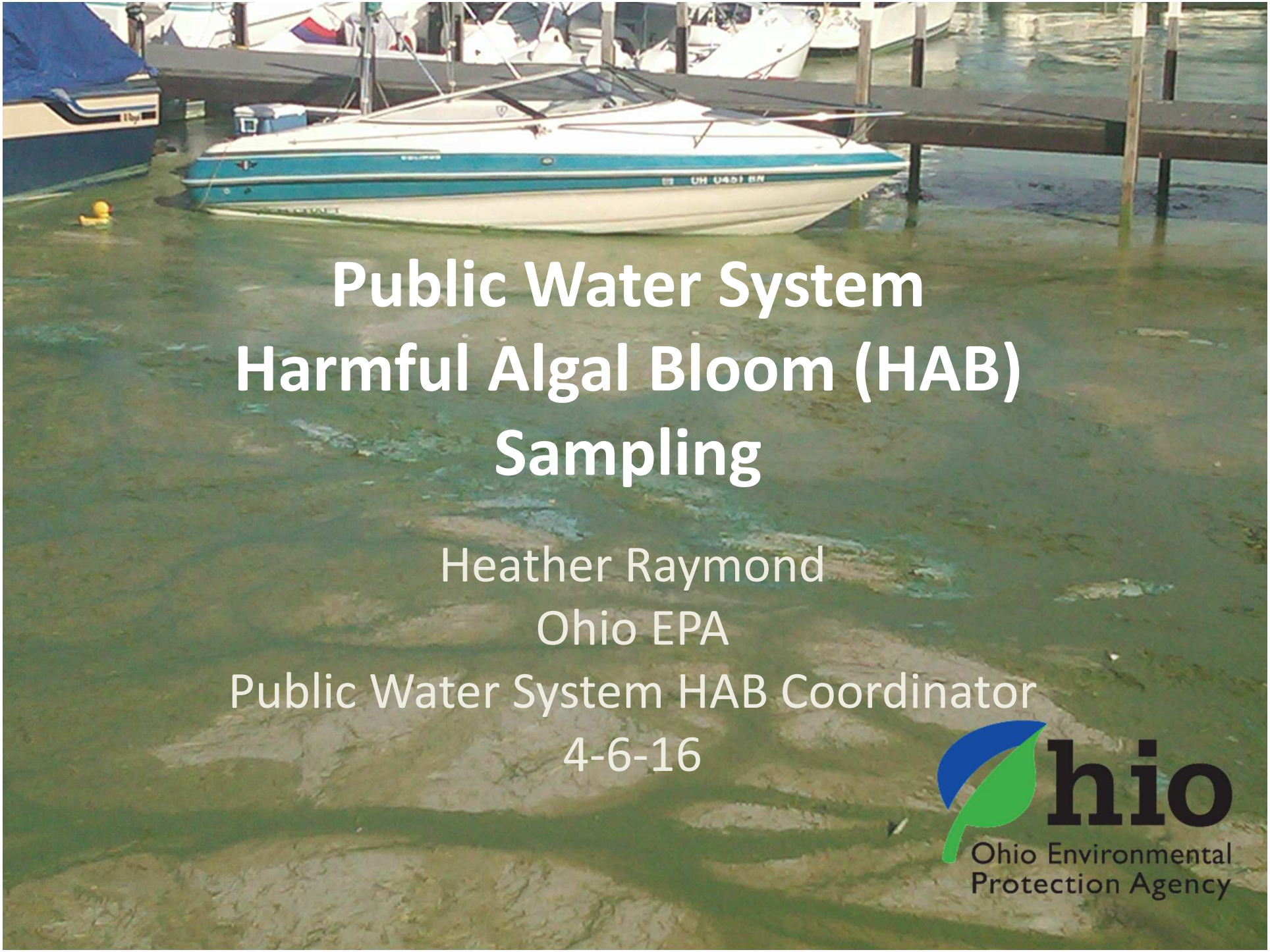

Public Water System Harmful Algal Bloom (HAB) Sampling

Heather Raymond
Ohio EPA
Public Water System HAB Coordinator
4-6-16

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Cyanotoxin Sampling at Ohio Public Water Systems (PWSs)

- **Cyanotoxins are Not Regulated under the Safe Drinking Water Act** - No Federal Monitoring Requirements
- **Ohio EPA began Sampling for Cyanotoxins at PWSs in 2010**
- **Ohio EPA worked with the Ohio Departments of Natural Resources and Health to create a State of Ohio HAB Response Strategy in early 2011**
  - Standardized definitions, sample collection procedures, cyanotoxin thresholds, and public notice language
- **In 2012 Created Separate PWS HAB Response Strategy (Updated Annually)** http://epa.ohio.gov/ddagw/HAB.aspx
- **In 2015 Ohio Senate Bill 1 Passed,** Directs Ohio EPA to Protect Against Cyanobacteria in the Western Basin of Lake Erie and in Public Water Supplies
- **In 2016, proposed HAB rules** that would require mandatory microcystins monitoring, cyanobacteria screening, and results reporting by all surface water public water systems.
  - Anticipated effective date: **June 1, 2016**

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HAB Rules - Overview

Proposed Rules: epa.ohio.gov/ddagw/rules.aspx

- PWS requirements - new rules in OAC Chapter 3745-90
  - Microcystins action levels in drinking water
  - Monitoring requirements
  - Treatment technique requirements
  - Public notification and Consumer Confidence Report (CCR) requirements
  - Recordkeeping requirements

- Laboratory Certification requirements – New OAC rule 3745-90-04 and amended rules in Chapter 3745-89
  - Laboratory certification
  - Analytical techniques
  - Reporting deadlines

http://epa.ohio.gov/Portals/28/documents/labcert/TotalMicrocystins.pdf

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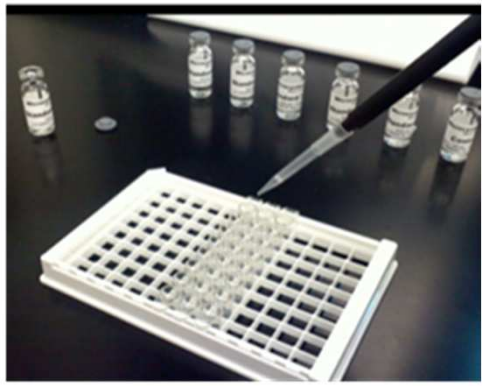
Monitoring Requirements

- Apply primarily to surface water systems (122)
- Routine monitoring for <u>microcystins</u>

  May – October:
  - Weekly raw and finished water monitoring
  - Raw water detections >5 ug/L and any finished water detections trigger additional sampling.

