Sampling Protocols

Barry H. Rosen, Ph. D.

Office of the Southeast Regional Director

(CFLWSC) Orlando, FL

brosen@usgs.gov

407-803-5508

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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

brosen@usgs.gov

Open-File Report 2015–1164

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

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Getting a Sample qualitative

Not dense: use a plankton net!

Dense: use a glove!

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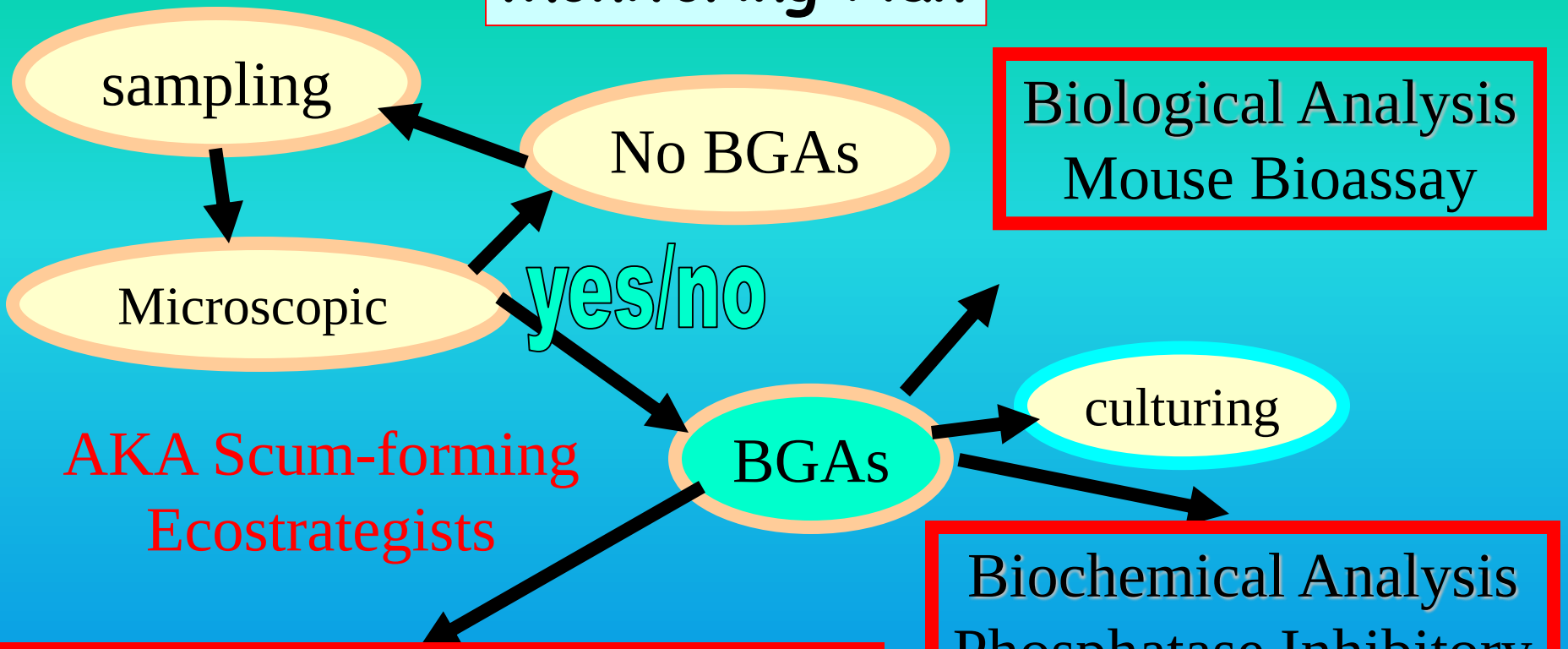
Monitoring Plan



Biological Analysis
Mouse Bioassay

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

Physio-chemical Analysis of Toxins
Test Kits for MC, CY, ANA and Sax
HPLC, LC/MS - microcystins, anatoxin-a & saxitoxin)

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Probes

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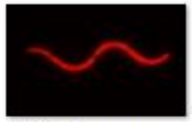
Qualitative: Diversity and **Potential** toxin producers


Cylindrospermopsis Mattamuskeet W. 10-22-15 WG curvy 2 cyano


Cylindrospermopsis Mattamuskeet W. 10-22-15 WG curvy cyano


Komvophoron 1 Mattamuskeet E. 7-22-15 Live 930 am cyano


Komvophoron 2 Mattamuskeet E. 7-22-15 930 am cyano


Komvophoron Mattamuskeet E. 9-10-15 Live 900 am cyano


Komvophoron Mattamuskeet W. 7 22 15 K. apiculatum me...


