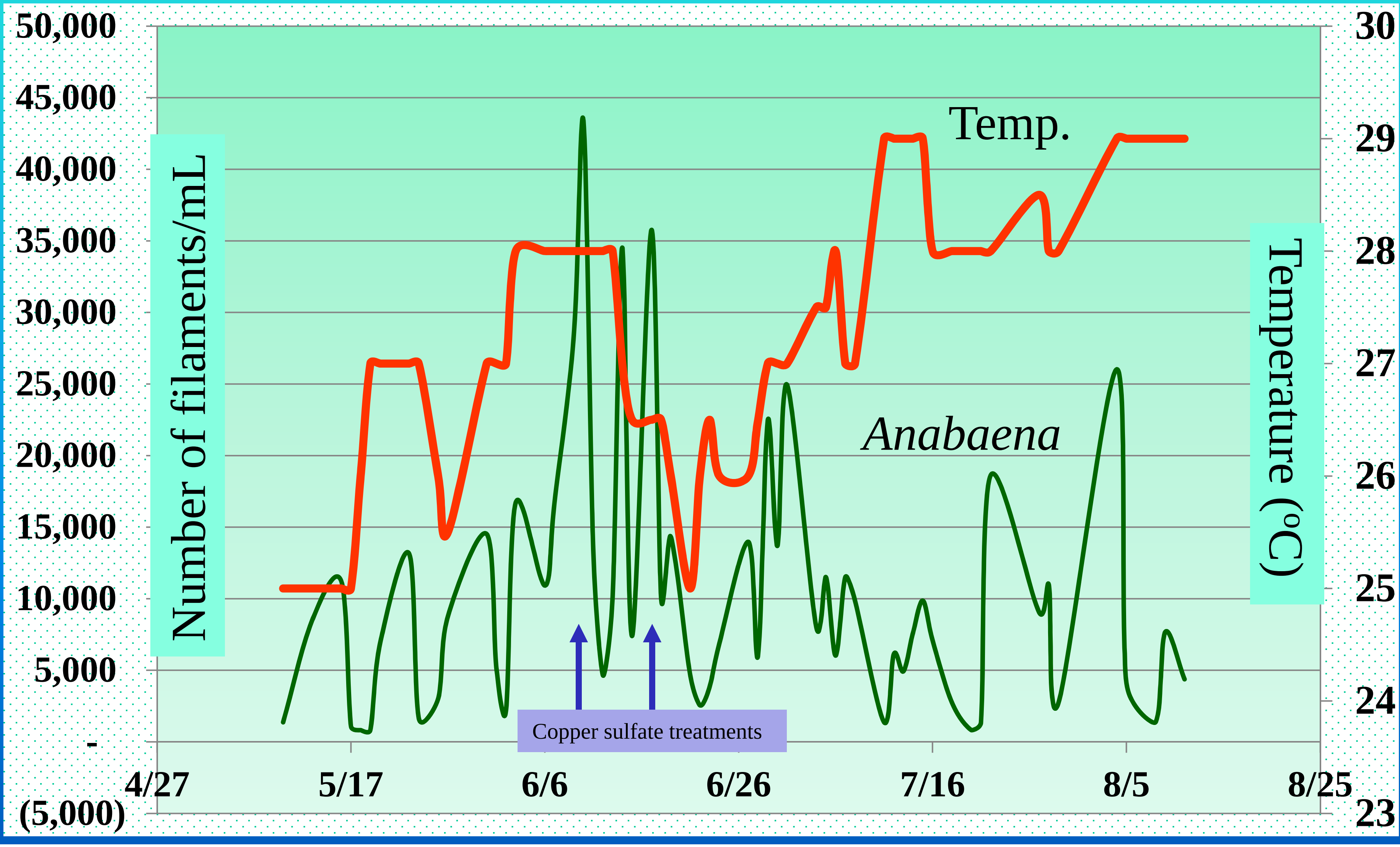
Rapid Growth



3 “doublings” or divisions every day

Ecological Strategies: Temperature Case Study

Anabaena circinalis in the Hillsborough River, FL



Why are we concerned about cyanoHABs?

Toxicity

Hypoxia

Taste and odors

Aesthetics

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Cyanotoxins

- **Hepatotoxins**
  - Disrupt proteins that keep the liver functioning, may act slowly (days to weeks)

microcystin (120+ variants)
nodularin
cylindrospermopsin

- **Neurotoxins**
  - Cause rapid paralysis of skeletal and respiratory muscles (minutes)

anatoxin -a
anatoxin -a (s)
saxitoxin
neosaxitoxin

- **Dermatotoxins**
  - Produce rashes and other skin reactions, usually within a day (hours)

lyngbyatoxin

- **b-*N*-methylamino-L-alanine**
  - Neurological: linked to ALS

BMAA

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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	200-250	Strychnine	2000
Nodularin	50		
Cylindrospermopsins	200		

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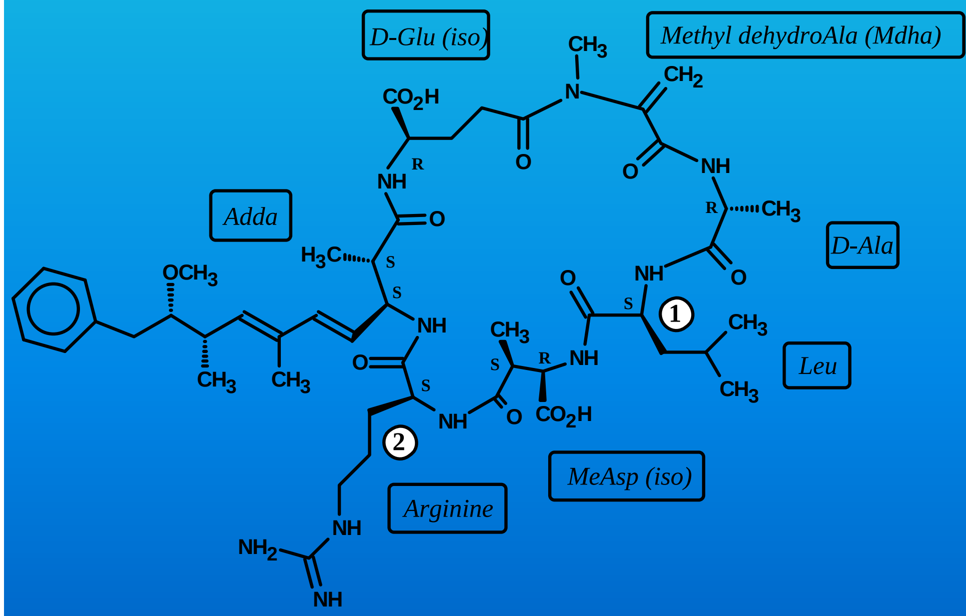
Microcystins

- Mostly *Microcystis aeruginosa* (very common)
  - also produced by a number of other species.
- Potent hepatotoxin
  LD-50: 25-60 µg kg^{-1}
- Called "fast death factor"
- Potent carcinogen
- Guide line values in water:
  - 0.3 micrograms per liter drinking water (10 days, younger than school age; 1.6 for other ages
  - Soon- recreational contact



- **Peptide Toxins:**
  120+ 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 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.

