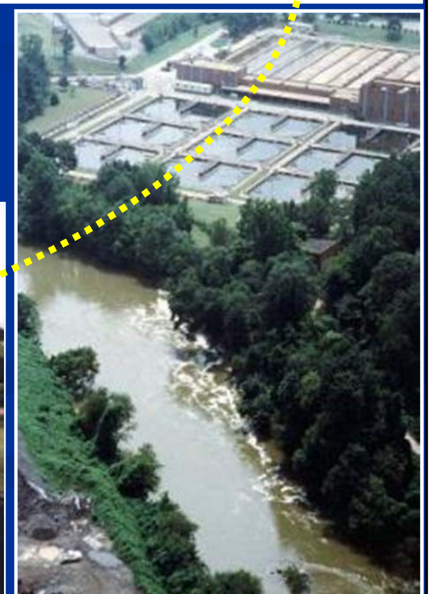
HAB Toxins "101 Course" – Surveillance Monitoring, Response Planning, and Implementation Basics.

Environmental Water Quality Integrators, Biological Endpoints, or Hazards?

- How do human behaviors influence natural processes?
- What are the relationships between climate and land use change, geology, ecosystem effects, human health, water quantity and quality, cyanobacteria, and toxins?

Keith Loftin, Jennifer Graham

USGS Kansas WSC



Organic Geochemistry Research Laboratory (OGRL) Mission

To investigate the occurrence, fate, transport, treatment, and effects of understudied anthropogenic and natural compounds with known or suspected biological effects.

- Pesticides
- Emerging Contaminants (e.g. antibiotics, endocrine disruptors)
- Harmful Algal Blooms, Toxins, and other metabolites

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Collaboration is vital.
Interdisciplinary Approach Required!

USGS

- Many Science Centers
- Toxic Substances Hydrology Program
- Office of Water Quality
- Climate and Land Use Change Mission Area
- Water Mission Area
- Cooperative Program
- SCA ASSIST

Many Federal and State Agencies and Tribes

- US EPA, US ACE, NOAA, CDC, US FDA
- KS, MO, IA, MN, OR, CA, TX, OK, MI, WI, AR, FL, GA, OH, AK,……
- Alaskan Native Tribal Health Consortium

Several Universities

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Harmful Algal Bloom Overview

- Introduction
- Analytical Methods for Algal Toxins
- Occurrence, Fate, and Transport of Algae and Toxins
- Big Picture

http://ks.water.usgs.gov/Kansas/studies/qw/cyanobacteria

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What Are Harmful Algal Blooms?

Harmful Algal Blooms are result of an over abundance of algae usually observed in fresh, brackish, and marine waters.

The "Harmful" designation comes from undesirable consequences caused by algae (e.g. toxin production, dissolved oxygen issues, aesthetics (e.g. taste, odor, etc.) .

Harmful algal blooms are a global issue with significant negative impacts!!!

- Economics (estimated losses are at least hundreds of millions of dollars annually)
- Global food supply
- Drinking water supply
- Human and ecological health impairment or death

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Harmful Algal Blooms (HABs)
Environmental Water Quality Integrators, Biological Endpoints, or Hazards?
Slide 1 of 2

- Environmental Water Quality Integrators:
  - Phytoplankton require varying amounts and/or types of sunlight, moisture, carbon, nutrients, metals, and trace elements.

- Biological Endpoints and Foodweb Dynamics:
  - Algae or phytoplankton (e.g. cyanobacteria, diatoms, dinoflaggelates, chlorophytes, chrysophytes, etc.) as primary producers are critical for all life to be sustained as we know it directly or indirectly.
  - Water quality, climate, geology, hydrology, predators, and human behaviors govern aquatic algal populations and diversity.

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Harmful Algal Blooms (HABs) Environmental Water Quality Integrators, Biological Endpoints, or Hazards?

Slide 2 of 2

- Hazards – Influenced by natural and anthropogenic processes/activities
  - Cases of animal and human poisonings and death have been recorded at least since the early 1800's.
    - Livestock and pet deaths fairly common. Veterinarians are more familiar with inland HAB hazards than physicians are in the United States.
    - 1800's to present – Multiple community-wide gastroenteritis outbreaks reported in the United States associated with insufficiently treated drinking water.
    - 1990's - Brazil – deaths of several dialysis patients tied to microcystin tainted water.

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Toxicity of Known Cyanotoxins

- **Acute Toxicity**
  - Cytotoxic
  - Neurotoxic
  - Hepatotoxic
  - Dermatoxic
  - Respiratory Distress
- **Chronic Toxicity**
  - Carcinogen
  - Tumor Promotion
  - Mutagen
  - Teratogen
  - Embryolethality

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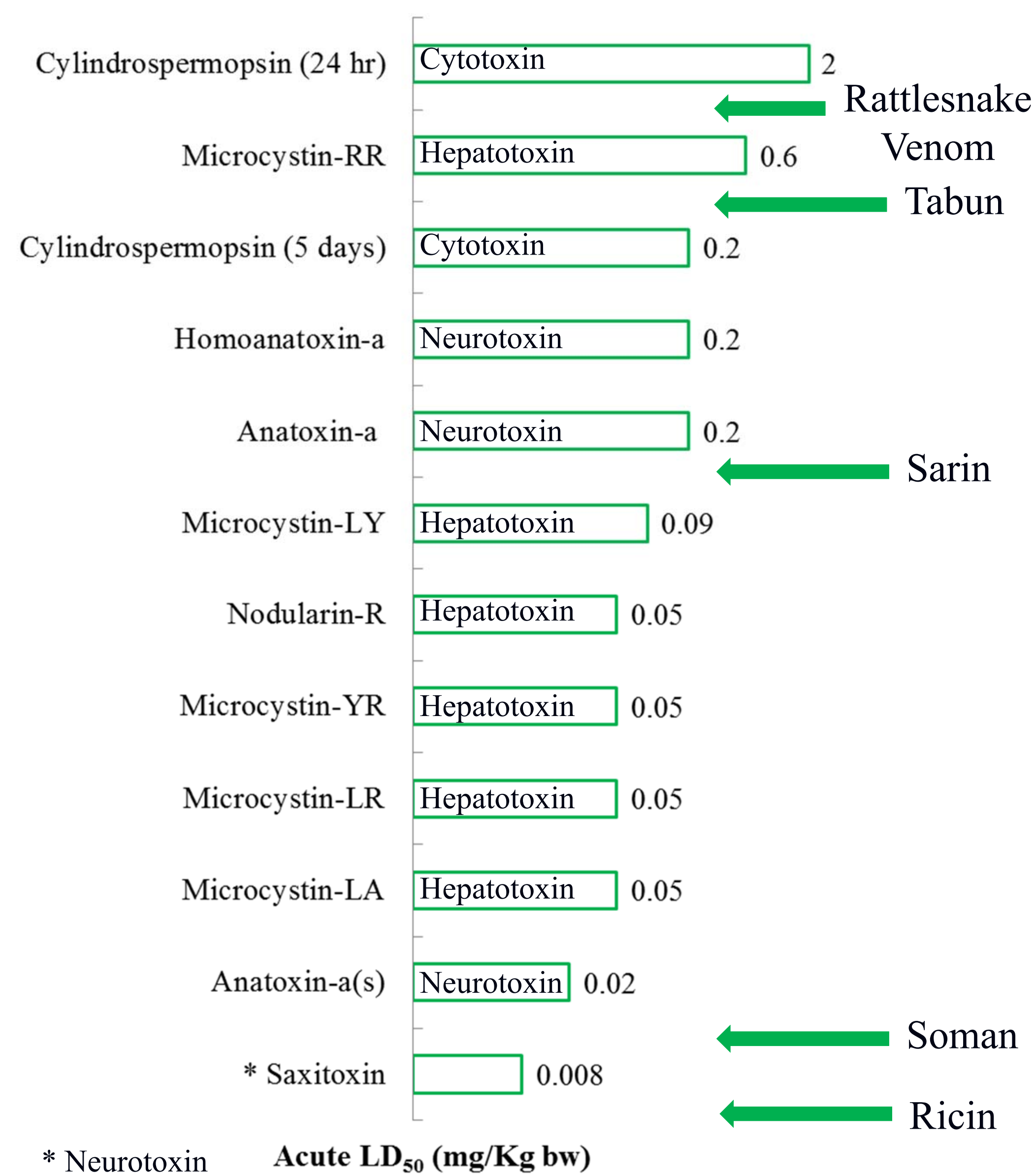

Cyanotoxins Have Been Implicated in Both Human and Animal Poisonings

-Global and national issue

-Cyanotoxins found in:

surface water
sediments
fish tissues
molluscs
plants….

What's in Your Newspaper?

Graham et al., 2009

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Ecologic, Economic, and Public Health Concerns Surrounding Cyanobacterial Harmful Algal Blooms are a Reality

Summer 2011 Headlines

Livestock producers beware: Watch for toxic blue-green algae Updated: 9:44 PM Jun 1, 2011

Watch the Water as Algae Bloom Seas

Milford Lake release sends algae to Kansas River
MARIA SUDEKUM FISHER, Associated Press
Published 09:10 p.m., Wednesday, September 21, 2011

DANGER

OF LAKE

In case of contact with harmful algae, call your doctor or veterinarian if you or your animals experience nausea, vomiting, diarrhea, skin rash, eye irritation, respiratory symptoms, or other unexplained illness.

Veterinarians warn dog dangers of blue-green algae

'It's going to be a bad year' for algae blooms in area lakes

Concerns about blue-green algae shut down Kaw River Water Treatment Plant
By Christine Metz on September 15, 2011

BLUE-GREEN A
NINE KANSAS L
Most facilities open for recreation,

Inhofe blames illness on Grand Lake algae

Grand Lake blue-green algal scare could cause long-term economic damage

Pig peril – boars asphyxiated by algae
9:36 AM Friday Jul 29, 2011

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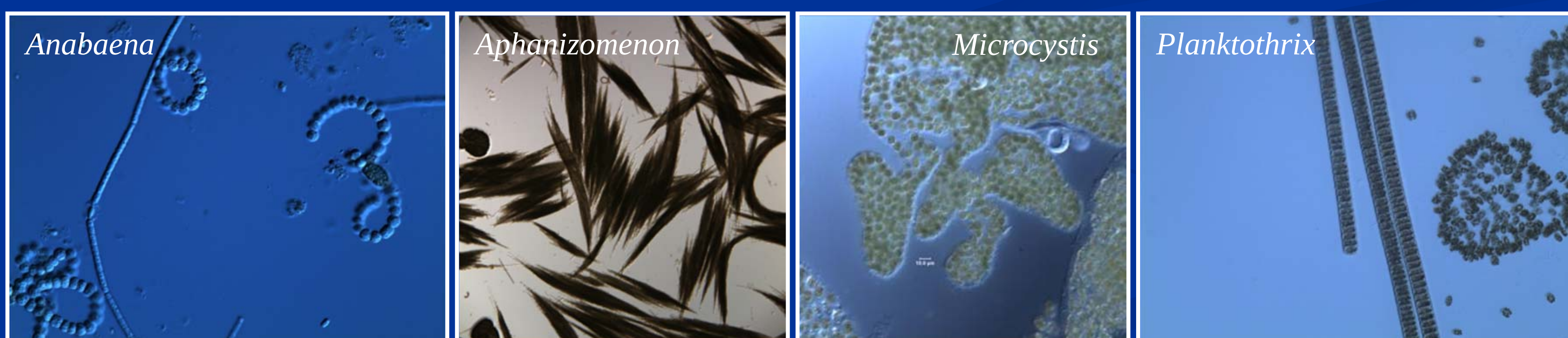
Cyanobacterial Toxins and Taste-and-Odor Compounds

	Hepatotoxins		Neurotoxins		Dermatoxins	Taste/Odor	
	CYL	MC	ANA	SAX		GEOS	MIB
Anabaena	X	X	X	X	X	X	?
Aphanizomenon	X	?	X	X	X	X	
Microcystis		X			X		
Oscillatoria/Planktothrix		X	X	X	X	X	X



Photos courtesy of A. St. Amand

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After Graham and others, 2008, TWRI Chapter 7.5
http://water.usgs.gov/owq/FieldManual/

Important Regulations and Thresholds

- No Current U.S. Federal or State Regulations for Recreation or Drinking Water Exposure except Oklahoma (2012).
  - Occurrence and Risk Assessment Phase
  - US EPA Office of Water listed Anatoxin-a, Cylindrospermopsin , and Microcystin-LR or selected producers on CCL2 and CCL3 under the Clean Water Act
- Some states have implemented or implemented variations of the World Health Organization suggested Microcystin Recreational threshold values.