Komvophoron Mattamuskeet W. 7-22-15, culture 8-3-15 cyano


Komvophoron Mattamuskeet W. 7-22-15, culture 8-7-15 1a cyano

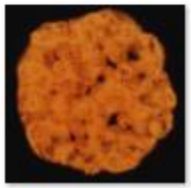

Merismopedia Mattamuskeet E. 7-22-15 Live 930 am cyano

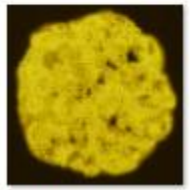

Merismopedia Mattamuskeet W. 7-22-15, culture 8-3-15 cyano

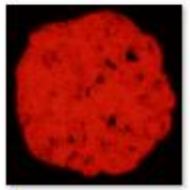

Merismospedia Mattamuskeet E. 7-22-15 930 am cyano


Merismospedia Mattamuskeet W. 7-22-15, culture 8-7-15 cyano

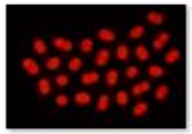

Microcystis culture 1a LM M. aeruginosa cyano


Microcystis culture 1a UV M. aeruginosa cyano

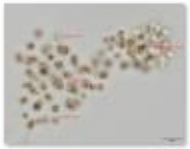

Microcystis culture 1a WB M. aeruginosa cyano


Microcystis culture 1a WG M. aeruginosa cyano


Microcystis Mattamuskeet W. 10-22-15 LM mea M. aeruginosa c...


Microcystis Mattamuskeet W. 10-22-15 WG M. aeruginosa cyano


Microsystis Mattamuskeet W. 10 22 15 M. aeruginosa cyano


Microsystis Mattamuskeet W. 10 22 15 mea M. aeruginosa cyano


Phormidium Mattamuskeet E. 8 13 15 1a cyano


Phormidium Mattamuskeet E. 8 13 15 1b cyano


Phormidium Mattamuskeet E. 8 13 15 c cyano


Phormidium Mattamuskeet E. 8 13 15 c mea cyano

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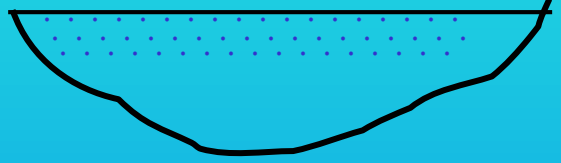
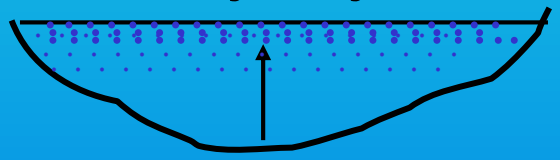
Beware of this phenomenon

initial distribution



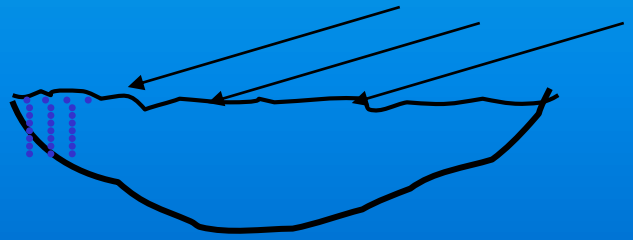
100,000 cells/L;
20 µg/L toxin

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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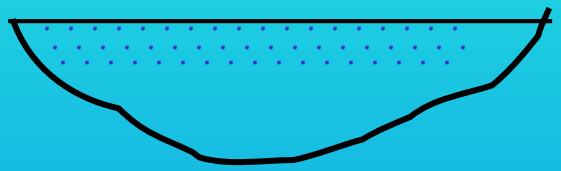
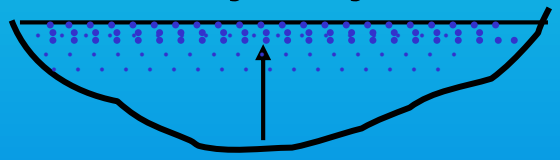
Where do I sample?

