U.S. Environmental Projection Agency, Office of Research and Development

SAFE AND SUSTAINABLE WATER RESOURCES RESEARCH PROGRAM

Analysis and Monitoring of Algal Toxins in Fresh and Coastal/Estuarine Environments

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US EPA Cincinnati OH

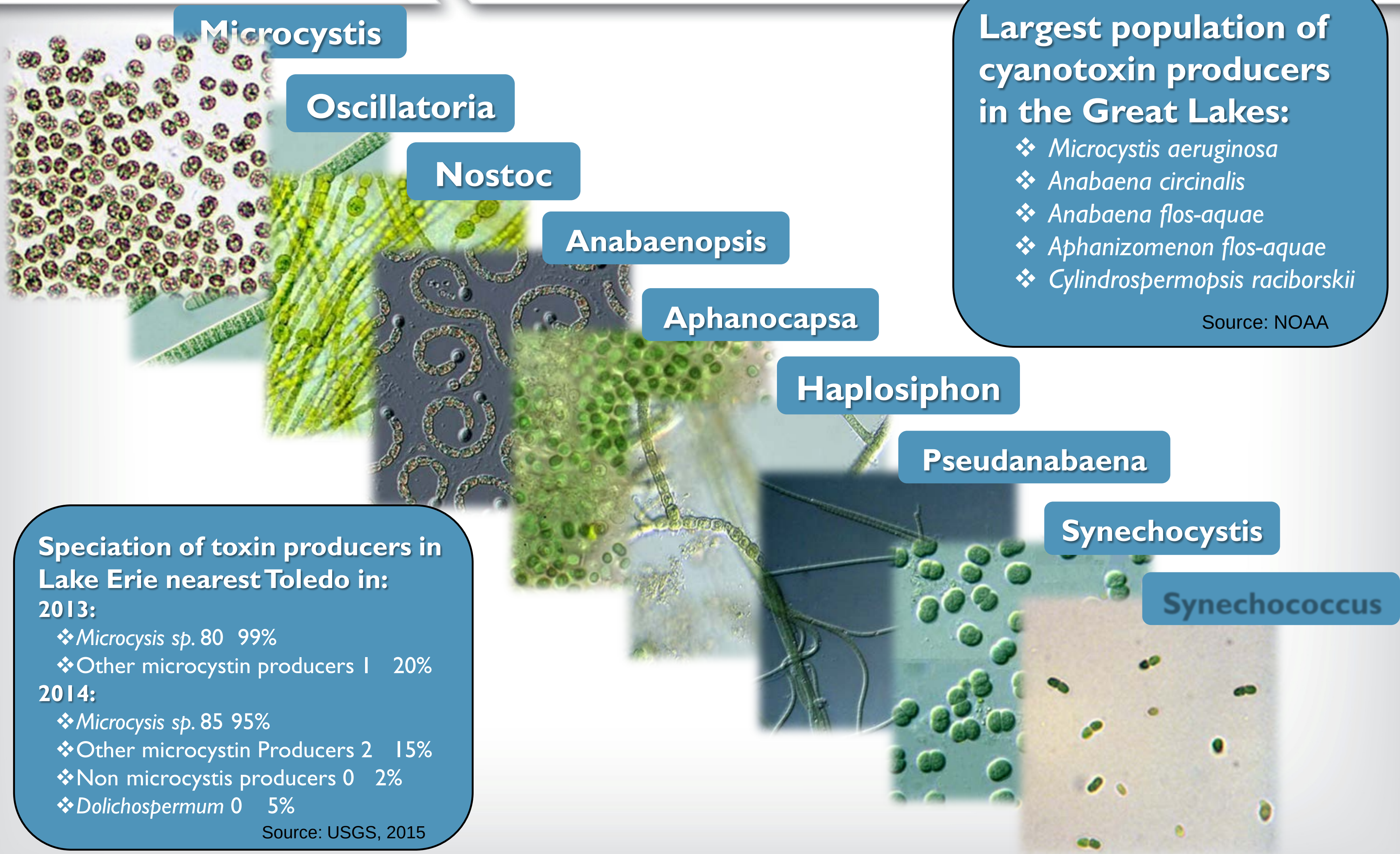
14th Annual EPA Drinking Water Workshop

August 22, 2017

Harmful Algal Blooms (HABs)

- HABs result in approximately $2.2B of associated costs annually in the US, from restricted use of recreational waters, declining waterfront real estate value, spending on recovery of biodiversity, and drinking water treatment.
- Wide variety of taxa can produce blooms
- Typically detrimental to the aquatic system and can be harmful to humans and land animals (contact and consumption)
- Blooms are dependent on numerous factors, including nutrient loading, temperature, water flow and weather patterns

Microcystin and other Cyanotoxin Toxin Producers



Largest population of cyanotoxin producers in the Great Lakes:

- ❖ *Microcystis aeruginosa*
- ❖ *Anabaena circinalis*
- ❖ *Anabaena flos-aquae*
- ❖ *Aphanizomenon flos-aquae*
- ❖ *Cylindrospermopsis raciborskii*

Source: NOAA

Speciation of toxin producers in Lake Erie nearest Toledo in:

2013:

- ❖ *Microcysis sp.* 80 99%
- ❖ Other microcystin producers 1 20%

2014:

- ❖ *Microcysis sp.* 85 95%
- ❖ Other microcystin Producers 2 15%
- ❖ Non microcystis producers 0 2%
- ❖ *Dolichospermum* 0 5%

Source: USGS, 2015

Source Water Impacts on Drinking Water

Problems facing drinking water treatment:

- Excessive nitrogen and phosphorous levels can cause harmful algal blooms
  - Agriculture (non-point source) is often the largest contributor of nitrogen load into waterways
- Forecasting is difficult because algal/cyanobacteria strains bloom under different conditions at different times
- Additionally, an algal bloom may not necessarily produce toxins
  - Treatment is still impacted due to biofouling, taste and odor concerns, increasing disinfection by-product potential, etc.

Algal blooms put pressure on drinking water facilities, requiring:

- Immediate operational changes (i.e. PAC addition) can be costly, with varying effectiveness
- Possible shut-off of services, public relations challenges
- Costly facility upgrades

Current Regulations/Guidance

21 states have recreational water guidelines for harmful algae blooms

Three states (MN, OH, and OR) have implemented standards or guidelines that apply to cyanotoxins in drinking water

EPA's Office of Water has released its Health Advisory Level for microcystin-LR and cylindrospermopsin

EPA's informational webpage
http://www2.epa.gov/nutrient policy data/cyanobacterial harmful algal blooms cyanohabs

Current Regulations/Guidance

- State Health Advisory Levels (MYCs)
  - OH
    - "Do not Drink" Advisories
      - 0.3 µg/L Tot MYC Child < 6 yr & Sensitive Pop
      - 1.6 ug/L Tot MYC Child > 6 yr & Adults
    - "Do Not Use" Advisory
      - 20 ug/L
  - MN 0.04 µg/L as MYC LR
  - OR 1.0 µg/L as MYC LR

- USEPA Health Advisory Level for microcystin-LR and Cylindrospermopsin

  "Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins"