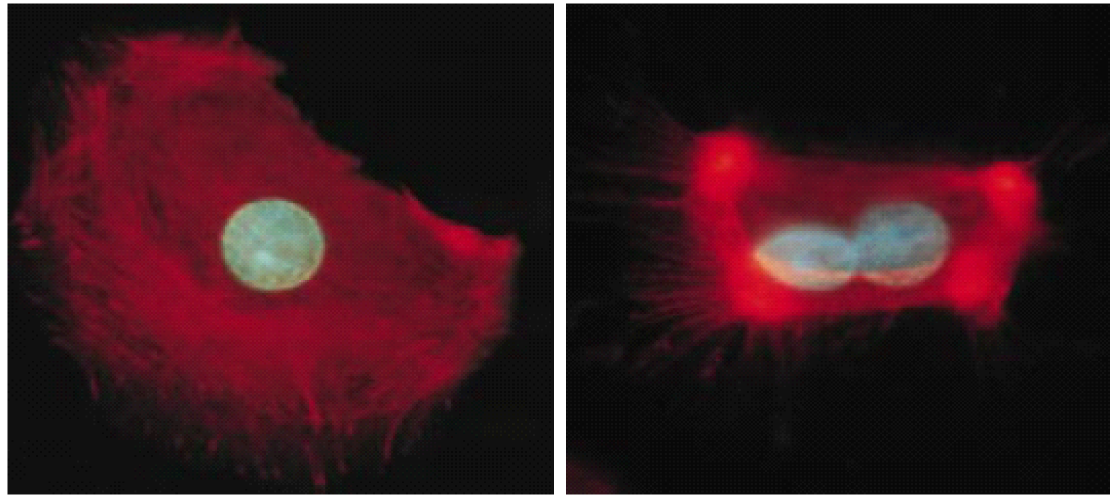
Potential health effects from longer exposure to higher levels of algal toxins in drinking water include gastroenteritis and liver and kidney damage. The health advisory values are based on exposure for 10 days.

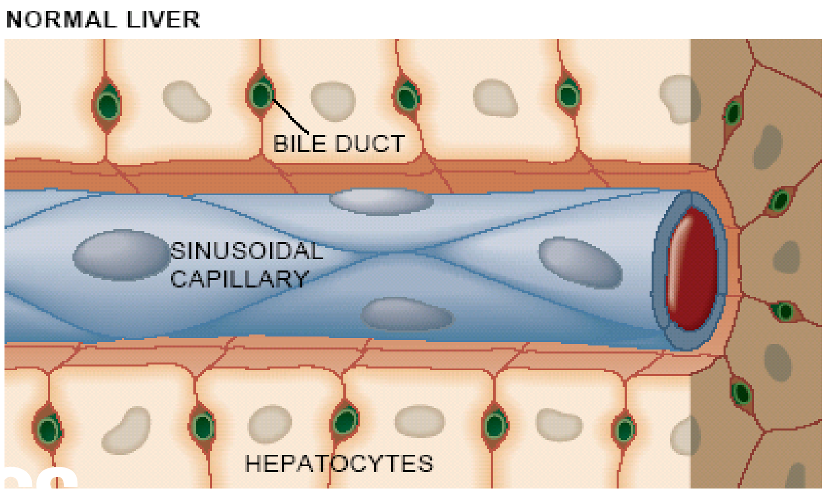
Microcystin exposure: response

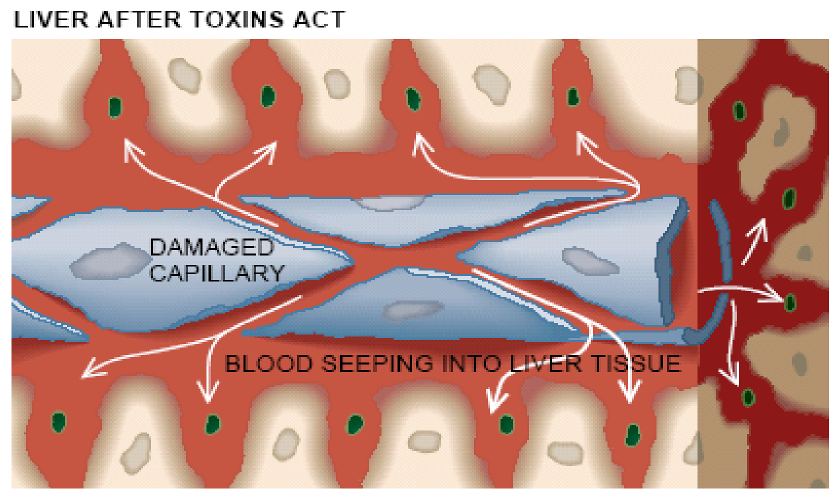
- Uptake by bile acid transporter
- Inhibit protein phosphatases 1 and 2A
- Affects cytoskeleton, cell cycle, general metabolism, apoptosis