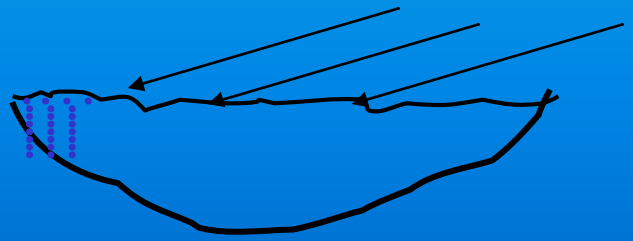
initial distribution

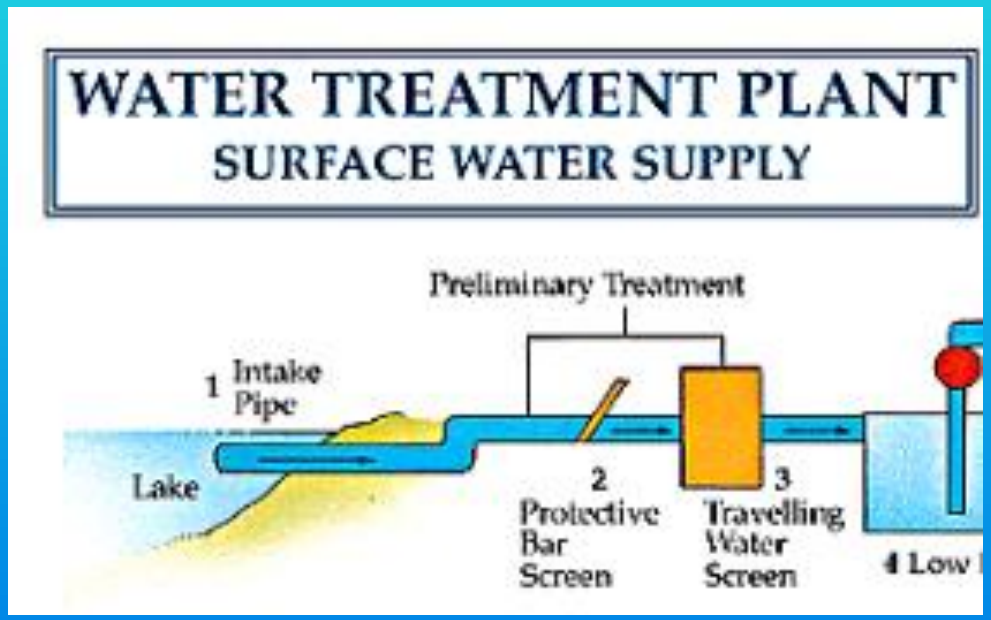


buoyancy



wind





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Getting a Sample quantitative