  "Drinking Water Health Advisory for the Cyanobacterial Toxin Cylindrospermopsin "
  - 0.3 µg/L MYC (0.7 µg/L CYL) 10 Day Infants/Young Children
  - 1.6 µg/L MYC (3.0 µg/L CYL) 10 Day Adults

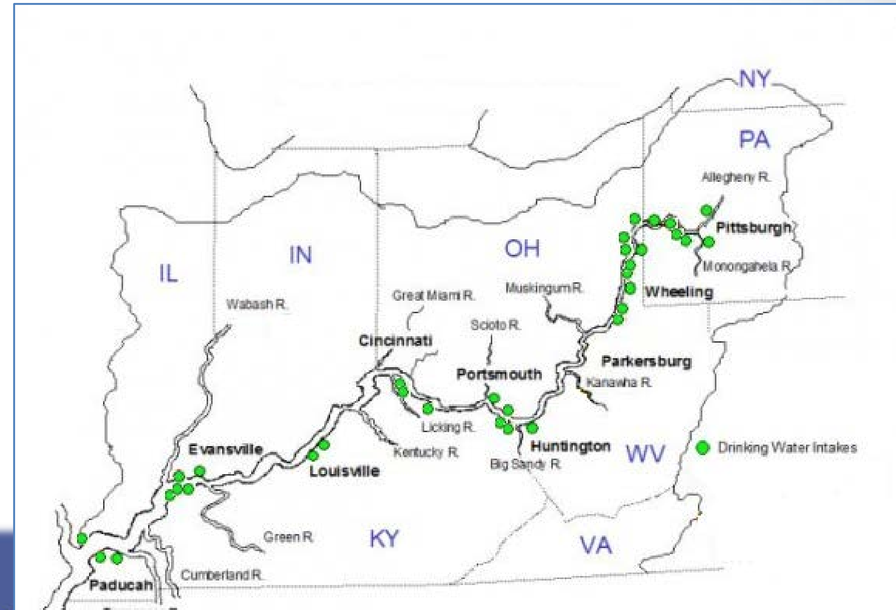
- The Harmful Algal Bloom and Hypoxia Research and Control Amendments Act of 2014
  - Delegates primary responsibility to NOAA in advancing the scientific understanding and ability to detect, monitor, assess, and predict HAB and hypoxia events in marine and freshwater

- Safe Drinking Water Act
  - Contaminant Candidate List 4 (draft) includes cyanotoxins
    - Microcystin LR, Anatoxin a, Cylindrospermopsin
  - Unregulated Contaminant Monitoring Rule (2018 2020)
    - US EPA Method 544

Lake Erie and the Ohio River are Major Drinking Water Sources

29 Ohio and 2 Michigan communities intake water directly from Lake Erie (Western and Central Basins)

Subsequent communities purchase either treated or untreated water from these primary DWTPs and may subject the water to further treatment



Source: ORSANCO

The Ohio River is a major source of drinking water along its entire reach

Ohio In 2013 and 2014

- **Celina (population 10,400)**

  Summer 2013: > 100 µg/L total microcystins and nodularin in treatment plant influent