  November – April:
  - Raw water only every other week
  - Detections trigger additional monitoring
- Routine raw water genomic cyanobacteria screening (every other week)
  - Information will be used to determine if monitoring for cyanotoxins other than microcystins needs to be conducted by Ohio EPA (or voluntarily by the PWS)
- Option for a decreased monitoring schedule



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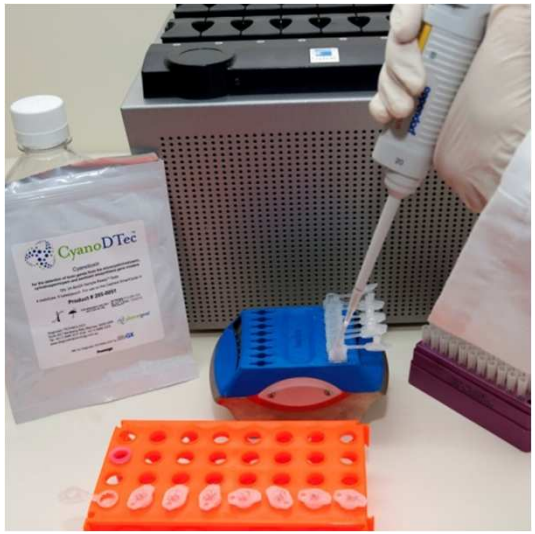
Cyanobacteria Screening: Multiplex qPCR

- Cyanobacteria screening
  - Quantitative polymerase chain reaction (qPCR) – identifies and quantifies the presence of genes unique to:
    - Cyanobacteria (16S rDNA, good correlation with cell counts)
    - Microcystin and Nodularin production (mcyE gene)
    - Cylindrospermopsin production (cyrA gene)
    - Saxitoxin production (sxtA gene)
  - Test completed within 2-3 hours (includes extraction)
  - Scalable
  - Cost-effective
  - Utilizes certified reference material
  - Specific: no gene, no toxin
- Method and certification beginning in 2017
- Until there is sufficient capacity at certified laboratories to perform this method, Ohio EPA's lab will conduct these analyses
- www.phytoxigene.com/products/



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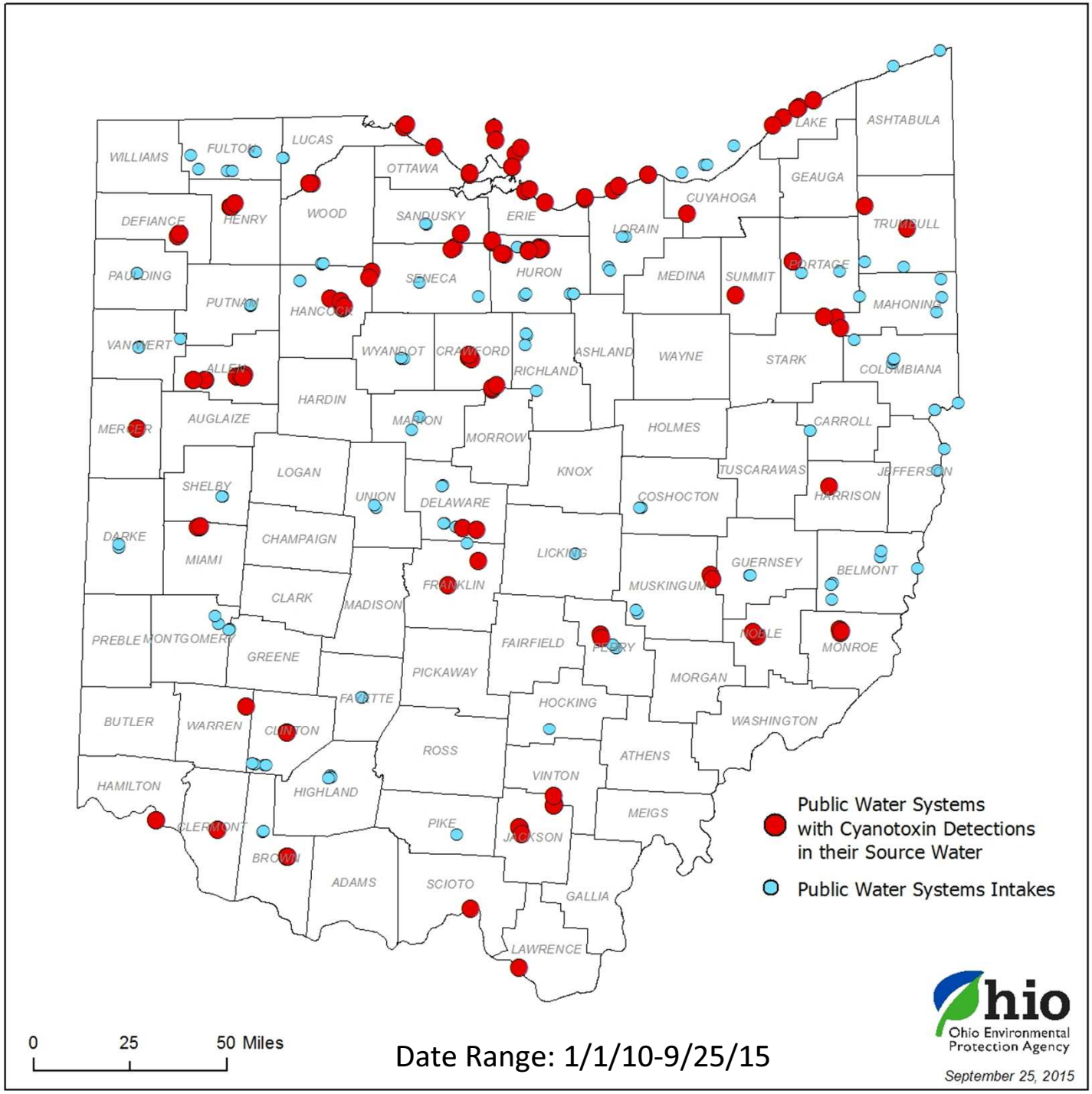