Van Dorn

Depth Integrated Sampling

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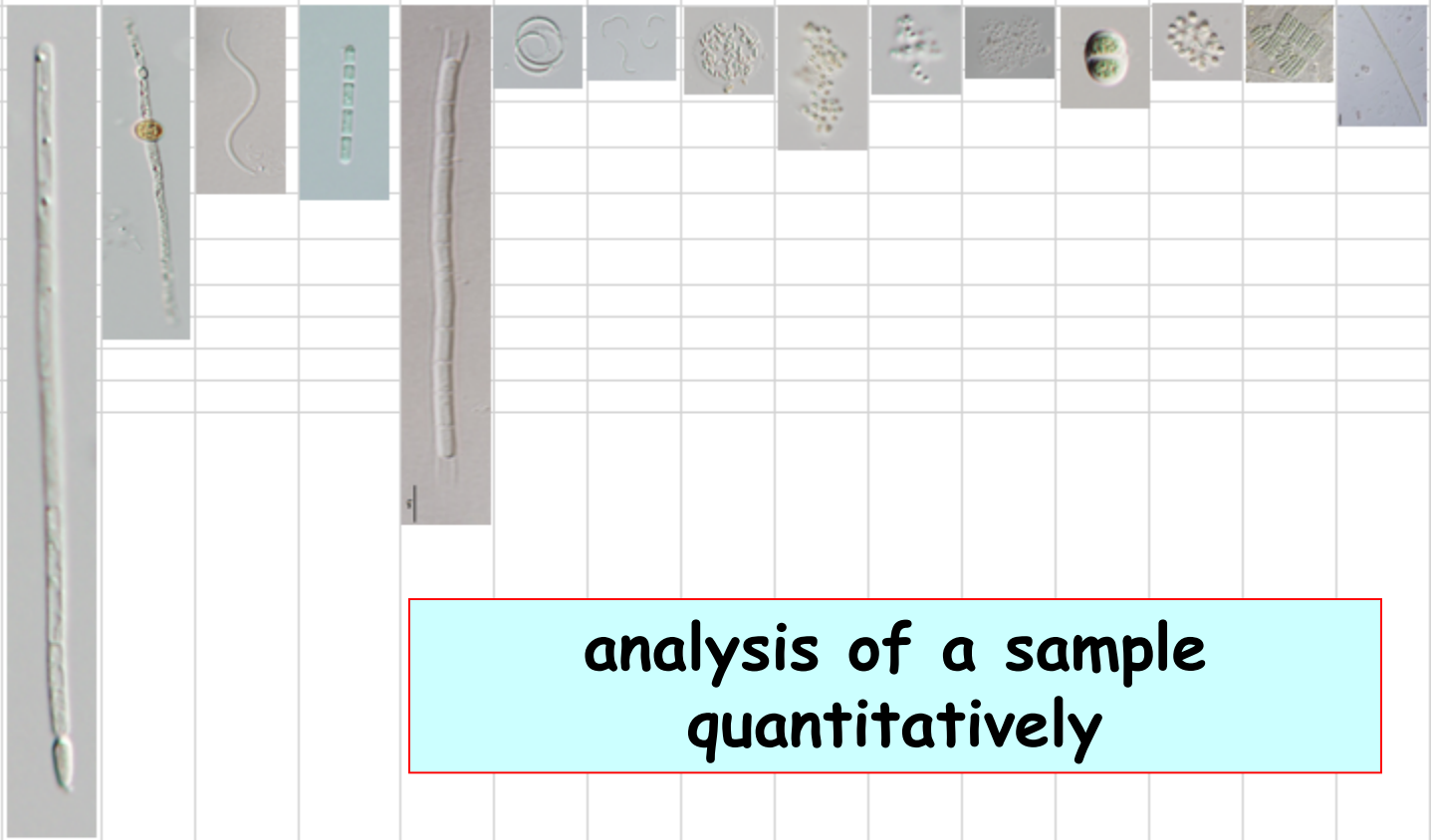
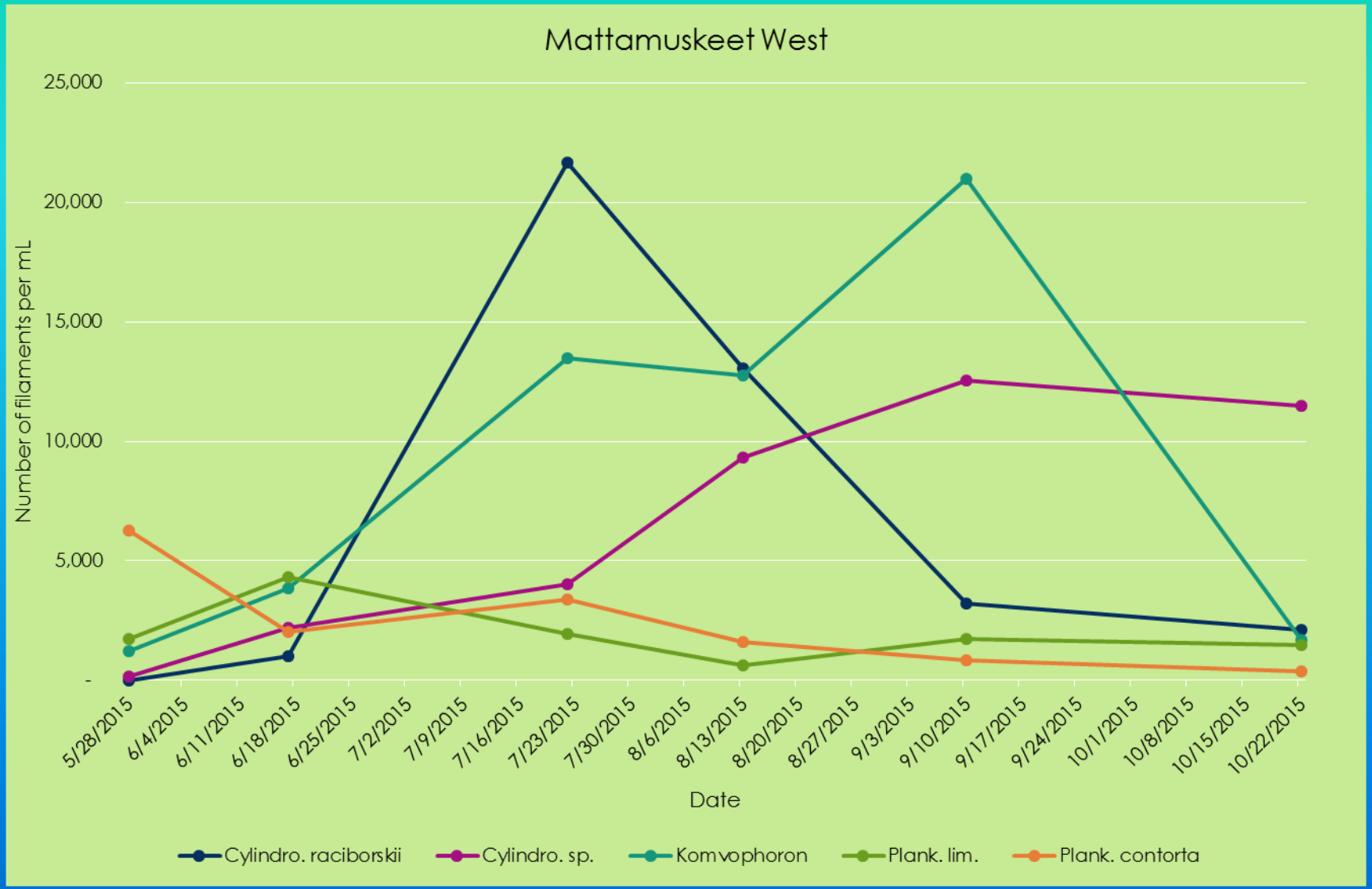