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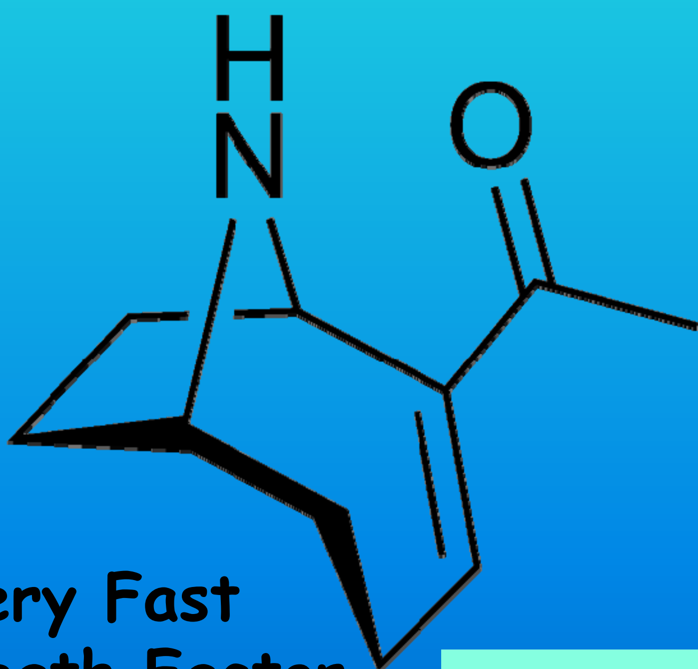
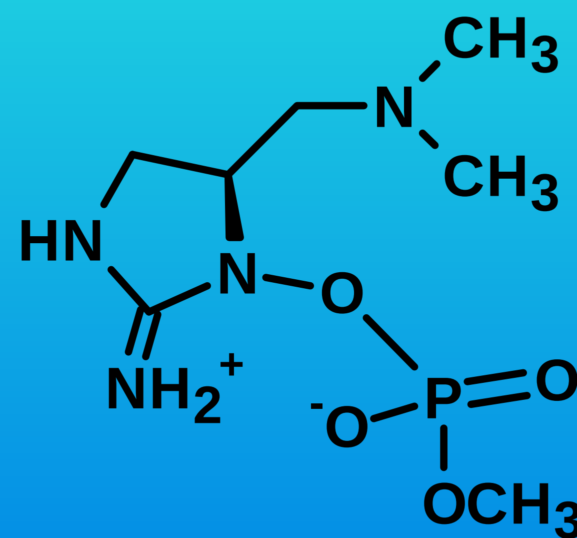
Wayne Carmichael ISOC-HAB Ch. 4, Scientific American, January, 1994

Anatoxins

Anatoxin-a
actylcholine agonist



Anatoxin-a(S)
acetylcholinesterase inhibitor



Very Fast Death Factor

Dolichospermum flos-aquae & lemmermannii

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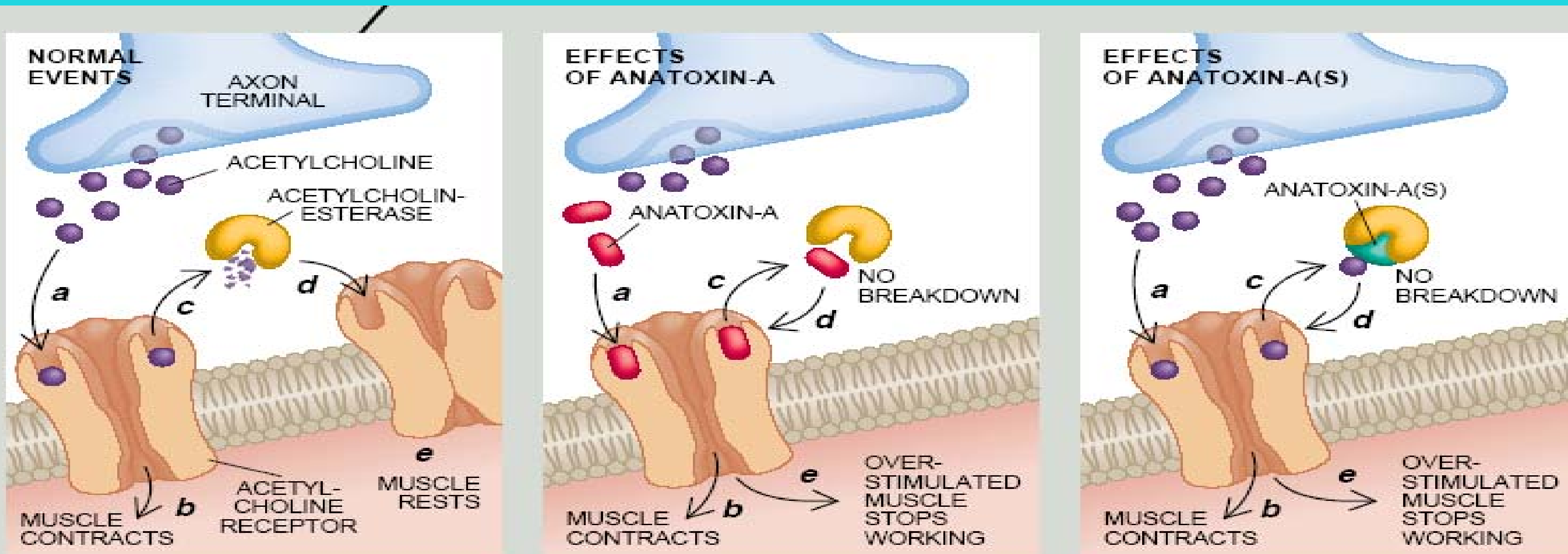
Anatoxin-a and a(s)

Anabaena

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

Neurotoxicity



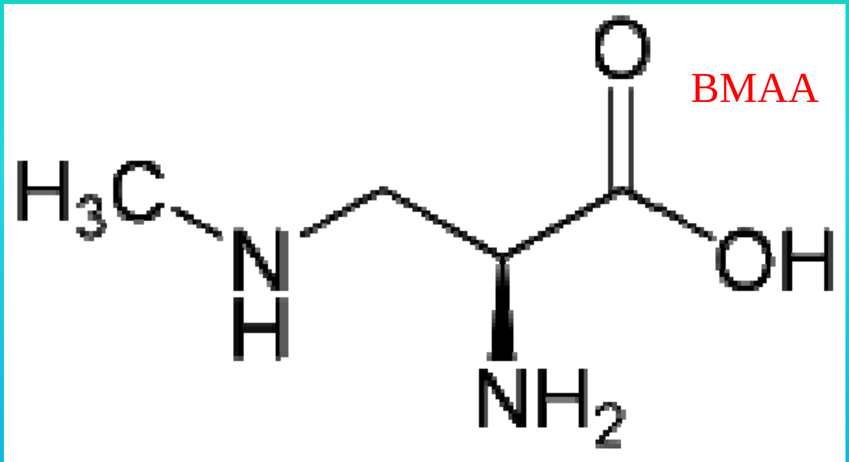
Anatoxin-a and anatoxin-a(s) (*center and right panels*) overexcite muscle cells by disrupting the functioning of the neurotransmitter acetylcholine. Normally, acetylcholine molecules (*purple*) bind to acetylcholine receptors on muscle cells (*a in left panel*), thereby inducing the cells to contract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

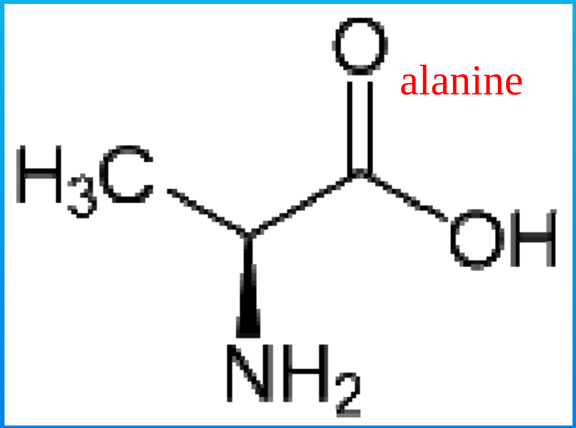
Wayne Carmichael ISOC-HAB Ch. 4, Scientific American, January, 1994

