Sampling Methods

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

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

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Open-File Report 2015–1164

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

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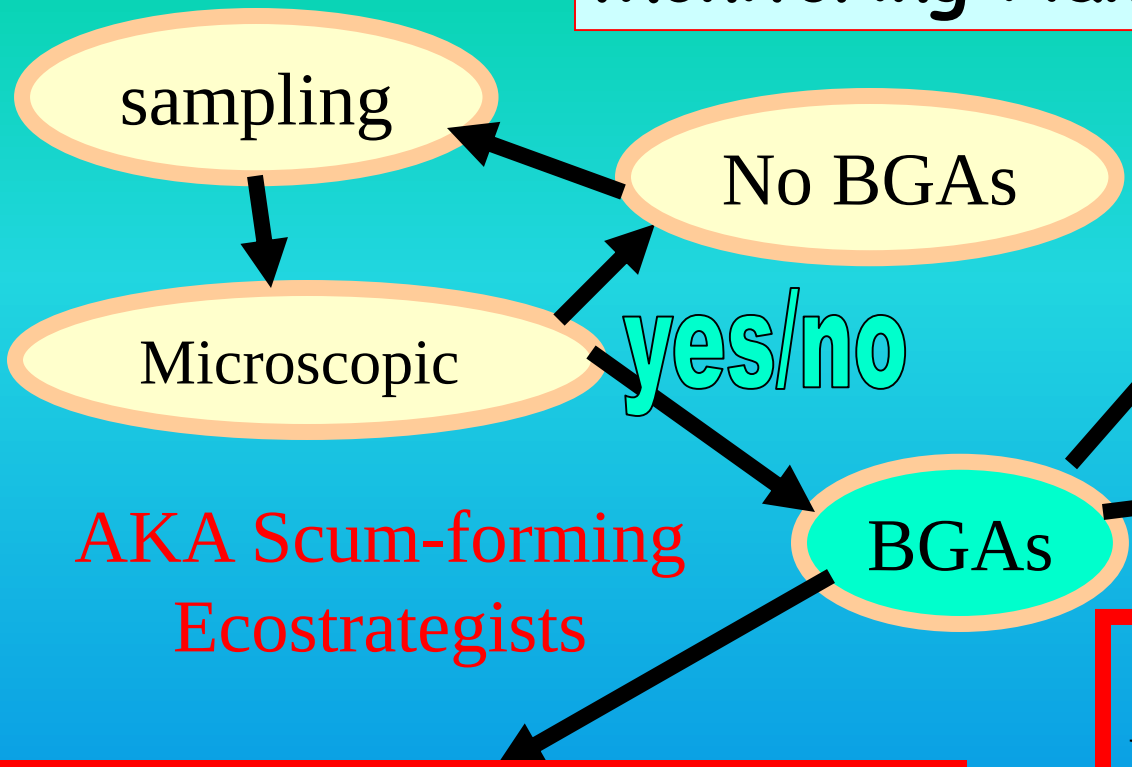
Monitoring Plan



Biological Analysis
Mouse Bioassay

AKA Scum-forming
Ecostrategists

Physio-chemical Analysis of Toxins
HPLC, LC/MS - microcystins, anatoxin-a & saxitoxin)

Biochemical Analysis
Phosphatase Inhibitory Assay (PPIA)
microcystin, cylindrosperopsin, immunoassay

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What are the toxins I might want to assess?

Compounds & LD_{50} (ug/kg)

Saxitoxin	9
Anatoxin-a(s)	20
Microcystin LR	50
Anatoxin-a	200-250
Nodularin	50
Cylindrospermopsins	200

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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

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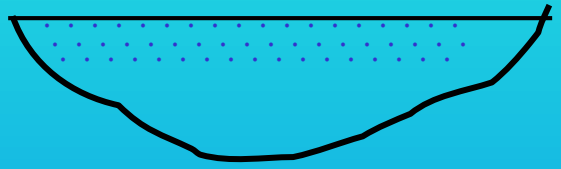
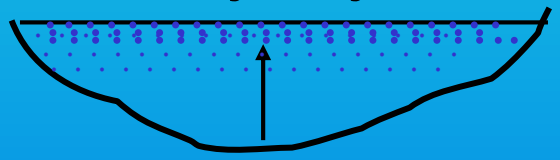
Beware of this phenomenon