analysis of a sample quantitatively

				Cylindrospermopsis raciborskii	Chrysosporum ovalis	Cylindrospermopsis cf. catemaco	Komvophoron sp.	Planktothrix limnetica	Planktolyngbya contorta	Romeria sp.	Aphanothece stagnina	Microcystis aeruginosa	Aphanocapsa nibilum	Aphanocapsa sp.	Chroococcus sp.	Snowella sp.	Merismopedia sp.	Sphaerospermopsis aphanizomenoides
plankton/ peri	Concentration factor	number of fields	cell count															
plankton	10x	6	311	0	0	4	3	44	127	0	3	1	3	69	0	2	28	0
plankton	10x	6	318	0	0	3	23	32	117	0	3	0	0	71	6	20	14	1
plankton	10x	7	307	9	5	33	17	67	53	1	1	0	0	59	6	14	8	0
plankton	10x	6	343	19	1	41	72	81	38	2	14	1	0	40	0	11	6	0



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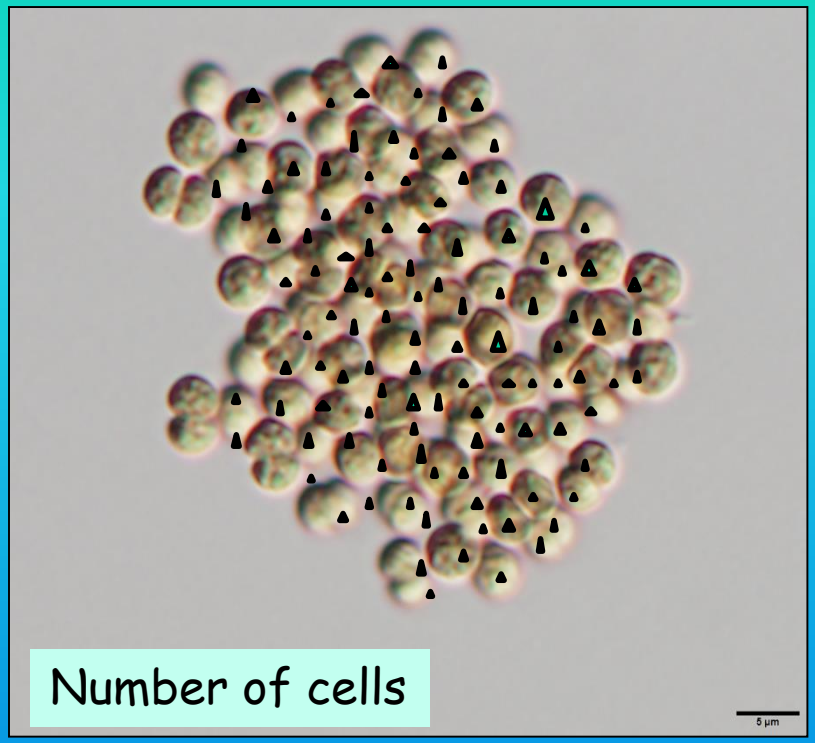
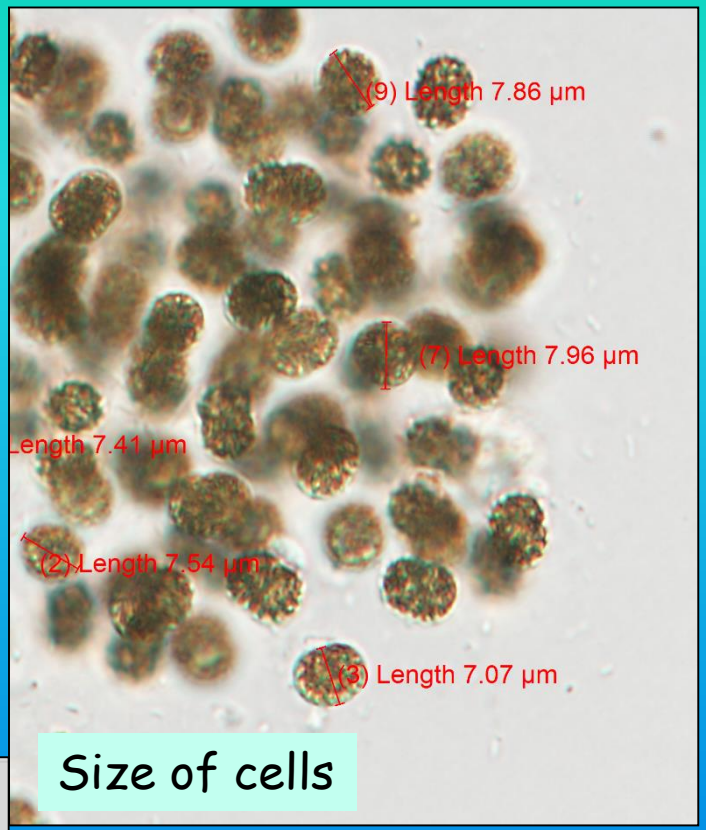
Biovolume per mL