Relative Probability of Acute Health Effects	Cyanobacteria (cells/mL)	Microcystin-LR (µg/L)	Chlorophyll-a (µg/L)
Low	< 20,000	< 10	< 10
Moderate	20,000 - 99,999	10 - 19.9	10 - 49.9
High	100,000-9,999,999	20 - 1999	50 - 4999
Very High	≥ 10,000,000	≥ 2000	≥ 5000

Chorus and Bartram, 1999

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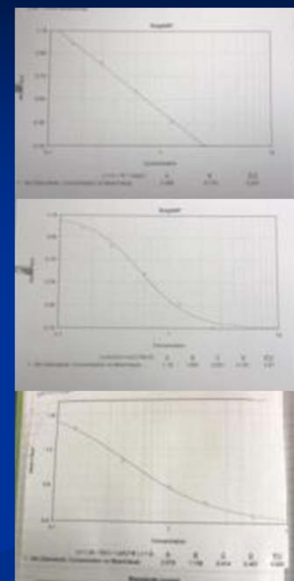
The Cyanotoxin Data Acquisition Process:

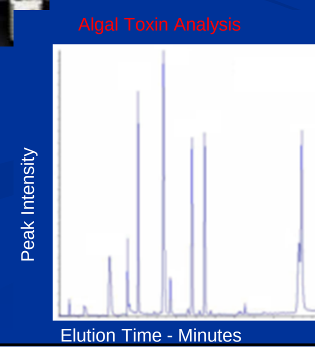
Sample Collection

Laboratory Processing

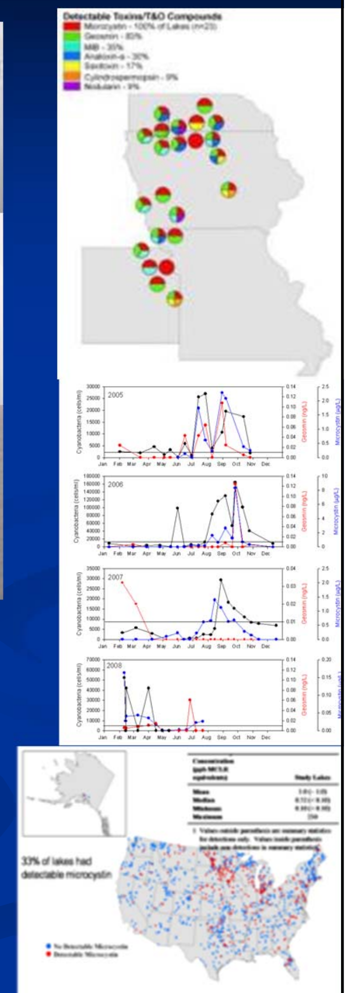
Analysis

Data Reduction





Interpretation



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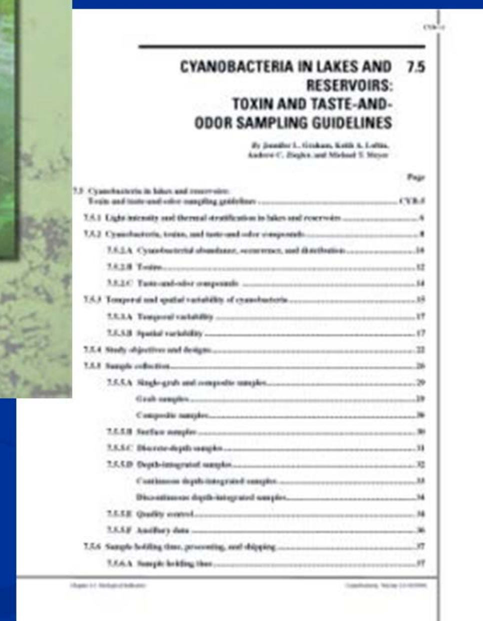
Study Design and Field Manual Resources

- SIR 2008-5038 Guidelines for Design and Sampling for Cyanobacterial Toxin and Taste-and-Odor Studies in Lakes and Reservoirs

- USGS National Field Manual Chapter 7.5 Cyanobacteria in Lakes and Reservoirs: Toxin and Taste-and-Odor Sampling Guidelines



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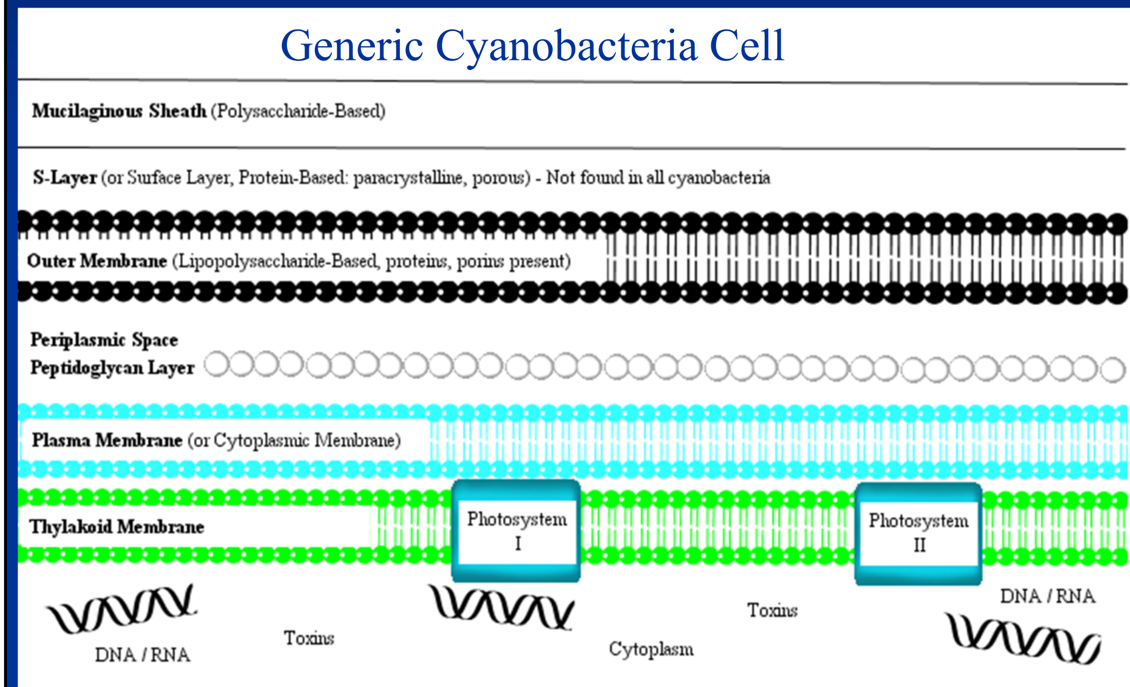
http://pubs.usgs.gov/sir/2008/5038/
http://water.usgs.gov/owq/FieldManual/Chapter7/7.5.html

Cyanobacteria Cell Structure: A Tough Nut To Crack…

Current thought indicates that cyanotoxins are contained in the cytoplasm of intact cyanobacteria (intracellular) and that toxins are released to the surrounding environment (extracellular - dissolved or bound) upon cell membrane disruption by natural (e.g. senescence, apoptosis, etc.), in source treatment (algicides) or laboratory processes.



Cyanobacteria are oxygenic prokaryotic bacteria and are different from other prokaryotes due to the presence of chlorophyll for photosynthesis.

Cyanobacterial cell structure is not as well understood as for other bacteria.

However, we do know that:

Cyanobacteria have three membranes:

1. Thylakoid membrane –where photosynthesis and respiration occur.
2. Plasma membrane
3. Outer membrane

Some cyanobacteria also have an S-layer and/or a mucilaginous sheath of varying degrees of thickness.

Based on existing research scientists think:

Cyanobacteria do not have membrane bound Organelles (e.g. no nucleus therefore genetic material is believed to be in the cytoplasm).

Cyanotoxins are also believed to be stored in the cytoplasm.

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Age and stress also play a role in cell structure!

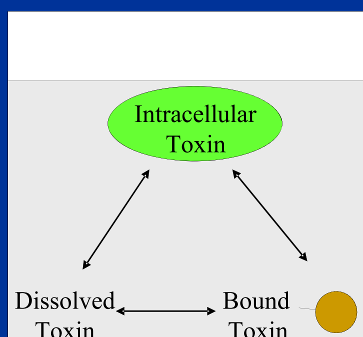
Cyanotoxin Measurement Background

Total Toxin = Extracellular Toxin (dissolved and bound) + Intracellular Toxin

Total Toxin

Dissolved-Phase Toxin (Extracellular)

Particulate / Bound Toxin – meaning depends on whether cells are lysed.



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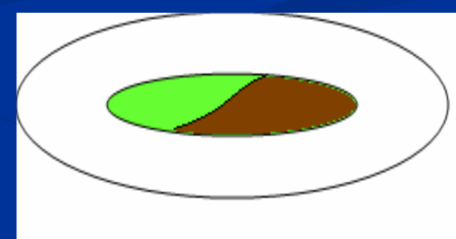

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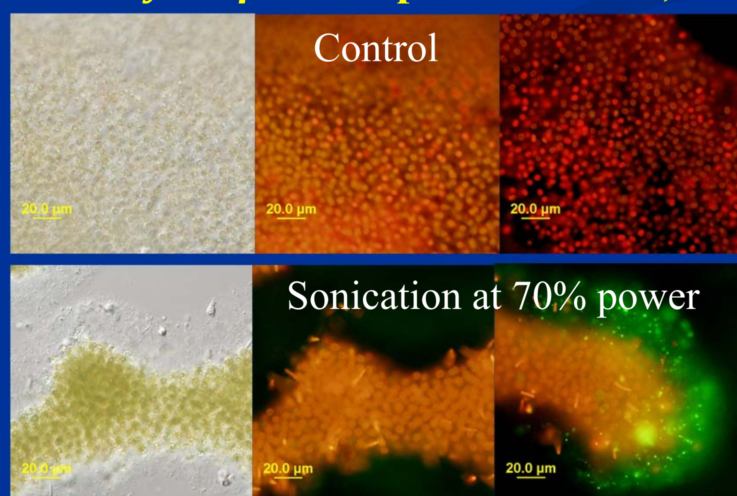
Cyanobacterial Cell Lysis in the Laboratory – All Methods Are Not Created Equal

Standardized Sample Preparation Techniques

- Lysis of cyanobacteria is a required step in laboratory sample preparation to obtain intracellular cyanotoxin concentrations.
- It is important to measure the maximum intracellular cyanotoxin concentration to characterize exposure.
- The degree of lysis can be visualized with genetic stains (fluorescent green color indicates genetic material is leaking from cells).

Microcystis from **Copco Reservoir, OR**



Images courtesy of Barry Rosen, USGS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Prepared in cooperation with the U.S. Environmental Protection Agency

Microphotographs of Cyanobacteria Documenting the Effects of Various Cell-lysis Techniques

20.0 µm

Open-File Report 2010–1289

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

http://pubs.usgs.gov/of/2010/1289/

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What Methods Are Available For Cyanotoxin Measurement?

Biological Assays:

Animal Tests (e.g. Mice)

Enzyme-Linked Immunosorbent Assays (ELISA)
Protein Phosphatase Inhibition Assays (PPIA)
Neurochemical assays (e.g. acetylcholinesterase-based)

Chromatographic Methods:

Gas Chromatography with Flame Ionization Detection (GC/FID) or Mass Spectrometry (GC/MS)

Thin Layer Chromatography (TLC)

Liquid Chromatography / Ultraviolet-Visible Detection (HPLC or LC/UV)
Liquid Chromatography / Fluorescence (LC/FL) – usually with post column oxidation prior to detection
Liquid Chromatography Ion Trap Mass Spectrometry (LC/IT MS)
Liquid Chromatography Time-of-Flight Mass Spectrometry (LC/TOF MS)
Liquid Chromatography Single Quadrupole Mass Spectrometry (LC/MS)
Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC/MS)

What Methods Are Available For Cyanotoxin Measurement?

