Sampling Cyanobacteria – From Cells to Toxins

Jennifer L. Graham

U.S. Geological Survey

U.S Environmental Protection Agency CyanoSymposium 2023

October 16, 2023

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

Cyanobacteria and Cyanotoxins are Diverse

	Hepatotoxins		Neurotoxins		Dermatoxins
	CYL	MC	ANA	SAX	
Anabaena/Dolichospermum	X	X	X	X	X
Aphanizomenon	X	?	X	X	X
Microcystis		X			X
Oscillatoria/Planktothrix		X	X	X	X

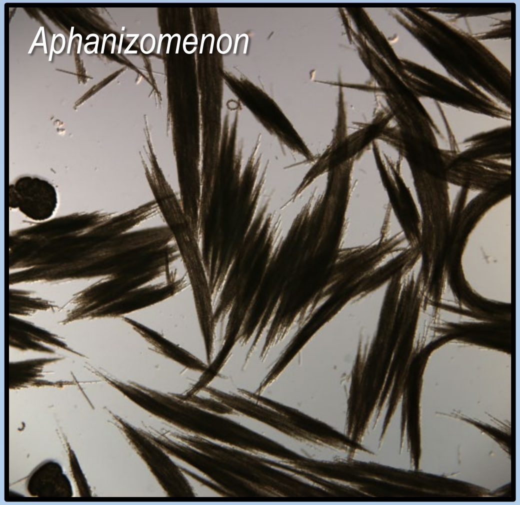


Photo Credit: A. St. Amand, PhycoTech

After Graham and others, 2008

The Spatiotemporal Variability of Cyanobacteria Poses Unique Challenges to Monitoring and Assessment

Rock Creek Lake, Iowa
2006 Beach Closure Event

Beach Area
Monday
July 31

Photo Credit: IA DNR

Photo Credit: IA DNR

Beach Area
Thursday
August 3

Photo Credit: J. Graham, USGS

Boat Ramps
Friday
August 11

USGS
science for a changing world

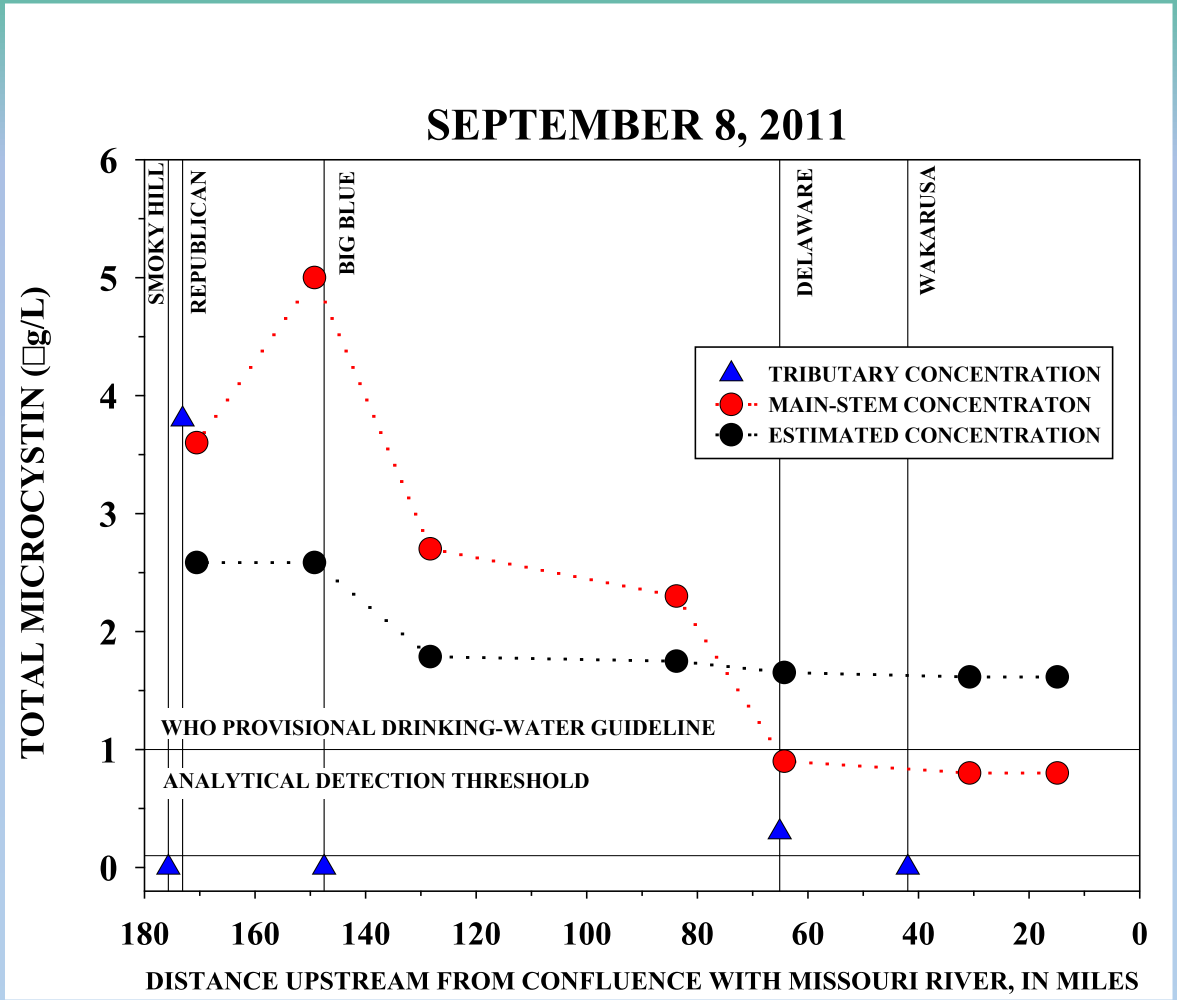
The Spatiotemporal Variability of Cyanobacteria Poses Unique Challenges to Monitoring and Assessment

Photo Credit: K. Bouma-Gregson, USGS

Photo Credit: K. Carpenter, USGS

USGS
science for a changing world

Cyanobacteria and Cyanotoxins May Be Transported for Long Distances Downstream of Source Areas



Graham et al., 2012

OPEN ACCESS Freely available online PLoS one

Evidence for a Novel Marine Harmful Algal Bloom: Cyanotoxin (Microcystin) Transfer from Land to Sea Otters

Melissa A. Miller[1,2]*, Raphael M. Kudela[2], Abdu Mekebri[3], Dave Crane[3], Stori C. Oates[1], M. Timothy Tinker[4], Michelle Staedler[5], Woutrina A. Miller[6], Sharon Toy-Choutka[1], Clare Dominik[7], Dane Hardin[7], Gregg Langlois[8], Michael Murray[5], Kim Ward[9], David A. Jessup[1]

1 Marine Wildlife Veterinary Care and Research Center, California Department of Fish and Game, Office of Spill Prevention and Response, Santa Cruz, California, United States of America, 2 Ocean Sciences Departm
California Department of Fish and Game, C
Center, United States Geological Survey, Lo
States of America, 6 Department of Patholog
of America, 7 Applied Marine Sciences, Live
America, 9 Division of Water Quality, State

Photo Credit: Getty Images

USGS science for a changing world

Overview

- ***Why*** sample?
- ***When*** to sample?
- ***Where*** to sample?
- ***How*** to sample?
- ***What*** to sample?
  - Direct measures
    - Cyanobacteria
    - Cyanotoxins
  - Indirect measures
    - Cameras
    - Pigments
    - Genetics

Photo Credit: J. Graham, USGS

USGS
science for a changing world

Why **Sample?**

- **Public Health Protection**
  - Recreational Areas
  - Drinking Water Supplies
  - Mitigation and Management
- **Reconnaissance**
  - Occurrence
  - Distribution
  - Concentration
  - Status and Trends
- **Research**
  - Fate and Transport
  - Environmental Drivers
  - Ecosystem Effects


Photo Credit: B. Brink


Photo Credit: D. Obrecht, University of Missouri

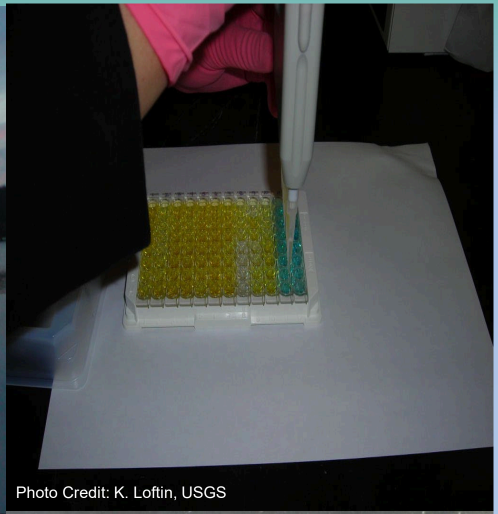


Photo Credit: K. Loftin, USGS


Photo Credit: J. Graham, USGS


Photo Credit: J. Graham, USGS


Photo Credit: B. Brink

The Why Informs All Other Aspects of Sample Collection

General objective	Site location	Sample frequency	Sample type
Recreational areas	Beaches Open water areas used for full-body contact recreation Bay or cove areas used for full-body contact recreation Public access sites	Routine basis during periods of peak recreational use • Daily • Weekly	Surface sample Integrated photic zone
Drinking-water supplies	Location relevant to the drinking-water intake(s)	Routine basis • Daily • Weekly During periods when events have historically occurred During events	Discrete depth Integrated photic zone Integrated epilimnion Integrated water column

Graham and others, 2008

When to Sample?

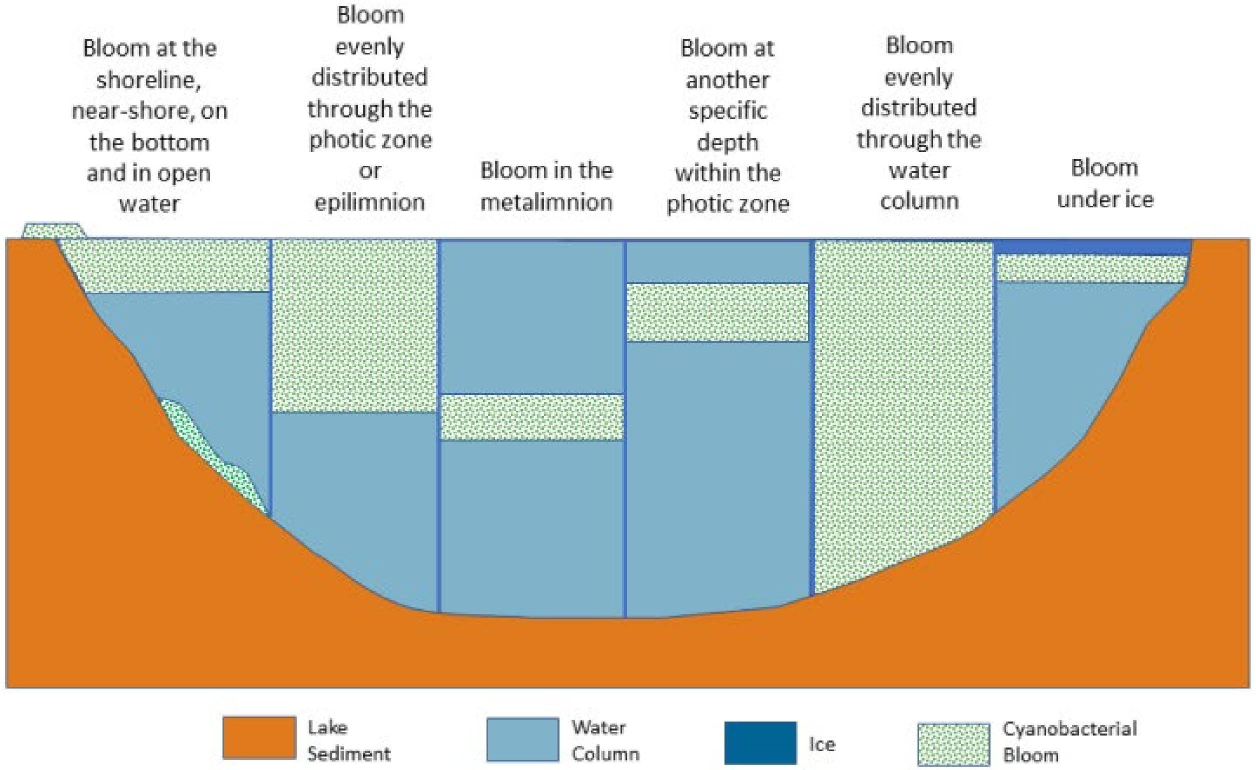
- **Recreational Areas**
  - Seasonal
  - Routine or Tiered Approach
  - Daily or Weekly
  - Event Based
- **Drinking-Water Supplies**
  - Routine or Tiered Approach
  - Daily or Weekly
  - Event Based
- **Other Types of Sampling**
  - Variable
  - Objective Driven

Do not try this at home (or anywhere else)!

Grand Lake St. Marys, Ohio
Source of Photos Unknown

USGS
science for a changing world

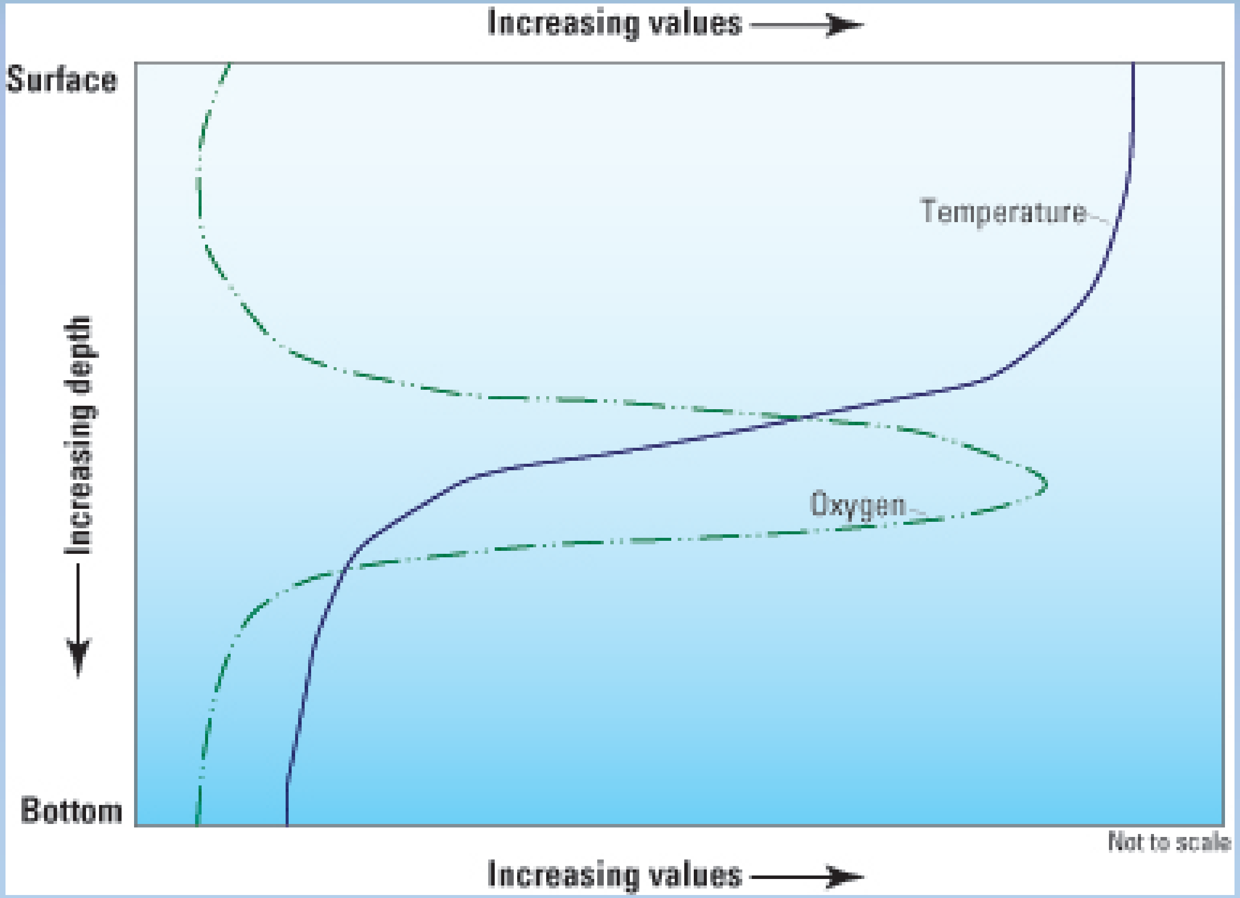
Where to Sample?