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)





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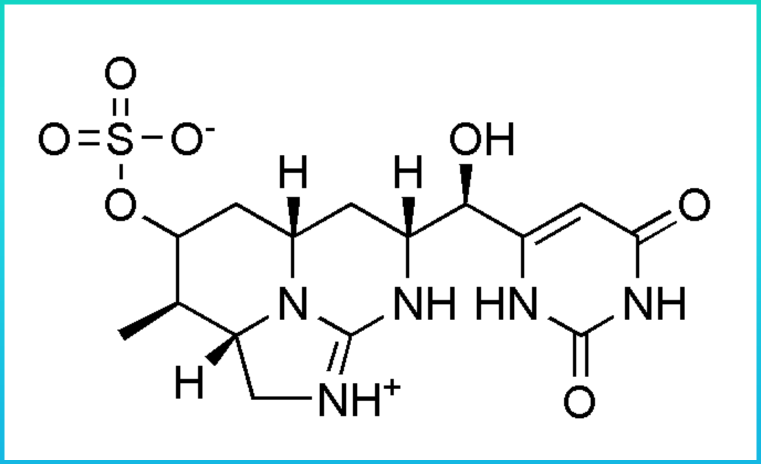
Cylindrospermopsin

Cylindrospermopsis



Alkaloid Toxin

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

- Covalently modify DNA and/or RNA
- Resistant to degradation by pH and temp-persistent

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Common Filamentous Cyanobacteria

Lake Mattamuskeet, NC (East and West)

July 22, 2015

Cylindrospermopsis raciborskii **(CYN)**

Chrysosporum ovalisporum **(CYN)**

Komvophoron (Pseudanabaena)

Planktolyngbya contorta **(MYC)**

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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?

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

brosen@usgs.gov

Open-File Report 2015–1164

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

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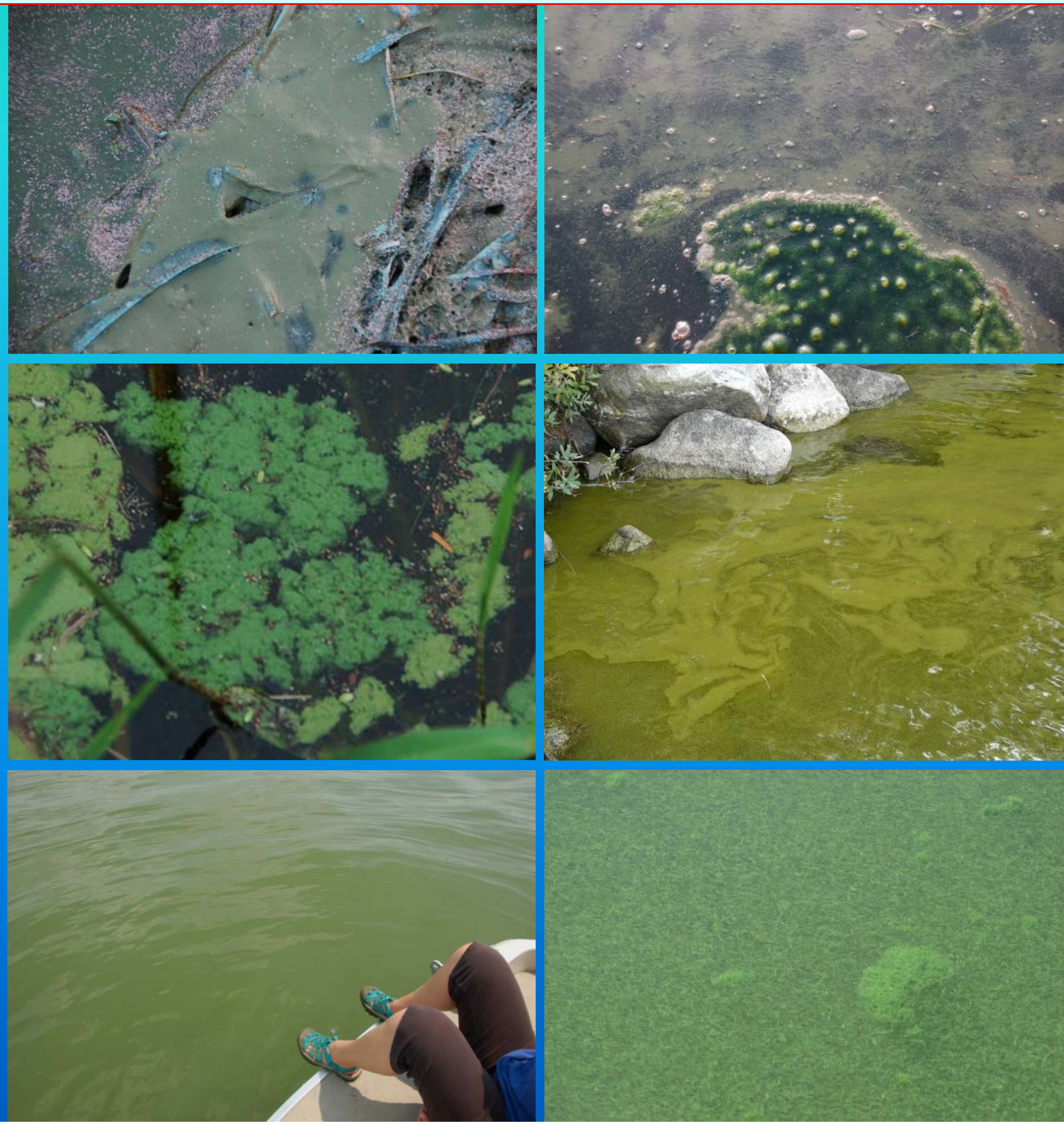
Recognizing a cyanobacteria bloom: field images (blue-green to greenish in color)



Getting a Sample
qualitative

Dense: use a glove!

Not dense: use a plankton net!

