

US EPA ARCHIVE DOCUMENT

Sampling Methods

Barry H. Rosen, Ph. D.

Office of the Southeast Regional Director
(CFLWSC) Orlando, FL

brosen@usgs.gov

407-803-5508

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



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

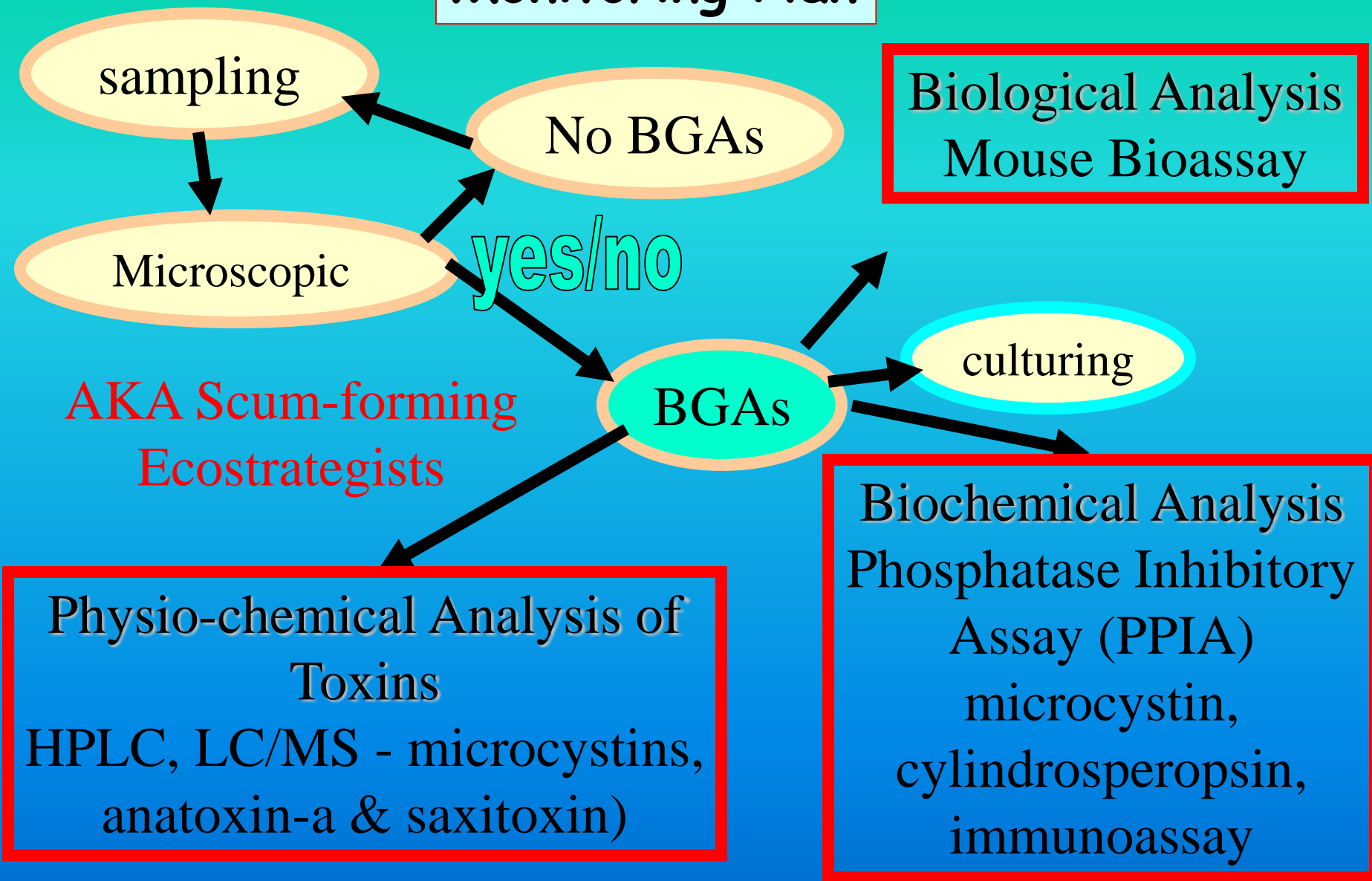


Open-File Report 2015–1164

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

brosen@usgs.gov

Monitoring Plan



What are the toxins I might want to assess?