Modified from Graham and others, 2008 by ITRC

Determining the Location of Cyanobacteria in the Water Column

- Visual assessment
- Vertical profiles
  - Photic depth
  - Stratification
  - Mixed depth
  - Photosynthetic activity
- Signs of photosynthetic activity include
  - Sharp increases in pH and dissolved oxygen
  - Increased fluorescence



Graham and others, 2008

USGS
science for a changing world

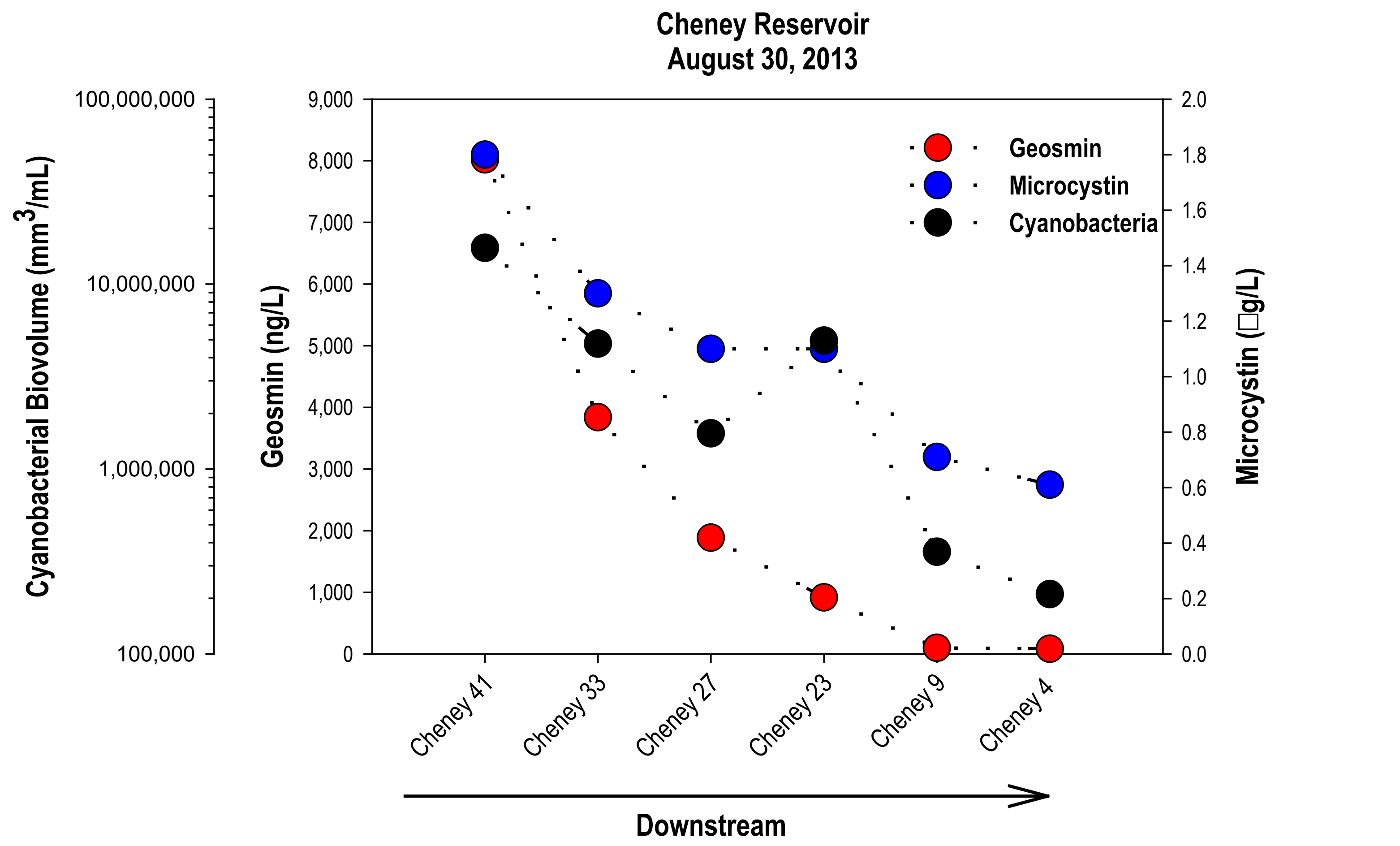
Concentrations of Cyanobacteria or Cyanotoxins May Vary Substantially at Different Sample Locations Within a Lake

Microcystin: 13 µg/L

Microcystin: 4 µg/L

Samples collected about 50 m apart

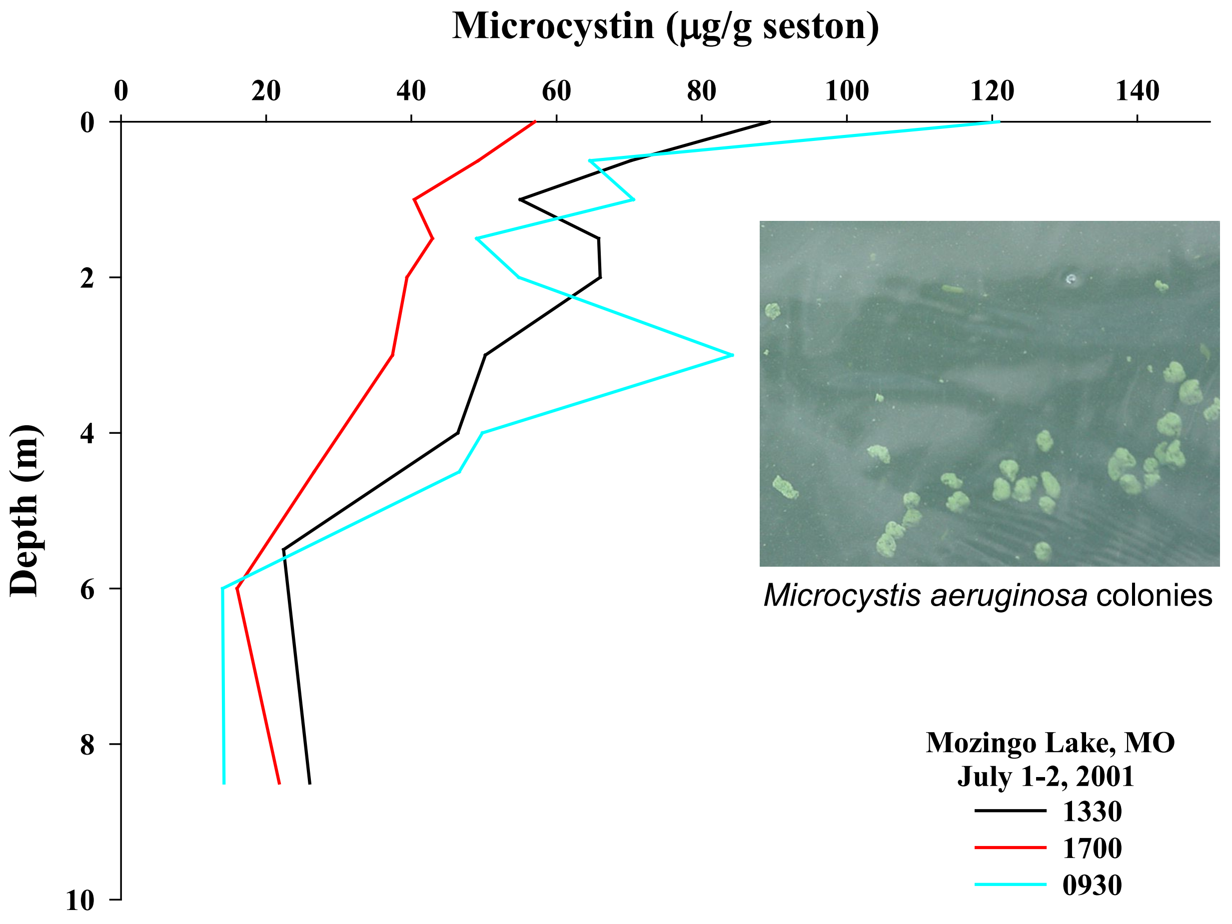
Concentrations of Cyanobacteria or Cyanotoxins May Vary Substantially at Different Sample Locations Within a Lake



After Otten et al., 2016

USGS
science for a changing world

Concentrations of Cyanobacteria or Cyanotoxins May Vary Considerably at a Single Site Depending on When and Where Samples Are Collected



Microcystis aeruginosa colonies

Time	Sample Type and Microcystin Concentration (µg/g seston)			
	Surface	Integrated Photic Zone	Integrated Epilimnion	Integrated Water Column
0930	121	68	71	57
1330	89	58	66	55
1700	57	39	42	37

After Graham and others, 2006

How to Sample?

- Visual surveys
- Surface samples
- Discrete-depth samples
  - Location of the cyanobacterial community is known
  - Structure of interest at depth
  - Vertical water column distribution of interest
- Depth-integrated samples
  - Integrated photic zone
  - Integrated epilimnion
  - Integrated water column

How to Sample?

- Benthic samples
  - Visual surveys
  - Rock scrapes
  - Artificial substrates
- Patchiness is a challenge for representative sample collection
- Quantitative sampling is a challenge

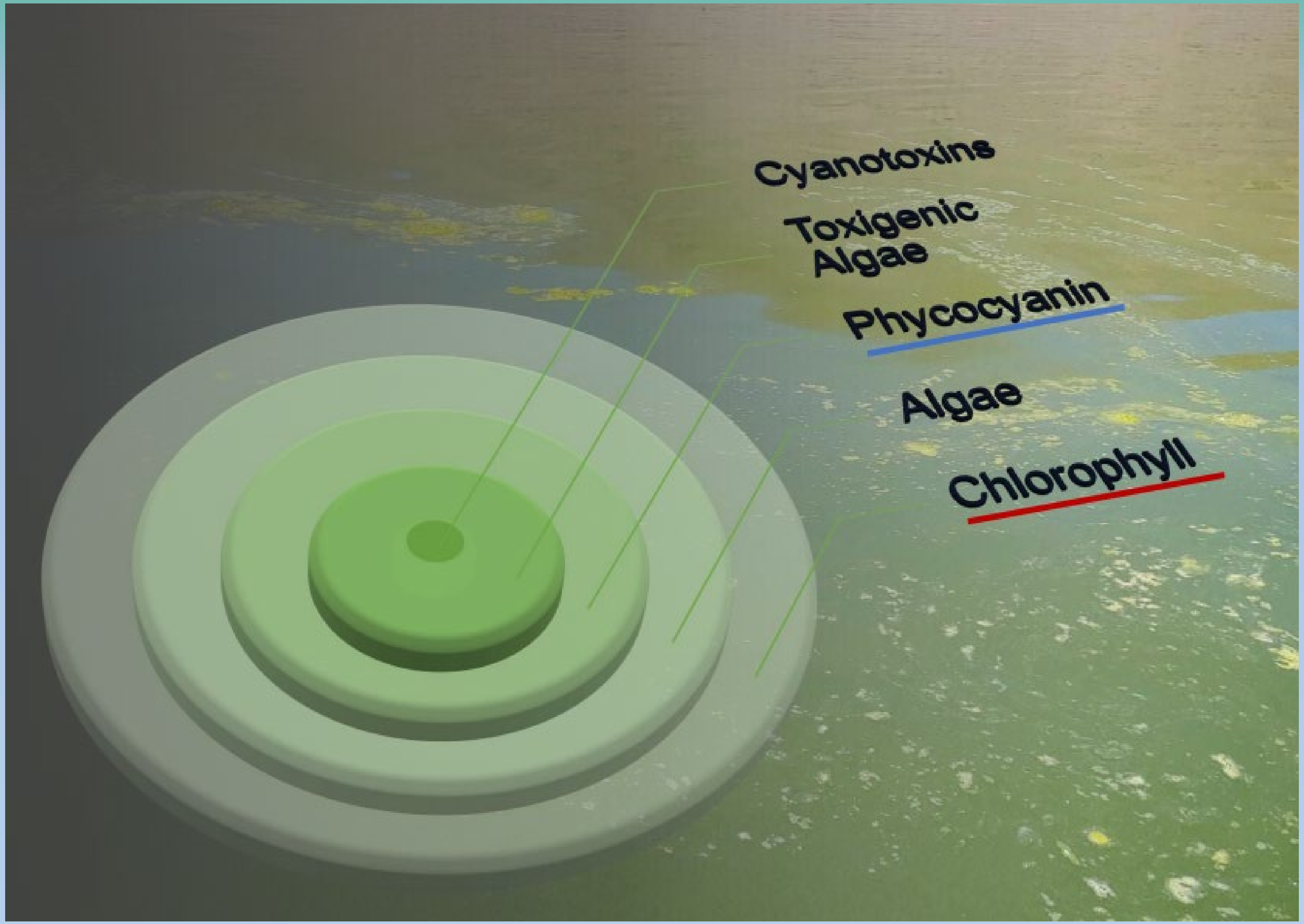
Considerations When Choosing Where and How to Sample

- Specific study objectives
- Stratification
- Spatial and/or water-column distribution of cyanobacteria
- Flexibility of sampling plans
  - Where and how to collect samples may be decided in the field
- Quality assurance and control
  - Spatial variability may influence field replicates
  - Cyanobacteria may influence split replicates

Photo Credits: J. Graham, USGS

What to Sample?