- **Carroll Township (population 2,000)**

  September 4, 2013 = 1.4 ug/L

  September 5, 2013 = 3.6 ug/L

  Switched to emergency connection with Ottawa County

  Began flushing distribution system

  Ohio EPA's first "Do Not Drink" advisory issued due to microcystin

  On Advisory 48 Hours

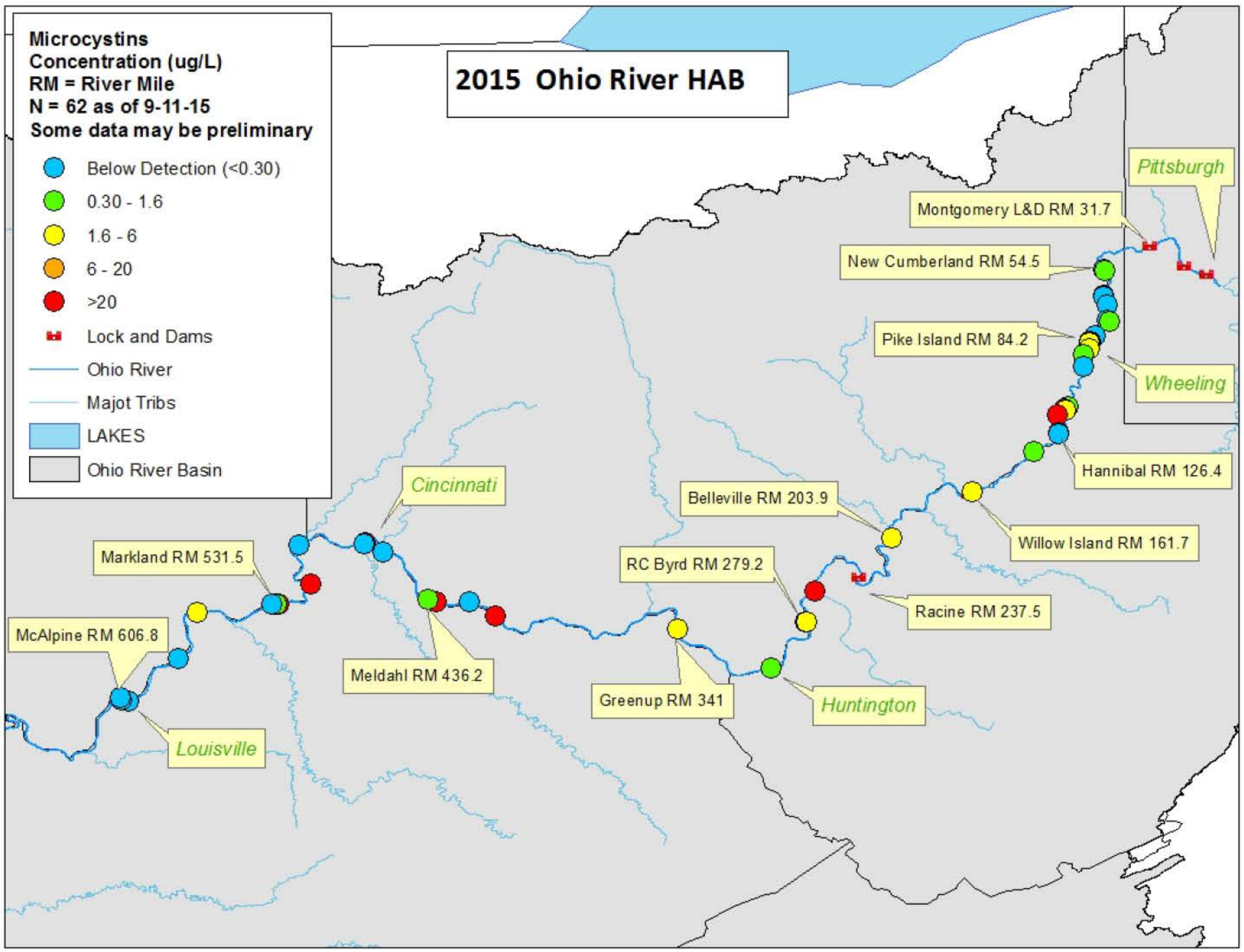
- **Toledo (population ~500,000)**

  September 2013: Detectable, but < 1 µg/L toxin in finished water

  August 2014: >1 µg/L total microcystins and nodularin in finished water,

  Ohio EPA "Do Not Drink" advisory

2015 Ohio River Bloom

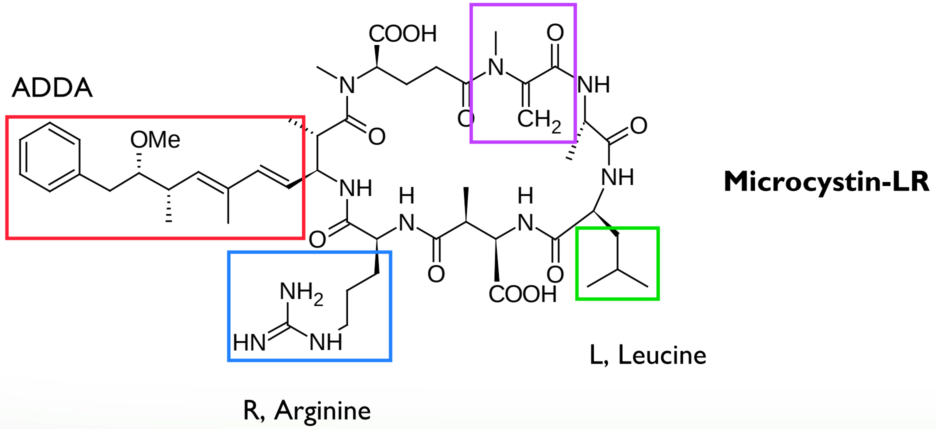


Source: Ohio River Sanitation Commission

- **Approximate Dates:**
  August 19th through October 29th, 2015
- **Main Contributor:**
  Microcystis aeruginosa
- ***$3x10^7$ cells/ml***
  $1x10^5$ typically referenced as level for water impairment
- **Numerous recreational water advisories**

Microcystin Toxin Variants

- MYCs are cyclic heptapeptides
- Varying strains produce different toxins at different rates and quantities
- Exist in multiple variants
  - 113+ known microcystin variants
  - Significant differences in hydrophobicity and pKa
  - Species can be multi-charged



All MYCs include the ADDA (3-amino-9-methoxy-2, 6, 8-trimethyl-10-phenyl-4(E), 6(E)-decadienoic acid, red) and methyldehydroalanine (MDHA, purple) modified amino acids. Leucine (green) and arginine (blue) residues are sites of structural diversity, referred to as positions X and Z, respectively.

Variants differ in potency

Estimated cytotoxic IC_{50} values

MC variants name	IC_{50} (μg/mL)
[D-Asp^3, *Z*-Dhb^7] MC-LR	0.053
[D-Asp^3, *Z*-Dhb^7] MC-HtyR	0.120
[D-Asp^3, *E*-Dhb^7] MC-LR	0.133
[D-Asp^3, Dha^7] MC-LR	0.217
[D-Asp^3] MC-LR	0.217
[Dha^7] MC-LR	0.217
[D-Asp^3, *E*-Dhb^7] MC-HtyR	0.327
[D-Asp^3] MC-HtyR	0.347
[Dha^7] MC-YR	0.418
MC-LR	0.800
MC-YR	1.48
[D-Asp^3, Dha^7] MC-RR	4.11
[D-Asp^3, *E*-Dhb^7] MC-RR	4.95
[Dha^7] MC-RR	5.33
[D-Asp^3] MC-RR	>10
MC-RR	>10

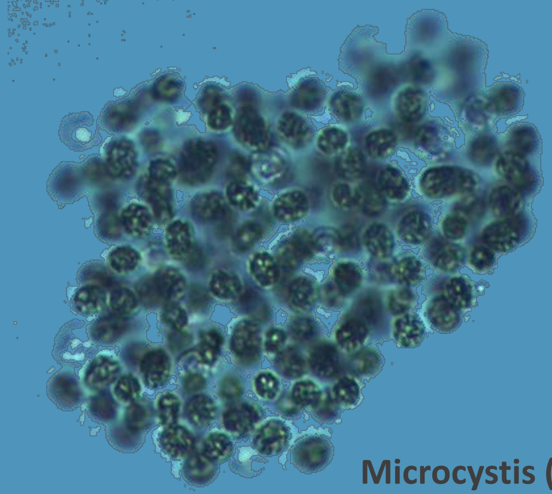
Shimizu, Kumiko, et al. *Toxins* 6.1 (2013): 168-179.

Dissolved or Particulate?

Toxin within the cell and those that are dissolved require different sample processing and treatment

Particulates (toxin in cell)

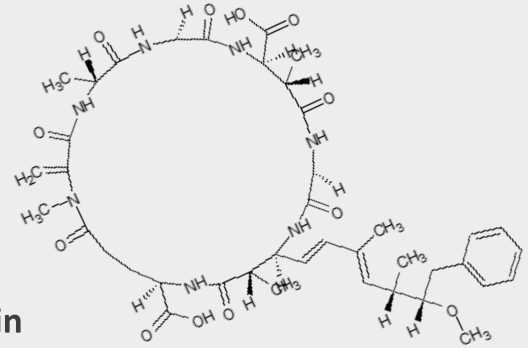
- Solids removal processes effective
- Do not want to lyse cell or toxin will be released



Microcystis (cells)

Dissolved (toxin released from cell)

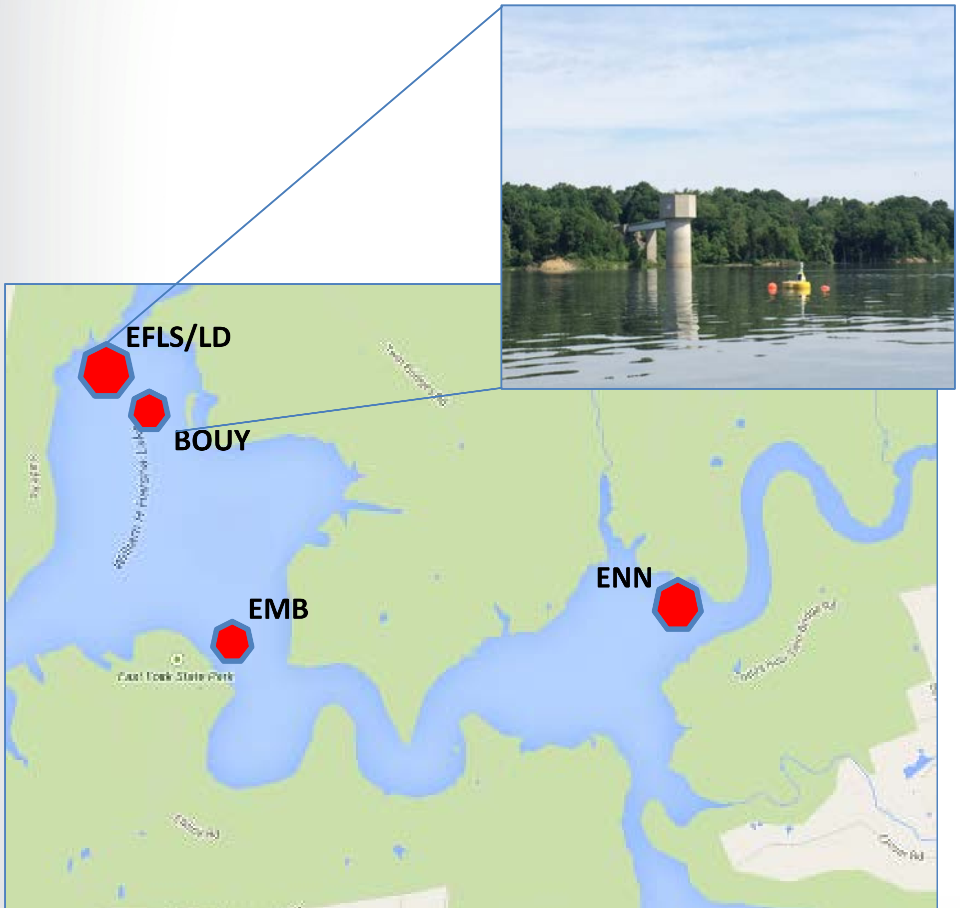
- Solids removal processes ineffective
- Typical disinfectants or dosages may not be effective (e.g., permanganate, chlorine)
- More effective treatments are expensive and plants typically do not have them in place (e.g., GAC)