Public Water Systems with Cyanotoxin Detections in their Source Water (54 Total)

Sampled 72 SW PWSs (59%)

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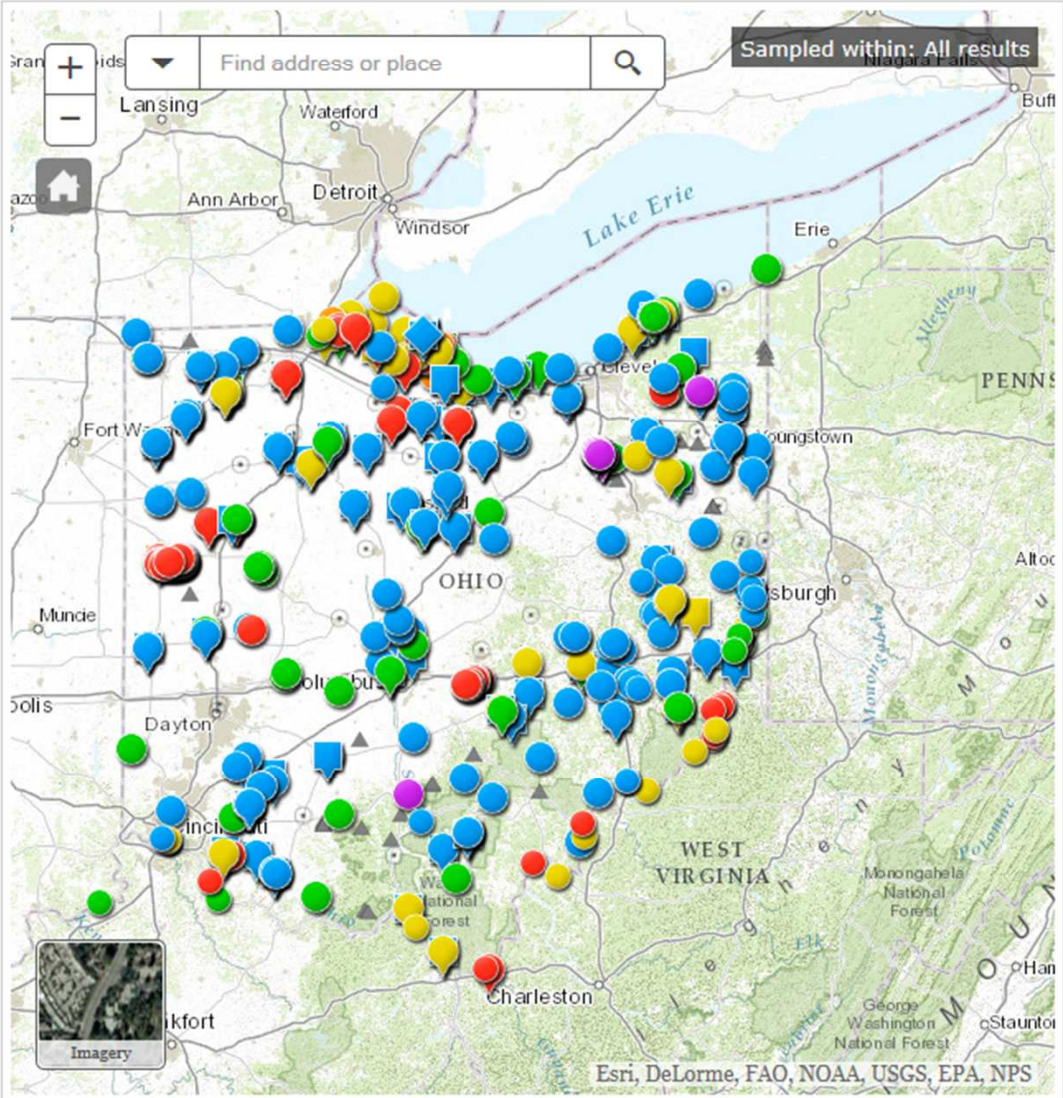
Ohio.gov State Agencies | Online Services

http://wwwapp.epa.ohio.gov/gis/mapportal/HAB_Monitoring.html

Harmful Algal Blooms: Cyanotoxin Monitoring in Ohio



Legend | Toxin Results | About this site

Filter the sampling sites by time period or type.