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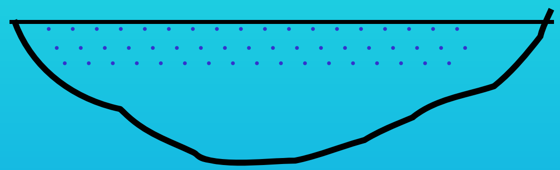
Getting a Sample quantitative

Van Dorn

Depth Integrated Sampling

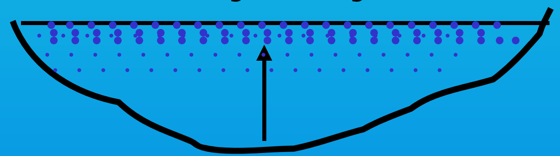
Beware of this phenomenon

initial distribution



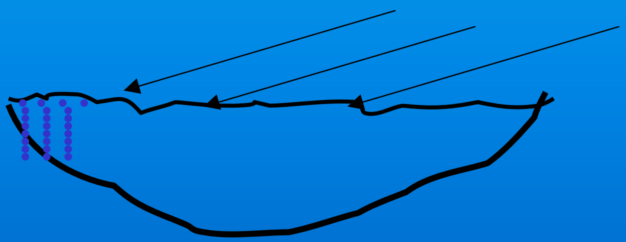
100,000 cells/L;
20 µg/L toxin

buoyancy



10,000,000 cells/L;
2000 µg/L toxin

wind

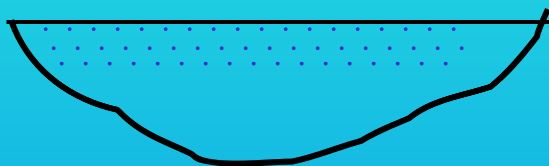


100,000,000 cells/L;
20,000 µg/L toxin

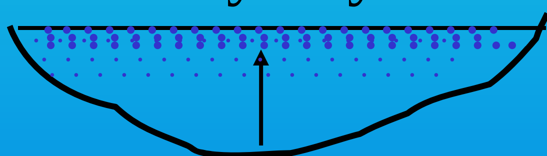
5,000-11,600 µg/kg bw causes liver damage = 2 mg in 10 kg child

Where do I sample?

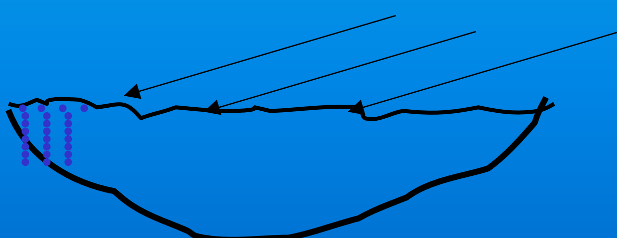
initial distribution

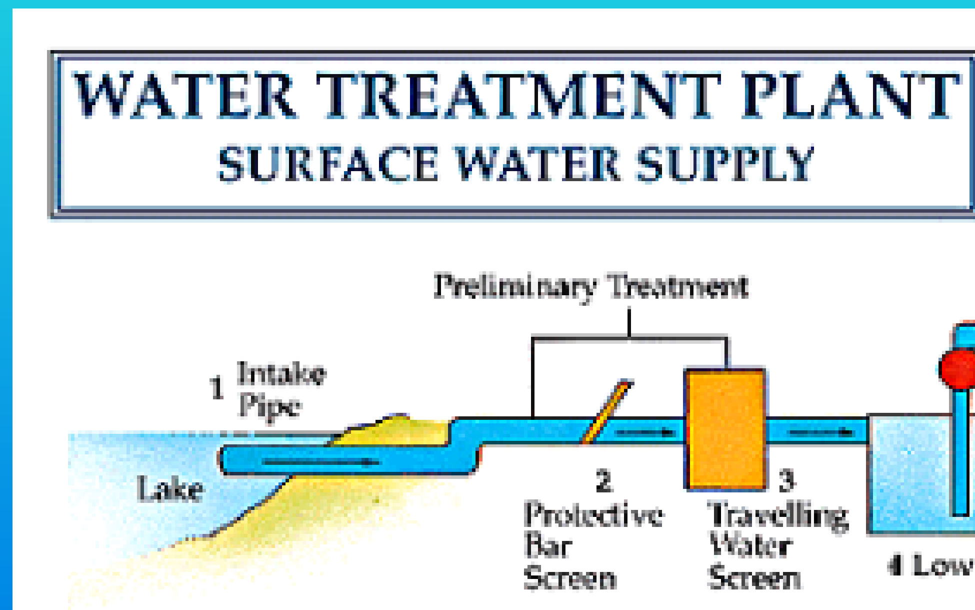


buoyancy



wind





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How much of a sample and how should I "save" it

1. **Collect 100 mL sample of a bloom live**

   Possible Methods:

   a) A whole water sample by simple immersing a 500 mL bottle (glass or plastic) into a waterbody. The small volume in a large bottle allows for ample gas exchange during shipping.

   b) A plankton tow of a bloom, which concentrates a sample, and a liquid volume of 10 mL in a 100 mL bottle.

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How much of a sample and how should I "save" it

2) Collect 100 mL sample of a bloom, preserved with Lugol's iodine

a) same procedures as step 1 to collect the samples

b) add 5% solution of Lugol's to turn the sample the color of tea. (5% (wt/v) iodine (I_2) and 10% (wt/v) potassium iodide (KI) mixed in distilled water and has a total iodine content of 126.5 mg/mL).

-alternatively, Povidone-iodine can be used.

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How much of a sample and how should I “save” it for toxin analyses

3) Collect 1000 mL sample of a bloom, freeze it!

ABRAXIS

Cyanotoxins in Raw Water
Sample Collection Quick Reference Guide

Analyte	Collection/Storage Container	Preservation (at time of sampling)
Anatoxin-a	Amber glass *Avoid exposure to light, as this will degrade the toxin.*	Immediately upon collection, freshwater samples should be preserved with 10X Concentrated Sample Diluent to prevent adsorptive loss of toxin. *Preservation is necessary for freshwater samples only. Saltwater samples do not require additional reagents for preservation.* *Avoid exposure to high pH conditions, as this will degrade the toxin.*
BMAA	Clear glass Polyethylene terephthalate glycol (PETG) High density polyethylene (HDPE) Polycarbonate (PC) Polypropylene (PP) Polystyrene (PS) *Avoid amber glass, as toxin will be lost due to adsorption to container surface.*	Freeze *Samples should be analyzed immediately or frozen to avoid degradation of toxin.*
Cylindrospermopsin	Clear or amber glass Polyethylene terephthalate glycol (PETG) High density polyethylene (HDPE) Polycarbonate (PC) Polypropylene (PP) Polystyrene (PS)	None
Microcystins	Clear or amber glass Polyethylene terephthalate glycol (PETG) *Avoid all plastic containers other than PETG, as toxin will be lost due to adsorption to container surface.*	None
Saxitoxin	Clear or amber glass Polyethylene terephthalate glycol (PETG) High density polyethylene (HDPE) Polycarbonate (PC) Polypropylene (PP) Polystyrene (PS)	Immediately upon collection, freshwater samples should be preserved with 10X Concentrated Sample Diluent to prevent adsorptive loss of toxin. *Preservation is necessary for freshwater samples only. Saltwater samples do not require additional reagents for preservation.*

Unless otherwise indicated, samples can be stored refrigerated for up to 5 days. If samples must be held for greater than 5 days, samples should be stored frozen. If samples are to be shipped, they should be shipped overnight, on ice.