initial distribution



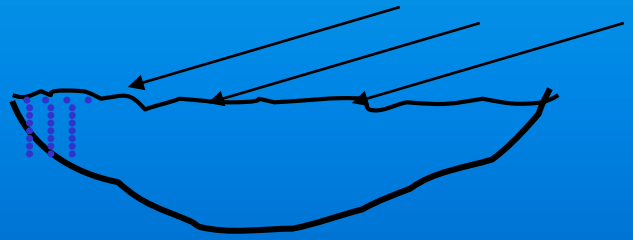
100,000 cells/L;
20 µg/L toxin

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

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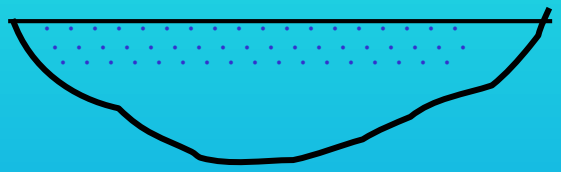
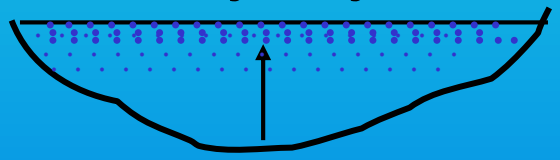
Where do I sample?