	Freshwater Cyanotoxins				
	Anatoxins	**Cylindrospermopsins**	**Microcystins**	**Nodularins**	**Saxitoxins**
Bioloigcal Assays (Class Specific Methods at Best):					
Mouse	Y	Y	Y	Y	Y
PPIA	N	N	Y	N	N
Neurochemical	Y	N	N	N	Y
ELISA	?	Y	Y	Y	Y
Chromatographic Methods (Compound Specific Methods):					
Gas Chromatography:					
GC/FID	Y	N	N	N	N
GC/MS	Y	N	N	N	N
Liquid Chromatography:					
LC/UV (or HPLC)	Y	Y	Y	Y	Y
LC/FL	Y	N	N	N	Y
Liquid chromatography combined with mass spectrometry can analyze cyanotoxins very specifically.					
LC/IT MS	Y	Y	Y	Y	Y
LC/TOF MS	Y	Y	Y	Y	Y
LC/MS	Y	Y	Y	Y	Y
LC/MS/MS	Y	Y	Y	Y	Y

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Y = Yes
N = No
? = It is being worked on

Method Specificity for Cyanotoxins

	Specificity
Biological Assays (Class Specific Methods at Best):	
Mouse	Non-specific, test must be rapid therefore endpoint usually death.
PPIA	Of the freshwater cyantoxins, only microcystins are known to inhibit protein phosphatase.
Neurochemical	Of the freshwater cyanotoxins, only anatoxins and saxitoxins are known to inhibit neurochemical processes.
ELISA	Compound and toxin class specificity dependent on antibody or mix of antibodies used.
Chromatographic Methods (Compound Specific Methods):	
Gas Chromatography:	
GC/FID	Only the anatoxins have been routinely measured. Derivitization is typically required.
GC/MS	Only the anatoxins have been routinely measured. Derivitization is typically required.
Liquid Chromatography:	
LC/UV (or HPLC)	Variable. Subject to interference with co-eluting matrix.
LC/FL	Variable. Subject to interference with co-eluting matrix.
Liquid chromatography combined with mass spectrometry can analyze cyanotoxins very specifically.	
LC/IT MS	Second in compound specificity only to LC/TOF MS.
LC/TOF MS	Accurate mass capability makes this technique the most specific.
LC/MS	Weaker cousin of LC/MS/MS. Fourth most specific.
LC/MS/MS	Third most specific technique routinely employed

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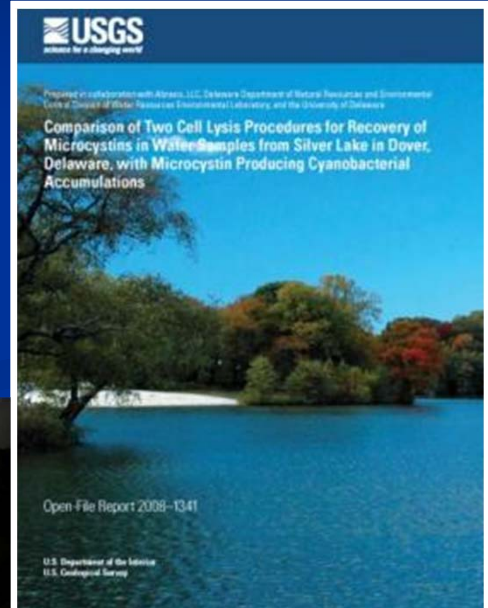
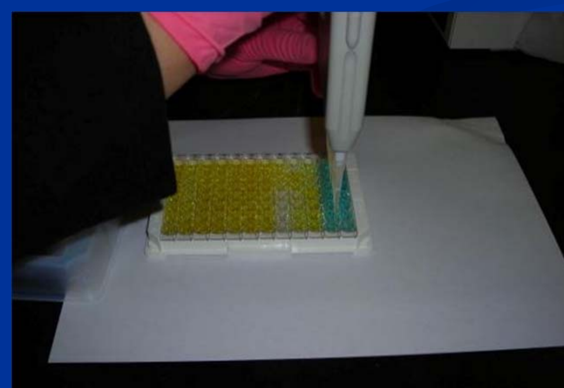
OGRL Analytical Methods

Analytical methods development is a large part of our research program. Without robust and sensitive analytical methods, research objectives can not be achieved.

Natural Toxins	Number of Parent Compounds	Number of Degradates or Conjugates	Total Number of Compounds	Maximum MRL (µg/L)
Cyanotoxins	11	1	12	0.010
Algal Toxins	2	0	2	0.010
Mycotoxins	Collaboration with Swiss Researchers			
Totals:	13	1	14	



http://pubs.usgs.gov/of/2008/1341/



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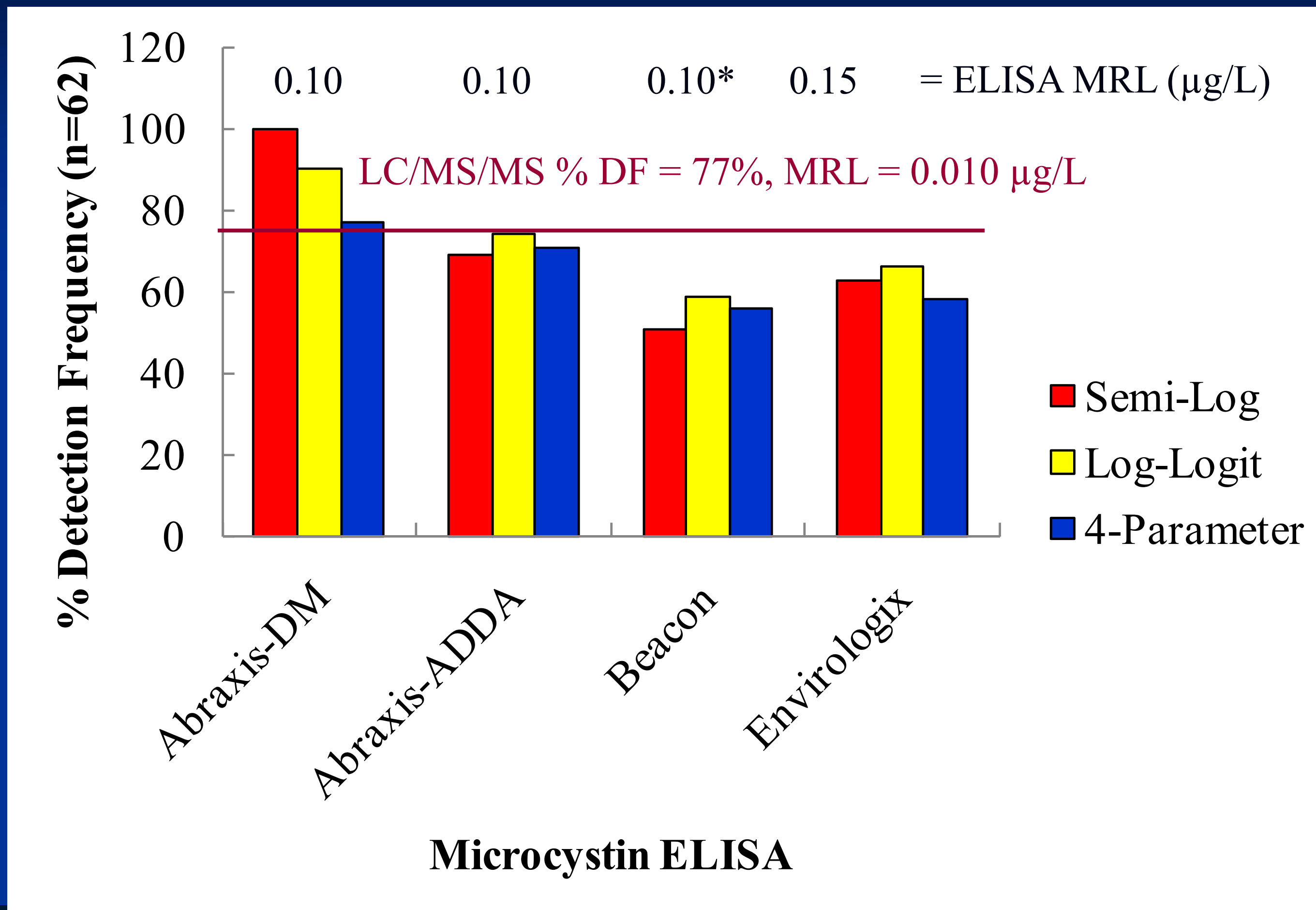
Working on sediment and saltwater methods as well.

Microcystin ELISAs Measure Different Things!



ELISA Cross-Reactivity Example for Microcystins

No ELISA is MCLR specific!

- With over 80+ microcystin and 10+ nodularins, most cross-reactivities are unknown!

Percent Cross-Reactivity

Microcystin Assays	MCLA	MCLF	MCLR	MCRR	MCLW	MCLY	MCYR	NODR
Monoclonal Assays								
Abraxis-DM	48	72	100	53	102	NA	64	76
Polyclonal Assays								
Abraxis-ADDA	125	108	100	91	114	NA	81	169
Beacon	5	NA	100	87	NA	NA	48	31
Envirologix	62	NA	100	54	NA	NA	35	69
Strategic Diagnostics	23	NA	100	97	NA	NA	82	66

- ELISA Response = Σ (Cross-Reactivity x Actual Congener Concentration) $_i$

- Example: Theoretical Concentration: 1 ppb MCLR + 1 ppb MCLA = 2 µg/L

Abraxis-DM	≈ 1.48 µg/L
Abraxis-ADDA	≈ 2.25 µg/L
Beacon	≈ 1.05 µg/L
Envirologix	≈ 1.62 µg/L
SDI	≈ 1.23 µg/L

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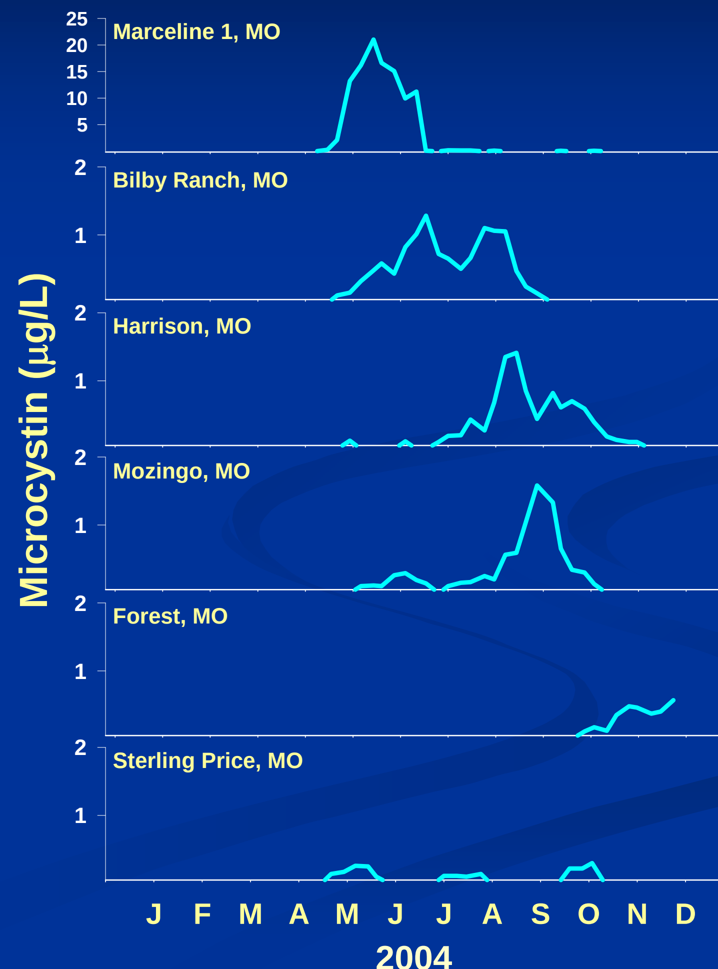
Spatial and Temporal Patterns

Seasonal Patterns in Microcystin Concentration are Unique to Individual Lakes and Peaks May Occur Anytime Throughout the Year

Weatherby Lake, Missouri
January, 2007

Mozingo Lake, Missouri
October, 2001



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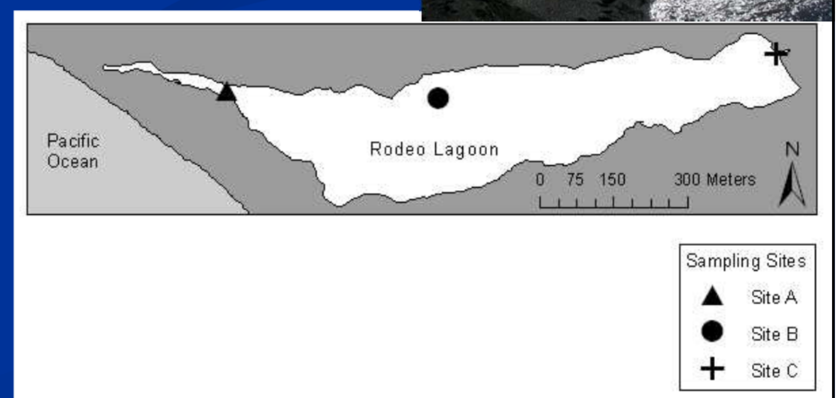
After Graham and others, 2006

Phytoplankton Succession and Cyanotoxin Production

Rodeo Lagoon, Golden Gate National Park, CA, April – December 2007

- Samples were collected, water chemistry measured, and phytoplankton characterized during 2007 weekly at multiple sites by UC-Berkeley and San Francisco State Univ.

- OGRL screened a subset of water samples by LC/MS/MS for cyanotoxins and algal toxins.



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Drake, J.L., et. al., 2010, Hydrobiologia, *654*, 177-192.