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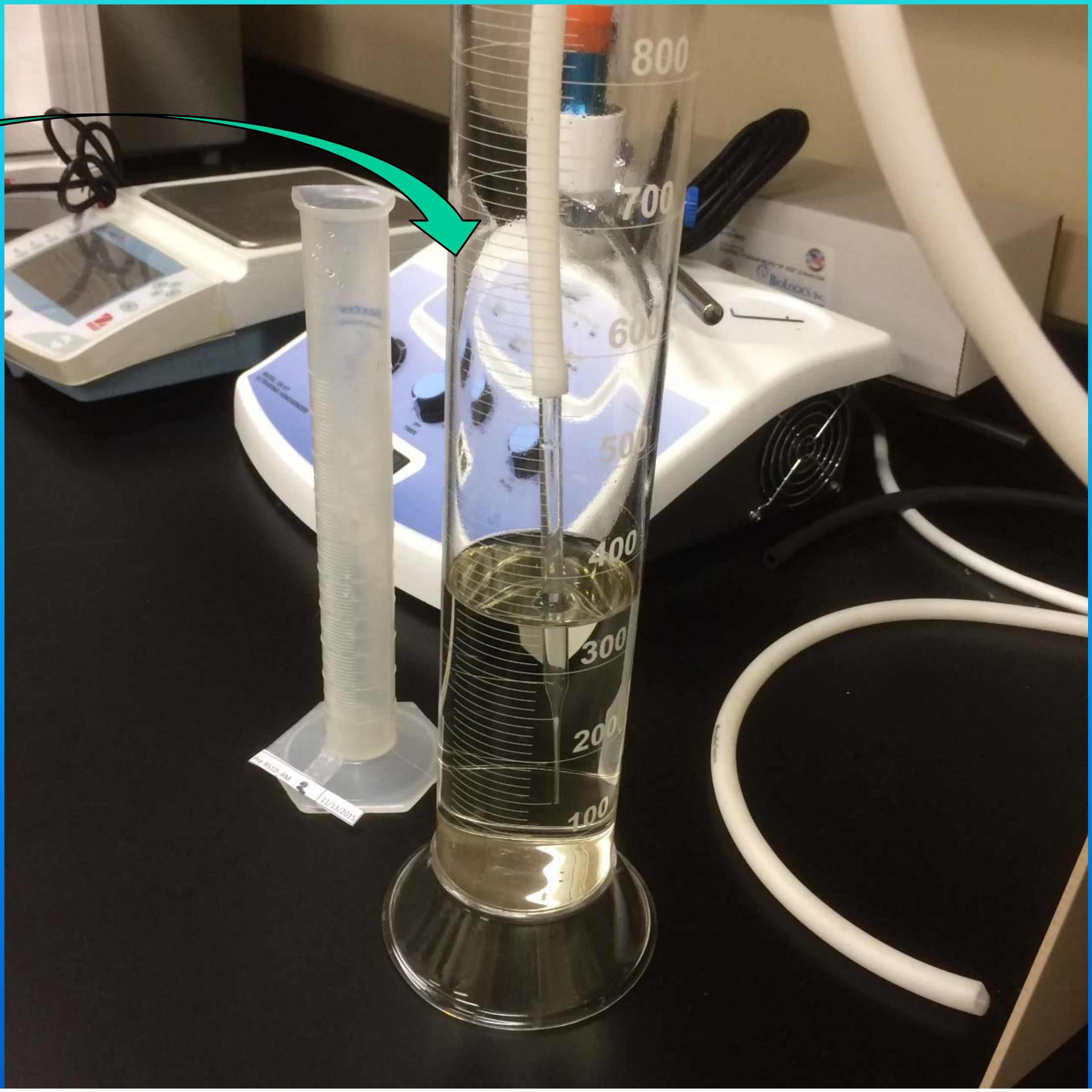
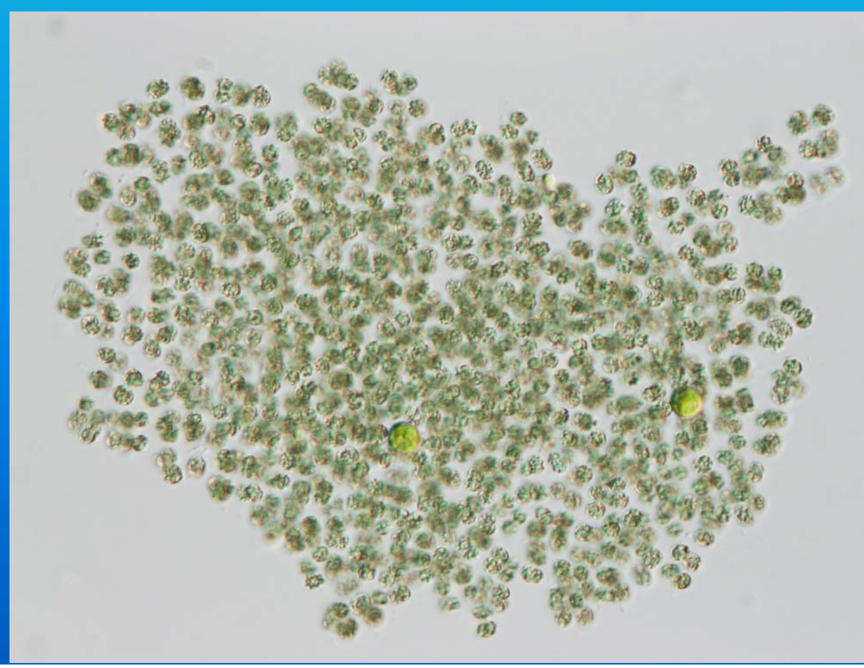
May need to concentrate a sample for IDs: settling method