Sites sampled within:
○ 7 days ○ 14 days ○ 30 days ○ 90 days ○ 1 year ◉ All

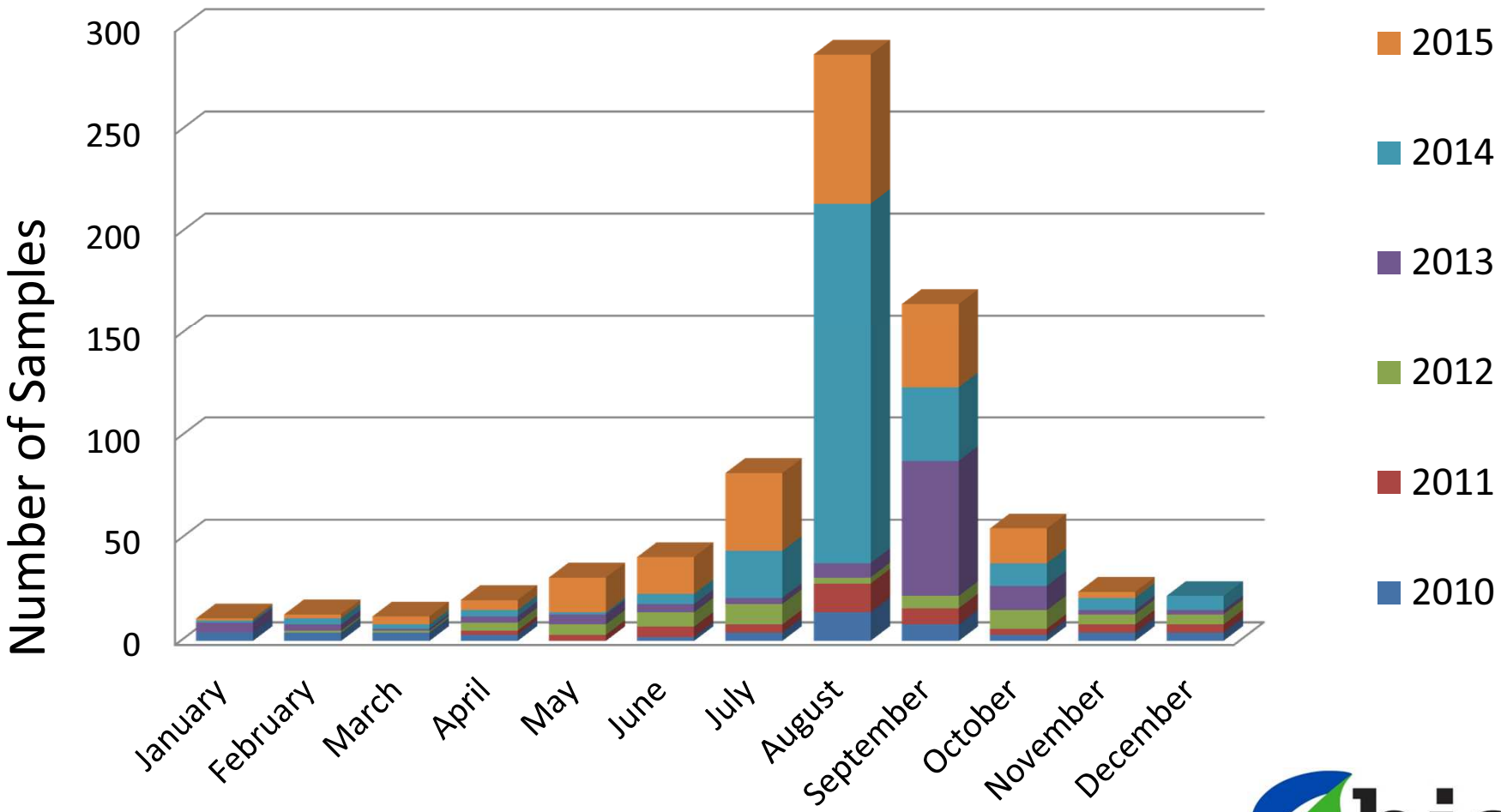
Type of Sampling Sites:
○ Lakes ○ Rivers ○ Groundwater ○ PWS Reservoirs/Intakes
○ Finished Drinking Water ○ ODNR Beaches ◉ All sites

Download all results (excel file).

Microcystins Concentration	Site Type
Below detection (<0.30 µg/l)	Public Water System (PWS) Finished Water
Between 0.30 - 1.6 µg/l	Reservoirs/Intakes (PWS)
Between 1.6 - 6 µg/l	Lakes & Rivers
Between 6 - 20 µg/l	Groundwater
Greater than 20 µg/l	PWS (no data)
Saxitoxin, Cylindrospermopsin, Anatoxin-a	ODNR State Park Beach (no data) Non-ODNR Beach (no data)

Markers on the map show sites sampled for microcystins during the selected time period. Marker color indicates that one or more results for that time period were within the range indicated by the marker color. Marker shape indicates the type of sampling site.
Concentration Units µg/l - micrograms per liter

Frequency of Ohio PWS Source Water Microcystins Detections > 1.6 ug/L



743 samples >1.6 ug/L microcystins, out of 3581 total samples (21 %).
44% of samples were > 0.30 ug/L microcystins.