Microcystin Toxin

Lake Harsha Cyanobacteria Bloom Study

Study Site Lake Harsha (East Fork State Park)



- 5 Sites
  - Inlet (ENN)
  - Beach (EMB)
  - Buoy near drinking water intake (BOUY)
  - Drinking water intake – Surface (EFLS)
  - Drinking water intake – Intake at depth (EFLS)

- Weekly sampling March-November
- 3x/week during observed bloom
- Bi-hourly sampling for 4 hours on representative days

Sample Handling and Analytical Methods

Sample Container

Preservative and dechlorination agents added
adapted from USEPA Method 544

Sample

Placed at 4ºC until Processed

Sample Filtered through 0.8 um Filter

Filtrate
Extracellular

Retentate-Solvent
Extracted

Combined
Total

Other Analysis

- Chlorophyll-a (USEPA 445.0)
- Phycocyanin
- Water Quality Parameters

  (i.e. Nutrients, Metals)

Enzyme Linked Immunosorbent Assay (ELISA)

- Broad-based method

  Total toxin concentration congeners/variants
- Abraxis Microcystins/Nodularins (ADDA), ELISA Kit, Microtiter Plate

LC/MS

- Two LC/MS/MS methods

  Triple quad and IT/HRMS (Orbitrap)
- Analytical methods includes 13 commercially-available MC congeners/variants
- Anatoxin A and Cylindrospermopsin (USEPA 545)
- MMPB Oxidation (Total MC methodology)

  (2-methyl-3-methoxy-4-phenylbutyric acid)

Microcystin Analytical Methods

- LC-IT/HRMS (Orbitrap)
  - Dionex Ultimate 3000 UPLC
    - 3400RS Analytical Pump
    - 3400SD Loading Pump
    - Equan Autosampler
  - Thermo Discovery Orbitrap
    - MS/MS product scan
    - Primary and confirmatory ions observed
    - Internal standards added to samples and standards
    - 8 point standard curve used (0.0 to 1000 ppt)
  - Internal Standards
    - $^{13}C,^{15}N_2$ 17-Amino Geldanamycin (225 ng/L)
    - Clarithromycin (56.25 ng/L)
    - $^{13}C_6$-Paclitaxel (562.5 ng/L)
    - Tacrolimus (56.25 ng/L)
    - Virustomycin A (1125 ng/L)
    - d_5-Atrazine (45 ng/L)

Mass Spectroscopy

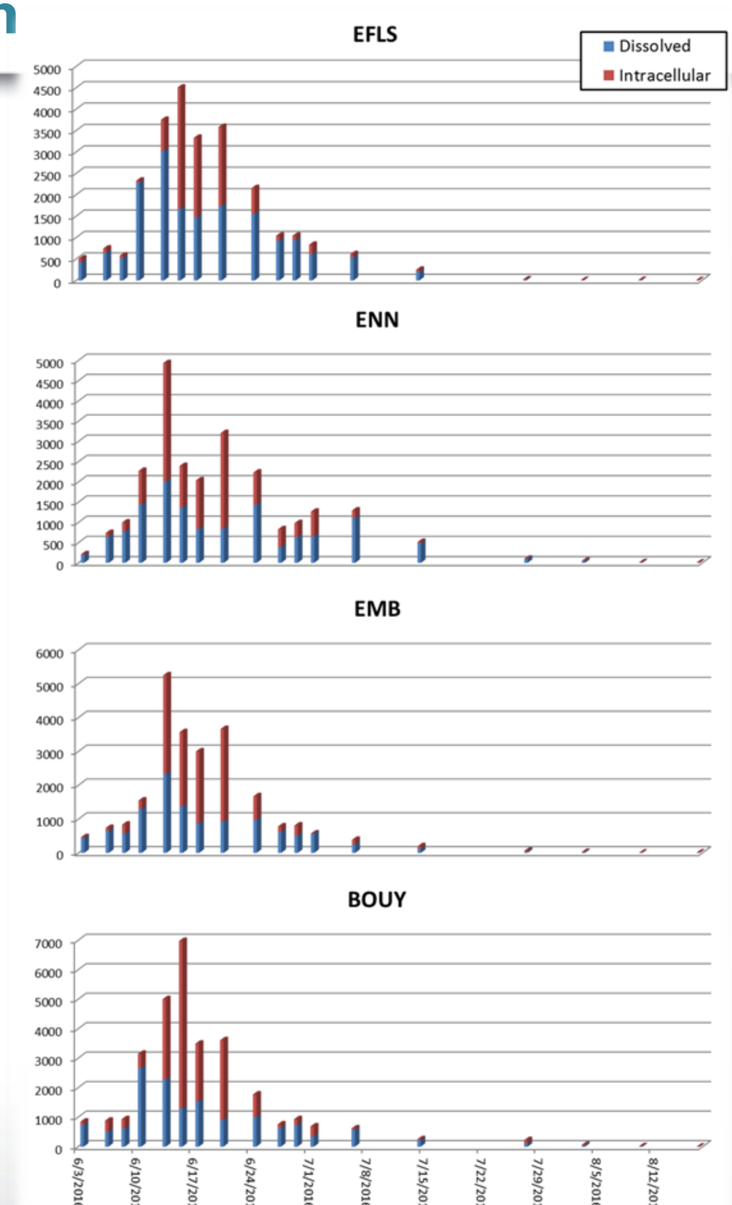
Algaltoxin	Formula	M_{mi} of Measured Species $[M+H]^+$ (Da)
Microcystin LF	$C_{52}H_{71}N_7O_{12}$	986.523
Microcystin LR	$C_{49}H_{74}N_{10}O_{12}$	995.556
Microcystin LW	$C_{54}H_{72}N_8O_{12}$	1025.532
Microcystin LY	$C_{52}H_{71}N_7O_{13}$	1002.518
Microcystin RR	$C_{49}H_{75}N_{13}O_{12}$	519.792[c]
Microcystin WR	$C_{54}H_{73}N_{11}O_{12}$	1068.543
Microcystin YR	$C_{52}H_{72}N_{10}O_{13}$	1045.535
Nodularin	$C_{41}H_{60}N_8O_{10}$	825.450
[D-Asp3-(E)-Hhb7] Microcystin-HphR	$C_{52}H_{72}N_{10}O_{12}$	1029.540
[D-Asp3-(E)-Dhb7] Microcystin-RR	$C_{48}H_{73}N_{13}O_{12}$	512.786[c]
Microcystin-N-Methyl-LR	$C_{50}H_{76}N_{10}O_{12}$	1009.572
Microcystin-HilR	$C_{50}H_{76}N_{10}O_{12}$	1009.572
Microcystin-HtyR	$C_{53}H_{74}N_{10}O_{13}$	1059.551
[D-Asp3] Microcystin-RR	$C_{48}H_{73}N_{13}O_{12}$	1024.557
[D-Asp3] Microcystin-LR	$C_{48}H_{72}N_{10}O_{12}$	981.540