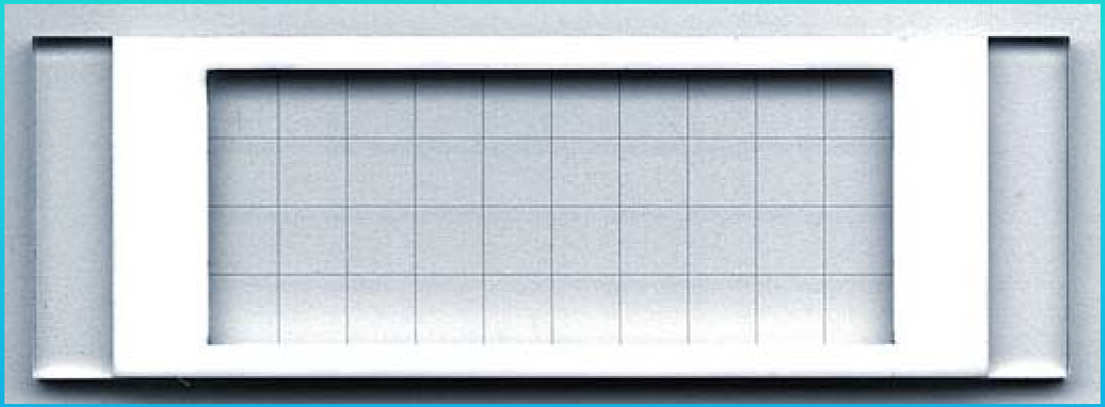
Under the microscope

Cylindrospermopsis raciborskii **(CYN)**

Chrysosporum ovalisporum **(CYN)**



Quantitative: know volume



Sedgwick-Rafter Counting Cell

nannoplankton chamber

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Quantitative: tally sheets

Epiphytic Algae

Site	Cage #	fresh vol.	preservative volume	dilution factor	tube wt. (check)	Constant strip area (12 in x 1.5 in) mm sq.	no. of strips	total area scrapped mm sq.	total volume (mL)	area (mm sq./mL)	subsample wt. (grams) (1 gr. = 1 mL)	area scrapped represented by the subsample	area subsample spread across on coverslip 22 mm sq.	each mm sq of the ocverslip equals of the original scrapped area mm sq.
CB1	4	1	0.0250	1.025	7.37	19,507	10	195,070	1.025	190,312	0.0407	7,746	484	16.00
DB2	8	1.5	0.038	1.025	8.79	19,507	4	78,028	1.5375	50,750	0.0399	2,025	484	4.18
New Zweig Slough	21	1	0.025	1.025	7.38	19,507	10	195,070	1.025	190,312	0.0148	2,817	484	5.82

Site	Constant: area of a field at 40x	No. of fields counted	Area counted (mm sq)	Number of organisms (this is for each column)	Number of organisms per unit area on the orginal strip No./ mm sq)	running total	Cyclotella	F. syngrotesca		Ulnaria	Achnanthes	Eunotia 1	Eunotia 2	Cymbella		Gomphonema	Mastogloea	Encynema	Navicula	Bra a
CB1	0.236719592	4	0.9469	2	34.65	322	1	86			7	1	4			10	23	122	1	
DB2	0.236719592	5	1.1836	2	7.25	295	p	75		1	22		1	2		6	13	101	6	
New Zweig Slough	0.236719592	9	2.1305	2	5.60	302		78		2	34	3	7	1		25	5	50	2	