Compounds & LD₅₀ (ug/kg)

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

Getting a Sample qualitative



Dense: use a glove!



**Not dense: use a
plankton net!**

Getting a Sample quantitative



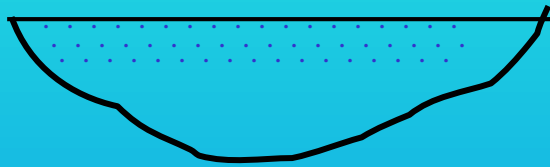
Van Dorn



Depth Integrated
Sampling

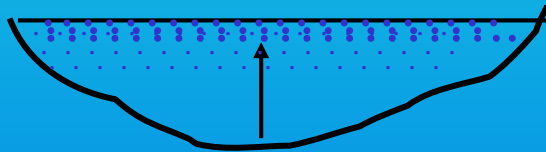
Beware of this phenomenon

initial distribution



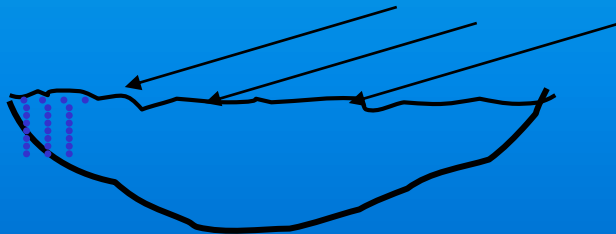
**100,000 cells/L;
20 $\mu\text{g/L}$ toxin**

buoyancy



**10,000,000 cells/L;
2000 $\mu\text{g/L}$ toxin**

wind

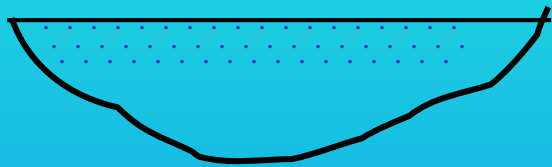


**100,000,000 cells/L;
20,000 $\mu\text{g/L}$ toxin**

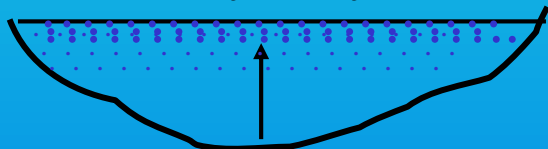
5,000-11,600 $\mu\text{g/kg}$ bw causes liver damage = 2 mg in 10 kg child

Where do I sample?

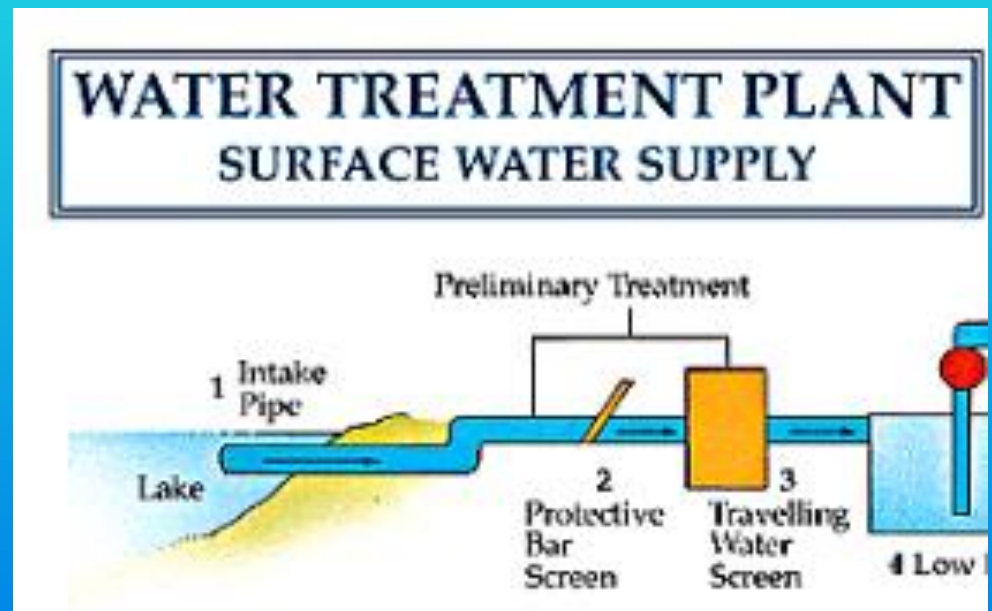
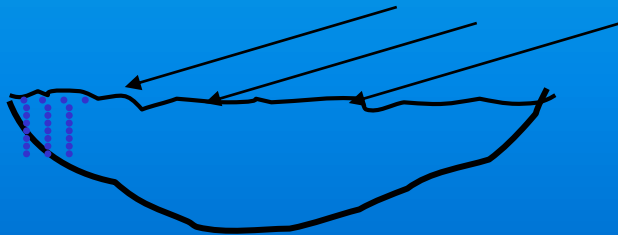
initial distribution