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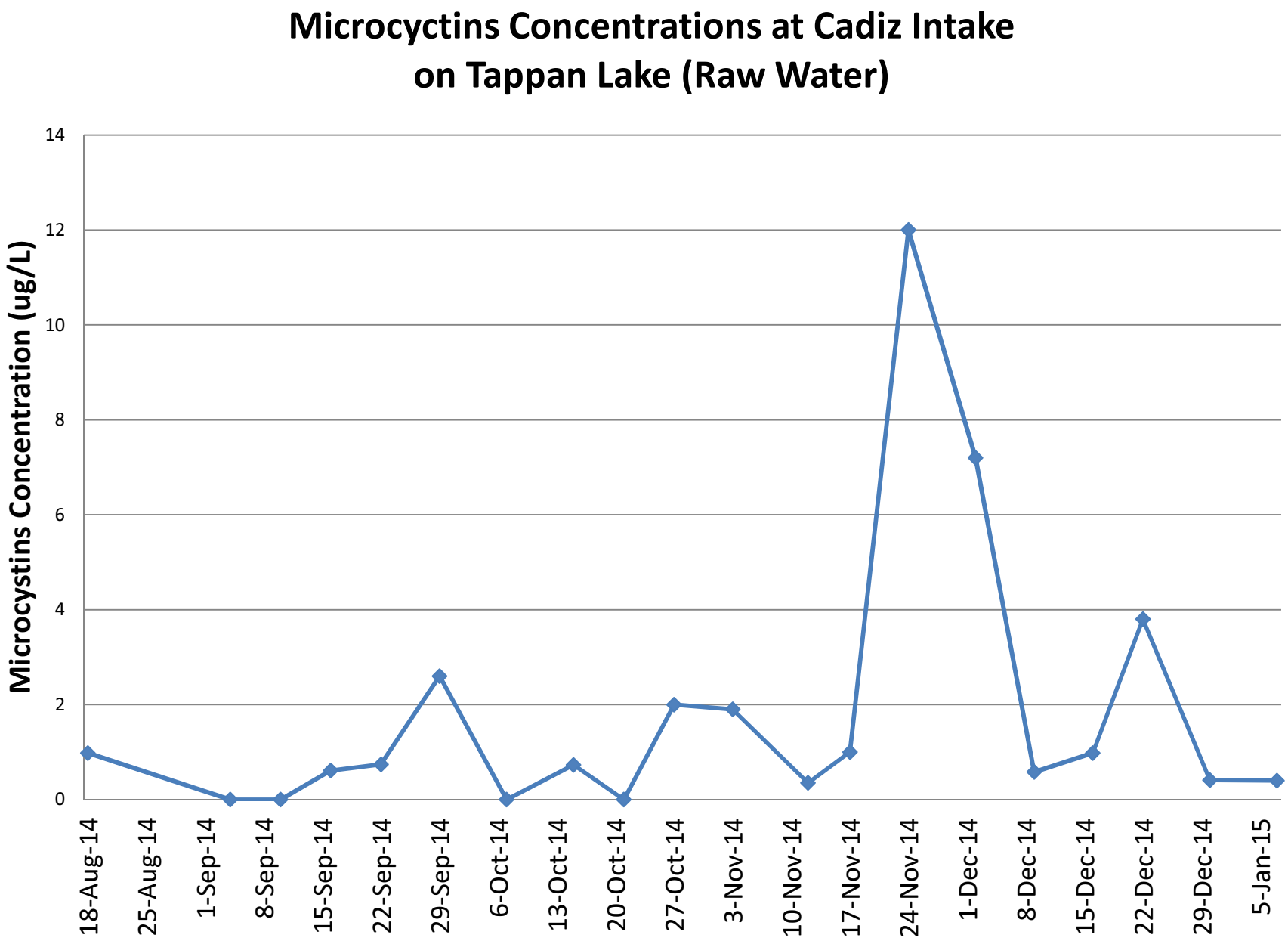
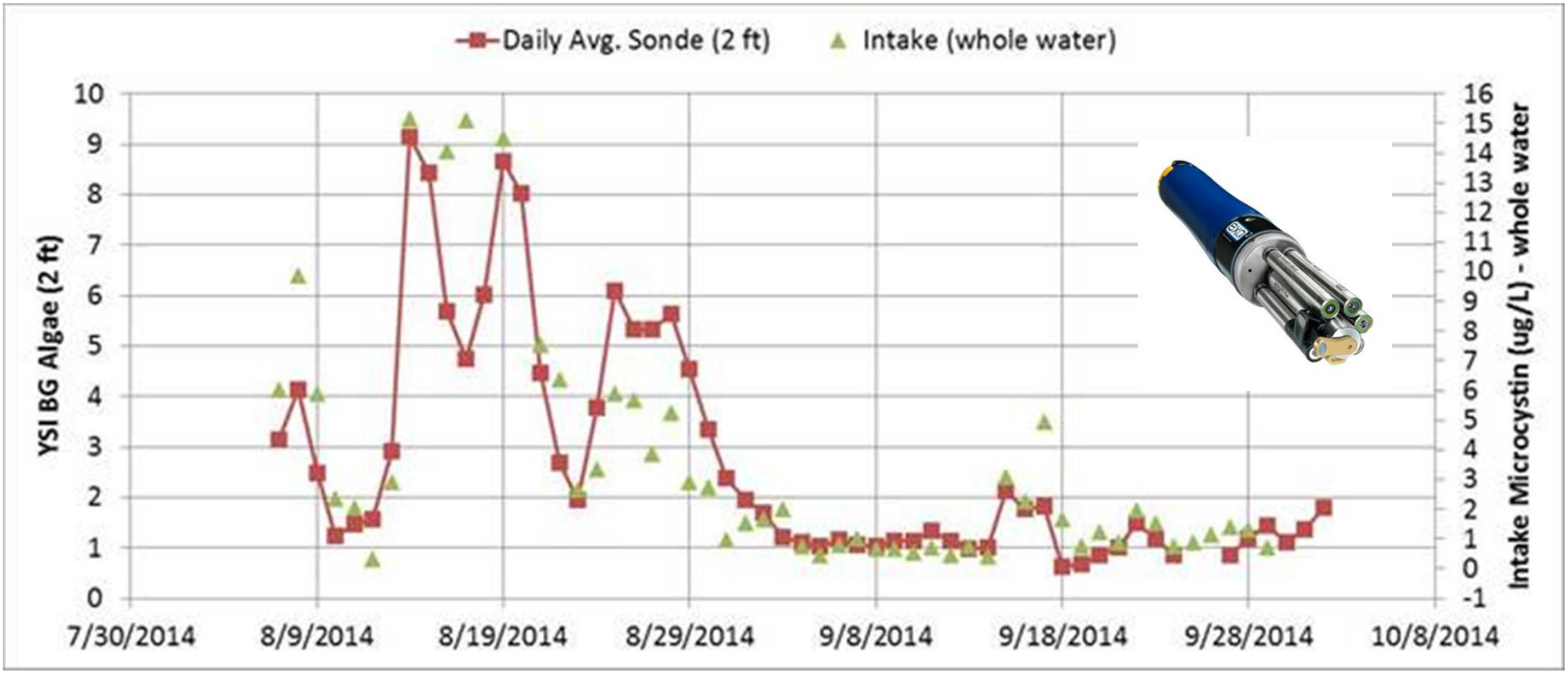

Microcyctins Concentrations at Cadiz Intake on Tappan Lake (Raw Water)
Microcystins Concentration (ug/L)
14
12
10
8
6
4
2
0
18-Aug-14
25-Aug-14
1-Sep-14
8-Sep-14
15-Sep-14
22-Sep-14
29-Sep-14
6-Oct-14
13-Oct-14
20-Oct-14
27-Oct-14
3-Nov-14
10-Nov-14
17-Nov-14
24-Nov-14
1-Dec-14
8-Dec-14
15-Dec-14
22-Dec-14
29-Dec-14
5-Jan-15