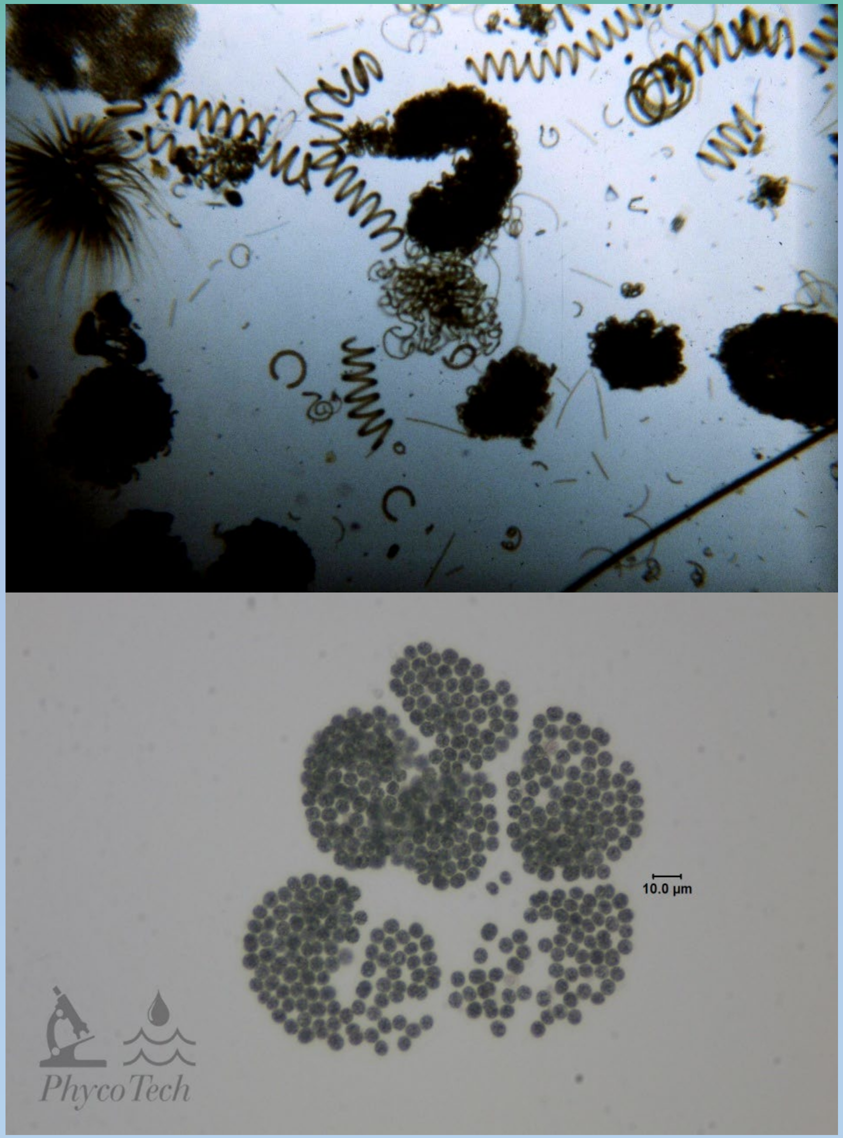
- **Direct measures**
  - Cyanobacteria
  - Cyanotoxins
- **Indirect measures**
  - Cameras
  - Pigments
  - Genetics



Courtesy of T. King, USGS

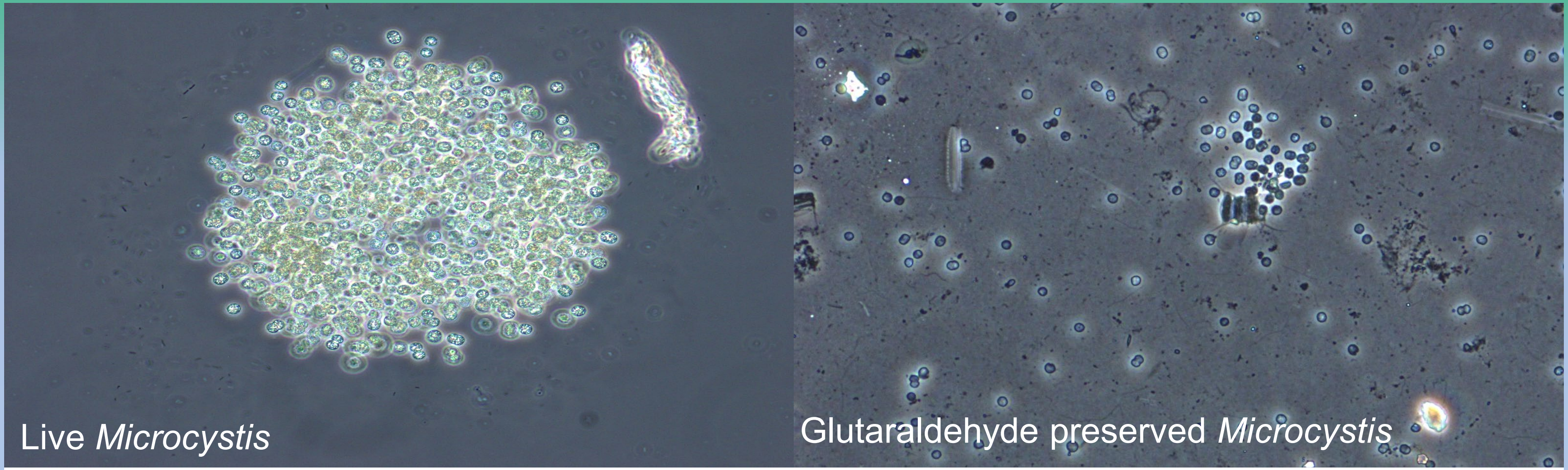
Cyanobacteria Sampling

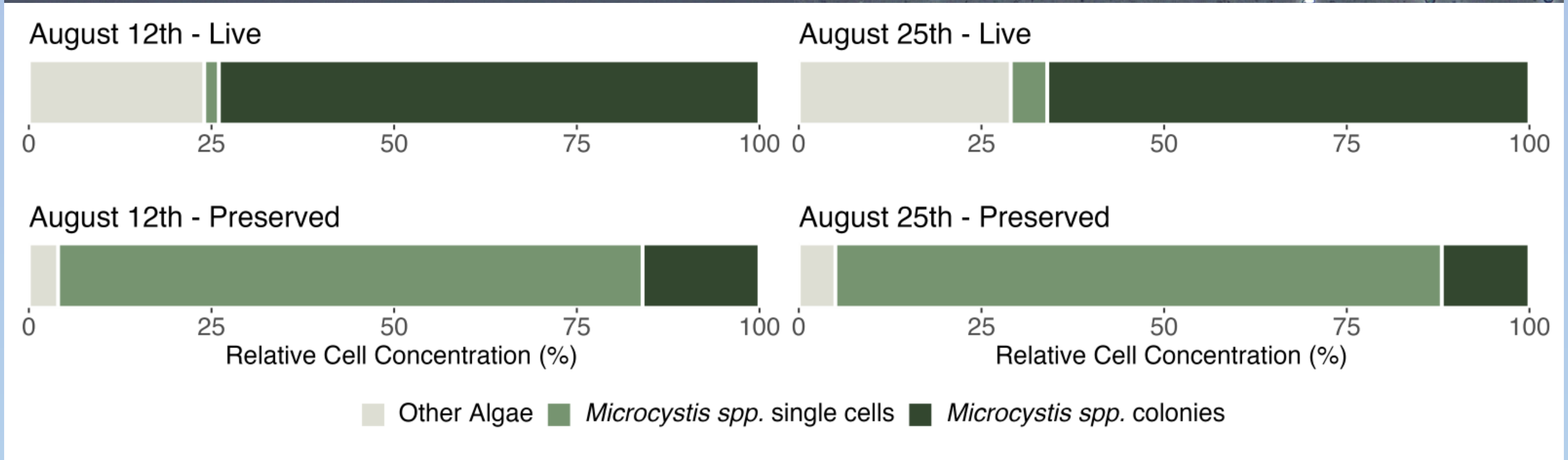
- Live or preserved
  - *Formaldehyde*: hazardous fumes, distorts cells, bleaches color
  - *Lugol's Iodine*: stains starch, may cause loss of flagella and cell distortion, loses preservative power over time
  - *Glutaraldehyde*: hazardous fumes, retains color and fluorescence, limited cell distortion
- Qualitative or quantitative
- Unit of measure
  - Natural units: NU/mL
  - Abundance: Cells/mL
  - Biovolume: µm3/mL



Courtesy of A. St. Amand, PhycoTech, Inc.

Preservation Effects



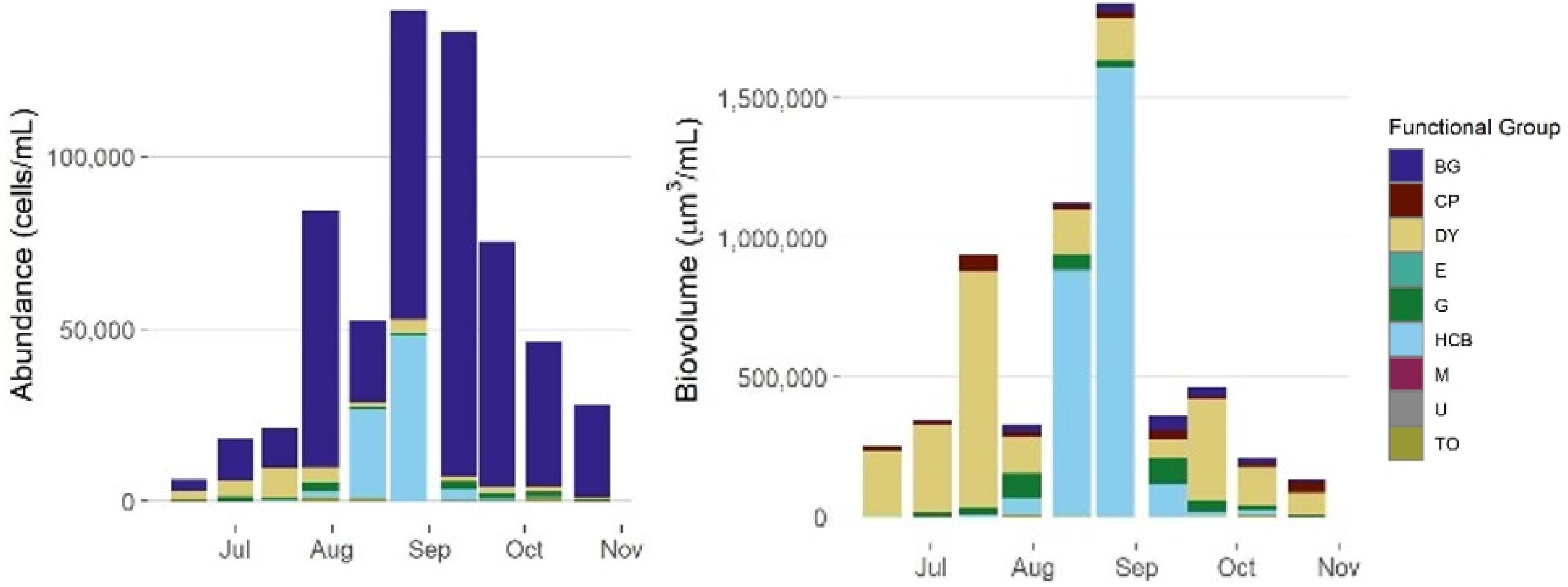


After Gifford et al., in review

USGS
science for a changing world

Cyanobacterial Abundance and Biovolume

Owasco Lake, New York – June through November 2020



After Gifford et al., in review

USGS
science for a changing world

Traditional Microscopy and Flow Cytometry

	Number of Samples per Day	Number of NU	Analysis Level	Size Range	Turnaround Time
Manual	6 - 8	400	Species	0.9um - 25+ mm	3-6 months
Flow Cytometry	12 - 20	1,000 - 10,000	Class	4 - 200um	24 hours

Courtesy of A. St. Amand, PhycoTech, Inc

Photo Credit: S. Gifford, USGS

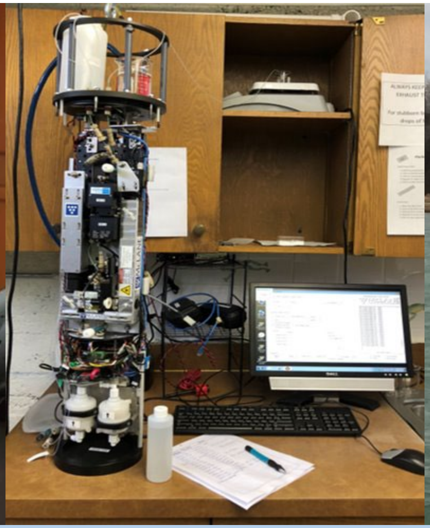


Photo Credit: A. St. Amand, PhycoTech, Inc

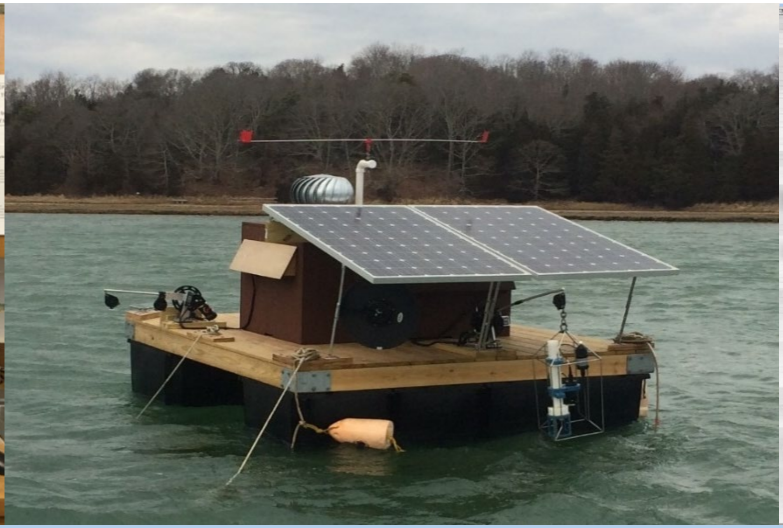


Photo Credit: M. Broshnahan, WHOI

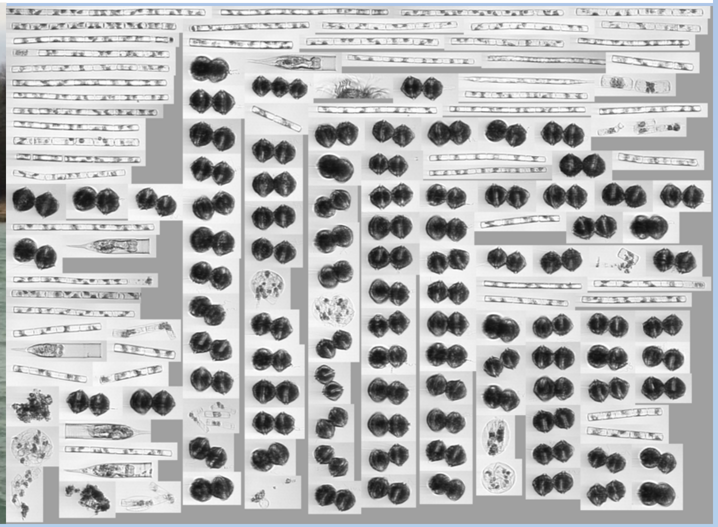
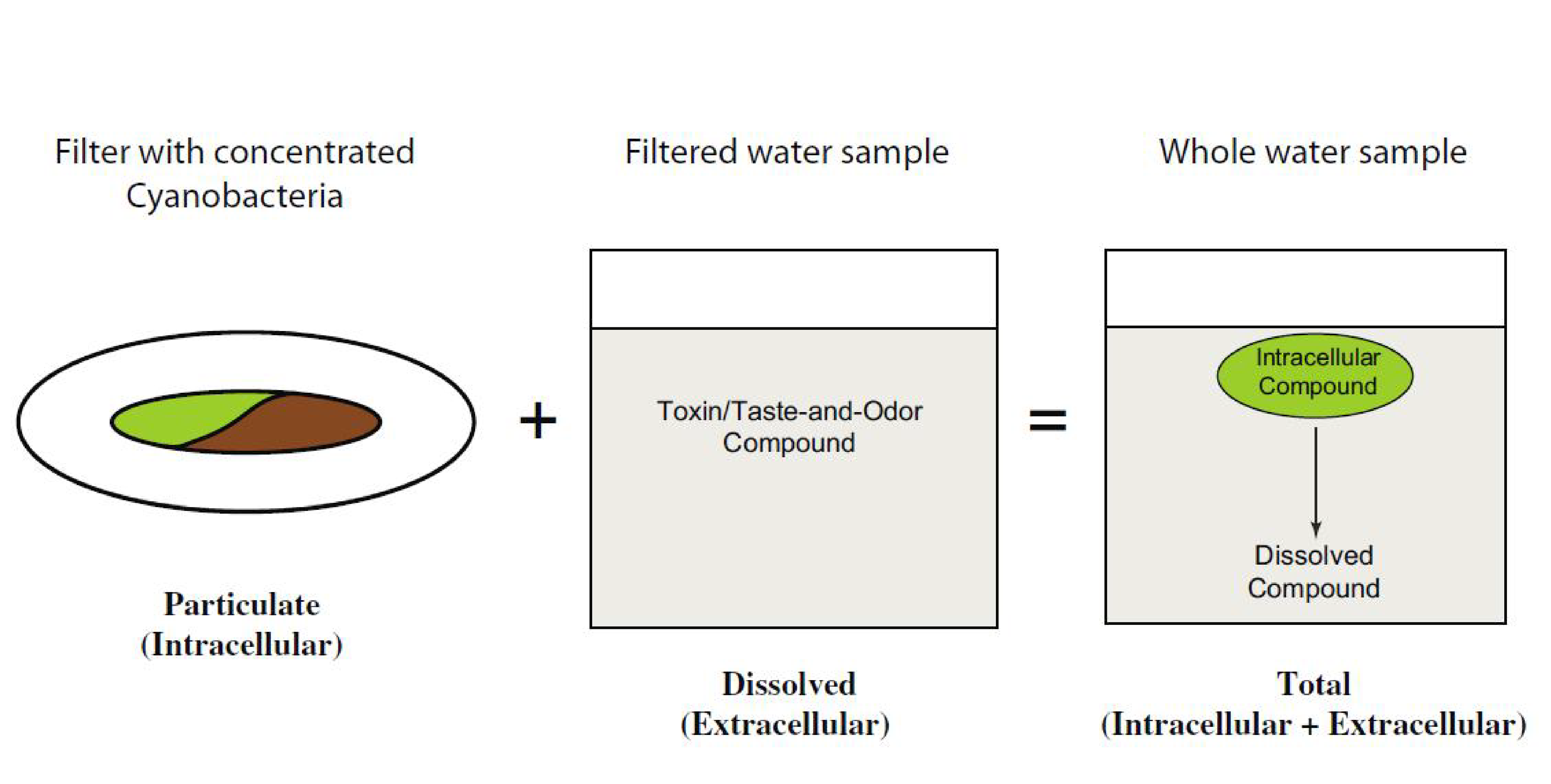