2016 Study Season – Total Targeted MC Concentration

Total Concentration of Known MYC Variants

– Similar targeted MCs concentration pattern seen between EMB and ENN sites

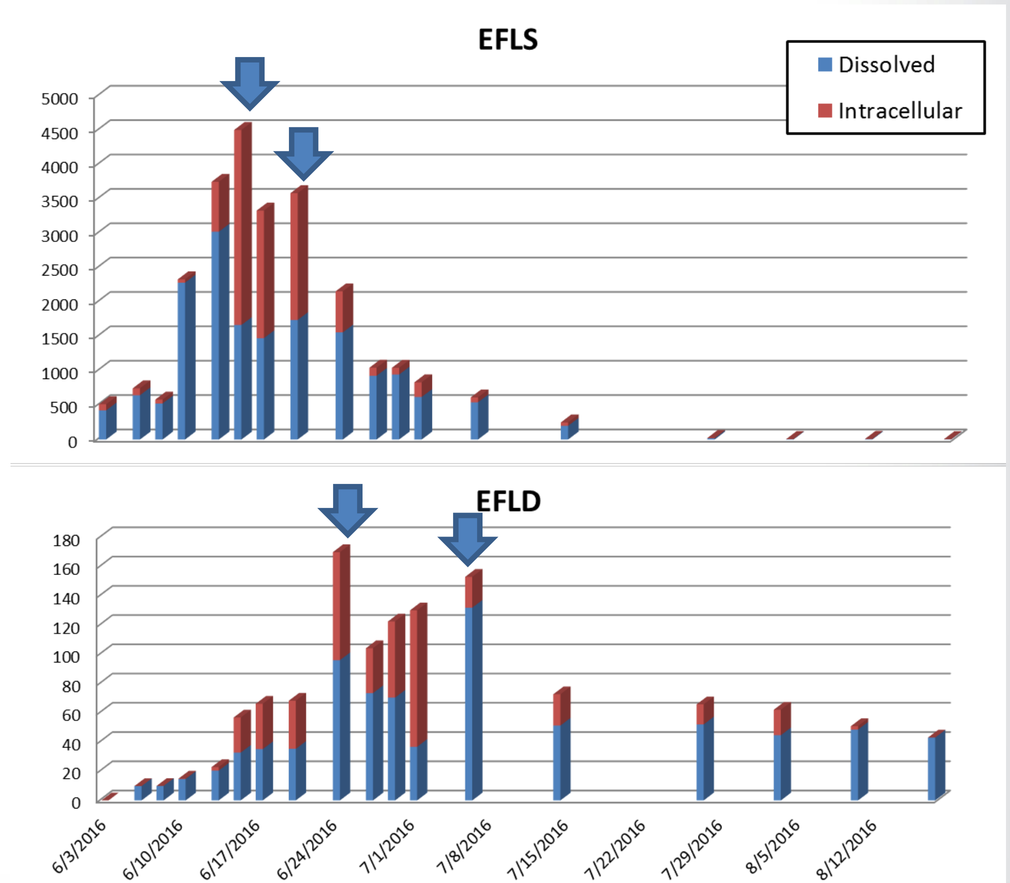
– Similar pattern appeared between EFLS and BOUY sites

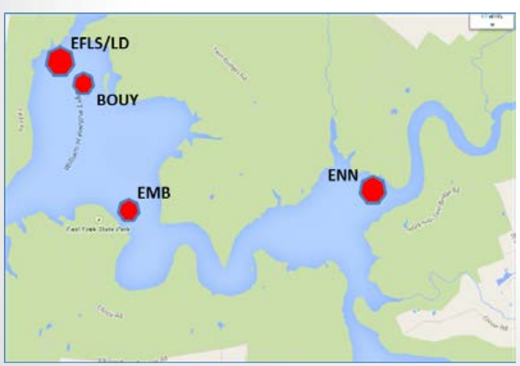


2016 Study Season – Total Targeted MC Concentration

Total Concentration of Known MYC Variants

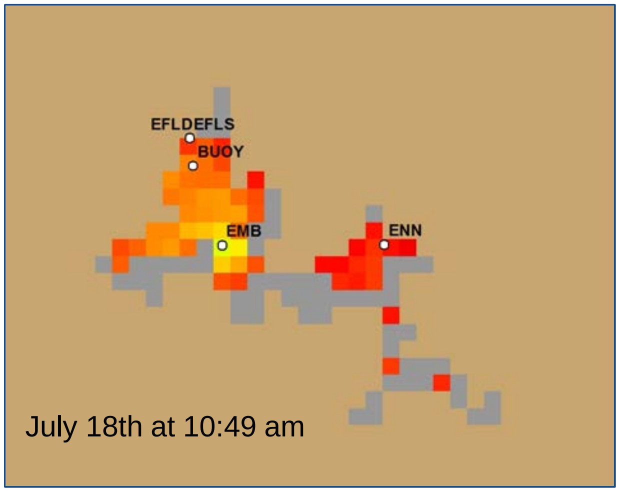
– Delayed response in peak MC concentrations between the surface (EFLS) and depth sample (EFLD)





2017 Lake Harsha Monitoring Study – Comparing Methodologies

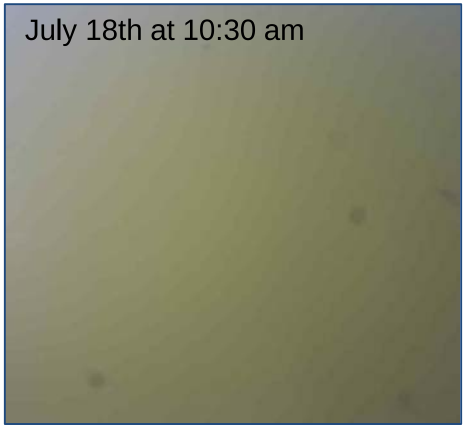
Satellite Imaging



Estimated Cell Counts

EFLD/EFLS = 1,023,293 cells/ml
BUOY = 676,083 cells/ml
EMB = 323,594 cells/ml
ENN = 1,258,925 cells/m