buoyancy



wind



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.

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.



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

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



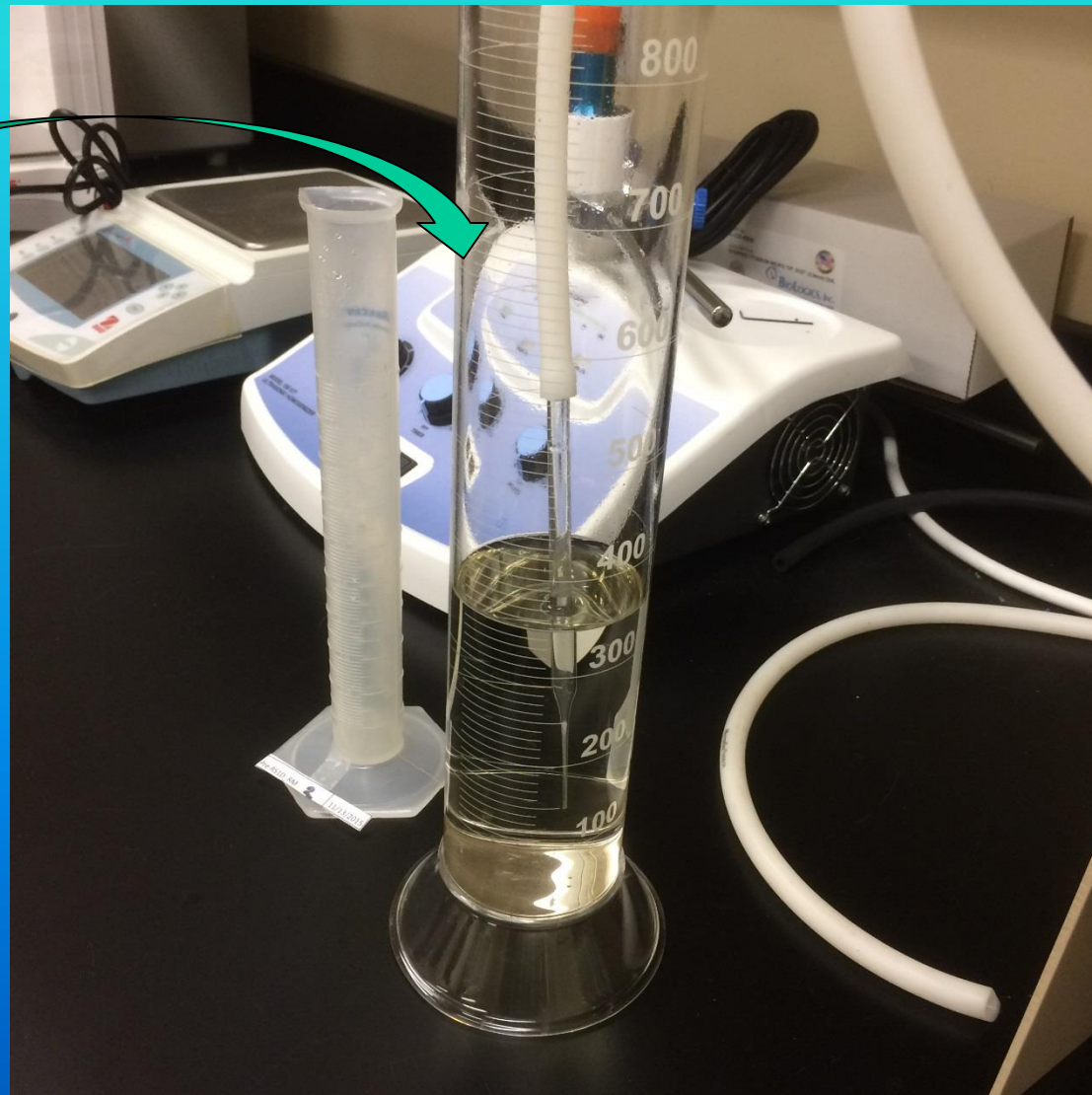
Cyanotoxins in Raw Water Sample Collection Quick Reference Guide