Photo Credit: Woods Hole Oceanographic Institute (WHOI)

USGS
science for a changing world

Cyanotoxin Sampling



USGS
science for a changing world

Sample Bottles, Preservatives, and Hold Times are Cyanotoxin and Analysis Specific

eurofins | Abraxis

Cyanotoxins in Water

Sample Collection Quick Reference Guide for ELISA Plate Assays

Analyte	Collection/Storage Container Use with PTFE lined screw caps	Sample Preservation**	Storage & Handling
Anabaenopeptins	• Glass – clear or amber • Polyethylene terephthalate glycol (PETG)	**Treated water:** *Immediately upon collection*, quench chlorine with ascorbic acid or sodium thiosulfate* (up to 1 mg/mL; recommended concentration 0.1 mg/mL)	Analyze immediately, refrigerate (4 °C) for up to 5 days, or freeze (-20 °C) Ship overnight on ice
Anatoxin-a	• Glass – amber only, do **NOT** use clear glass ***Anatoxin-a is light sensitive – avoid exposure to light, as this will degrade the toxin and produce inaccurate results***	**Treated water:** *Immediately upon collection*, quench chlorine with ascorbic acid* (up to 1 mg/mL; recommended concentration 0.1 mg/mL). ***Do NOT use sodium thiosulfate, as it will degrade the toxin and produce inaccurate results*** **Freshwater (treated & raw):** *Immediately upon collection*, preserve the sample by diluting 9 parts sample to 1 part 10x concentrated sample diluent & adjust pH to between 5-7 using 1N NaOH or HCl‡ **Marine water:** Adjust pH to between 5-7 using 1N NaOH or HCl‡ ***‡Avoid exposure to high pH conditions, as this will degrade the toxin and produce inaccurate results***	Analyze immediately, refrigerate (4 °C) for up to 28 days, or freeze (-20 °C) if greater than 28 days Ship overnight on ice
BMAA	• Glass – clear only, do **NOT** use amber glass ***Toxin will adsorb to amber glass, producing inaccurate results*** • Polyethylene terephthalate glycol (PETG) • High density polyethylene (HDPE) • Polycarbonate (PC) • Polypropylene (PP) • Polystyrene (PS)	**All samples:** Analyze samples *immediately upon collection* or **freeze (-20 °C) to avoid degradation of the toxin, which will produce inaccurate results**	Analyze or freeze (-20 °C) immediately upon collection Ship overnight on ice
Cylindrospermopsin	• Glass – clear or amber • Polyethylene terephthalate glycol (PETG) • High density polyethylene (HDPE) • Polycarbonate (PC) • Polypropylene (PP) • Polystyrene (PS)	**Treated water:** *Immediately upon collection*, quench chlorine with ascorbic acid or sodium thiosulfate* (up to 1 mg/mL; recommended concentration 0.1 mg/mL)	Analyze immediately, refrigerate (4 °C) for up to 5 days, or freeze (-20 °C) Ship overnight on ice
Microcystins/ Nodularins	• Glass – clear or amber • Polyethylene terephthalate glycol (PETG) ***Avoid plastic containers other than PETG, as toxin will adsorb to container surfaces, producing inaccurate results***	**Treated water:** *Immediately upon collection*, quench chlorine with sodium thiosulfate* (up to 1 mg/mL; recommended concentration 0.1 mg/mL). ***Do NOT use ascorbic acid, as this may degrade the toxin and produce inaccurate results***	Analyze immediately, refrigerate (4 °C) for up to 5 days, or freeze (-20 °C) Ship overnight on ice
Saxitoxins	• Glass – clear or amber • Polyethylene terephthalate glycol (PETG) • High density polyethylene (HDPE) • Polycarbonate (PC) • Polypropylene (PP) • Polystyrene (PS)	**Treated water:** *Immediately upon collection*, quench chlorine with ascorbic acid or sodium thiosulfate* (up to 1 mg/mL; recommended concentration 0.1 mg/mL) **Freshwater (treated & raw):** *Immediately upon collection*, preserve the sample by diluting 9 parts sample to 1 part 10x concentrated sample diluent **Marine water:** None required	Analyze immediately, refrigerate (4 °C) for up to 5 days, or freeze (-20 °C) Ship overnight on ice

*The use of solid quenching reagents is recommended. *Note: EPA Method 546 prohibits the use of liquid reagent solutions for sample quenching.*

**Additional sample preparation such as cell lysis and filtration may be required prior to analysis depending on analytical objectives (e.g. "total" versus "free" toxin). Refer to specific kit user's guide for additional information.

DISCLAIMER: Current recommendations by applicable regulatory agencies should always take precedence and should be followed in the event of a conflict. R20190826

USGS science for a changing world

Cyanobacteria Colonies or Filaments Large Enough to Be Retained on a Net Can Be A Useful Indicator But is a Semiquantitative Approach

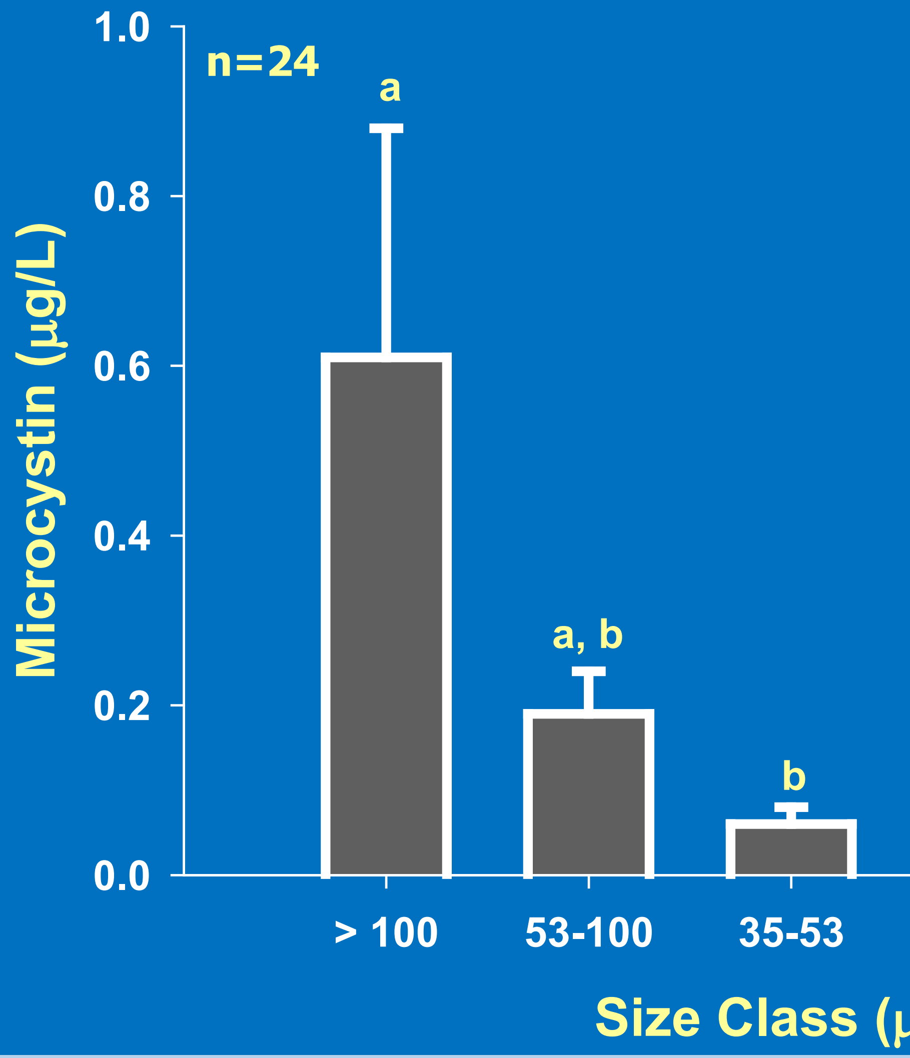


Photo Credit: S. Panken, University of Missouri

Photo Credits: J. Graham, USGS

USGS
science for a changing world

Solid Phase Adsorption Toxin Tracking (SPATT)

- Time-integrating (hours to weeks) passive sampler
- Microbead resins adsorb dissolved toxins (HP20 “Diaion” is common)
- Toxins extracted off resins
- Semiquantitative
- Complimentary, not interchangeable approach