Site	Cage #	fresh vol.	preservative volume	dilution factor	tube wt. (check)	Constant strip area (12 in x 1.5 in) mm sq.	no. of strips	total area scrapped mm sq.	total volume (mL)	area (mm sq./mL)	subsample wt. (grams) (1 gr. = 1 mL)	area scrapped represented by the subsample	area subsample spread across on coverslip 22 mm sq.	each mm sq of the ocverslip equals of the original scrapped area mm sq.	Constant: area of a field at 40x	No. of fields counted	Area counted (mm sq)	Number of organisms (this is for each column)	Number of organisms per unit area on the orginal strip No./ mm sq)	running total
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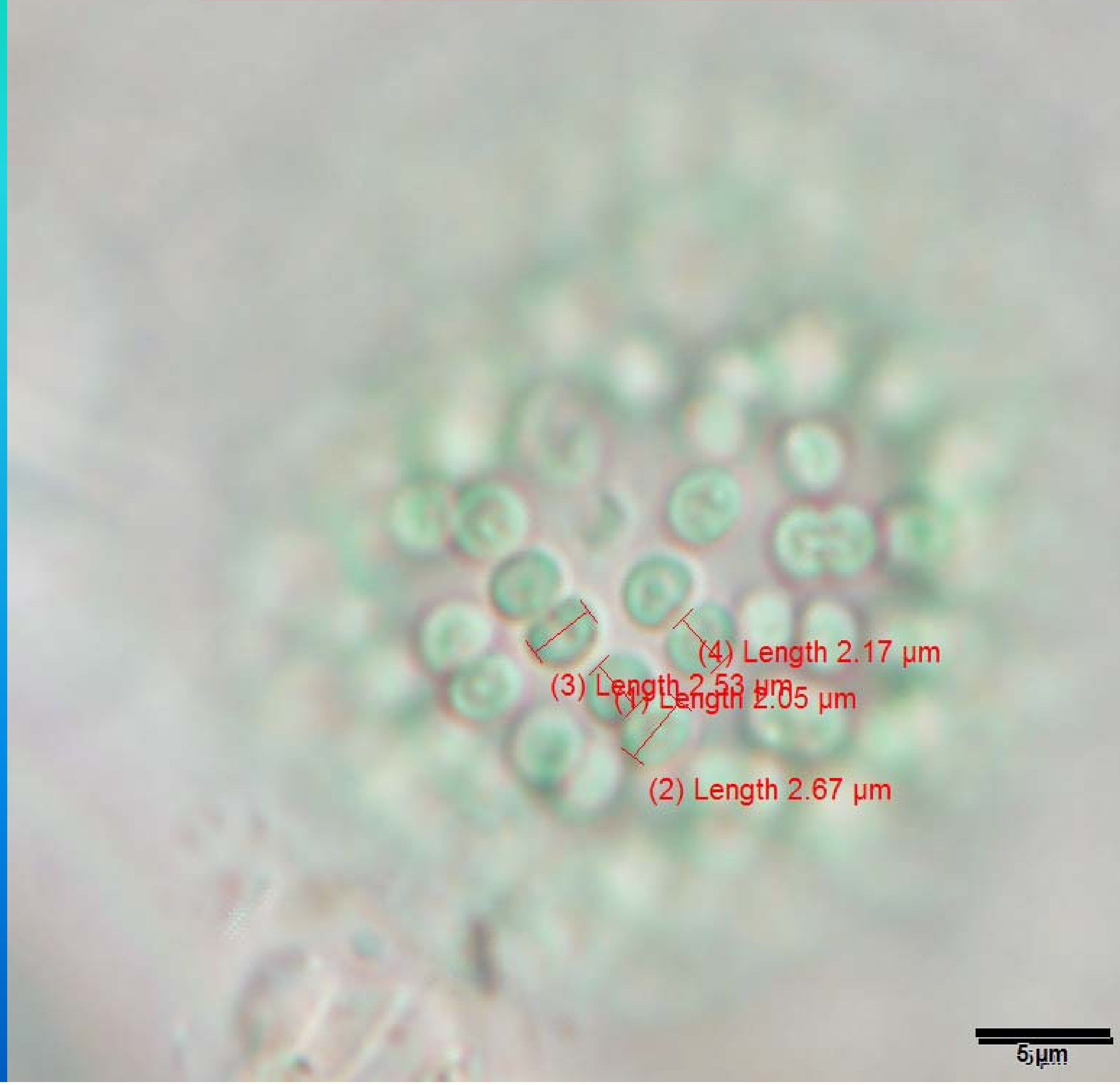
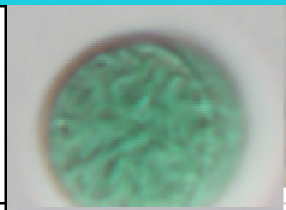
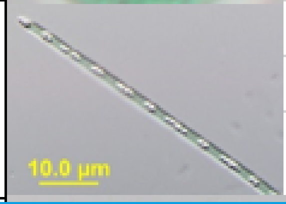
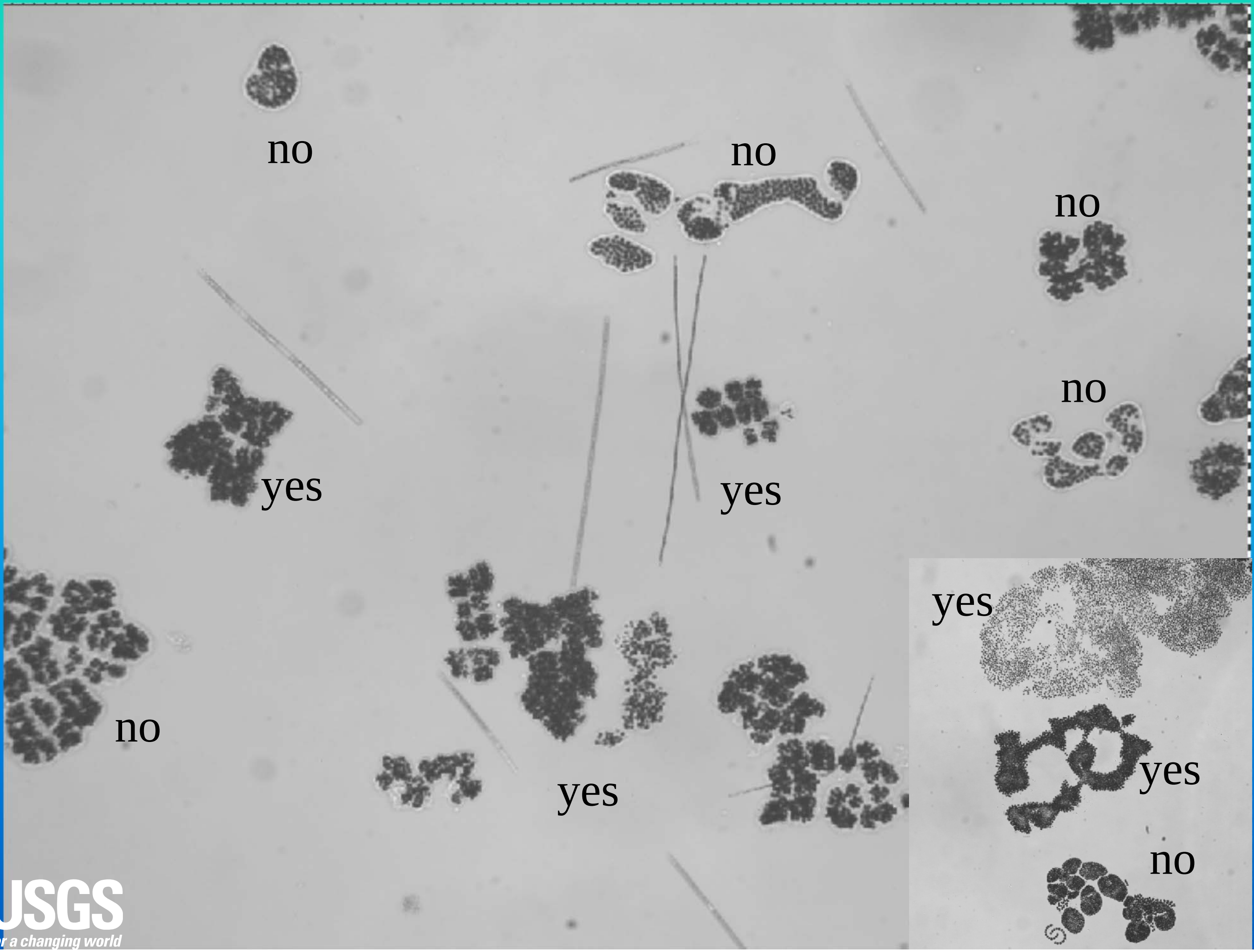
Quantitative: biovolume



Quantitative: biovolume

***Synechocycstis* sp.**		l	13.8	sphere	$V=\pi/6 * d^3$	1,369.39	13.78
		w	13.8				13.78
***Limnothrix* sp.**		l	5.3	Cylinder	$V=\pi/4 * d^2 * h$	34.62	5
		w	1.6				1.6

Can not use taxonomy to predict toxicity



Help Need

Sample from tribal waterbodies experiencing a cyanobacterial bloom: identification of key organisms* Need your help getting a sample

1. **Contact me: 407-803-5508; 407-738-0669 ; brosen@usgs.gov or text 407-738-0669**
2. **Follow standard sampling protocol (see next slide)**
3. **Ship live samples (overnight): Barry Rosen, USGS, 12703 Research Parkway, Orlando, FL 32779**

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Sample Protocol and Preparation

1. **Collect 100 mL sample of a bloom live**
   Possible Methods:
   a) A whole water sample by simple immersing a 500 mL bottle (glass or plastic) into a waterbody. The small volume in a large bottle allows for ample gas exchange during shipping.
   b) A plankton tow of a bloom, which concentrates a sample, and a liquid volume of 10 mL in a 100 mL bottle.

2. **Collect 100 mL sample of a bloom, preserved with Lugol's iodine**
   a) same procedures as step 1 to collect the samples
   b) add 5% solution of Lugol's to turn the sample the color of tea. (5% (wt/v) iodine (I_2) and 10% (wt/v) potassium iodide (KI) mixed in distilled water and has a total iodine content of 126.5 mg/mL). Alternatively, Povidone-iodine can be used.


WARNING
LIFEGUARD,
BACTERIOLOGIST,
MICROBIOLOGIST,
& TOXICOLOGIST
NOT ON DUTY
SWIM AT YOUR
OWN RISK

Thank You!