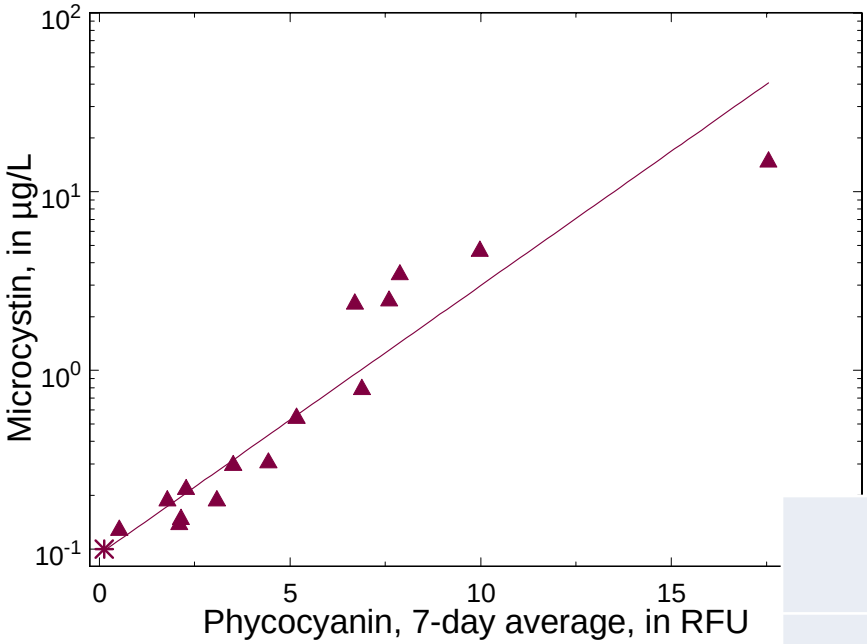
Ohio EPA Monitoring and Equipment Grants To Public Water Systems

- $1 million in grants, up to $30,000/water system
- Eligible items included water quality sensors, microscopes, ELISA-related analysis equipment and training



-Graph provided to Ohio EPA by Ed Verhamme, Limnotech.

Harsha Main 2014—
U.S. EPA Continuous Monitor



Spearman's correlation to microcystin concentrations	rho	p
Phycocyanin, 7-day average	0.98	<0.0001
Dissolved oxygen, 14-day average	0.88	<0.0001
pH, 7-day average	0.83	<0.0001
Temperature, instantaneous 10 a.m.	0.73	0.0031
Chlorophyll, 24-hour average	0.53	0.0358
Specific conductance, 3-day average	-0.20	0.4473

Data Courtesy:
Donna Francy, USGS

Phycocyanin Data Interpretation

- Phycocyanin concentrations vary based on type of cyanobacteria present, turbidity of the water and other factors.
- Relative/Raw Fluorescence Units (RFUs) better than Cell Counts.
  - Can calibrate to cell counts in source water, but this can change if cyanobacteria genera shift or turbidity changes.
- Evaluate trends, not absolute values.

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Using qPCR to Direct Reservoir Management

- Saxitoxins Detections in Finished Water from July 31, 2015 – September 21, 2015. Maximum concentration 0.039 ug/L. Maximum raw water concentration at intake 0.812 ug/L
- Extracellular saxitoxins predominated all samples.
- 10 different potential saxitoxin producing genera found in multiple habitat zones (pelagic, benthic, periphyton, etc.) in multiple locations.
- qPCR results indicated benthic source, data used to target algaecide application.

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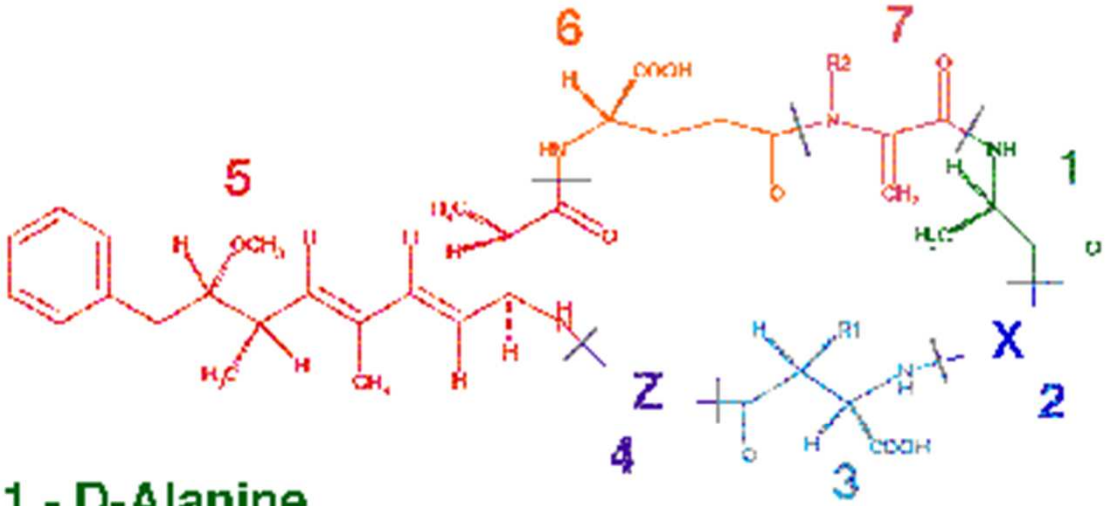
Microcystins Testing

No "Perfect" Analytical Method for Detecting Total Microcystins



1 - D-Alanine
2 - Variable L-amino acid
3 - D-Methylaspartic acid
4 - Variable L-amino acid
5 - 3-amino-9-methoxy-2,6,8-trimethyl -10-phenyldeca-4,6-dienoic acid (Adda)
6 - D-Glutamic acid
7 - N-Methyldehydroalanine