Number of cells



Size of cells

Sphere

Solve for volume

$$V = \frac{4}{3}\pi r^3$$

r Radius Enter value

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

1. **Collect 100 mL sample of a bloom <u>live</u>**

   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.

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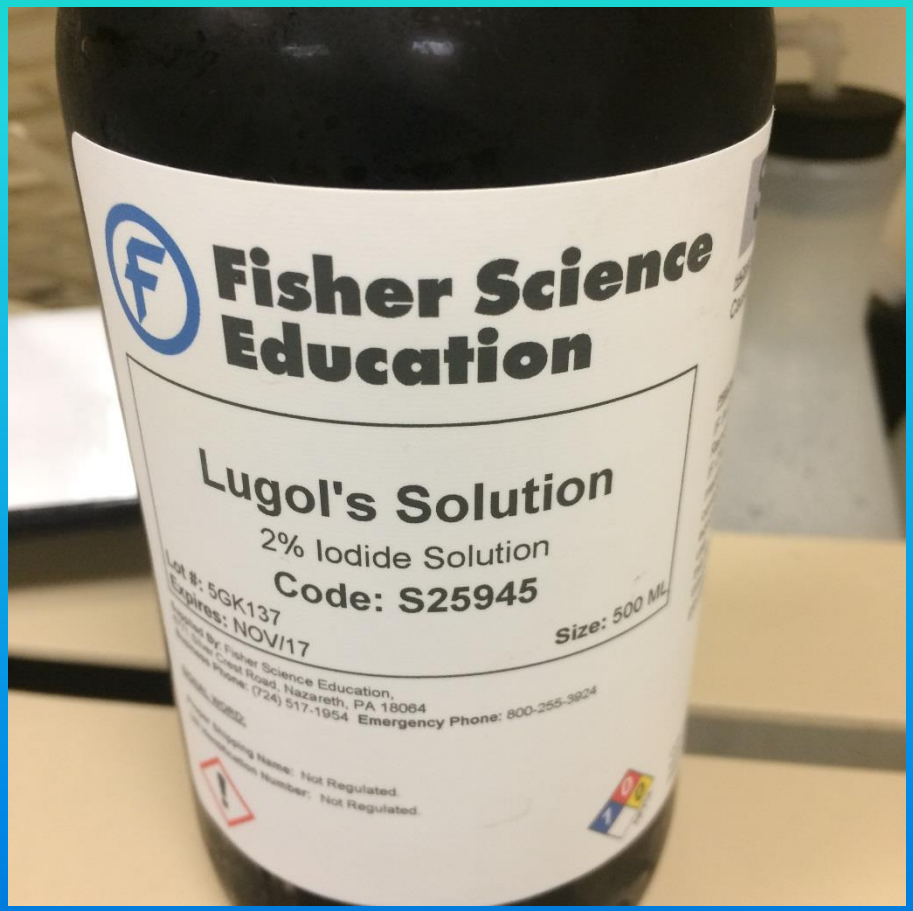
Lugol's and Settling