BOUY Site Fixed Camera Station



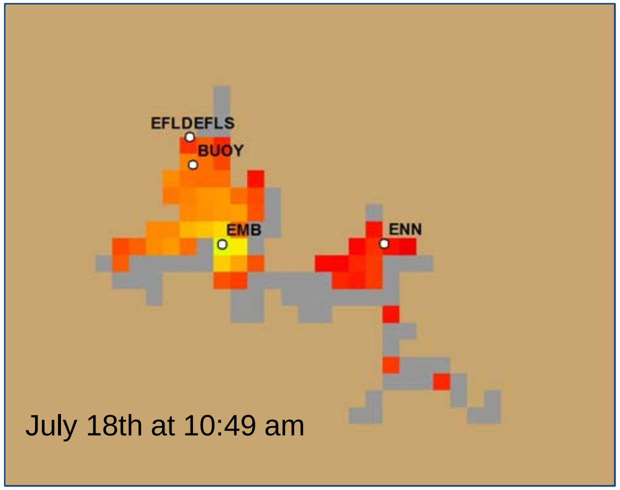
Fixed Camera Prediction of Cyanobacteria

- Camera Prediction:
- 10:30 am - 98.3% Probability of Bluegreens
- 11:30 am 100% Probability of Bluegreens

Courtesy of Jim Lazorchak/Blake Schaeffer

2017 Lake Harsha Monitoring Study

Satellite Imaging



Estimated Cell Counts

EFLD/EFLS = 1,023,293 cells/ml
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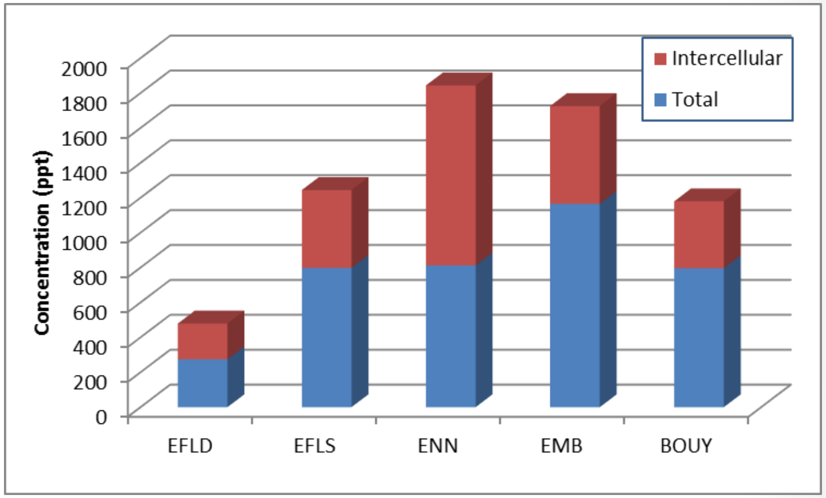
Targeted LC/MS/MS MC Concentration



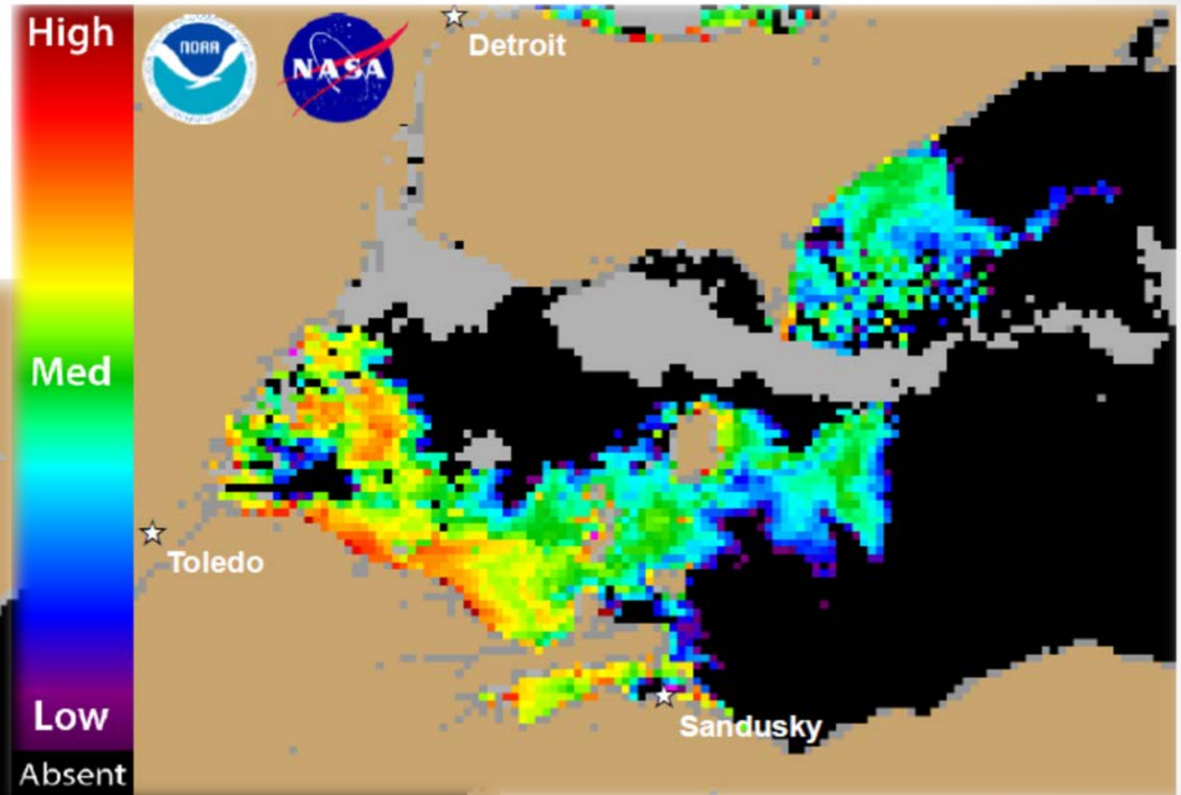
Total Targeted MC Concentration

EFLD/EFLS =1244.3 ppt
BUOY = 1179.7 ppt
EMB = 1725.3 ppt
ENN = 1843.8 ppt

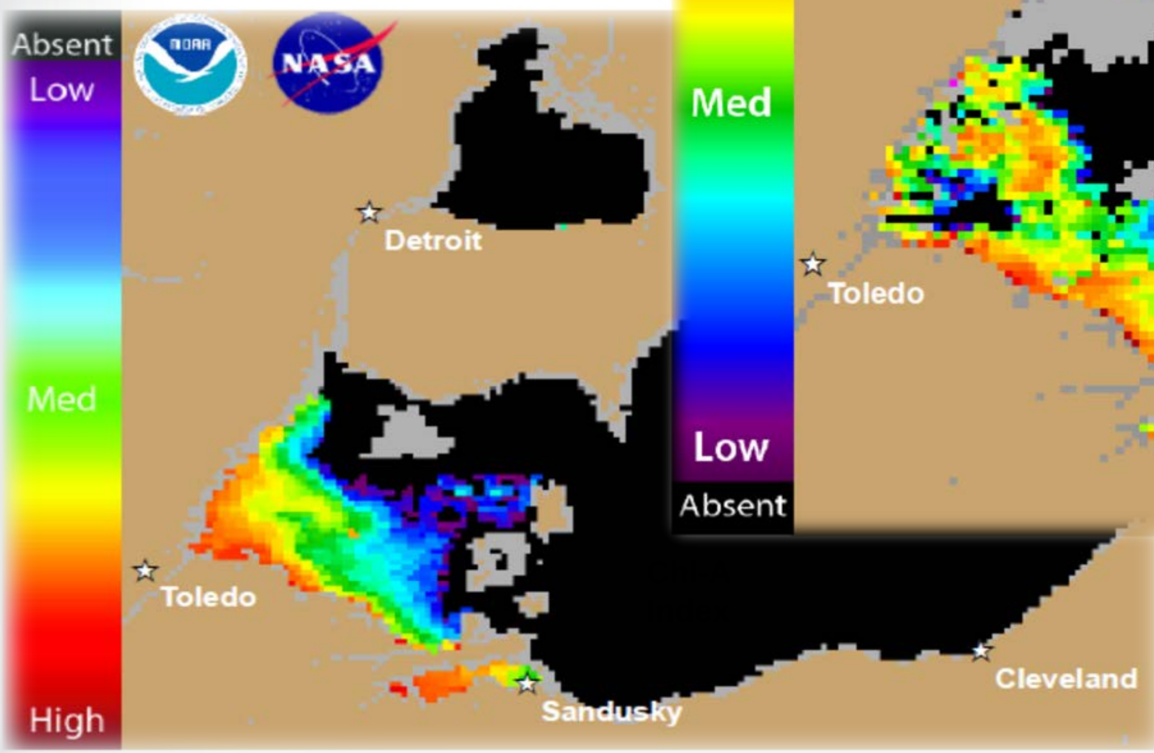
Lake Erie Drinking Water Treatment Plant Study