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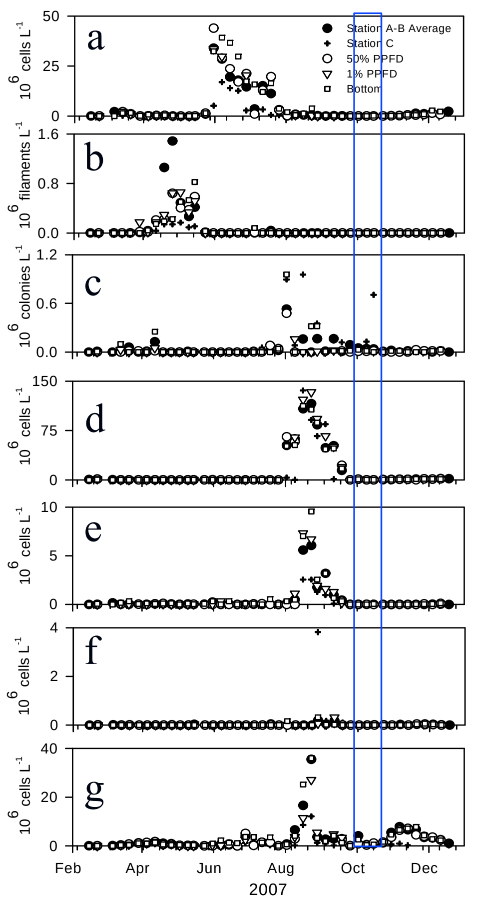
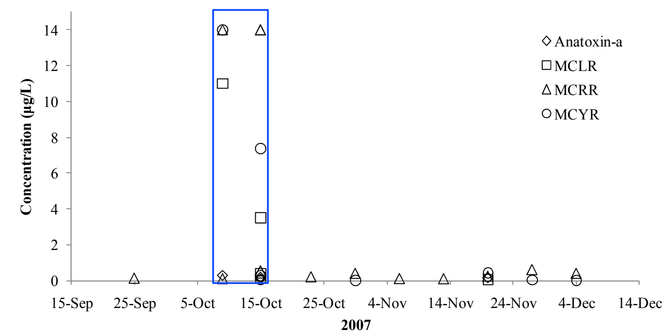


a – Centric diatoms
b – *Nodularia spumigena*
c – *Microcystis aeruginosa*
d – flagellated protozoa
e – pennate diatoms
f – dinoflagellates
g - chlorophytes



- All Cyanotoxin production was observed between 9/25/2007 and 12/4/2007
- Anatoxin-a was detected in 11% of samples
  - maximum concentration of 0.29 µg/L.
  - Anatoxin-a always co-occurred with microcystins
- Microcystins were detected in 40 % of samples
  - Up to 3 microcystins were detected in a given sample (microcystins – LR (11%), -RR (37 %), and – YR (23 %))

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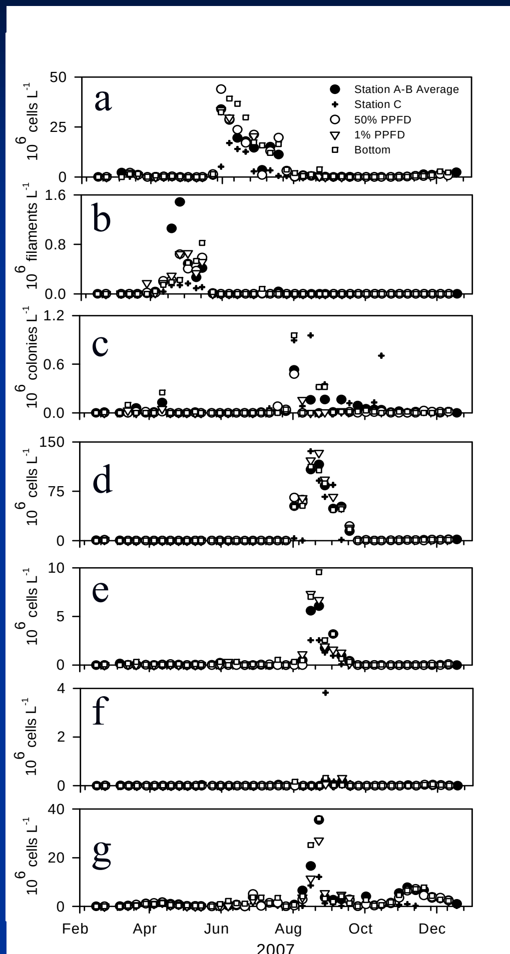

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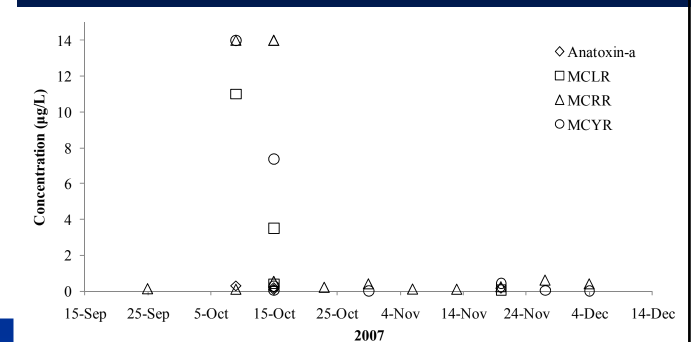


- Findings significant because "freshwater " cyanotoxins have historically been ignored in coastal waters. This study demonstrates that freshwater inflows can strongly influence phytoplankton populations and types of toxins measured in coastal waters.

USGS science for a changing world

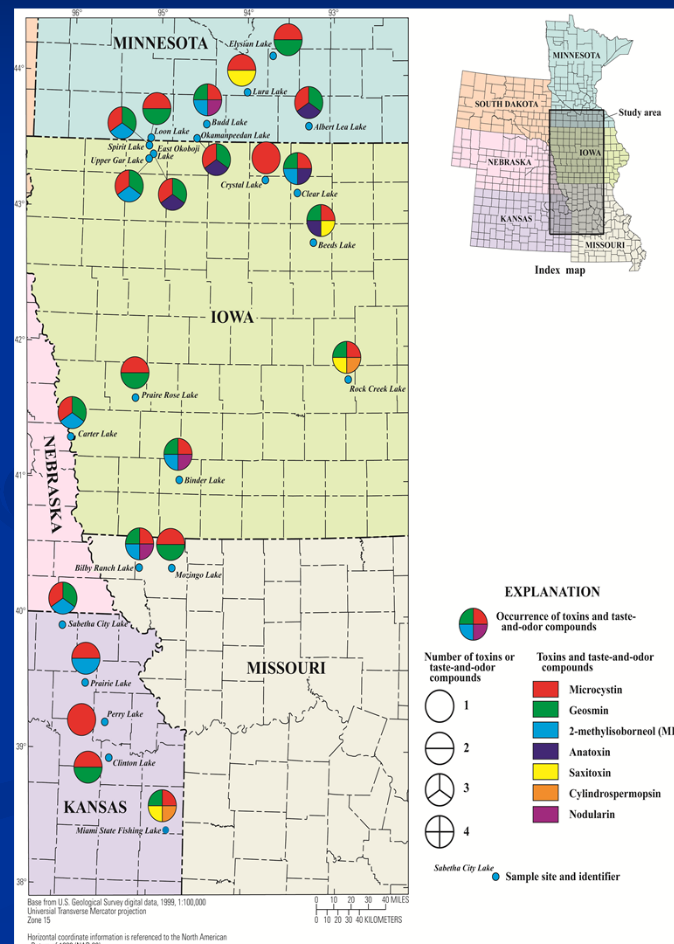
San Francisco State University

Berkeley University of California

Drake, J.L., et. al., 2010, Hydrobiologia, *654*, 177-192.

Co-Occurrence of Cyanotoxin Mixtures and Taste-and-Odor Compounds – August 2006

- Cyanotoxin mixtures were typical.
  - Anatoxin-a (30%)
  - Cylindrospermopsins (9%)
  - Microcystins (100%),
    - max value = 19,000 µg/L
  - Saxitoxins (17%)
- 17% of blooms had microcystin concentrations exceeding the WHO high risk recreational guideline of 20 µg/L.
- Toxins and taste-and-odor compounds co-occurred in 91% of bloom samples (n=23).
- Drinking water utilities frequently say that finished water is safe when taste-and-odor compounds are present.



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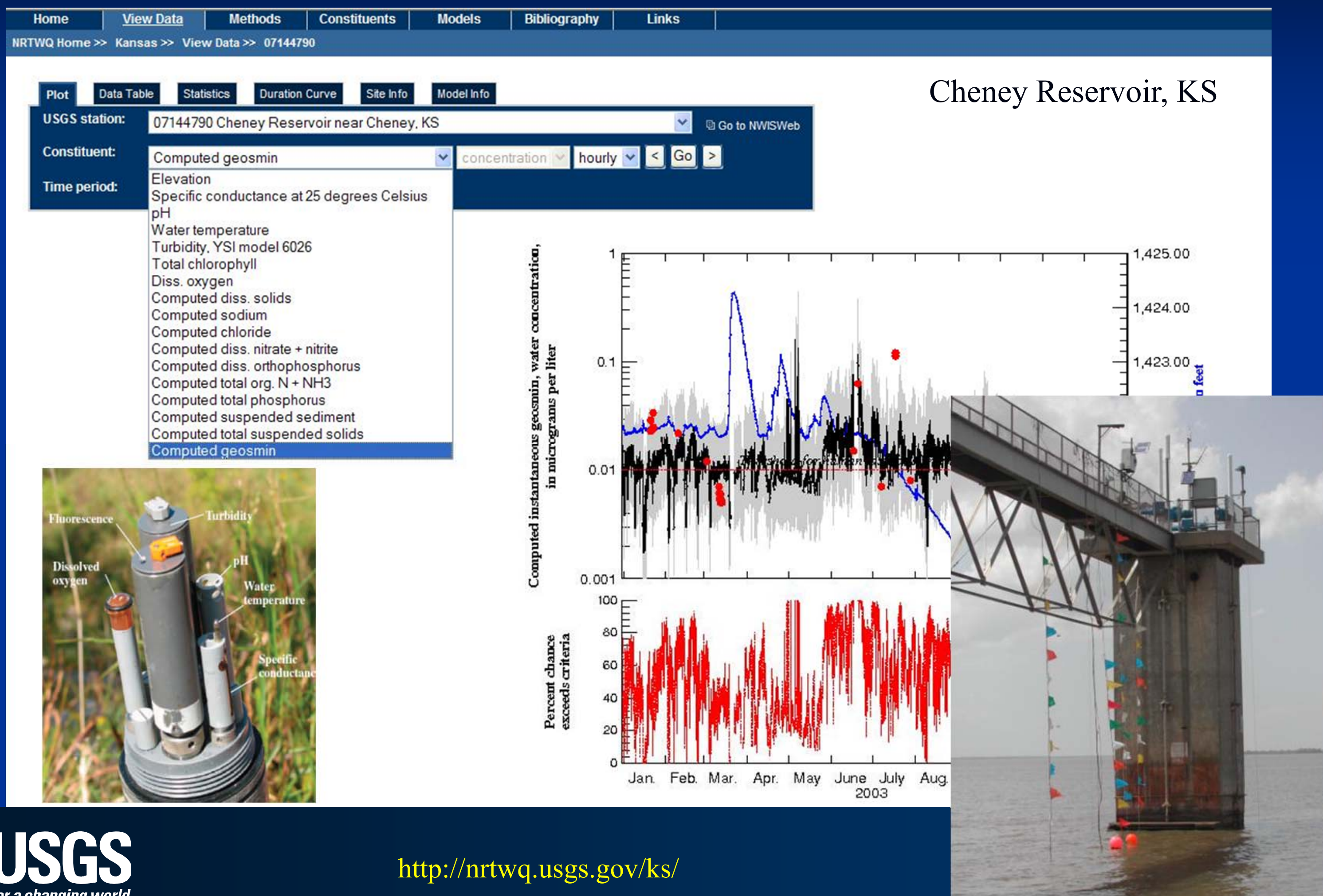
Graham, J.G., et al., 2010, ES&T, *44*, 7361-7368.

Environmental Influences

Continuous Water-Quality Monitors Can Be Used to Develop Models to Compute Geosmin Concentrations in Real Time

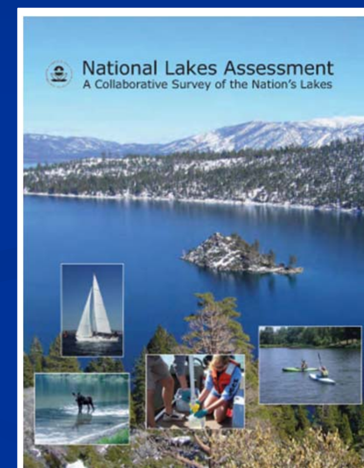
Cheney Reservoir, KS



http://nrtwq.usgs.gov/ks/

2007 National Lake Assessment and Cyanotoxins – Integrated Photic Zone

- First statistical survey to classify the water quality of U.S. lakes.
- Approximately, 1000 lakes sampled out of an estimated 50,000 natural and man-made lakes from May 2007 to October 2007.
- Lake defined as:
  - Greater than 10 acres
  - At least 1 meter deep
  - freshwater
- Collaborators: US EPA, States, Tribes, USGS, selected universities
- Cyanotoxins only one of many chemical, biological, and physical indicators measured.