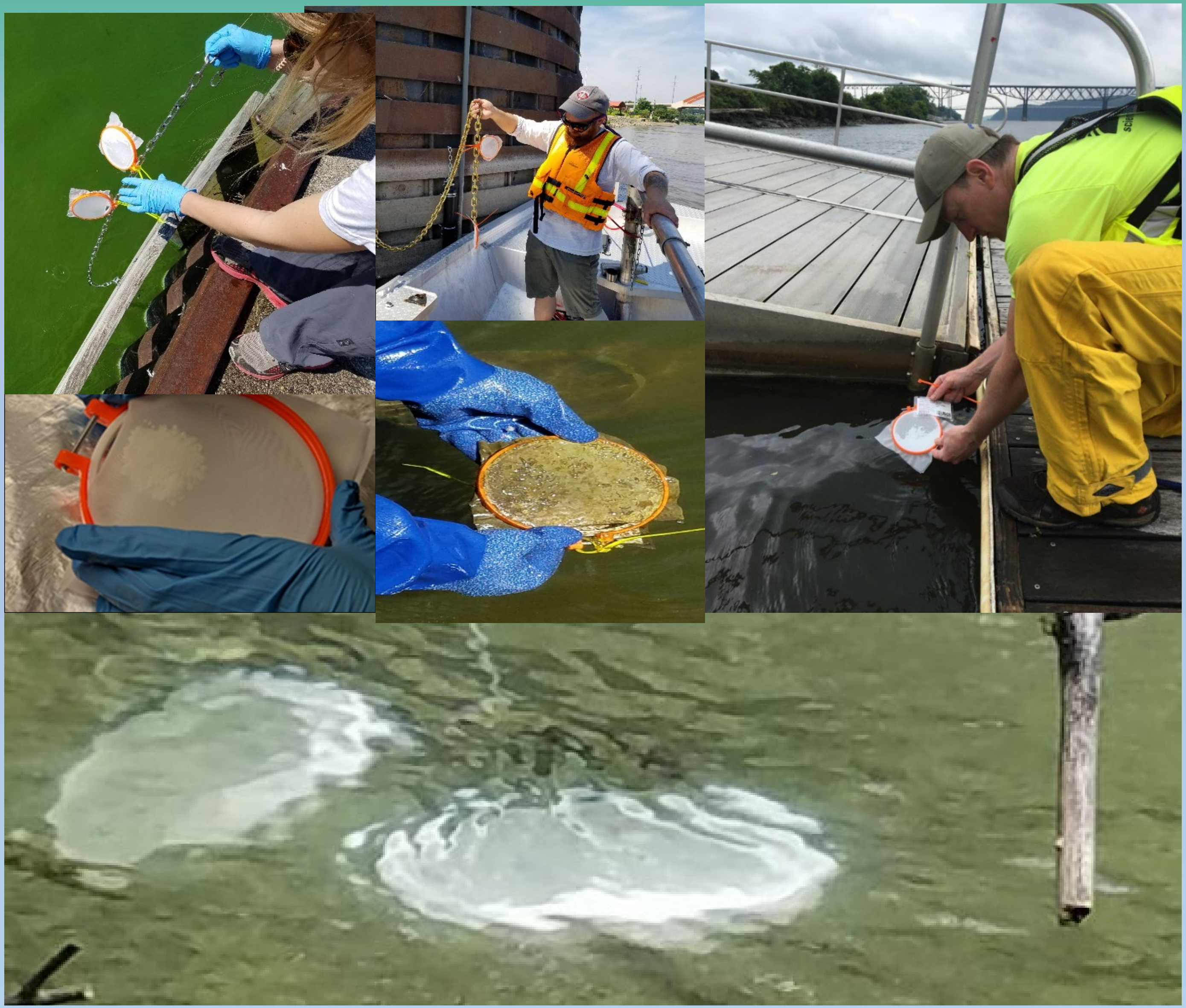


Photo credits: USGS

USGS
science for a changing world

Cameras Show Potential as Visual Monitoring Tools

Photo Credit: E. Emory, USACE

Photo Credit: C. Smith, USGS

July 20, 2016 at 3:54 pm

Photo Credit: G. Foster, USGS

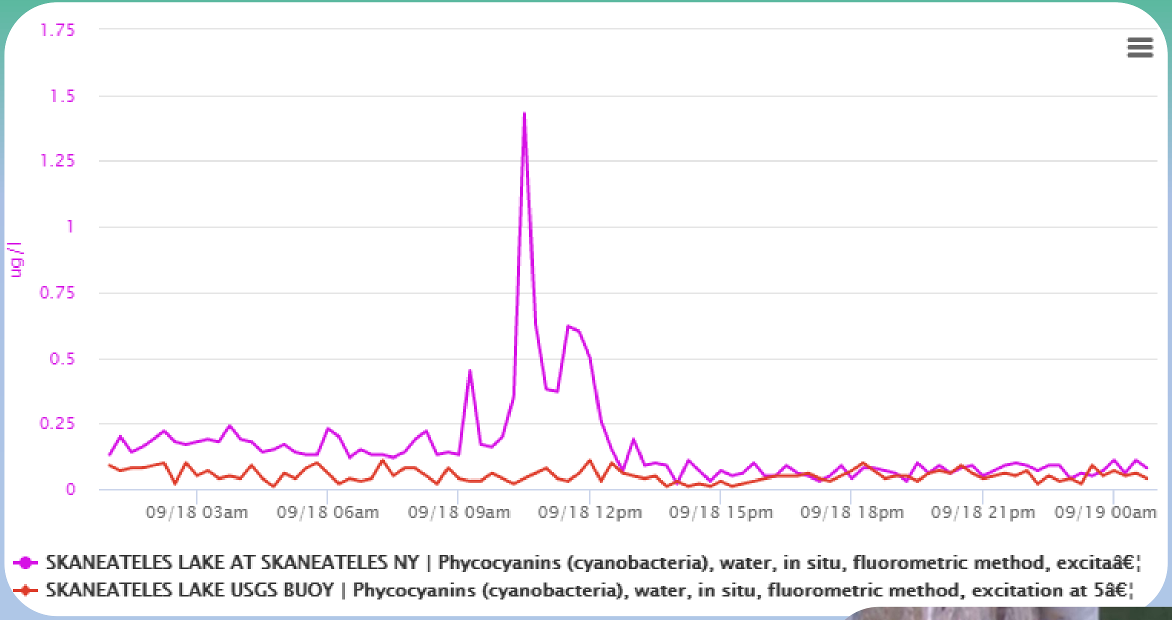
July 20, 2016 4:09 pm

Photo Credit: G. Foster, USGS

Milford Lake, KS

USGS
science for a changing world

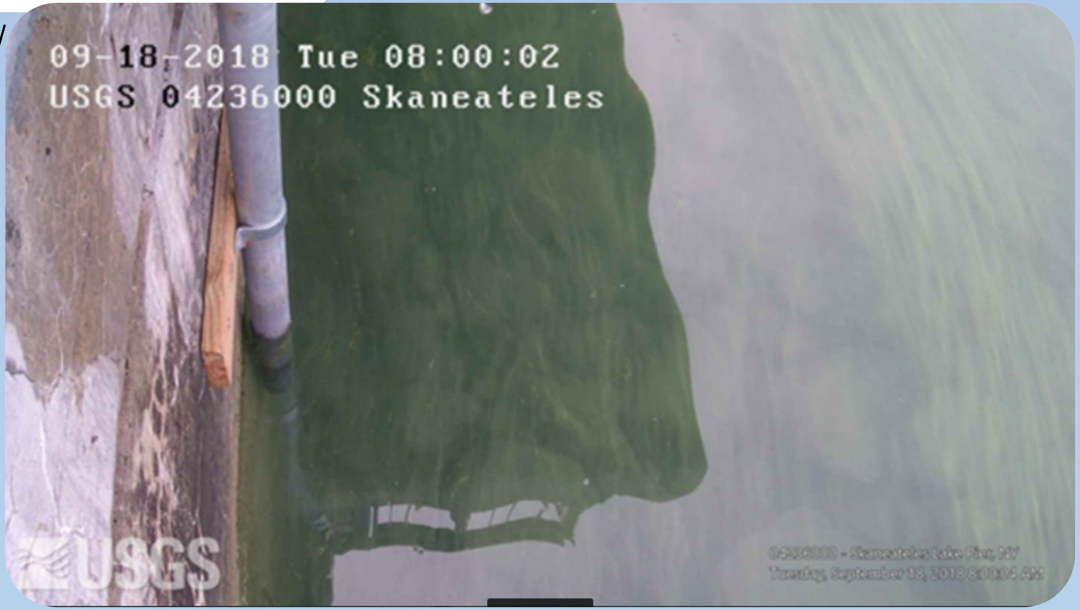
Cameras May Provide Additional Lines of Evidence When Interpreting Sensor-Based Data



https://ny.water.usgs.gov/maps/habs/

Photo Credit: J. Wernly, USGS

Photo Credit: J. Wernly, USGS



Courtesy of G. Foster, USGS

USGS
science for a changing world

Pigment Sampling

- Laboratory analysis
  - Chlorophyll
  - Phycocyanin
- *In Situ* sensors
  - Single channel
  - Dual channel
  - Multi channel
- Remote sensing

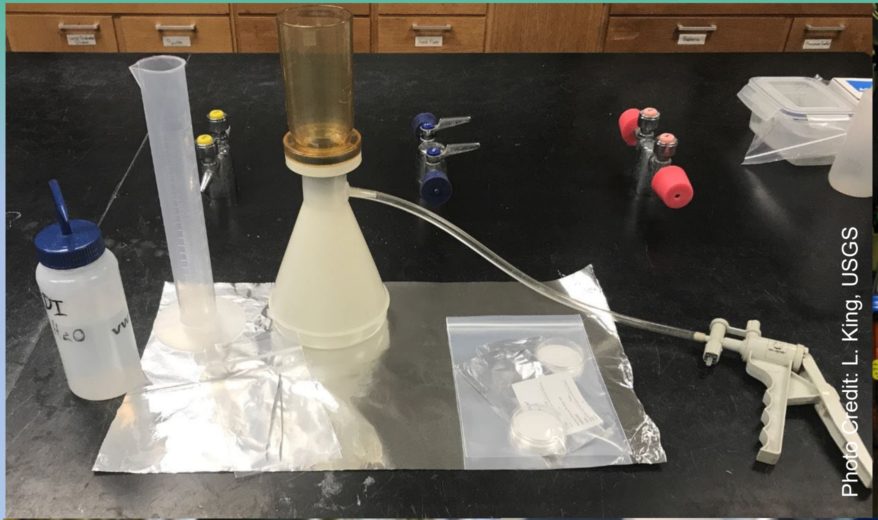


Photo Credit: L. King, USGS


Photo Credit: J. Graham, USGS


After Harris and Graham, 2015


Photo Credit: S. Gifford, USGS

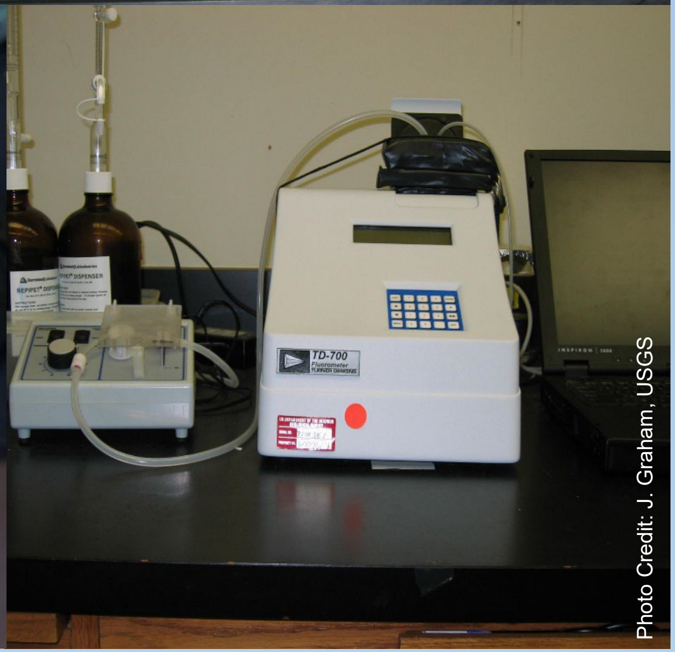
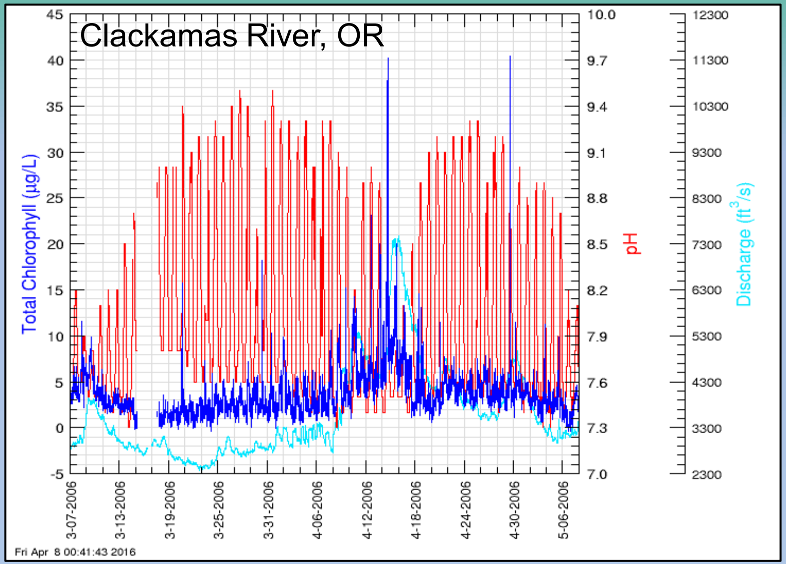


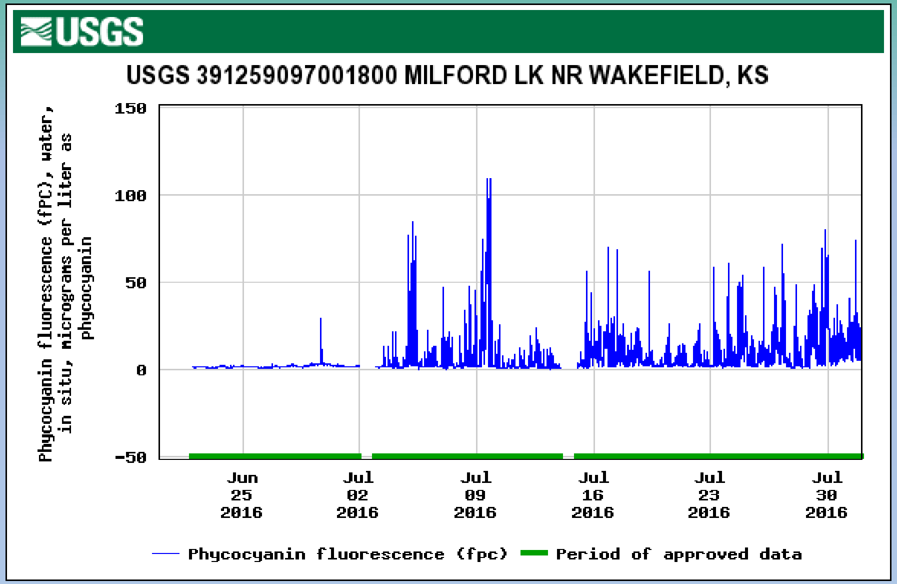
Photo Credit: J. Graham, USGS

In Situ Pigment Sensors Have a Wide Range of Applications

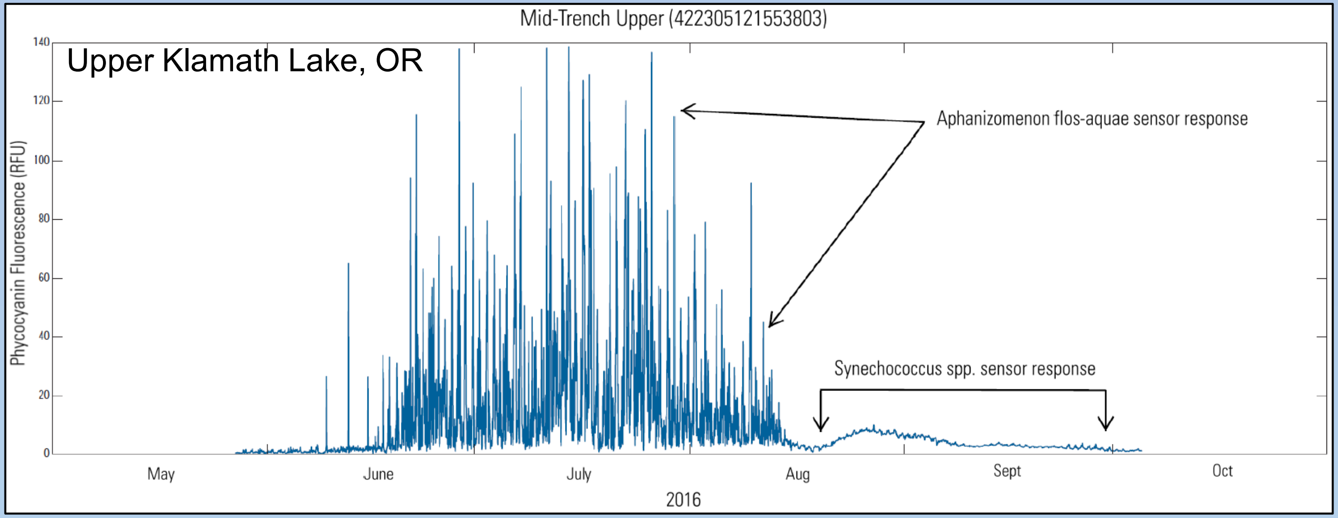
Diurnal or Noisy Patterns in Algal Fluorescence May Be Indicative of Potentially Harmful Algal Blooms