Lake Erie Blooms 2014 vs. 2015 - Toxic vs. Non-Toxic Producing Blooms

Chl-a Index



August 2014

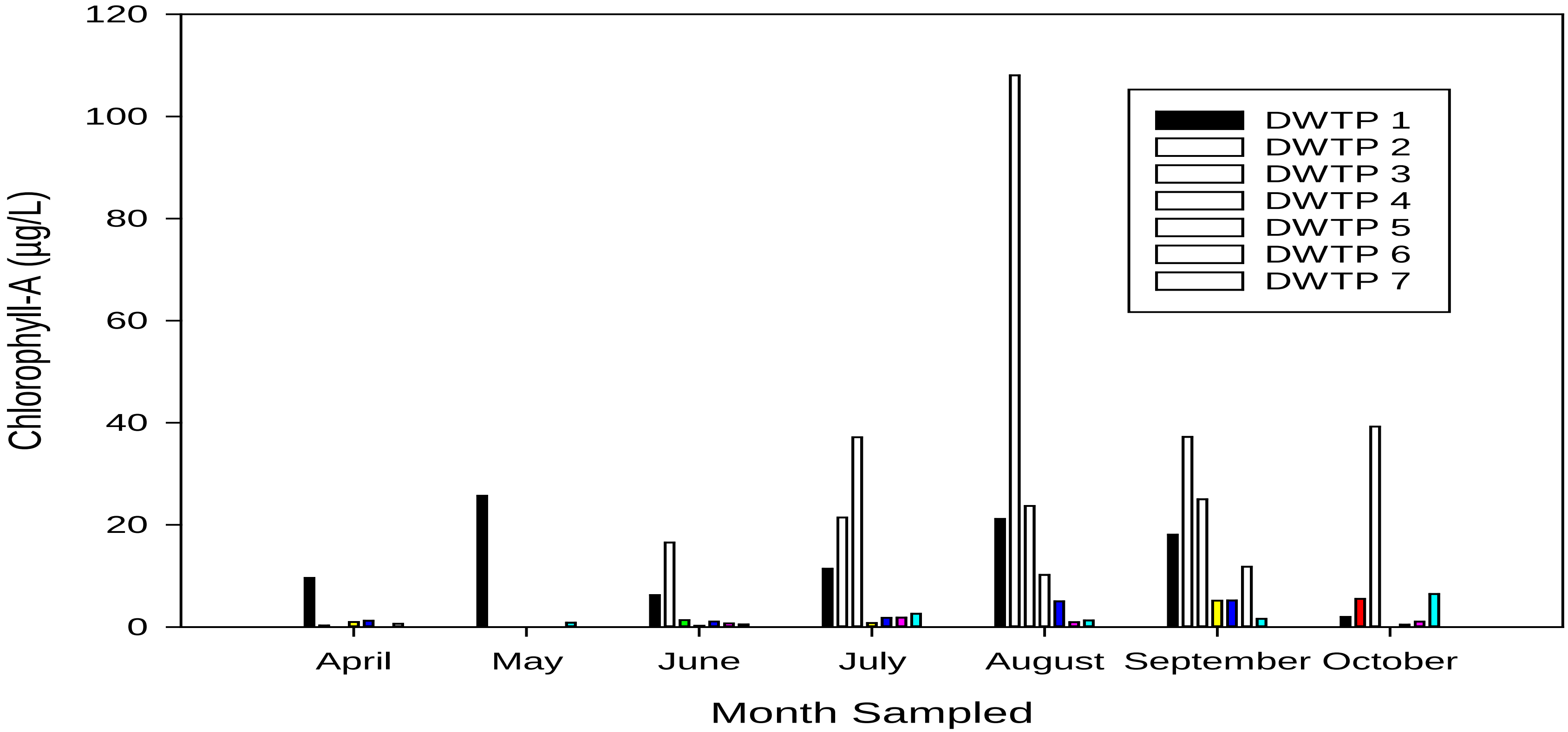


Chl-a Index

August 2015

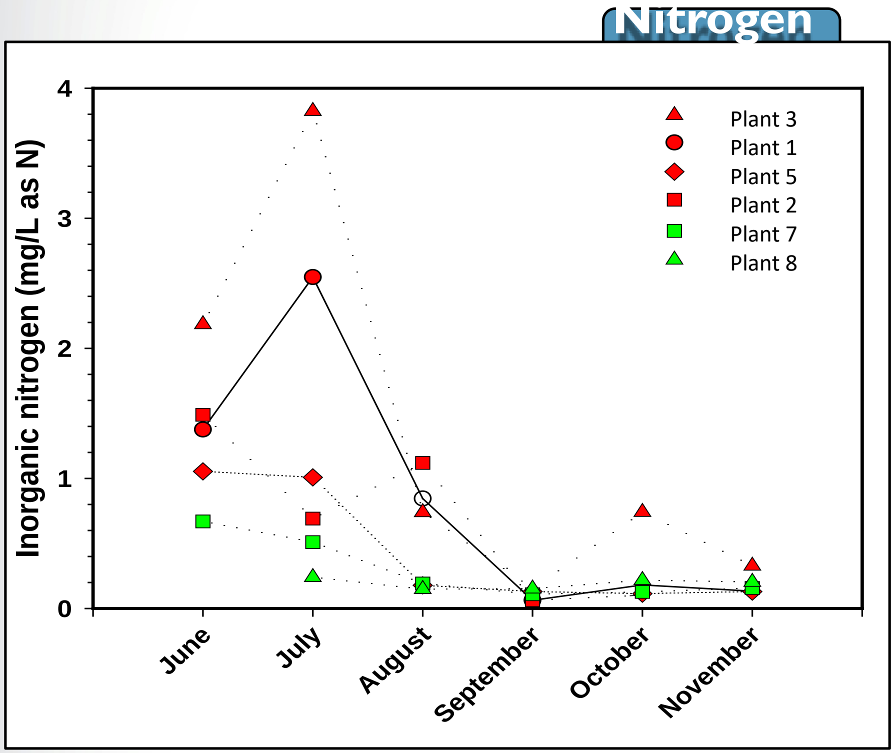
Source: NOAA/NASA

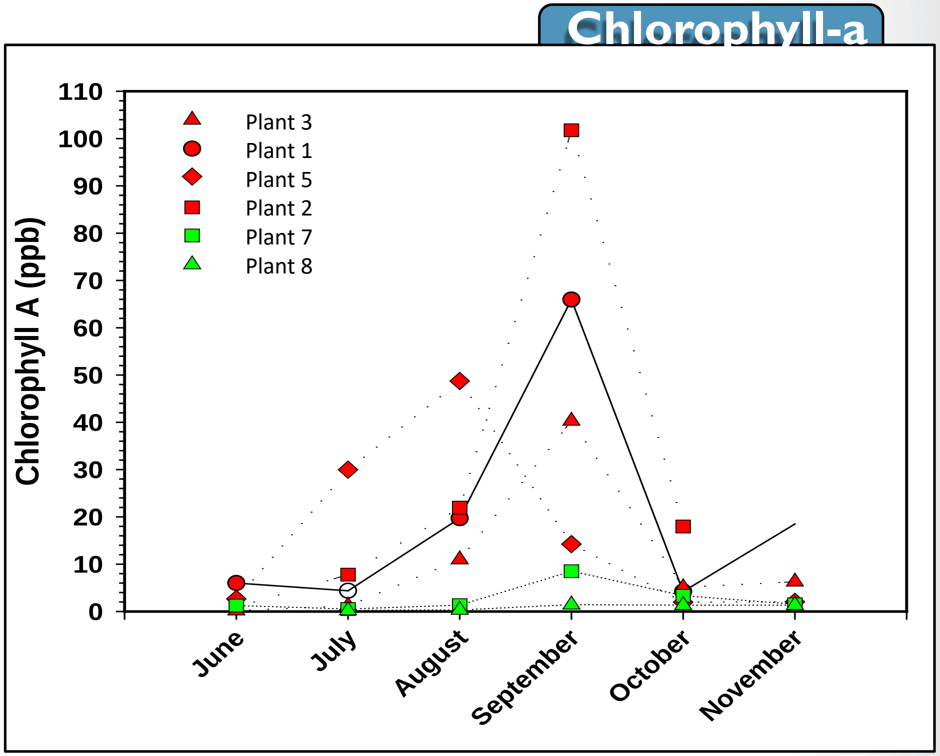
Chlorophyll-a Concentration Observed 2014



- Chlorophyll-a, a proxy indicator, concentrations peak in August-September, consistent with the observed HAB occurrence