Over 140 Microcystin Variants
Standards Not Available for Majority

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Analytical Method Comparison & Microcystin Variant Evaluation

- 11 Sites: 4 Up-ground Reservoirs, 2 In-stream Reservoirs, 2 Lake Erie locations, 2 Canal-feeder Lakes, and 1 River Source.
- 22 Samples from 2014 Selected to Help Evaluate Spatial and Temporal Variability Within Source Waters
- Variety of Cyanobacteria Genera Represented
- Each Sample Analyzed Using 5 Separate Analytical Methods

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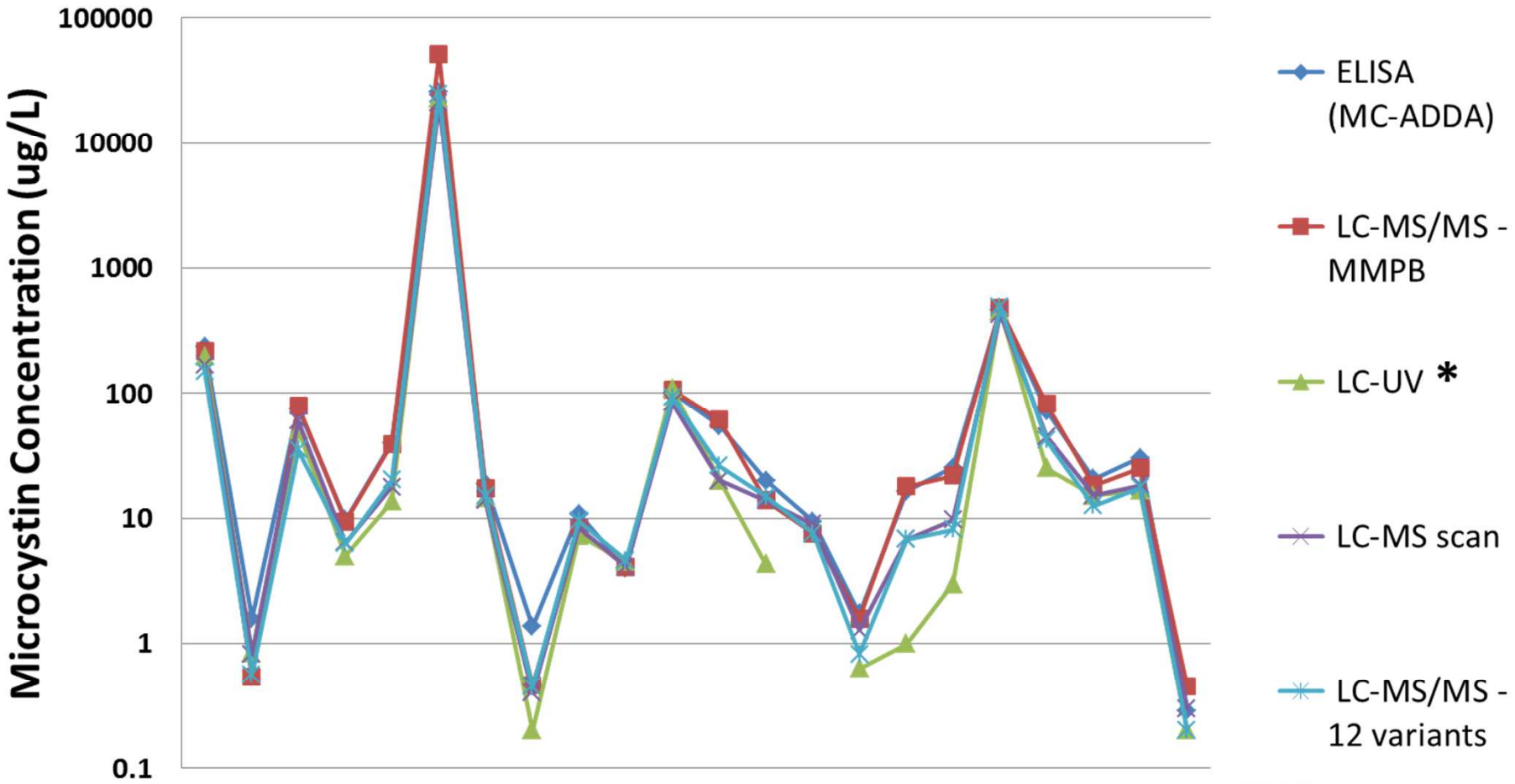
Results of Method Comparison



* LC-UV data presented does not include false-positives that were eliminated from total (Based on lack of confirmation with LC-MS methods).
Sample # 14 was non-detect using LC-UV.