Analyte	Collection/Storage Container	Preservation (at time of sampling)
Anatoxin-a	Amber glass <i>Avoid exposure to light, as this will degrade the toxin.</i>	Immediately upon collection, freshwater samples should be preserved with 10X Concentrated Sample Diluent to prevent adsorptive loss of toxin. <i>Preservation is necessary for freshwater samples only. Saltwater samples do not require additional reagents for preservation.</i> <i>Avoid exposure to high pH conditions, as this will degrade the toxin.</i>
BMAA	Clear glass Polyethylene terephthalate glycol (PETG) High density polyethylene (HDPE) Polycarbonate (PC) Polypropylene (PP) Polystyrene (PS) <i>Avoid amber glass, as toxin will be lost due to adsorption to container surface.</i>	Freeze <i>Samples should be analyzed immediately or frozen to avoid degradation of toxin.</i>
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) <i>Avoid all plastic containers other than PETG, as toxin will be lost due to adsorption to container surface.</i>	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. <i>Preservation is necessary for freshwater samples only. Saltwater samples do not require additional reagents for preservation.</i>

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.

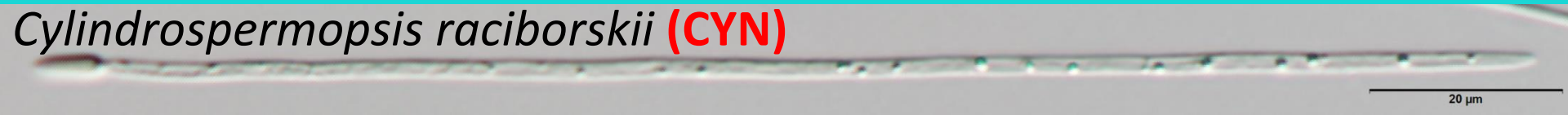
R021516

May need to concentrate a sample for IDs:
settling method

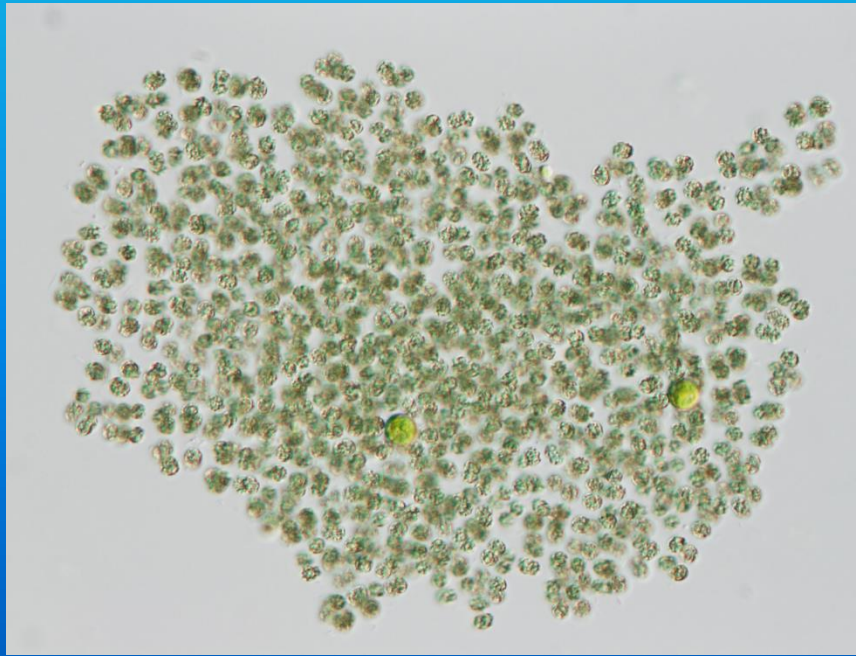
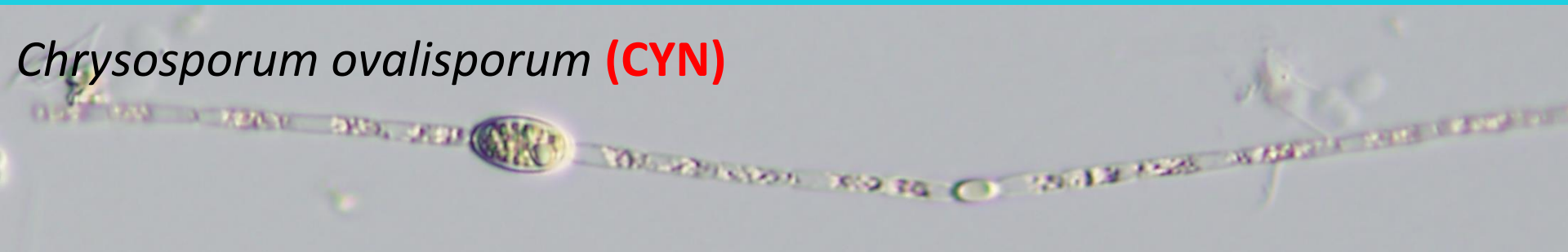


Under the microscope

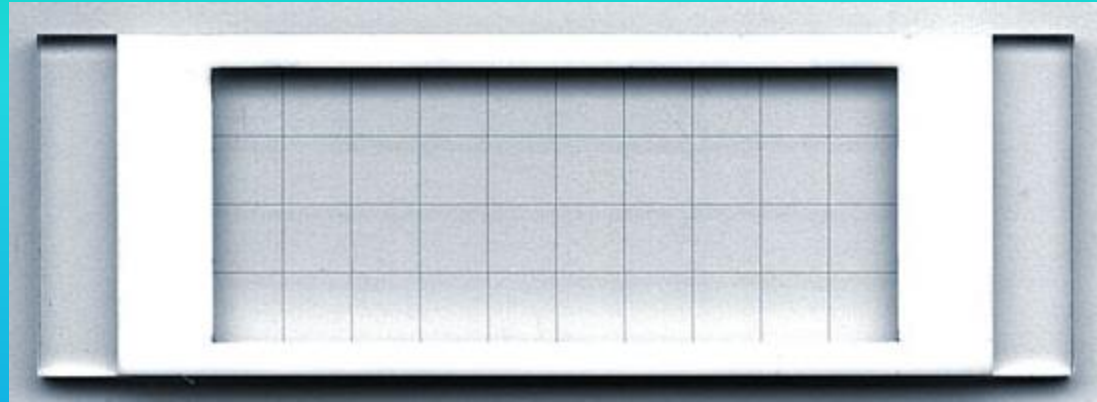
Cylindrospermopsis raciborskii (CYN)



Chrysosporum ovalisporum (CYN)