EPA 841-R-09-001
http://water.epa.gov/type/lakes/lakessurvey_index.cfm

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A Few Words on the 2007 National Lakes Assessment

- Larger reservoirs were not numerous due to study design.
- There is a temporal signal in the data set (samples collected May to October 2007).
- Weather patterns can be different from year to year for each region.
- This study represents a snapshot in time.
- It is the first attempt at a national assessment of cyanotoxins in the U.S.

- Cylindrospermopsin and Saxitoxin ELISA results are preliminary and are being completed outside of the National Lakes Assessment in collaboration with USGS Toxic Substance Hydrology Program, US EPA NHERL (Betsy Hilborn), and US EPA OWOW.

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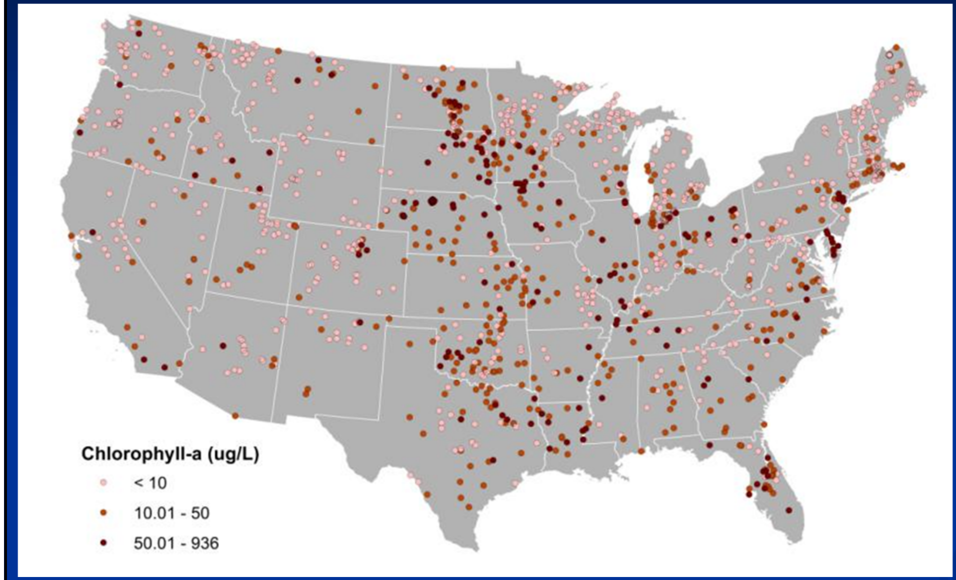
EPA 841-R-09-001

http://water.epa.gov/type/lakes/lakessurvey_index.cfm

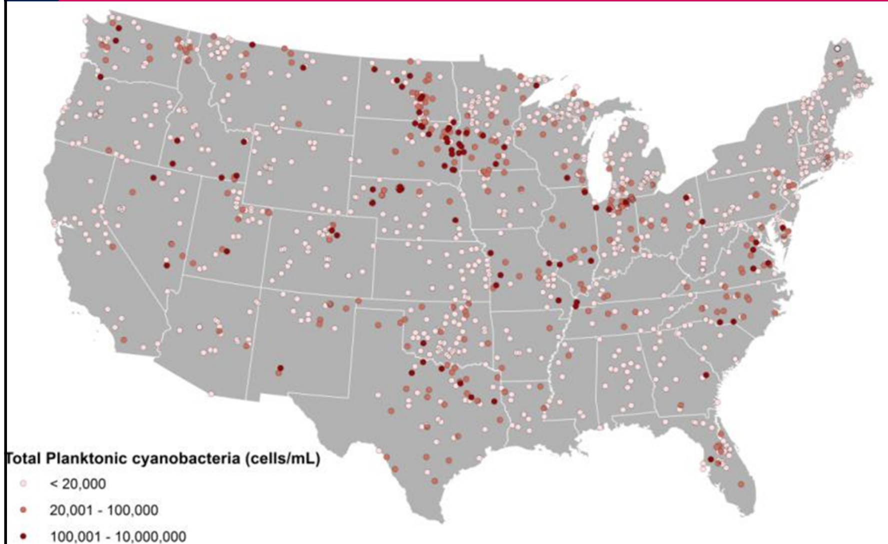
2007 National Lake Assessment and Cyanotoxins – Integrated Photic Zone - Chlorophyll



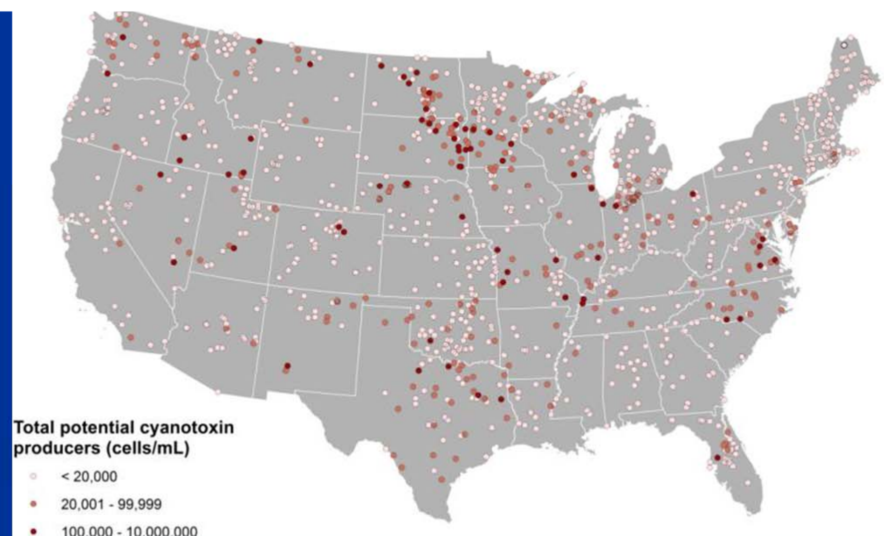
- Chlorophyll was detected most frequently and typically at larger concentrations East of the Rocky Mountains.

EPA 841-R-09-001
Loftin, K.A., et al., ES&T, in draft

2007 National Lake Assessment and Cyanotoxins
Integrated Photic Zone – Total Phytoplankton



- The most abundant phytoplankton species were usually potentially toxic cyanobacteria.
- This relationship is a function of seasonality (e.g. temperature and solar irradiance).
- Diatoms, green algae, and other organisms can be the most dominant during other seasons of the year.



EPA 841-R-09-001

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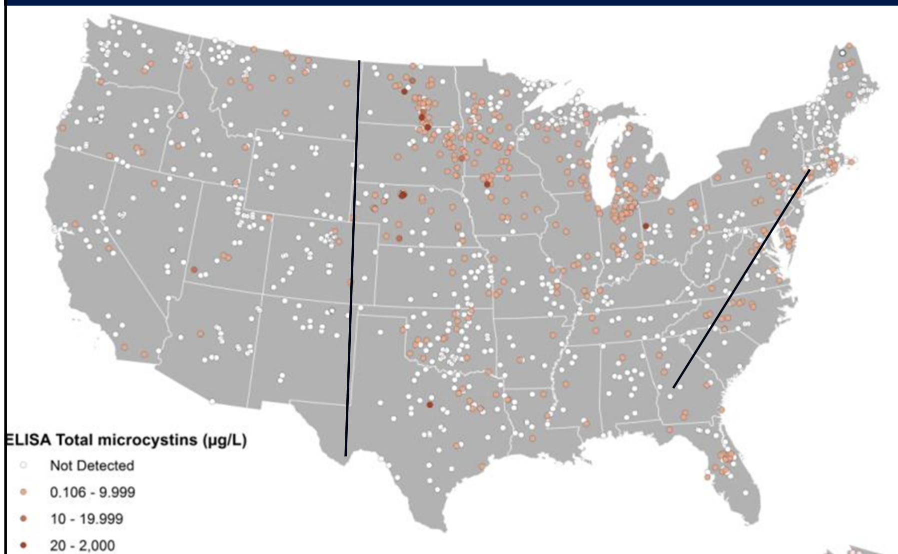
2007 National Lake Assessment and Cyanotoxins
Integrated Photic Zone – Microcystins (ELISA)



- Microcystins were detected in ~30% of lakes.
- Microcystin detections were approximately equal in natural and Man-made lakes.
- Microcystin Detection Summary Statistics:
  - Mean: ~1.0 µg/L
  - Maximum: 230 µg/L

- Known microcystin producing cyanobacteria occurred more frequently compared with detected microcystins.

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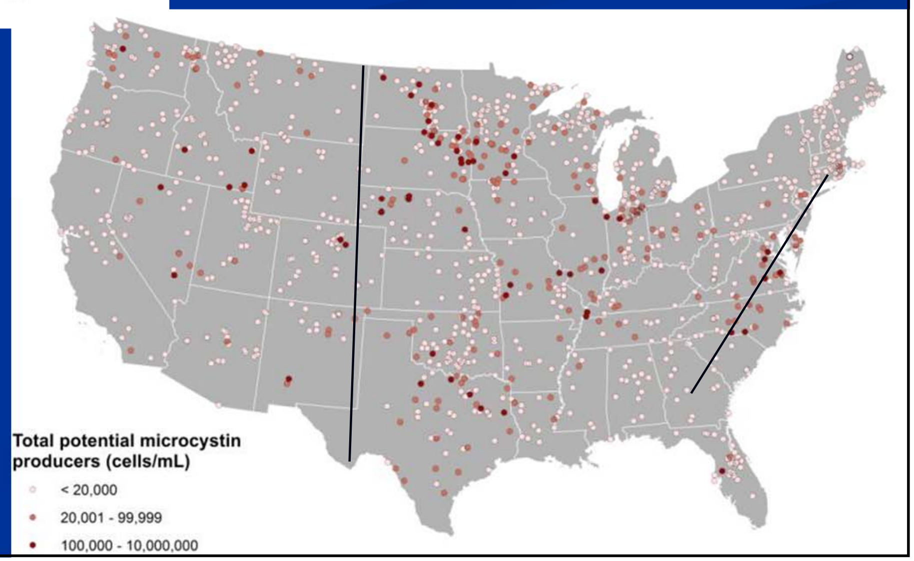
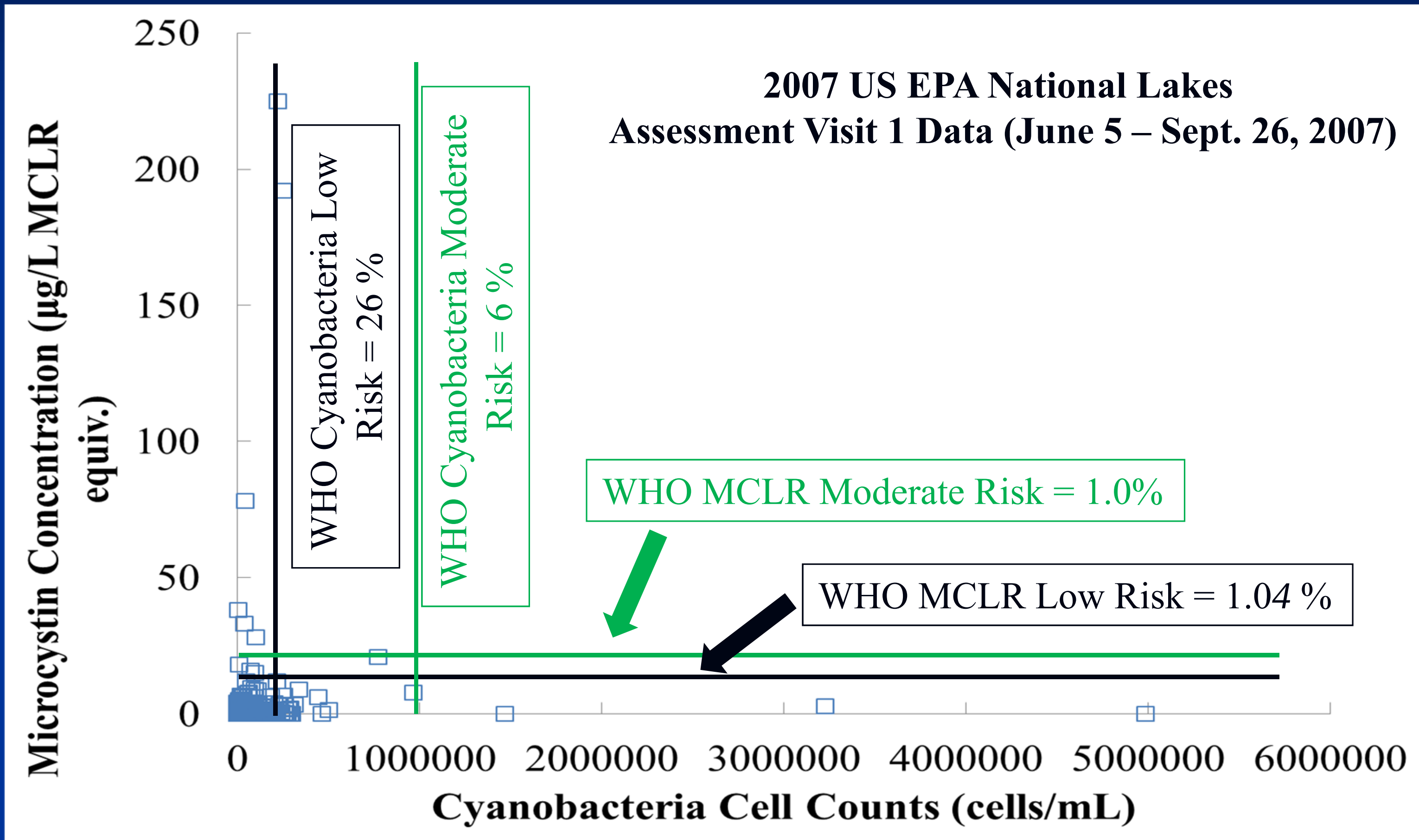


National Snapshot (2007 NLA) WHO Recreational Guideline Exceedances



WHO risk designations are probability of an adverse health risk during recreation.