Courtesy of K. Carpenter, USGS



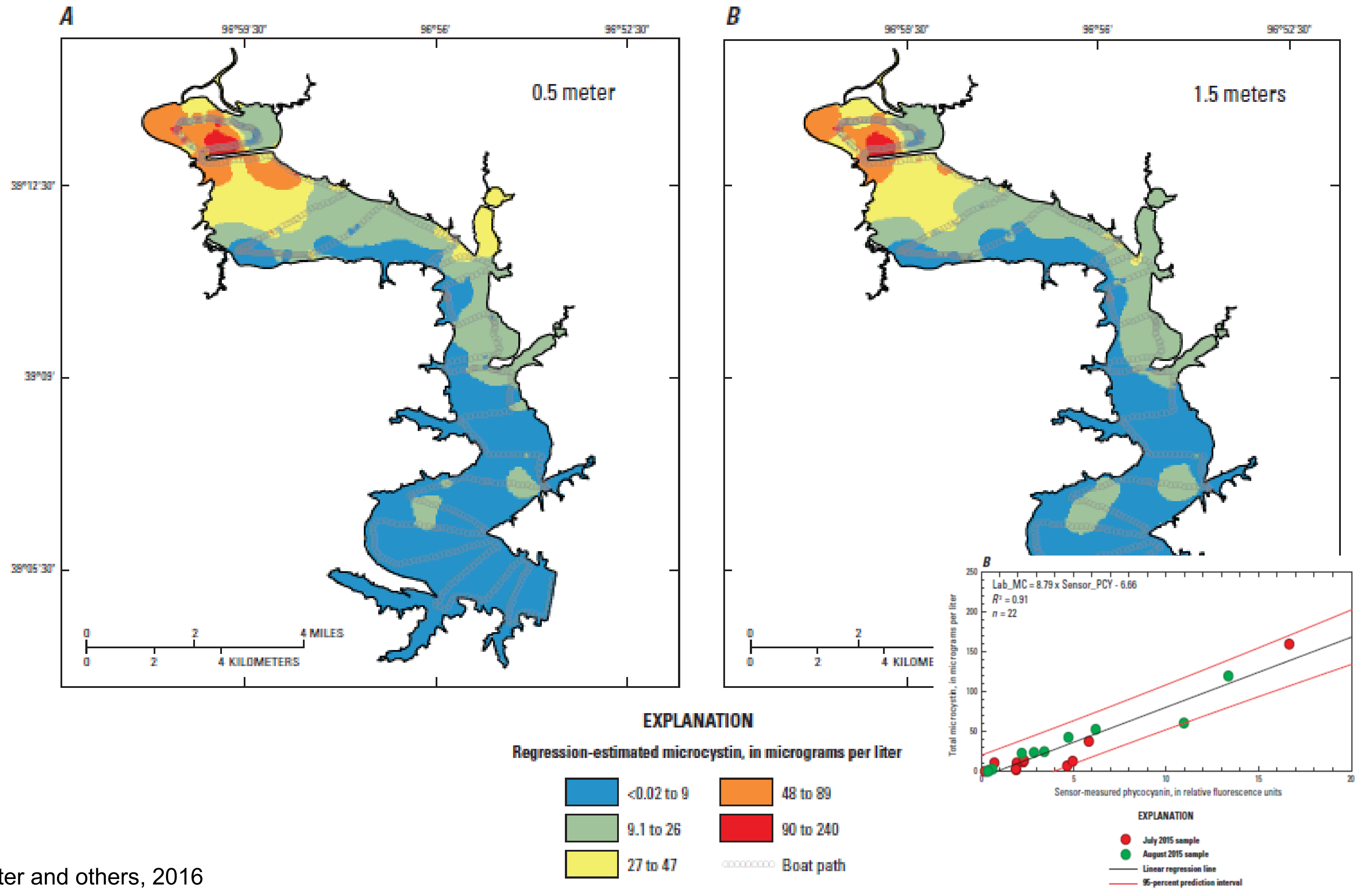
Courtesy of G. Foster, USGS



Courtesy of O. Stoken, USGS

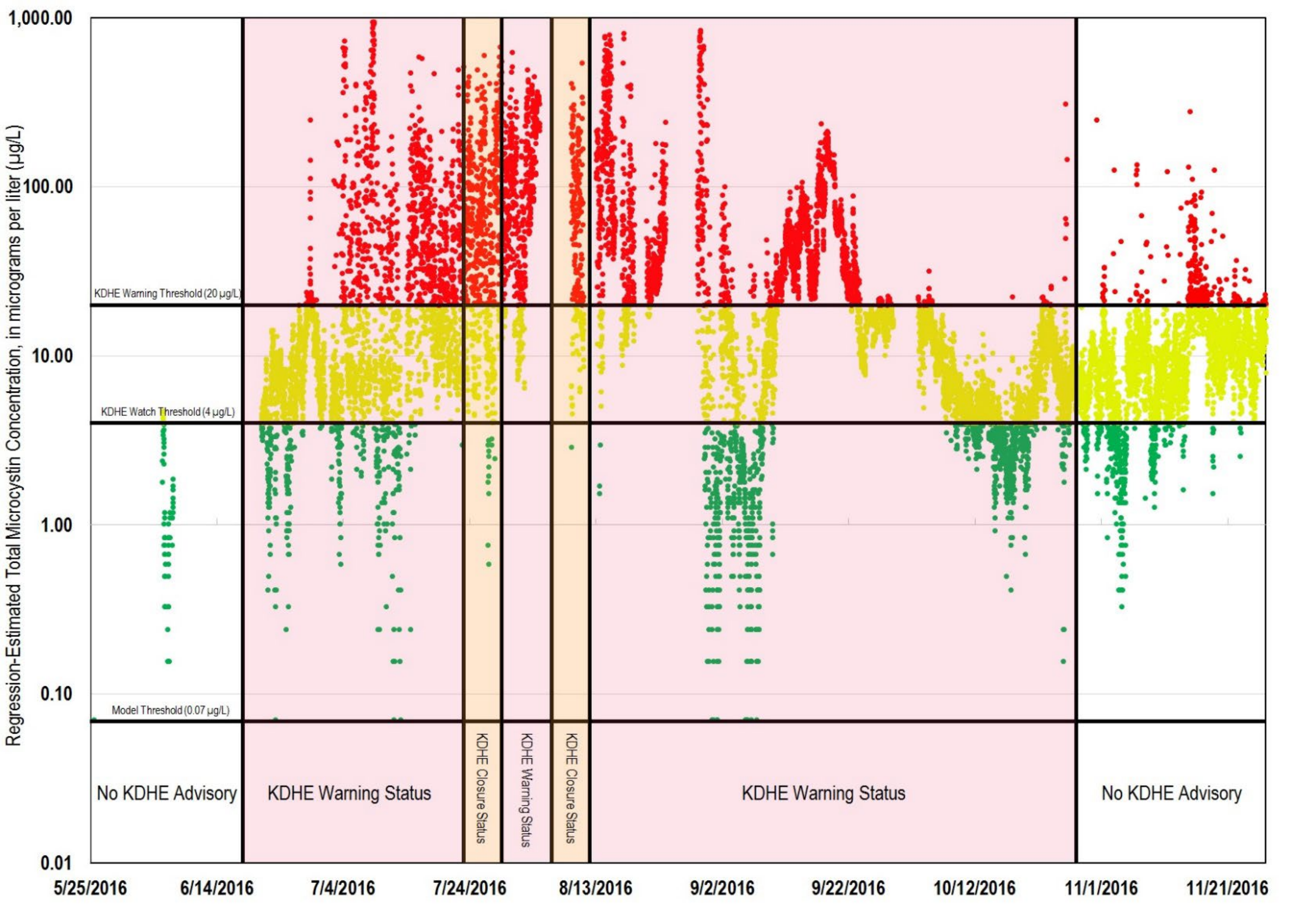
science for a changing world

Pigment Sensor Data Can Be Used to Develop Models to Estimate Cyanobacteria and Cyanotoxin Concentrations



Foster and others, 2016

Pigment Sensor Data Can Be Used to Develop Models to Estimate Cyanobacteria and Cyanotoxin Concentrations



After Foster et al., 2018

Genetic Sampling

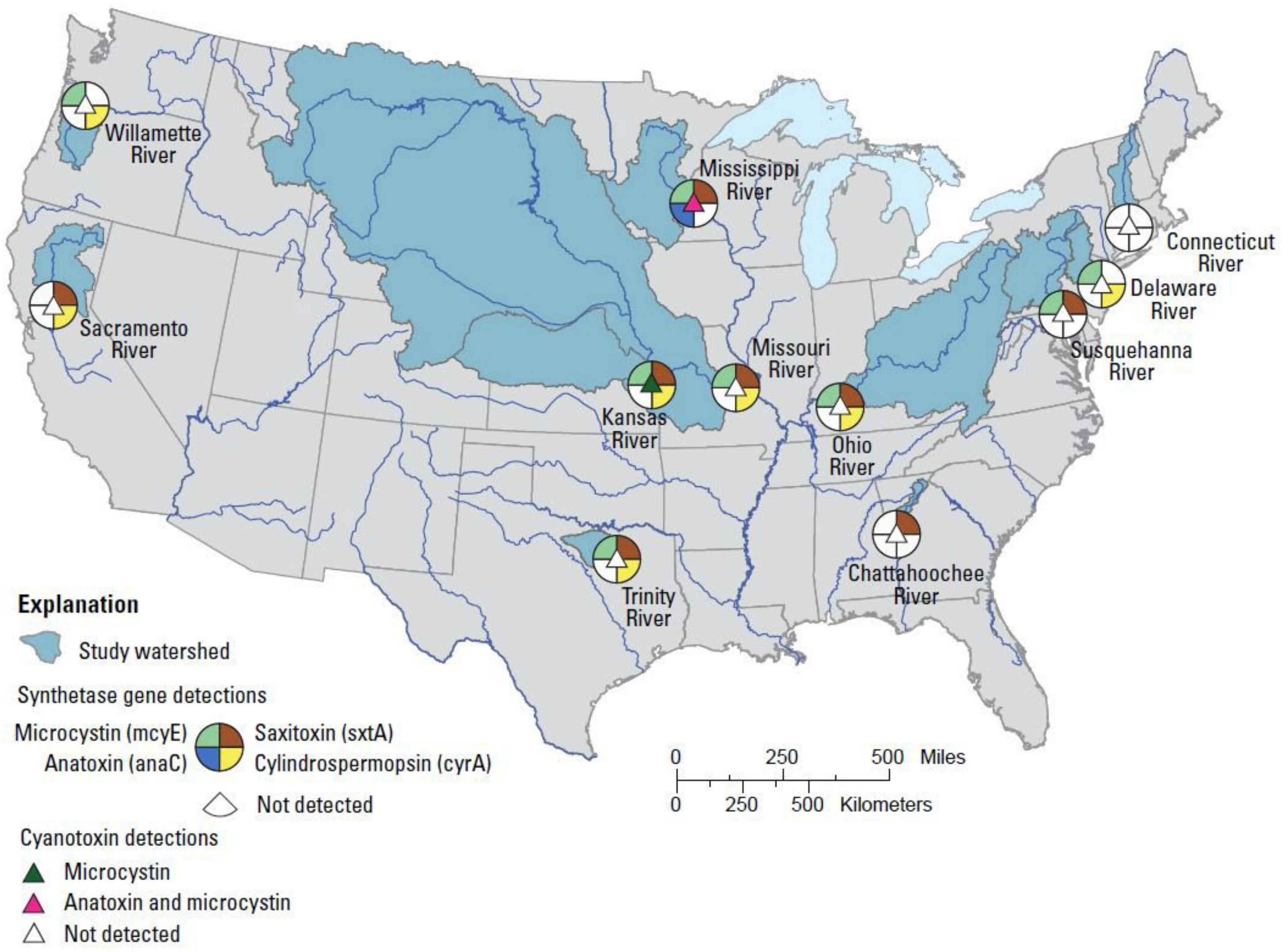
- qPCR and qRT-PCR
  - Targeted
  - Presence and abundance
- Omics
  - Structure, function, and dynamics

Photo Credits: USGS

Image Credit: biosistemika.com

Image Credit: dnatix.com

Cyanotoxin Synthetase Genes Occurred More Frequently Than Cyanotoxins in Large Rivers

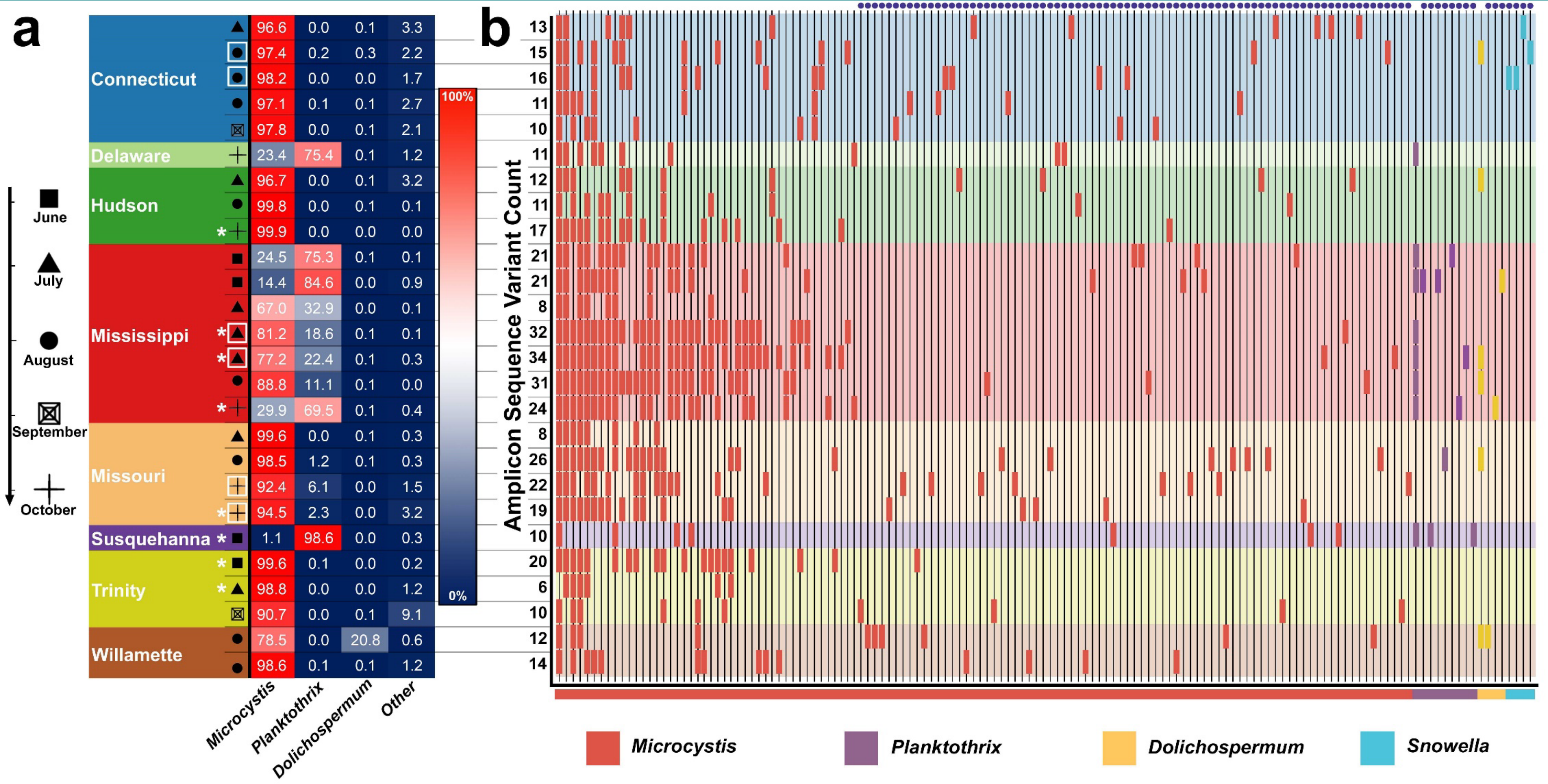


Graham et al., 2020

USGS
science for a changing world

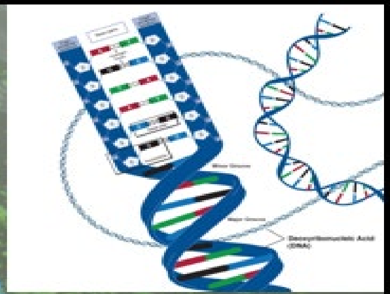
Advances in Genetic Approaches Allow Assessment of Occurrence in Novel Ways