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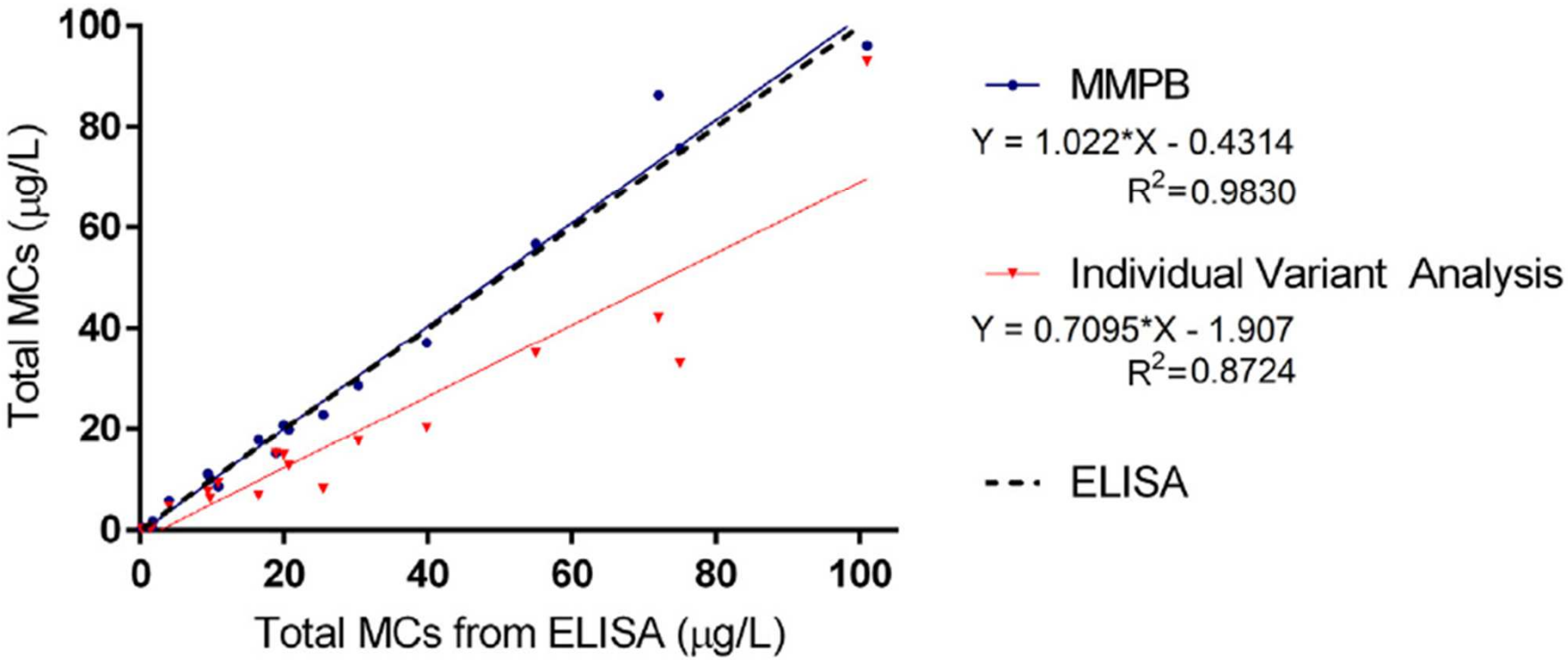
Results of LC-MS/MS MMPB and Individual Variant Analysis Compared to ELISA



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Spatial and Temporal Variability in Microcystin Variants

Lake Erie Microcystin Variants					
MC-Variant	Site 1 8/25/14	Site 2 8/4/14	Site 2 8/18/14	Site 2 9/29/14	Site 2 10/14/14
MC-RR	2.1	20	10	5.5	8.5
MC-YR	0.6	6	5	1.2	2.5
MC-LR	2.9	16	10	5.5	6.1
MC-WR	0.6		3-9	0.2-0.6	0.2-0.6
MC-LY			2-6		
8.7 min 1043.5 m/z			10-30	3.6	

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Inland Lake Microcystin Variants (Planktothrix)			
MC-Variant	Site 1 6/16/14	Site 2 6/16/14	Site 2 9/2/14
[DAsp3] MC-RR	5.3	6.1	17.5
[Dha7] MC-LR	1.1	1.4	1.5
MC-YR	0.2-0.6	0.2-0.6	1.2
MC-RR		0.1-0.3	

Inland Lake Microcystin Variants (Mixed Bloom)				
MC-Variant	Site 1 6/18/14	Site 2 6/18/14	Site 2 7/9/14	Site 3 6/30/14
[Dha7] MC-RR	2.9	3-9	1.0	0.08
MC-RR	1.4	39	1.0	0.01-0.03
MC-YR	1.1	15	1.0	
MC-LR	4.0	67	2.4	0.55
[DAsp3] MC-LR	0.6	18	0.4	0.03
[Dha7] MC-LR	3.6		1.0	0.05
MC-WR	0.2-0.6		0.2-0.6	
MC-LA	0.2-0.6			
MC-LY	0.2-0.6	6	0.2-0.6	0.10

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Ohio Microcystin Variant Data

MC-Variant	USEPA 544
MC-YR	Yes
[Dha7] MC-LR	No
[DAsp3] MC-RR	No
MC-LR	Yes
MC-RR	Yes
MC-LY	Yes
MC-WR	No
[DAsp3] MC-LR	No
MC-HilR	No
MC-LA	Yes
[Dha7] MC-RR	No
MC-FR	No
[DAsp3] MC-FR	No
6.9 min 1049.5 m/z	No
7.5 min 1029.5 m/z	No
8.7 min 1043.5 m/z	No
MC-LF	Yes

Key Findings

- LC-based Methods Confirmed ELISA Results
- 16 Different MC-variants were detected
- MC-LR was only detected at 5 of 11 sites (45%)
- Most common variants were: MC-YR, [Dha7] MC-LR and [DAsp3] MC-RR
- HPLC-PDA Methods Prone to Interference
- Generally, the Dominant/Co-Dominant Variant Did Not Vary Spatially nor Temporally
- Secondary and Minor Variants Present Did Vary Spatially and Temporally
- 91% of sites had MC-variants not detectable by USEPA Method 544

Foss and Aubel, *Using the MMPB technique to confirm microcystins concentrations in water measured by ELISA and HPLV (UV, MS, MS/MS),* Toxicon 104 (2015) 91-101

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Ongoing HAB Method Studies

- Microcystins 5 Method Comparison on 2016 Samples
- ELISA Matrix Interference Study with Abraxis
- ELISA Matrix Interference Study with USEPA (2016)
- qPCR Method Validation (Spring 2016)
- qPCR Method Refinement with USEPA (2016-2017)

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PWS HAB Response Strategy

Analytical Methods

	Microcystins (µg/L)	Cylindro-spermopsin (µg/L)	Saxitoxins (µg/L)	Anatoxin-a (µg/L)
Surveillance sampling	ELISA (MC-ADDA)	ELISA	ELISA	LC-MS/MS
Repeat sampling in response to a finished water detection	ELISA (MC-ADDA)	LC-MS/MS	LC-MS/MS	LC-MS/MS

ELISA: Enzyme-Linked Immunosorbent Assay

LC-MS/MS: Liquid Chromatography followed by tandem Mass Spectrometry

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Questions?

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