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Natural Toxins, Their Sources, Human Influences, and Exposure Scenarios

Higher Detection Frequencies and Higher Concentrations were Observed in Blooms and Accumulations than in Photic Zone Sampling.

	2007 EPA National Lakes Assessment	2006 USGS Midwestern US Reconnaissance
Sample Type	Photic Zone, Deepest	Blooms and Accumulations
% Microcystin Detections	32 % (n=1238)	100 % (n=23)
Total Microcystin Concentration (µg/L)		
Mean	3.0 (~1.0) [1]	600
Median	0.52 (< 0.10)	3
Minimum	0.1 (< 0.10)	< 0.10 [2]
Maximum	230	13000

1 Values outside of parenthesis are summary statistics for detections only.
Values inside of parenthesis are summary statistics that include non-detections also.

2 Microcystin detections were measured below ELISA threshold of 0.10 µg/L by Liquid Chromatography Triple Quadrupole Mass Spectrometry resulting in a detection frequency of 100%.

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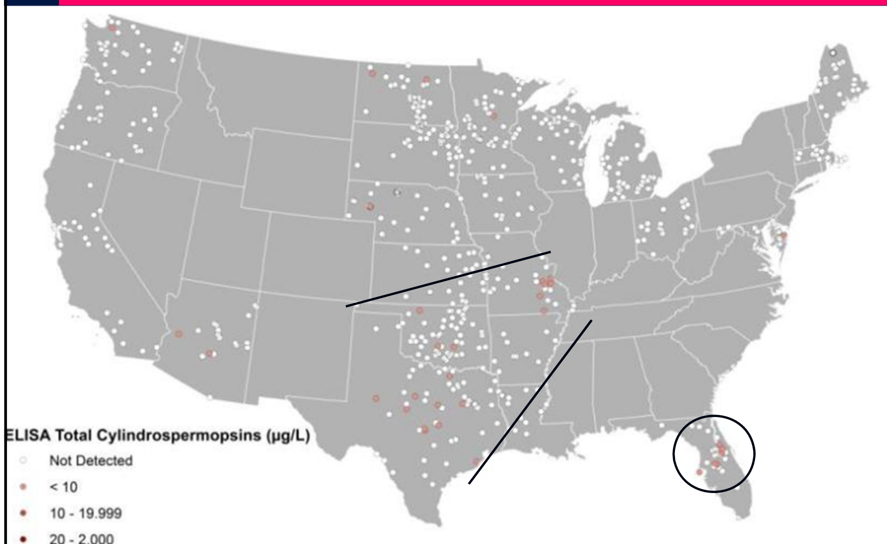
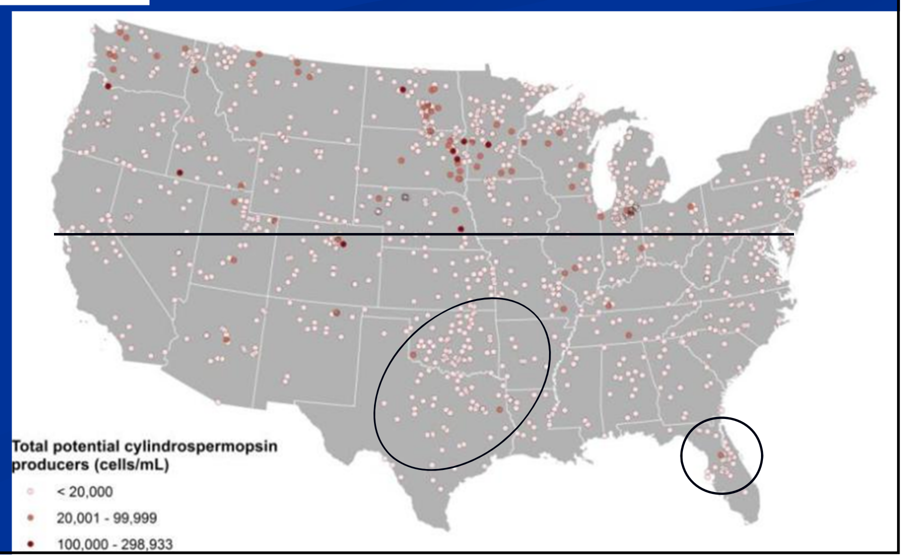
2007 National Lake Assessment and Cyanotoxins
Integrated Photic Zone – Preliminary Cylindrospermopsins (ELISA)



- Cylindrospermopsins were detected in ~ 5 % of lakes of analyzed lakes (n= 659).
- Cylindrospermopsin Detection Summary Statistics:
  - Mean: 0.44 µg/L
  - Maximum: 3.4 µg/L

- Known cylindrospermopsin producing cyanobacteria occurred more frequently than cylindrospermopsin was detected.
- Cylindrospermopsis blooms tend to be subsurface.



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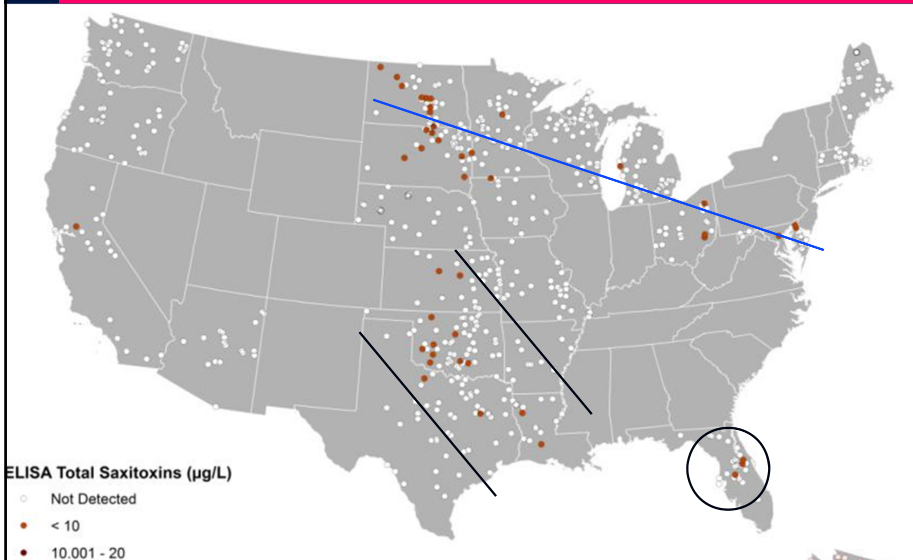
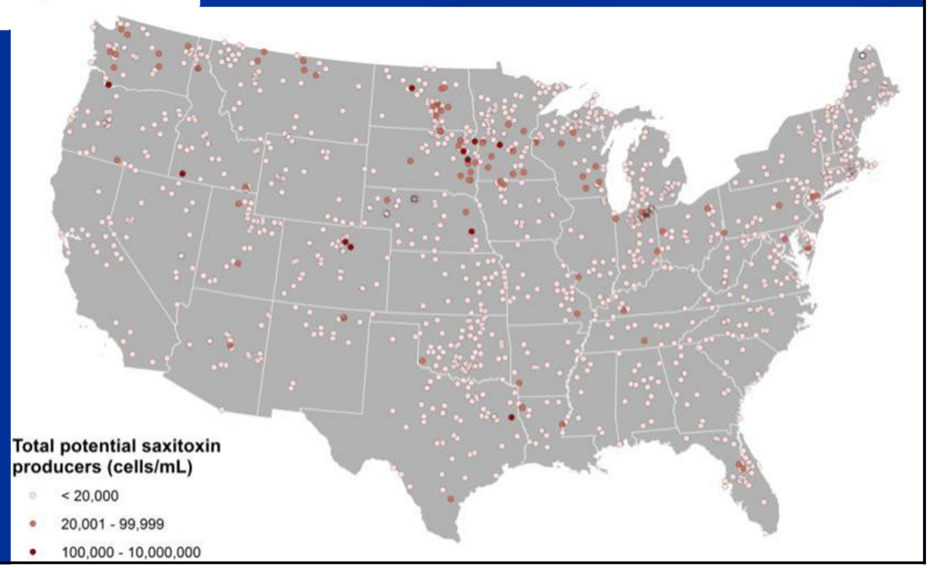
2007 National Lake Assessment and Cyanotoxins
Integrated Photic Zone – Preliminary Saxitoxins (ELISA)



- Saxitoxins were detected in 8% of lakes of analyzed lakes (n=678).
- Traditionally, thought of as marine toxins. Perhaps not anymore….
- Saxitoxin Detection Summary Statistics:
  - Mean: 0.05 µg/L
  - Maximum: 0.38 µg/L

- Known saxitoxin producing cyanobacteria occurred more frequently than saxitoxin was detected.



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2007 National Lake Assessment and Cyanotoxins Integrated Photic Zone – Microcystins and Nodularin-R (LC/MS/MS)

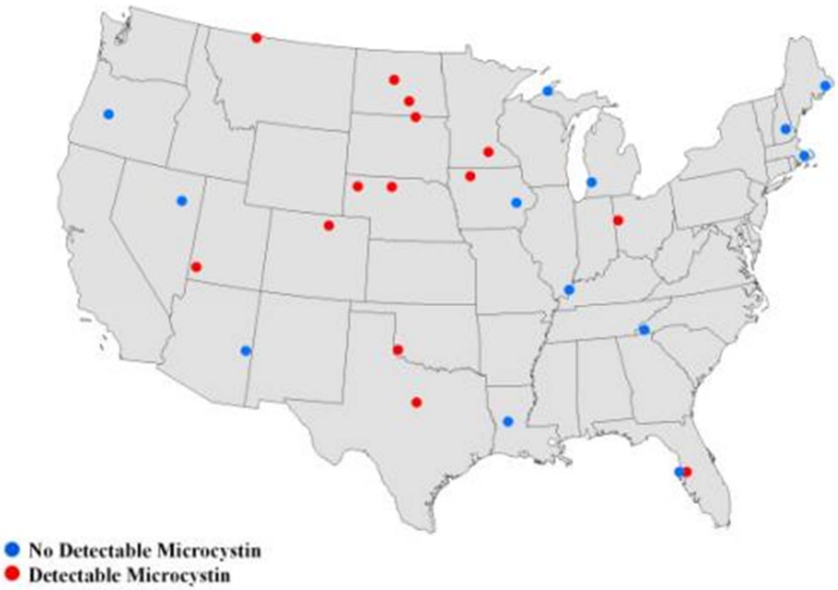
Sample selection included 13 of 27 (48%) detections by Microcystin ELISA

LC/MS/MS Results:

Microcystins detected:
14 of 27 (59%) samples

Nodularin-R detected:
1 of 27 (3.7%) in Texas

Nodularin-R is known as a brackish water toxin.