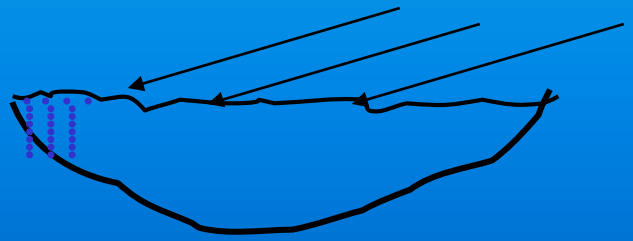
initial distribution

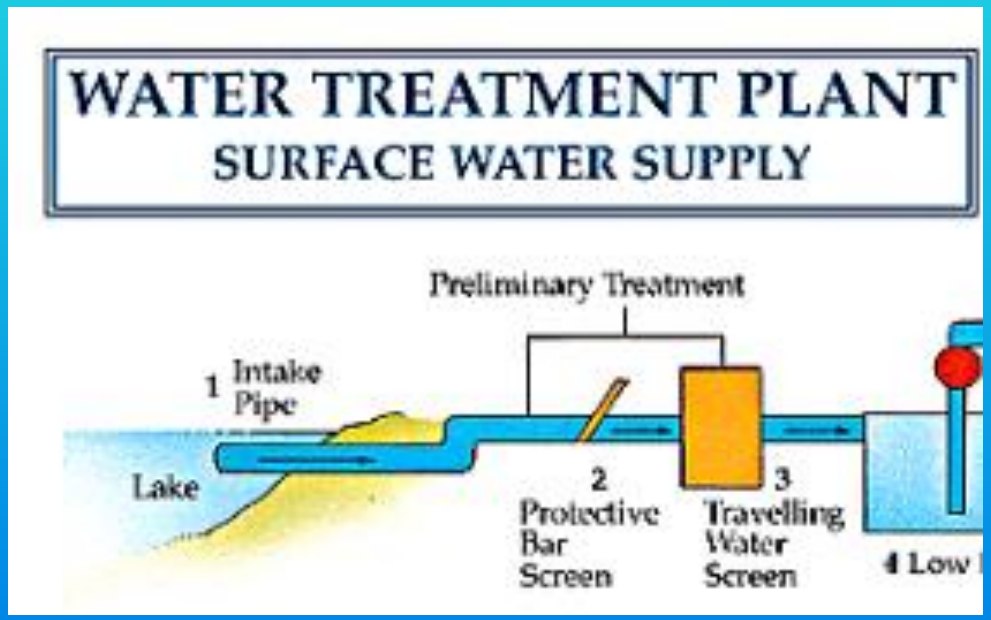


buoyancy



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

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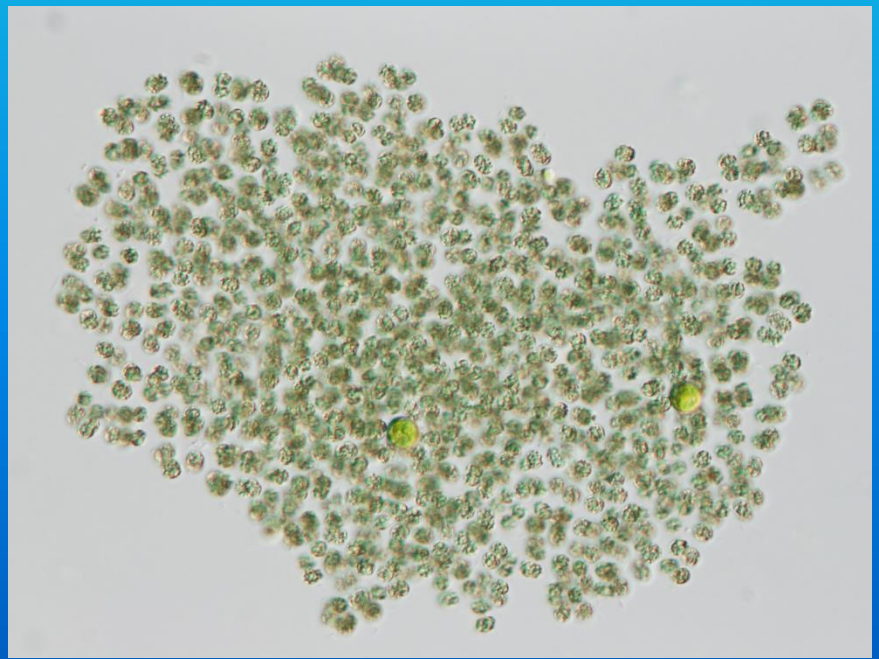
Under the microscope

Cylindrospermopsis raciborskii **(CYN)**

Chrysosporum ovalisporum **(CYN)**



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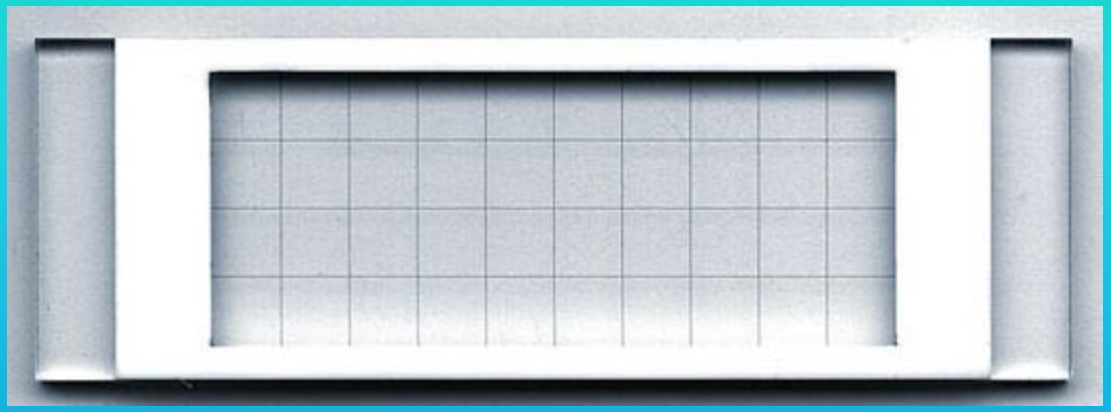
Quantitative: know volume