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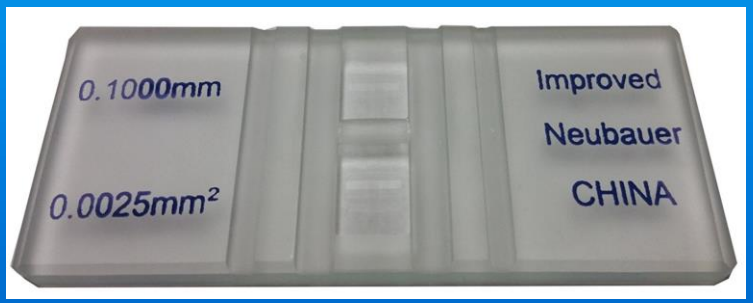
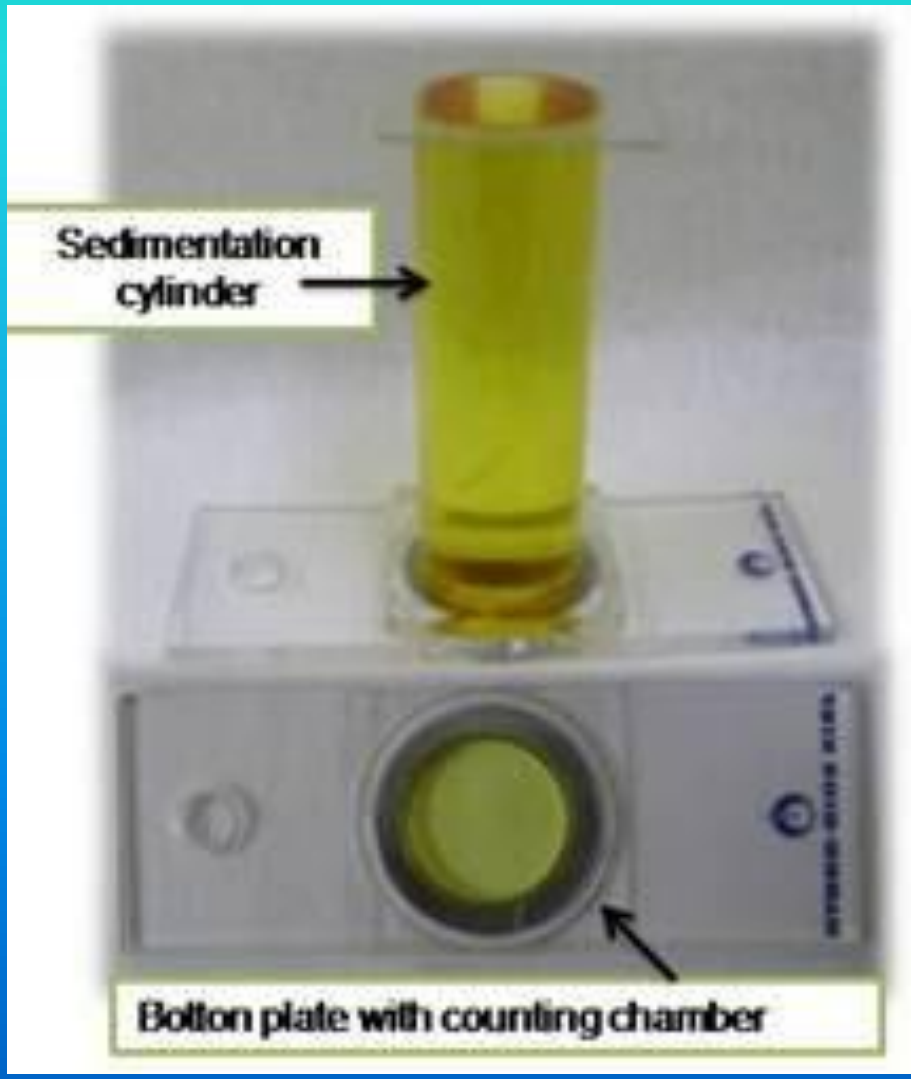
Quantitative Counting





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Semi-Quantitative Counting and Toxins