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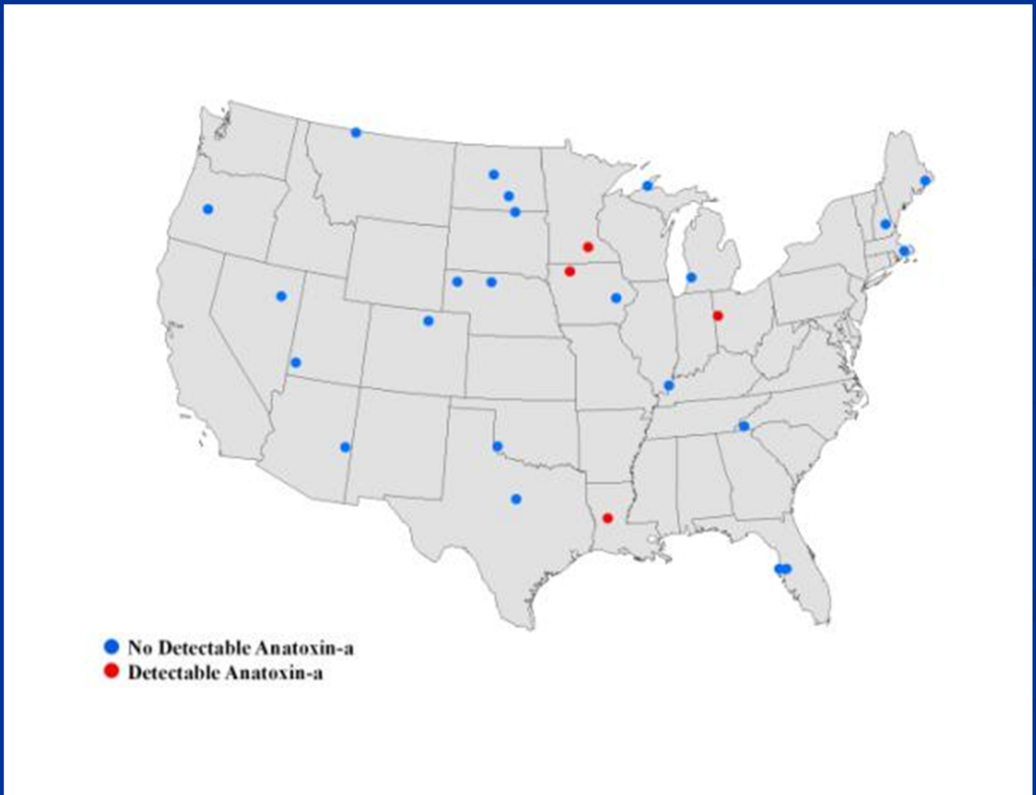
2007 National Lake Assessment and Cyanotoxins Integrated Photic Zone – Anatoxin-A (LC/MS/MS)

Sample selection included 13 of 27 (48%) detections by Microcystin ELISA



Anatoxin-a detected:
4 of 27 (15%) samples

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2007 National Lake Assessment and Cyanotoxins – Summary

- First ever national lake survey of cyanotoxins in the United States!

-Cyanotoxin detections and their concentrations were more prevalent than expected.

-Microcystins – 30%

-Saxitoxins ~ 8% (so far) –

-Cylindrospermopsins ~ 5 % (so far)

-Anatoxin-a and Nodularin-R detected.

- Mixtures are common on a national scale.

-The largest detection frequencies tended to occur East of the Rocky Mountains in the central plains.

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Microcystin Transport: Milford Lake Floodwater Released to Kansas River - 2011

Milford Lake release sends algae to Kansas River
MARIA SUDEKUM FISHER, Associated Press
Published 09:10 p.m., Wednesday, September 21, 2011

Concerns about blue-green algae shut down Kaw River Water Treatment Plant
By Christine Metz on September 15, 2011

Topeka water safe despite Milford Lake discharge
Posted: September 15, 2011 - 12:19pm

http://pubs.er.usgs.gov/publication/sir20125129

USGS science for a changing world

Microcystin Transport: Milford Reservoir Floodwater Released to Kansas River (Preliminary)

- Lessons Learned:
  - Microcystins and intact cyanobacteria can travel over 170 stream miles.
  - Topeka, the state capital of Kansas, pulls water from the Kansas River and does not have an alternate drinking water source.
  - Lawrence, Johnson County, and Kansas City have alternate water supplies, but need sufficient notice to change source.

USGS science for a changing world

http://pubs.er.usgs.gov/publication/sir20125129

Cyanotoxin Survey in Alaska, Guam, Hawaii, and Puerto Rico– Characteristics of Each Area

- Each area is quite diverse:
  - Topography
  - Geology
  - Climate

Source: U.S. Census Bureau, various websites

Source: Several websites

	Puerto Rico	Alaska	Guam	Hawaii
Relationship with U.S.	Commonwealth	State	U.S. Territory	State
Area (mi^2)	3515	570,640.95	212	6422.63
Description	Mountainous, Tropical	Moutainous, Arctic, Subarctic, Volcanic	Coralline Limestone Plateau, Volcanic Hills	Volcanic, Tropical
Population (2010)	3,977,663	710,231	175,877 (2008)	3,725,789
% Population Change (2000 to 2010)	0.3	13.3	1.3	12.3
% of Population Native:	?	14.8	37	10
Major Economy:	Services, Pharmaceuticals, electronics, Agriculture, Tourism	Natural Resources, Tourism	Tourism, Military	Tourism,

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Cyanotoxin Survey in Alaska, Guam, Hawaii, and Puerto Rico

- Collaborators:
  - USGS Caribbean, Alaska, and Pacific Islands WSC's, University of Puerto Rico
- Hypotheses:
  - Cyanotoxins and their potential producers will be observed in Puerto Rico, Alaska, Guam, and Hawaii.
  - Larger cyanotoxin concentrations, greater cyanotoxin diversity, and greater phytoplankton diversity will be observed in tropical locations of Puerto Rico, Guam, and Hawaii compared with Arctic/subarctic locations such as Alaska.

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Study supported by USGS Water Mission Area, Climate and Land Use Change Mission Area, Toxic Substances Emerging Contaminant Program

Cyanotoxin Survey in Alaska, Guam, Hawaii, and Puerto Rico

- Reasons for Study:
  - U.S. states, commonwealths, and protectorates are commonly ignored for water quality studies due to difficulty and cost of studies compared to the contiguous U.S..
  - Water quality comparisons of tropical, arctic, and subarctic regions may give us insight in more temperate regions when subjected to changes in climate and land use associated with those climate changes.
  - Many of the economies of these areas thrive on global tourism, food exports, and/or are of strategic military importance.
- Site Selection:
  - Targeted natural and manmade lakes, rivers, wetlands, and coastal lagoons.
  - Most samples have low salinity (e.g. less than 2.5 PPT).
  - Representative of typical environment, land use, climate, etc. for that region.
  - Accessibility

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Cyanotoxin Survey in Alaska (Preliminary)

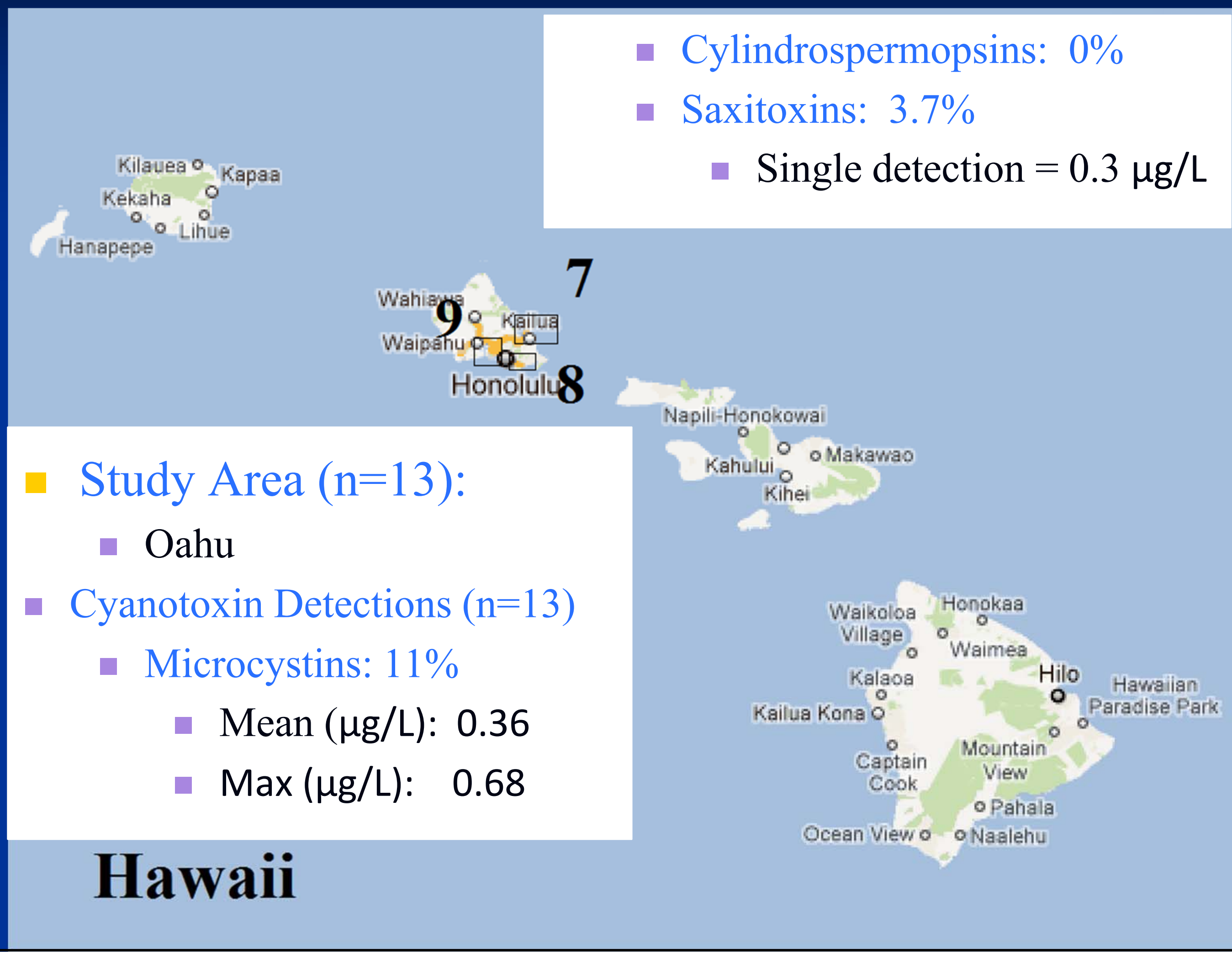
- Study Areas (# of sites):
  - 1. Point Hope (6)
  - 2. Shishmaref (6)
  - 3. Yukon Flats River Basin (5)
  - 4. Yukon Flats River Basin (1)
  - 5. Anchorage (4)

- Cyanotoxin Detections (n=27)
  - Microcystins: 22%
    - Mean (µg/L): 11
    - Max (µg/L): 59 in 11 ° C water
  - Cylindrospermopsins: 3.7%
    - Single detect = 0.06 µg/L
  - Saxitoxins: 0%

Cyanotoxin Survey in Hawaii (Preliminary)

- Cylindrospermopsins: 0%
- Saxitoxins: 3.7%
  - Single detection = 0.3 µg/L

- Study Area (n=13):
  - Oahu
- Cyanotoxin Detections (n=13)
  - Microcystins: 11%
    - Mean (µg/L): 0.36
    - Max (µg/L): 0.68



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Cyanotoxin Survey in Puerto Rico (Preliminary)

- Study Areas (6 of 20 sites sampled).
- No cyanotoxins detected to date.
- High frequency of tropical storms in 2011 during sampling.
- More sampling planned for 2012.

- Precipitation was 10" more than normal in June 2011 and 6" more than normal in July 2011 (2^{nd} wettest month on record.
- Likely led to resuspension of diatoms.

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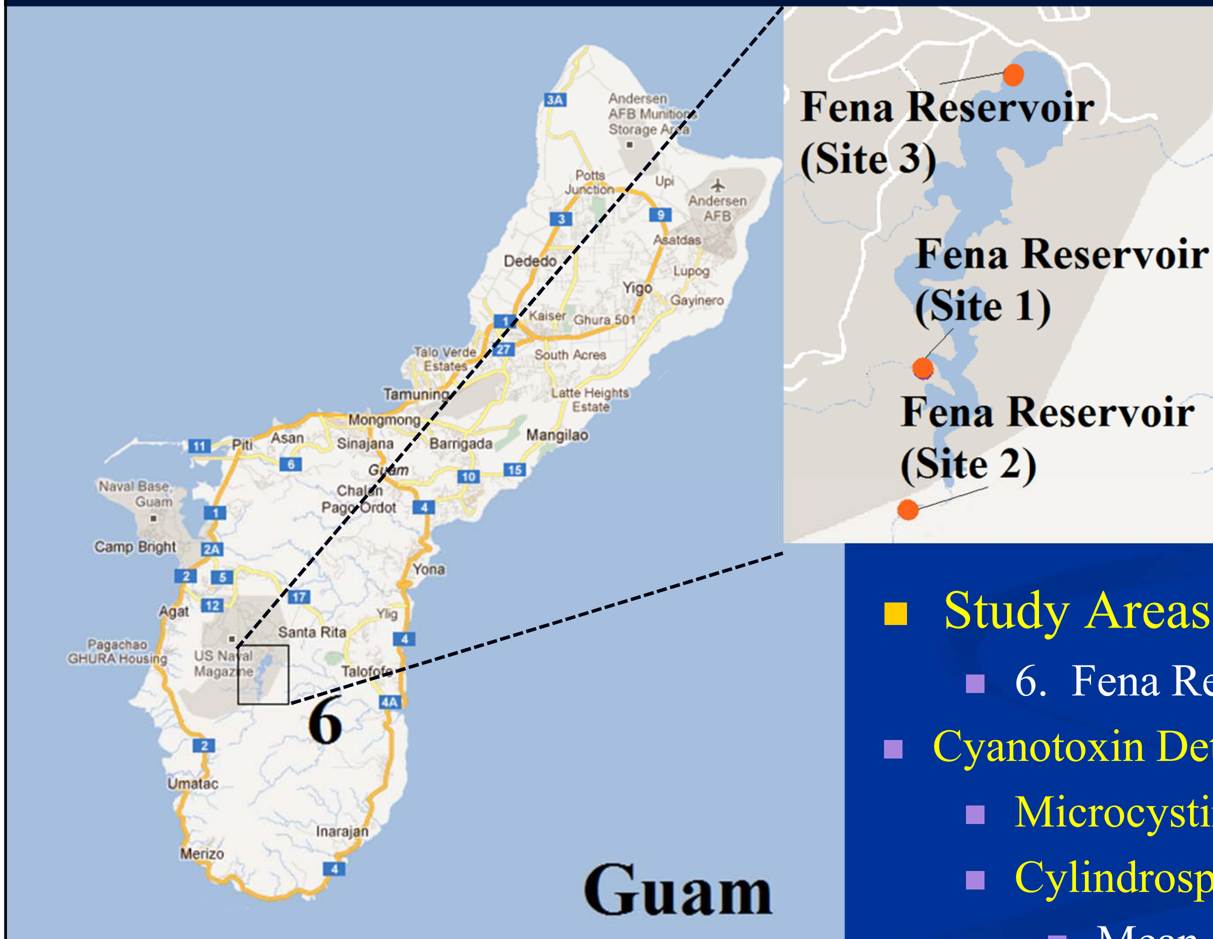
Cyanotoxin Survey in Guam (Preliminary)