Quantitative: know volume



Sedgwick-Rafter Counting Cell



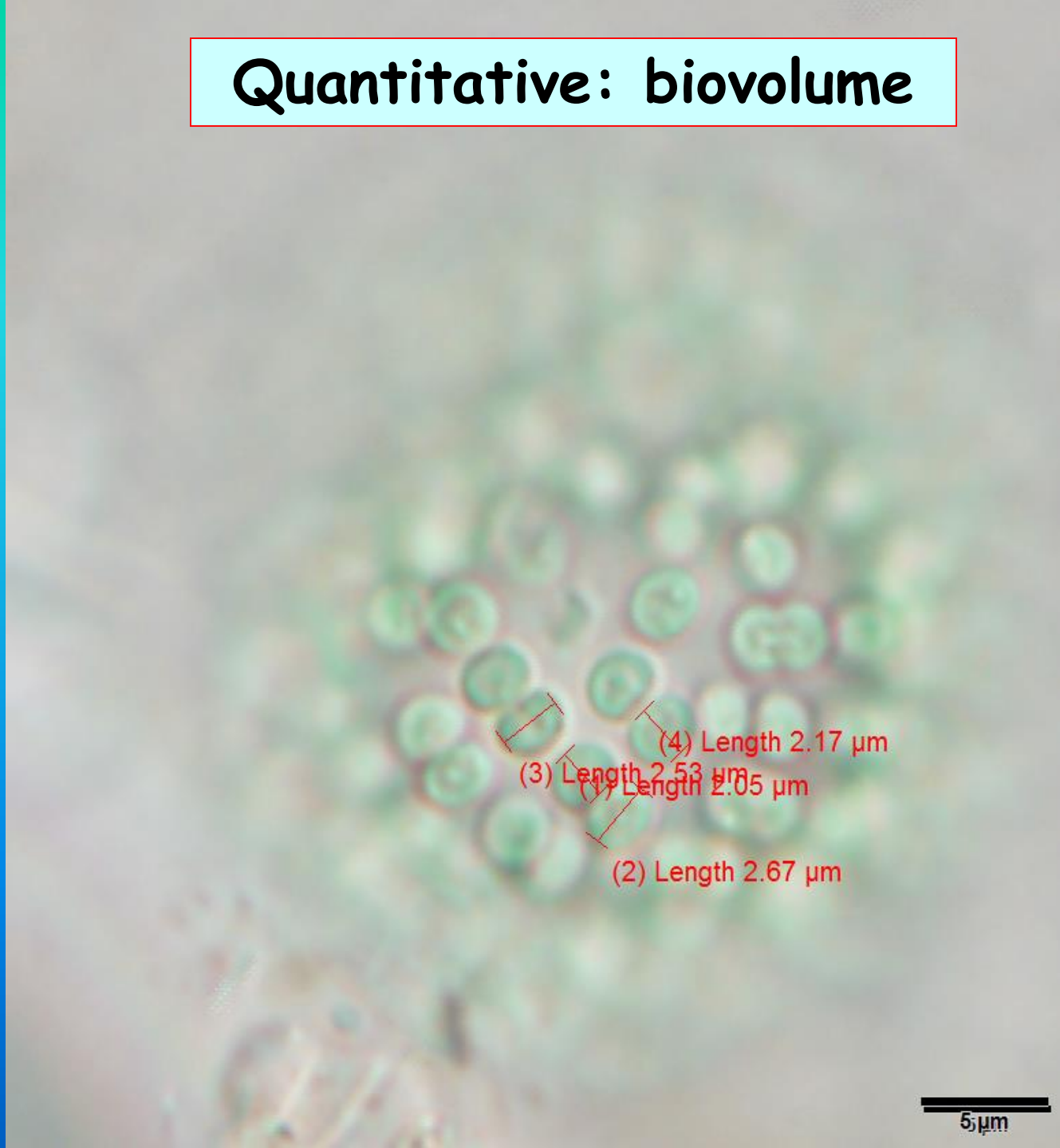
nanoplankton chamber

Quantitative: tally sheets


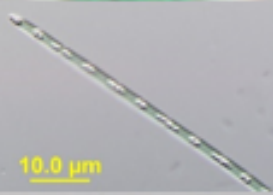
[illegible]

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)	subsampling wt. (grams) (1 gr. = 1 mL)	area scrapped represented by the subsample	area subsampled spread across on coverslip 22 mm sq.	each mm sq of the coverslip 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 original 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

Quantitative: biovolume



Quantitative: biovolume

<i>Synechocystis</i> sp.		l	13.8	sphere	$V = \pi/6 * d^3$	1,369.39	13.78
		w	13.8				13.78
<i>Limnothrix</i> 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**

Sample Protocol and Preparation

1. Collect 100 mL sample of a bloom live

Possible Methods:

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Thank you!