Lab Instruments

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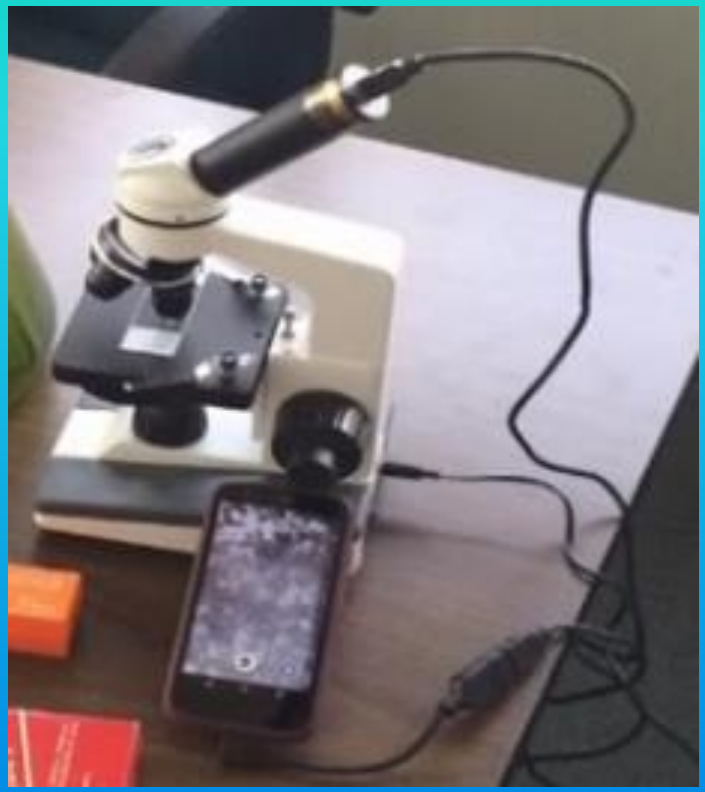
Lab Instruments



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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.

R021516

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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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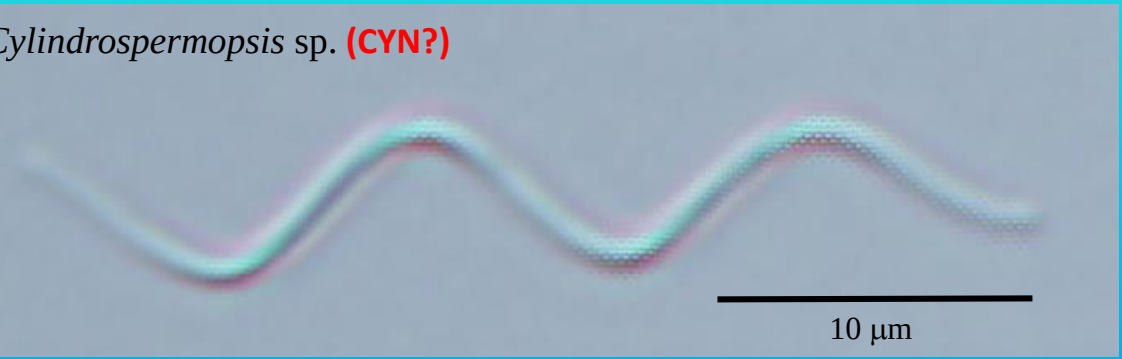
Thank You!

Cylindrospermopsis raciborskii **(CYN)**

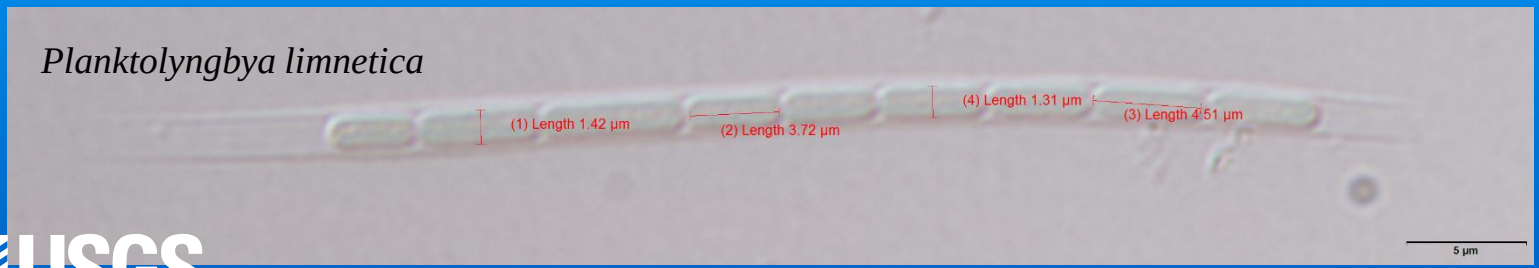
Cylindrospermopsis sp. **(CYN?)**



Pseudanabaena contorta **(MYC)**

Komvophoron sp. (*Psuedanabaena*)

Planktolyngbya limnetica



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