- Study Areas (n=3):
  - 6. Fena Reservoir
- Cyanotoxin Detections (n=3)
  - Microcystins: 0%
  - Cylindrospermopsins: 3.7%
    - Mean (µg/L): 0.18
    - Max (µg/L): 0.20
  - Saxitoxins: 0%

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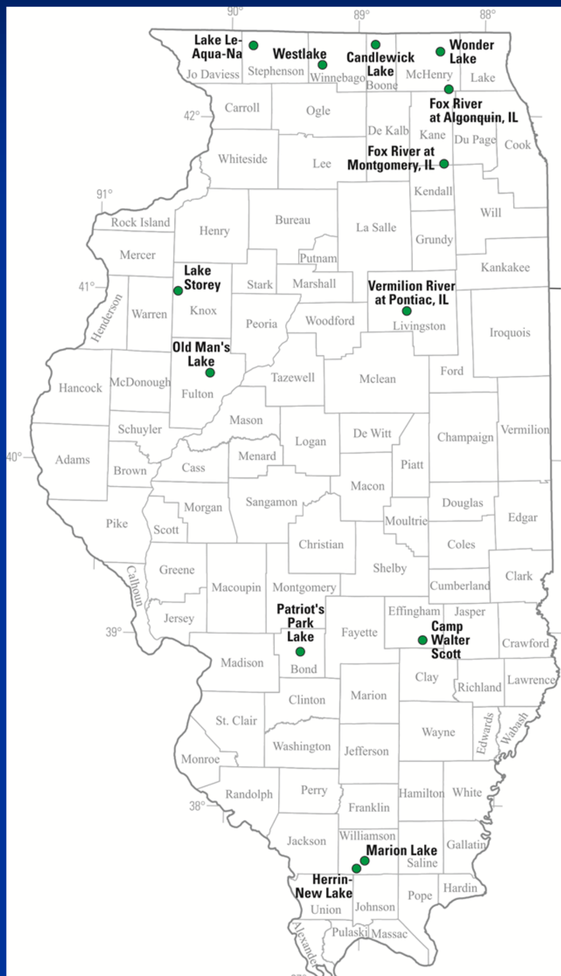
Illinois Reconnaissance Study 2012
A Collaboration with IL EPA, Local Lake Managers



- 26 samples analyzed by ELISA for:
  - Cylindrospermopsins (MRL < 0.05 µg/L)
    - Det. Frequency = 0%
  - Saxitoxins (MRL < 0.02)
    - Det. Frequency = 0%
  - Microcystins
    - Det. Freq. = 88% (n=26)
    - Minimum = < 0.10 µg/L
    - Mean = 360 µg/L
    - Median = 1.4 µg/L
    - Maximum = 4800 µg/L

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Initial Results from a Reconnaissance of Cyanobacteria and Associated Toxins in Illinois, August–October 2012

By Paul J. Terrio[1], Lenna M. Ostrodka[1], Keith A. Loftin[2], Gregg Good[3], and Teri Holland[3]

Abstract

Ten lakes and two rivers in Illinois were sampled in August–October 2012 to determine the concentrations and spatial distribution of cyanobacteria and associated cyanotoxins throughout the State. The reconnaissance was a collaborative effort of the U.S. Geological Survey and the Illinois Environmental Protection Agency. Sample results indicated that concentrations of both total cyanobacterial and microcystin cells were commonly at levels likely to result in adverse human health effects, according to World Health Organization guidance values. Concentrations generally decreased from August to October following precipitation events and lower temperatures.

Introduction

Cyanobacteria, also known as blue-green algae, can be found in surface waters throughout the United States. These microscopic organisms, when present in high concentrations, can cause the water to have a pea-soup appearance or they can accumulate as floating masses of blue- or green-colored scum, commonly called cyanobacterial blooms. Elevated nutrient concentrations, slow-moving water, warm temperatures, and sunlight are all thought to be conducive to the growth of cyanobacteria.

The presence of cyanobacteria in lakes and rivers can pose risks to human and ecological health. Some species of cyanobacteria produce toxins, known as cyanotoxins, which can cause gastroenteritic problems if ingested or inhaled. Cyanotoxins can also cause allergic reactions following bodily contact (Graham and others, 2009). In addition to toxin production, cyanobacteria also can produce taste-and-odor compounds, which increase the cost of water treatment (Graham and others, 2008). Consequently, cyanobacteria in Illinois lakes and rivers may hinder recreational activities, contaminate drinking-water supplies, and pose health risks.

A reconnaissance was conducted by the U.S. Geological Survey (USGS) and the Illinois Environmental Protection Agency (EPA) during August–October 2012 to (1) confirm recent detections of high cyanotoxin concentrations, (2) assess the spatial extent, concentrations, and characteristics of cyanobacterial blooms in Illinois, and (3) provide data to support state and local agencies in managing water resources to protect human, animal, and ecological health. In late August 2012, concerns arose after samples analyzed from a private lake indicated elevated concentrations of cyanotoxins and several observations of cyanobacterial blooms in northern Illinois were reported. The two agencies made an effort to collect a substantial number of samples prior to both the Labor Day weekend and imminent rainfall and cooler temperatures (remnants of Hurricane Isaac) which could potentially alter the cyanobacterial communities and cyanotoxin concentrations. Illinois EPA field screening for cyanotoxins earlier in 2012 and in previous years did not detect concentrations of concern. But, an extended drought in 2012 and accompanying high temperatures might have provided conditions facilitating cyanobacterial dominance and associated cyanotoxin production.

Methods

Study design

Between August 29 and September 4, 2012 10 lakes and two rivers were sampled for cyanobacteria, cyanotoxins, nutrients, and chlorophyll *a* (fig. 1). The water bodies sampled were selected based on reports of current cyanobacterial blooms, where cyanobacterial blooms had been observed in the past, where other field efforts already were being conducted, and where additional sites would improve longitudinal distribution of sample locations throughout Illinois. Additional samples were collected on October 24, 2012 at four sites (three lakes and one river) where some of the highest cyanotoxin concentrations were determined in August–September samples.

Field measurements

Field measurements of water temperature, specific conductance, dissolved oxygen, and pH were collected by using multi-meter sensors. All sensors were calibrated according to individual agency protocols the day of the sampling. Field measurements were made approximately 1 foot below the water surface and within 10 feet of the water- sampling location, so as not to disturb the water column at the point of sample collection. Field measurement values reported here are considered preliminary pending agency review and approval.

Sample collection

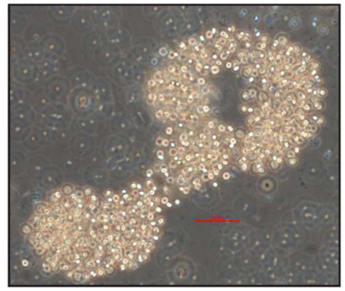
Cyanobacterial samples from lakes were collected at or near the shoreline or from a dock. Samples from rivers were collected near the banks or from a bridge at the center of the stream. All samples were collected from observed cyanobacterial blooms or areas of cyanobacterial accumulation, if present. Samples were collected by immersing and subsequently filling polyethylene bottles by hand or by using a weighted-bottle

[1]U.S. Geological Survey, Illinois Water Science Center, Urbana, Illinois.
[2]U.S. Geological Survey, Kansas Water Science Center, Lawrence, Kansas.
[3]Illinois Environmental Protection Agency, Springfield, Illinois.

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

USGS Open-File Report XXX
January 2013

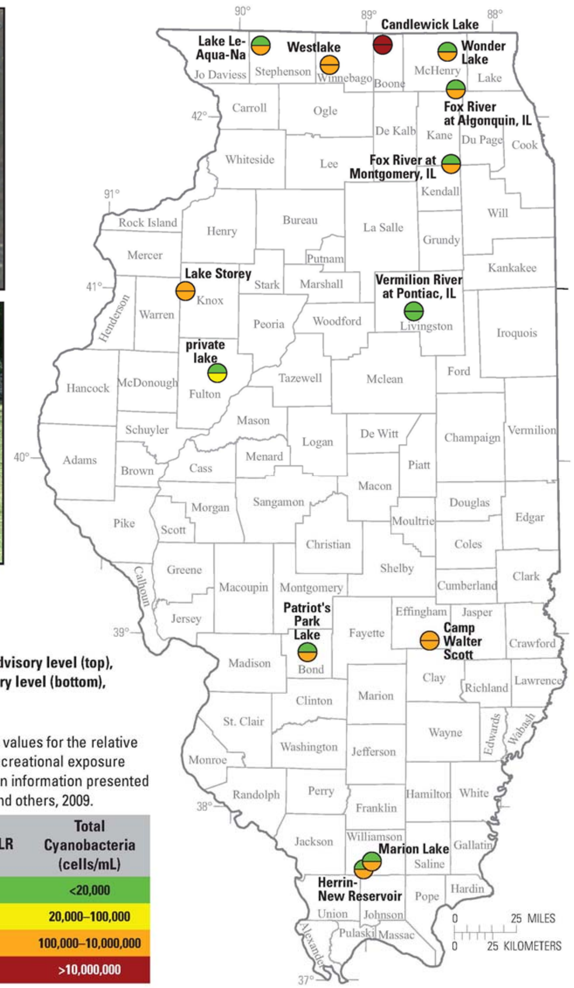
See Paul Terrio, USGS for copy of report.



Top: *Cylindrospermopsis raciborskii* (photo reproduced with permission from Greenwater Laboratories)

Middle: *Microcystis aeruginosa* (photo reproduced with permission from Greenwater Laboratories)

Bottom: Algae on Patriot's Park Lake (photo by Mike Bundren, Illinois EPA)



EXPLANATION

Marion Lake — Site with Microsystin-LR advisory level (top), Total Cyanobacteria advisory level (bottom), and name

World Health Organization (WHO) guidance values for the relative probablility of acute health effects during recreational exposure to microcystins and cyanobacteria, based on information presented in Chorus and Bartram, 1999, and Graham and others, 2009.

Relative Probability of Acute Health Effects (Advisory Level)	Microsystin-LR (ug/L)	Total Cyanobacteria (cells/mL)
Low	<10	<20,000
Moderate	10–20	20,000–100,000
High	20–2,000	100,000–10,000,000
Very High	>2,000	>10,000,000

Figure 1. Cyanobacteria sampling sites in Illinois showing relative probability of acute health effects from microcystins and cyanobacterial cells based on maximum levels found in samples collected in 2012.

Big Picture on HABs

- Cyanotoxins have been known to be lethal to humans and animals under certain exposure scenarios and concentrations.
- Federal and state agencies and tribes are appreciative of our efforts on cyanotoxins and harmful algal blooms because there has been a lack of capability and data on the presence and persistence of cyanotoxins in the United States to adequately prevent exposure of humans and ecosystems.
- Algal toxin mixtures are commonly found in the United States and at concentrations of concern for human and ecosystem health. Taste and odor compounds can be associated frequently with toxins.

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Significant progress on Harmful Algal Blooms is needed still!

- Why , when, where, and how do HABs produce toxins?
- How do human behaviors enhance/detract from HAB occurrence and toxin production?
- Predictive capabilities and early warning systems are needed.
- Drinking water assessment
- Global food supply assessment
- Ecosystem impacts need to be evaluated.
- Current risk assessments for microcystins only….. Need toxin mixture risk assessments.

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http://ks.water.usgs.gov/studies/qw/cyanobacteria/

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