mcyE Amplicon Sequencing



Linz et al., 2023

Photo Credit: A. Horner, USGS



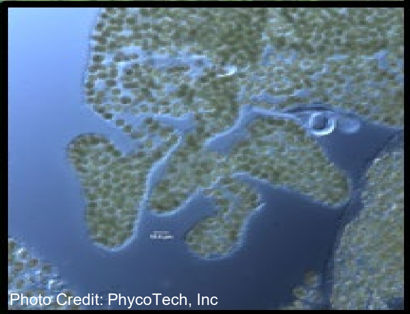


Photo Credit: PhycoTech, Inc

New and Emerging Technologies Allow Ground-to-Space Assessment of HABs

Landsat 8, Public Domain

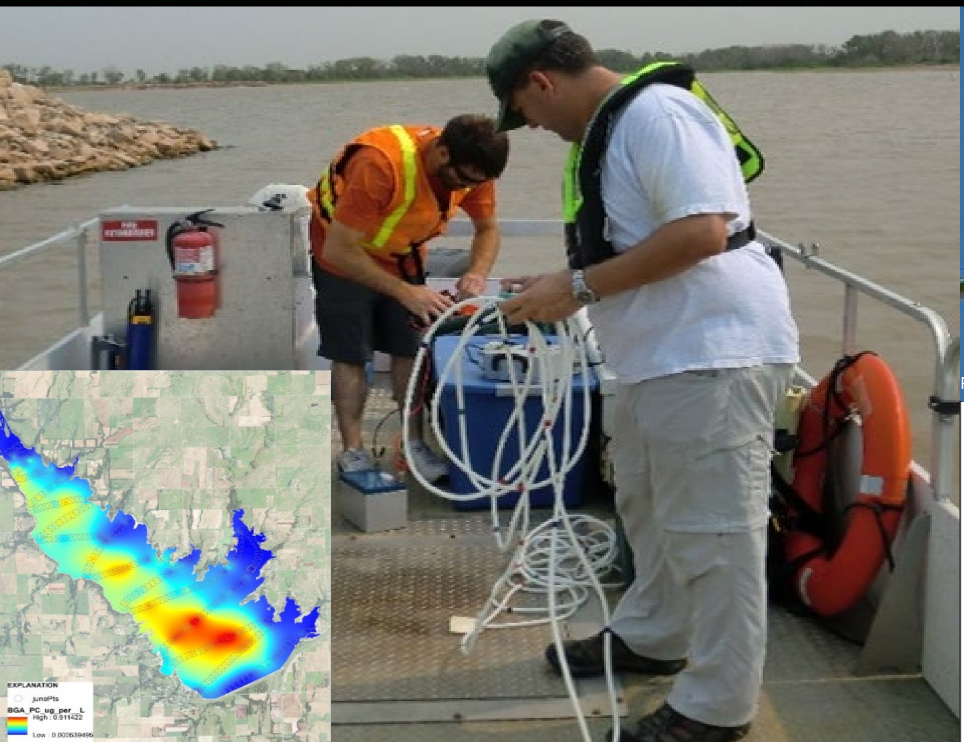


Photo Credit: USGS

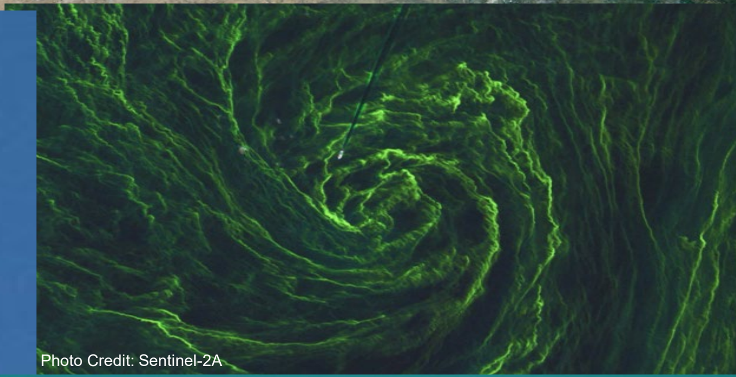
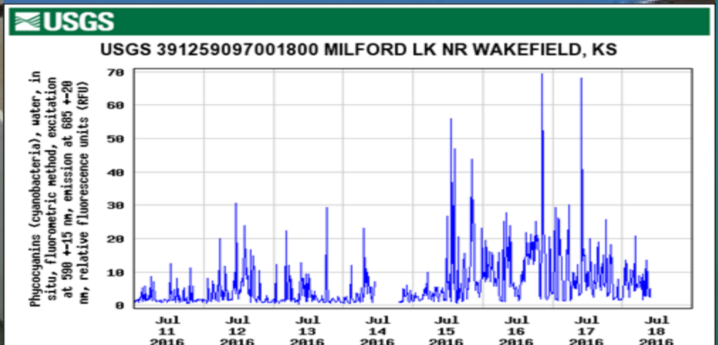
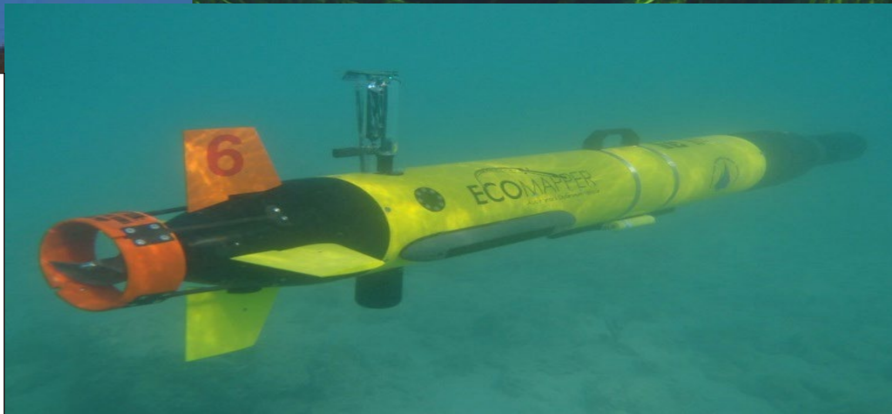
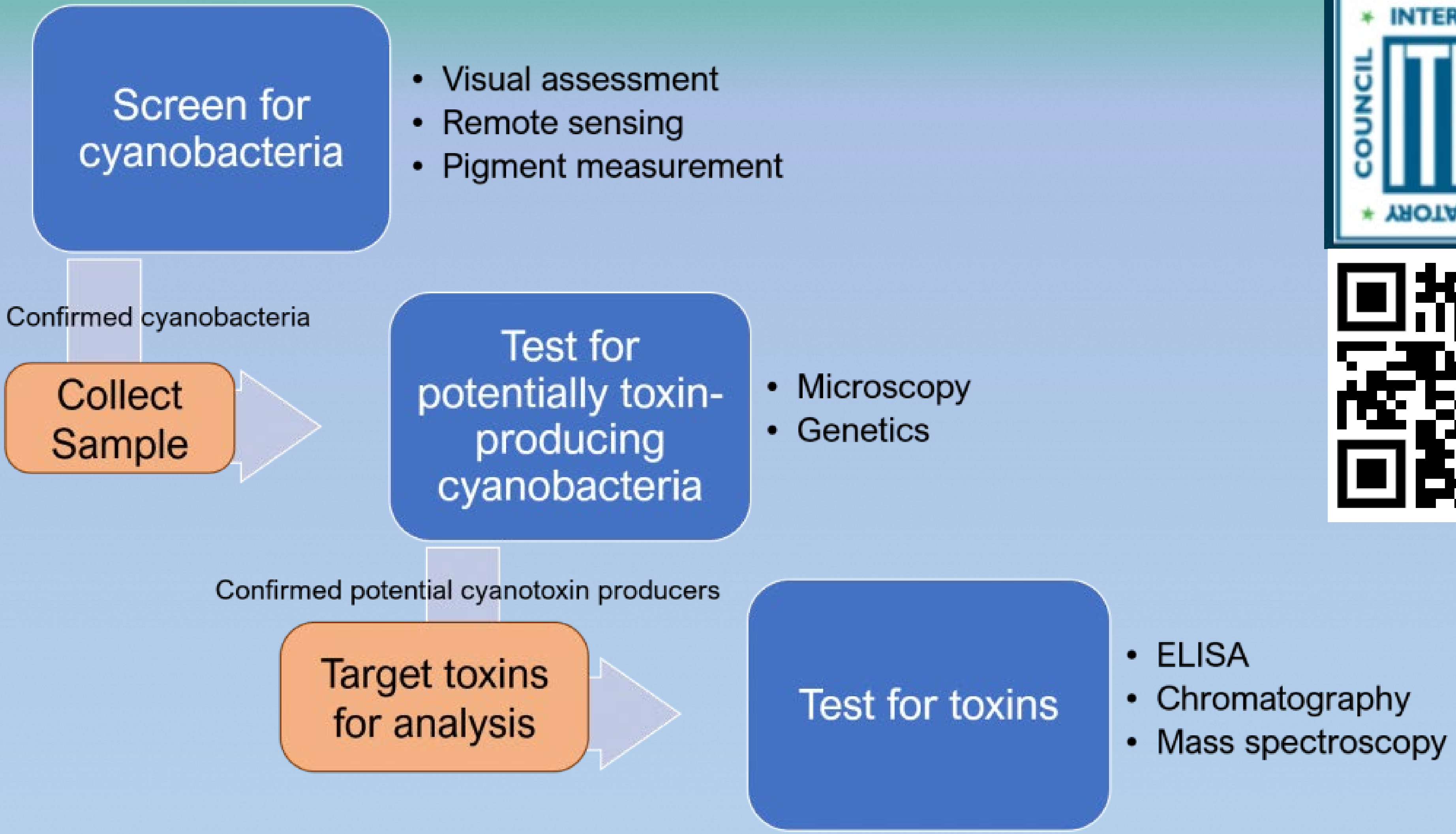


Photo Credit: Sentinel-2A



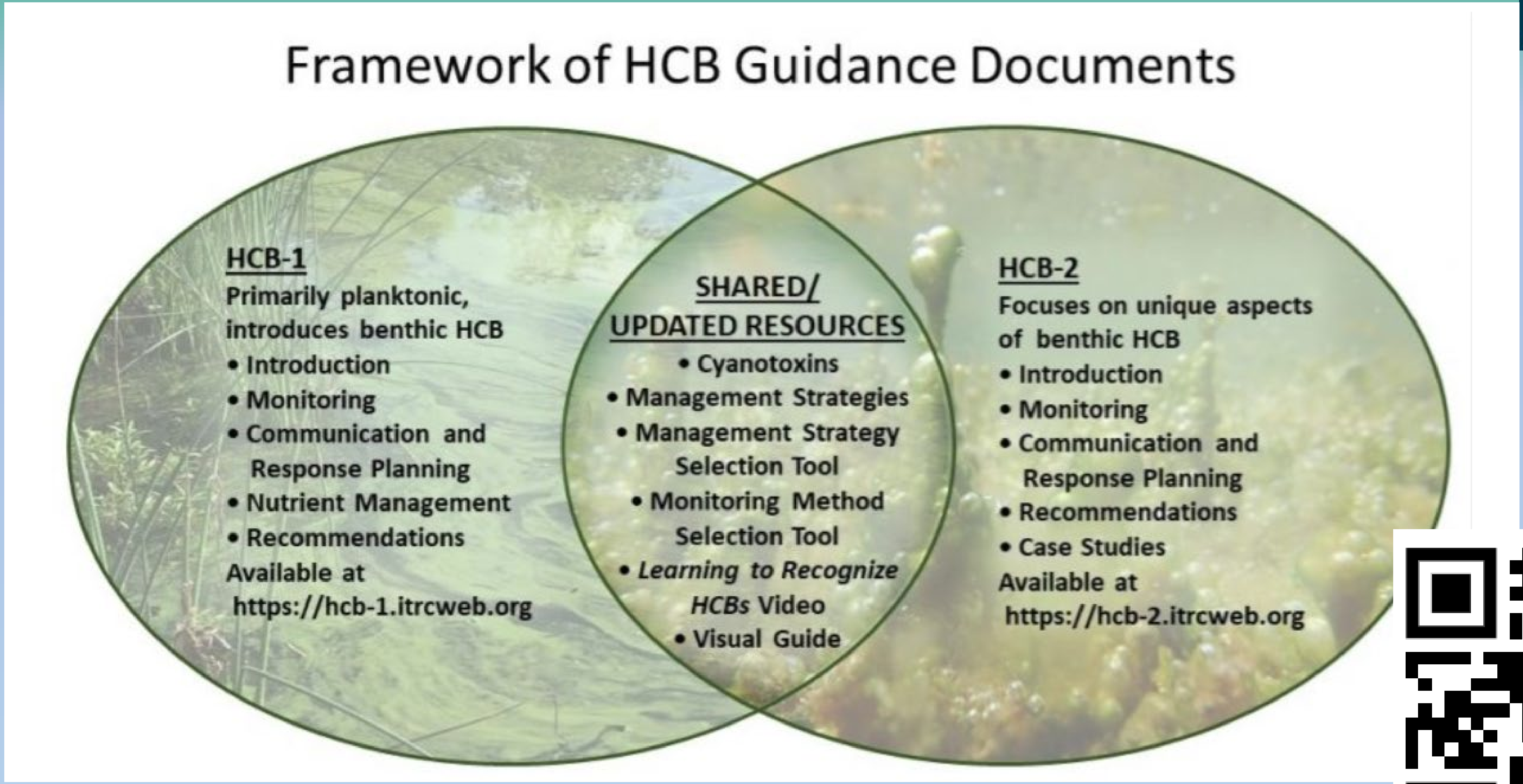


Tiered Sampling Approaches Can Be Cost Effective



https://hcb-1.itrcweb.org/

Resources: ITRC Guidance

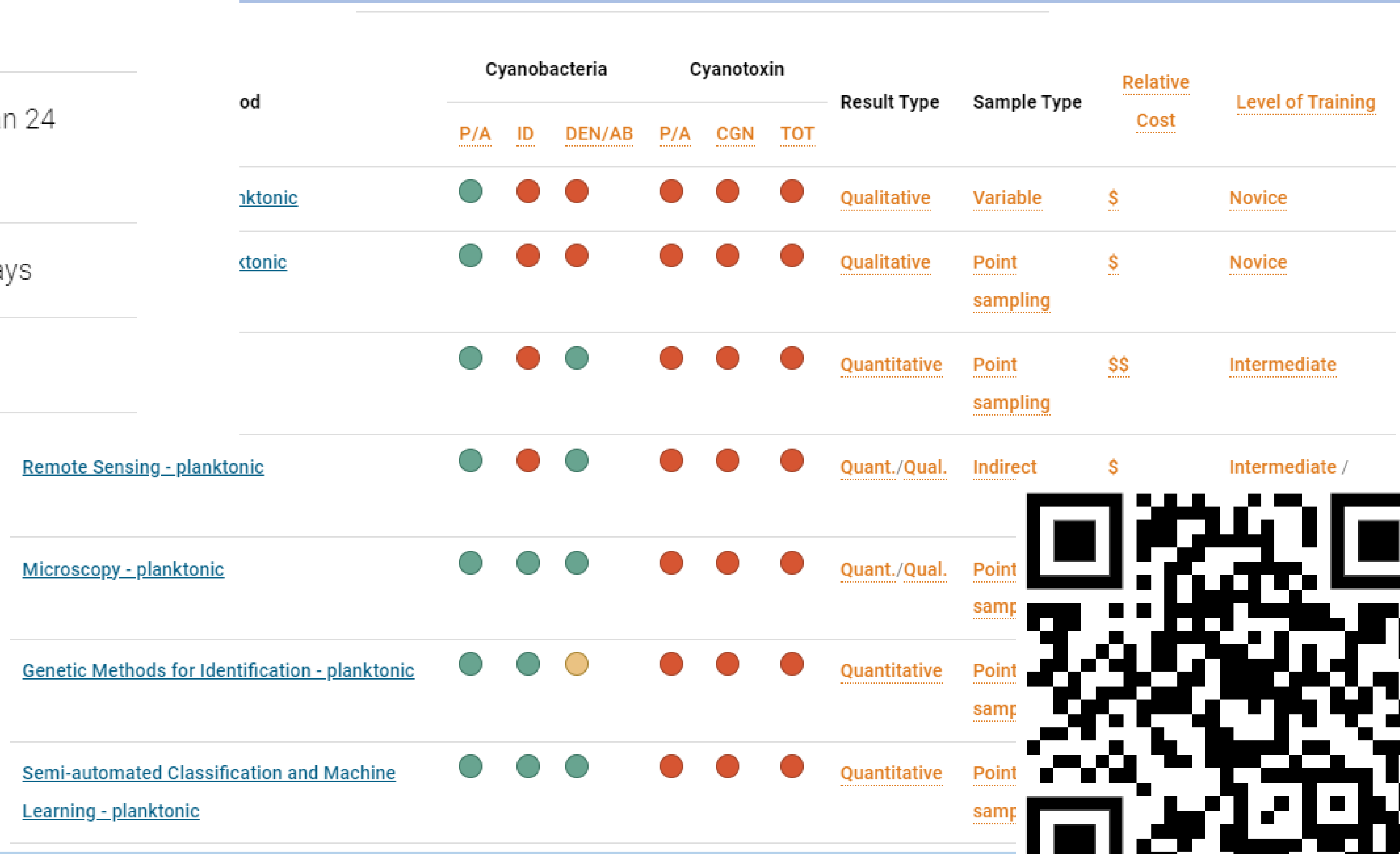


https://hcb-1.itrcweb.org/

Resources: ITRC Guidance

Select your monitoring requirements:

Target Analyte		Lab Required		Turnaround Time	
☑	Planktonic Cyanobacteria	☑	Yes	☑	Less than 24 hours
☑	Benthic Cyanobacteria	☑	No	☑	1 to 3 days
☑	Cyanotoxin				

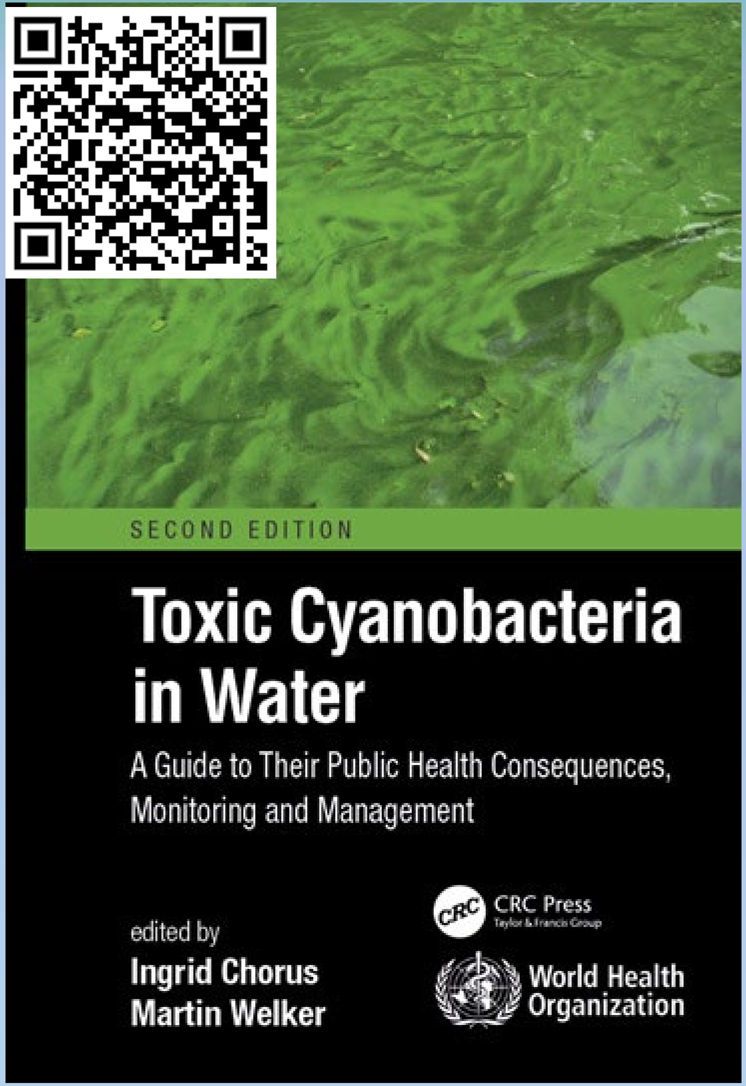


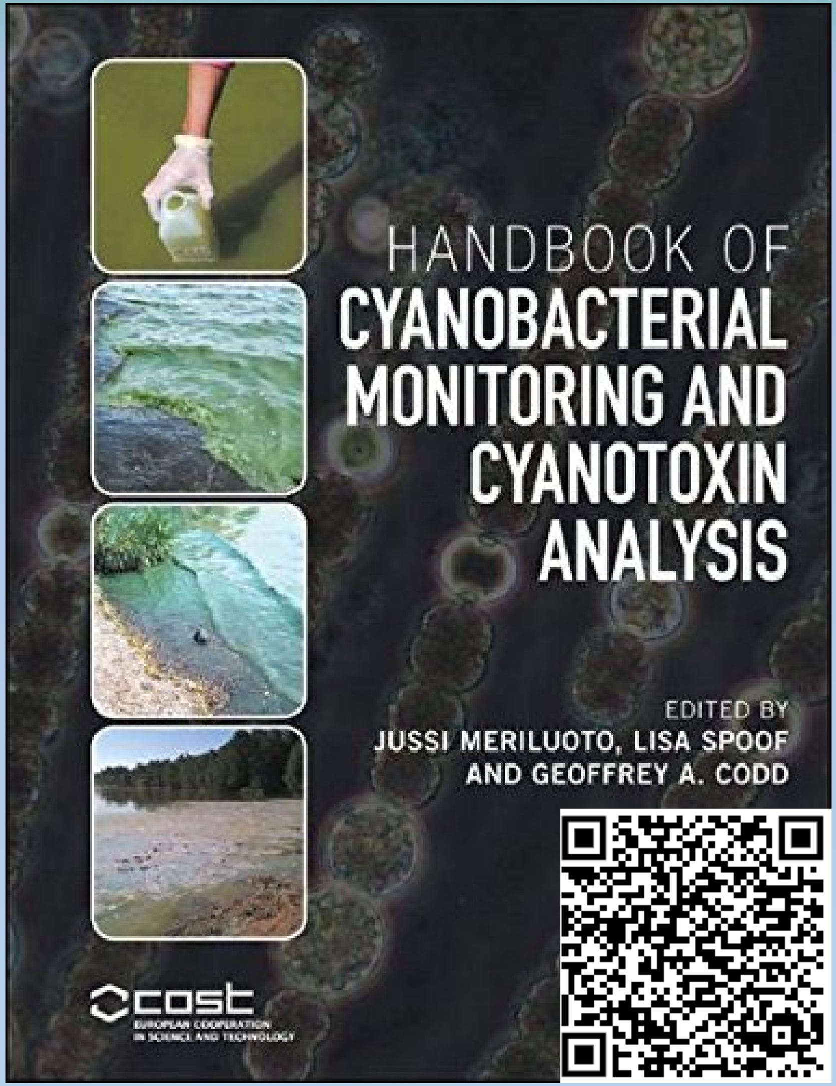
od	Cyanobacteria P/A	Cyanobacteria ID	Cyanobacteria DEN/AB	Cyanotoxin P/A	Cyanotoxin CGN	Cyanotoxin TOT	Result Type	Sample Type	Relative Cost	Level of Training
ıktonic	●	●	●	●	●	●	Qualitative	Variable	$	Novice
ktonic	●	●	●	●	●	●	Qualitative	Point sampling	$	Novice
	●	●	●	●	●	●	Quantitative	Point sampling	$$	Intermediate
Remote Sensing - planktonic	●	●	●	●	●	●	Quant./Qual.	Indirect	$	Intermediate /
Microscopy - planktonic	●	●	●	●	●	●	Quant./Qual.	Point samp		
Genetic Methods for Identification - planktonic	●	●	●	●	●	●	Quantitative	Point samp		
Semi-automated Classification and Machine Learning - planktonic	●	●	●	●	●	●	Quantitative	Point samp		

https://hcb-2.itrcweb.org/monitoring-tool/

USGS science for a changing world

Resources: World Health Organization and European Cooperation in Science and Technology Guidance





USGS
science for a changing world

Resources: USEPA Guidance

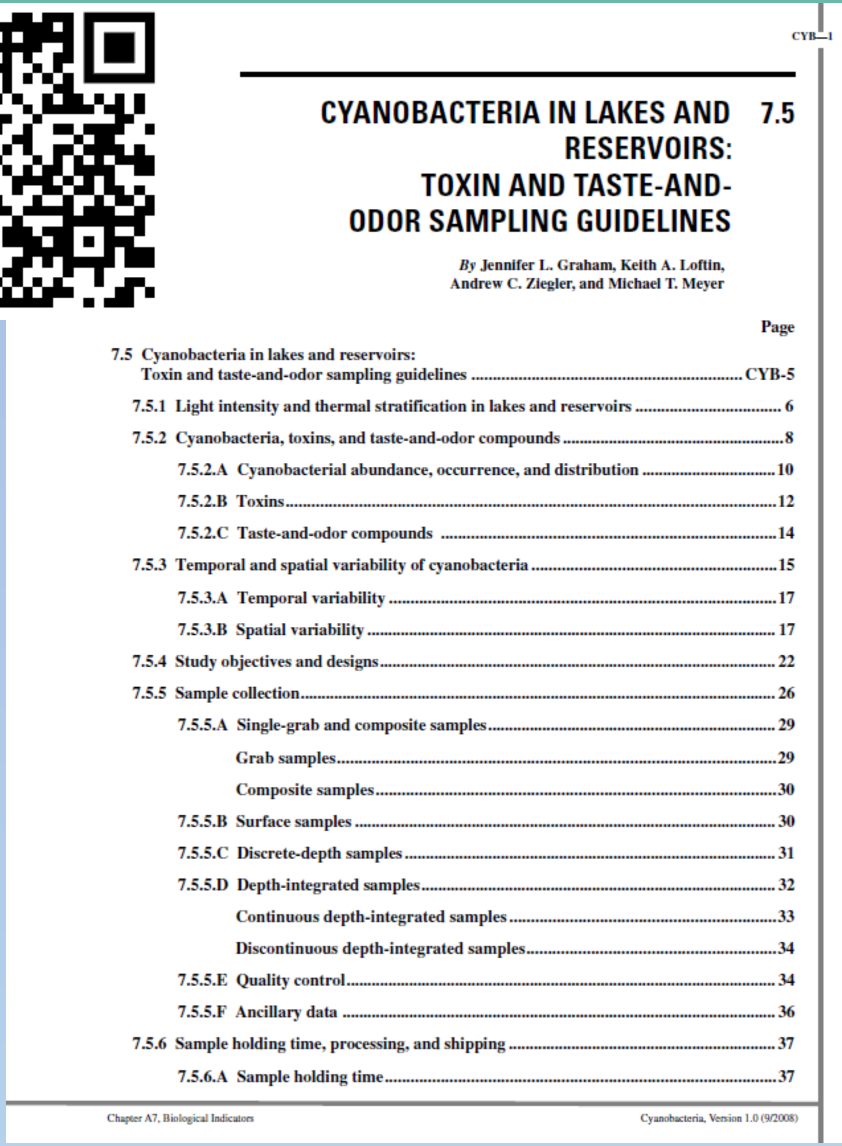
Cyanobacterial Harmful Algal Blooms (CyanoHABs) in Water Bodies

https://www.epa.gov/cyanohabs

Monitoring and Analysis

- Determination of Cyanotoxins in Drinking and Ambient Freshwaters
- Laboratories that Analyze for Cyanobacteria and Cyanotoxins
- State HABs Monitoring Programs

Resources: USGS Field and Laboratory Guidance

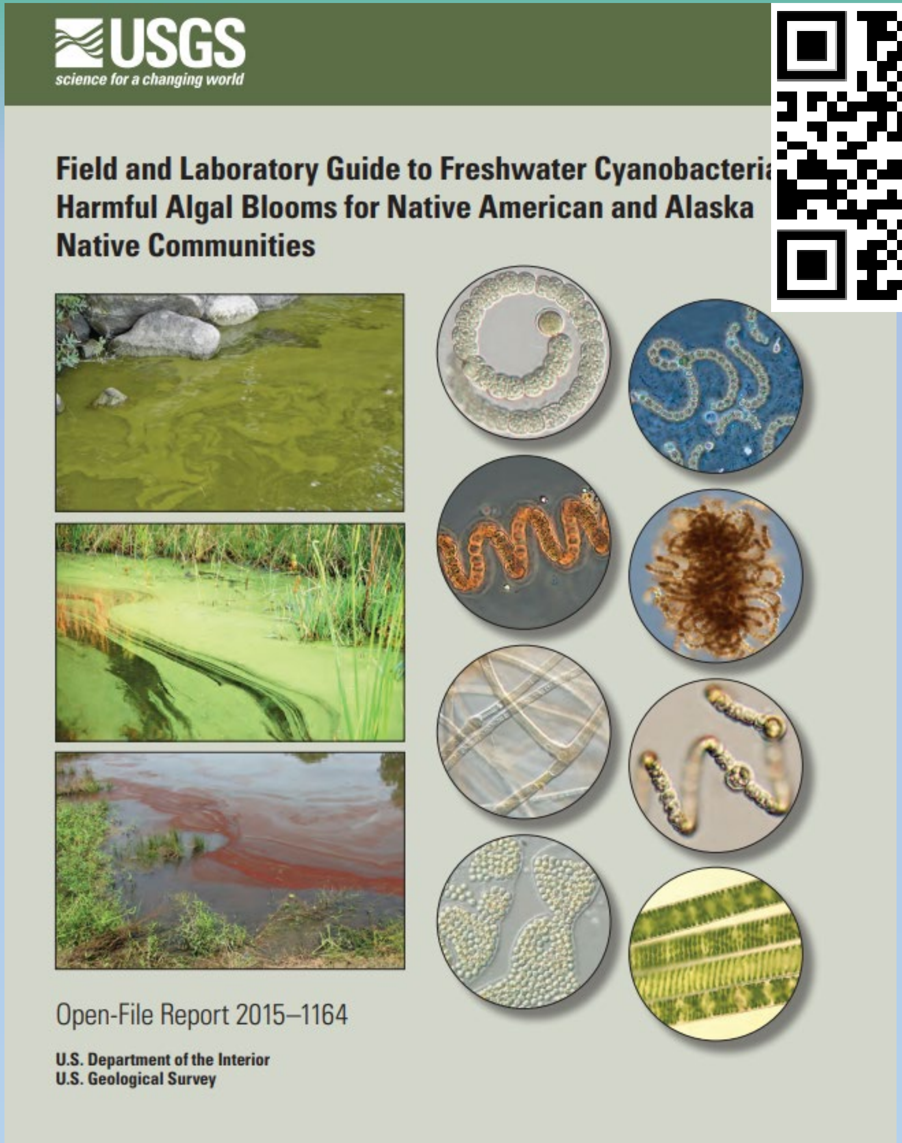


CYANOBACTERIA IN LAKES AND RESERVOIRS: TOXIN AND TASTE-AND-ODOR SAMPLING GUIDELINES 7.5

By Jennifer L. Graham, Keith A. Loftin, Andrew C. Ziegler, and Michael T. Meyer

Page

7.5 Cyanobacteria in lakes and reservoirs: Toxin and taste-and-odor sampling guidelines CYB-5
7.5.1 Light intensity and thermal stratification in lakes and reservoirs 6
7.5.2 Cyanobacteria, toxins, and taste-and-odor compounds 8
7.5.2.A Cyanobacterial abundance, occurrence, and distribution 10
7.5.2.B Toxins 12
7.5.2.C Taste-and-odor compounds 14
7.5.3 Temporal and spatial variability of cyanobacteria 15
7.5.3.A Temporal variability 17
7.5.3.B Spatial variability 17
7.5.4 Study objectives and designs 22
7.5.5 Sample collection 26
7.5.5.A Single-grab and composite samples 29
Grab samples 29
Composite samples 30
7.5.5.B Surface samples 30
7.5.5.C Discrete-depth samples 31
7.5.5.D Depth-integrated samples 32
Continuous depth-integrated samples 33
Discontinuous depth-integrated samples 34
7.5.5.E Quality control 34
7.5.5.F Ancillary data 36
7.5.6 Sample holding time, processing, and shipping 37
7.5.6.A Sample holding time 37



USGS
science for a changing world

Jennifer Graham
jlgraham@usgs.gov

USGS
science for a changing world