Sedgwick-Rafter Counting Cell

nannoplankton chamber

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Quantitative: tally sheets

																					Cyclotella	F. syngrotesca		Ulnaria	Achnanthes	Eunotia 1	Eunotia 2	Cymbella		Gomphonema	Mastogloea	Encyonema	Navicula	Braa
Epiphytic Algae																																		
Site	Cage #	fresh vol.	preservative volume	dilution factor	tube wt. (check)	Constant stip 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														
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	0.236719592	4	0.9469	2	34.65	322	1	86			7	1	4			10	23	122	1	
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	0.236719592	5	1.1836	2	7.25	295	p	75		1	22		1	2		6	13	101	6	
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	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 stip 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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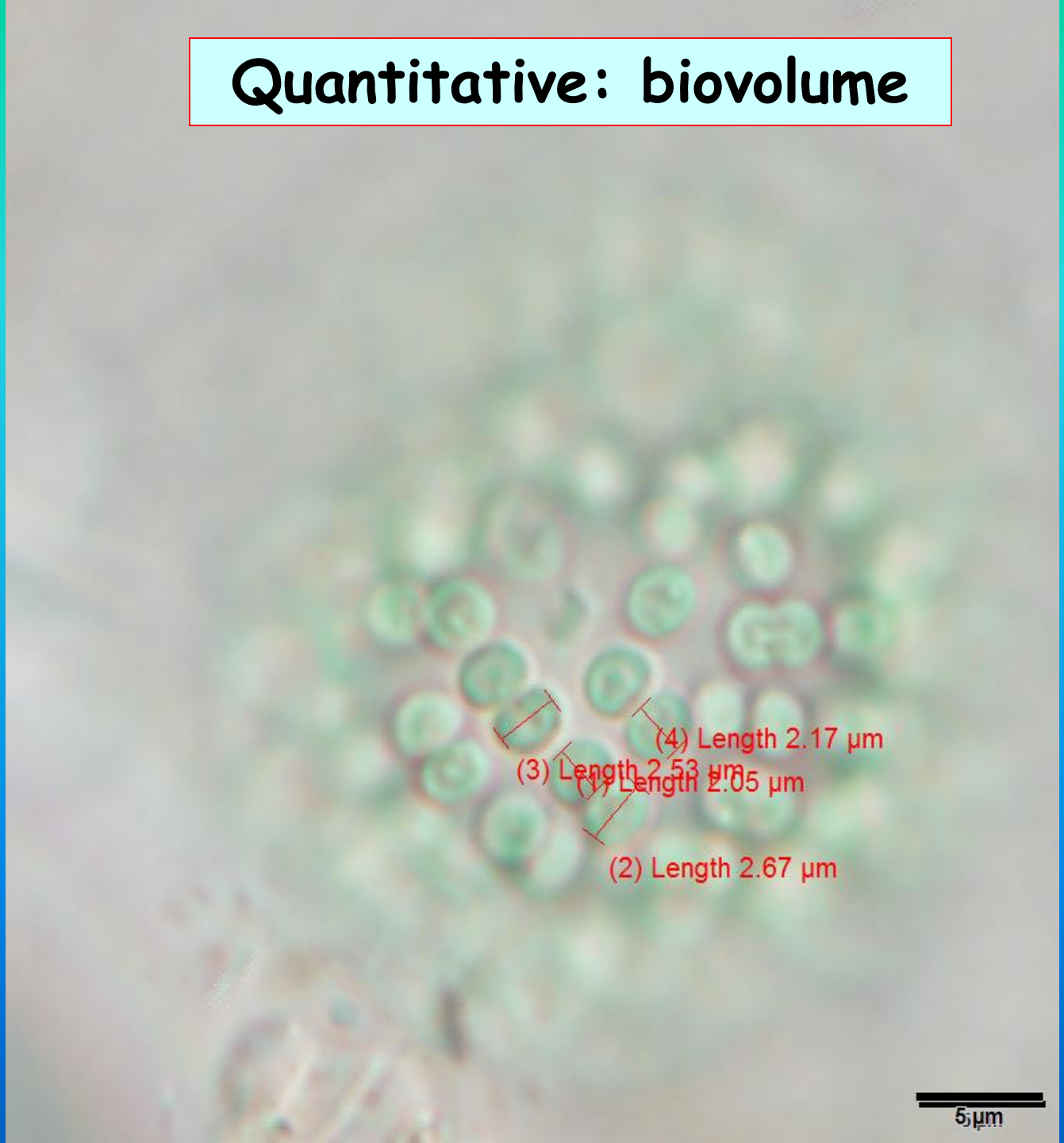
Quantitative: biovolume



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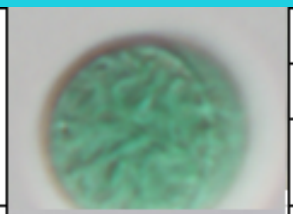
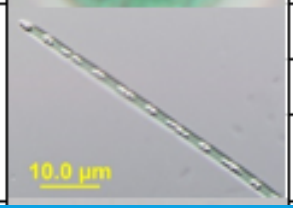
Quantitative: biovolume

***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

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Can not use taxonomy to predict toxicity

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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**

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Thank you!