- Large fluctuations show need for an increased sampling frequency

Nutrient and Bloom Event Flux 2014

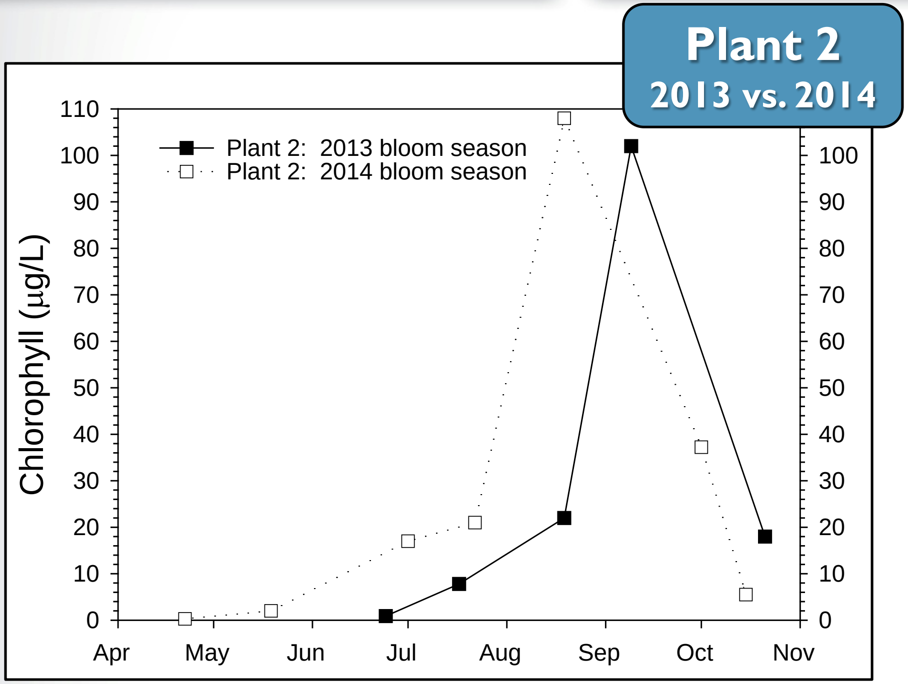




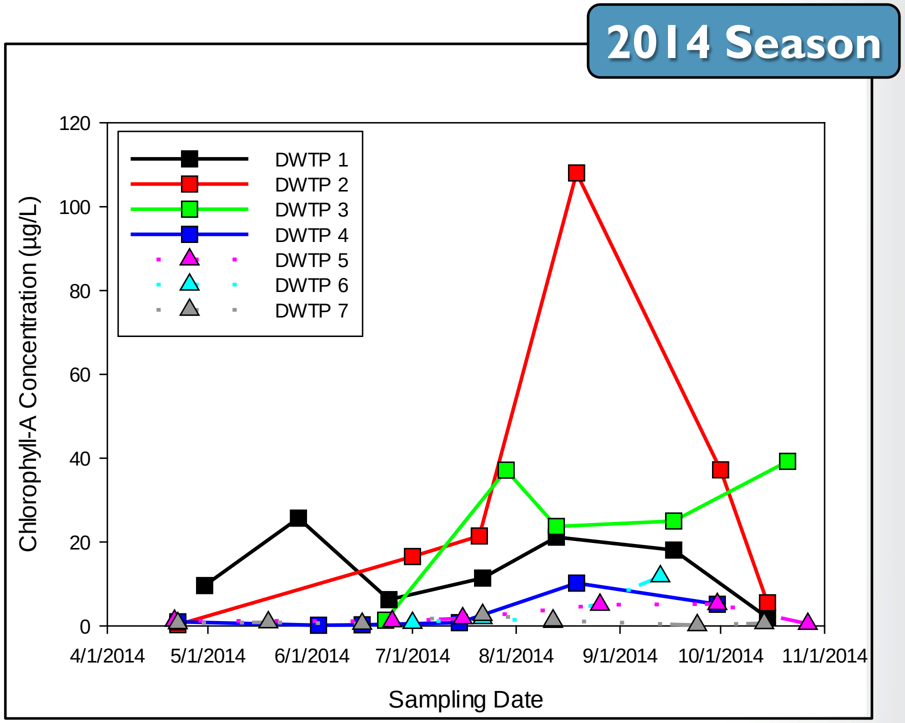
Western basin intakes had higher nutrient loadings in early summer

Western basin intakes had higher blooms levels later in summer

Seasonal Bloom Dynamics

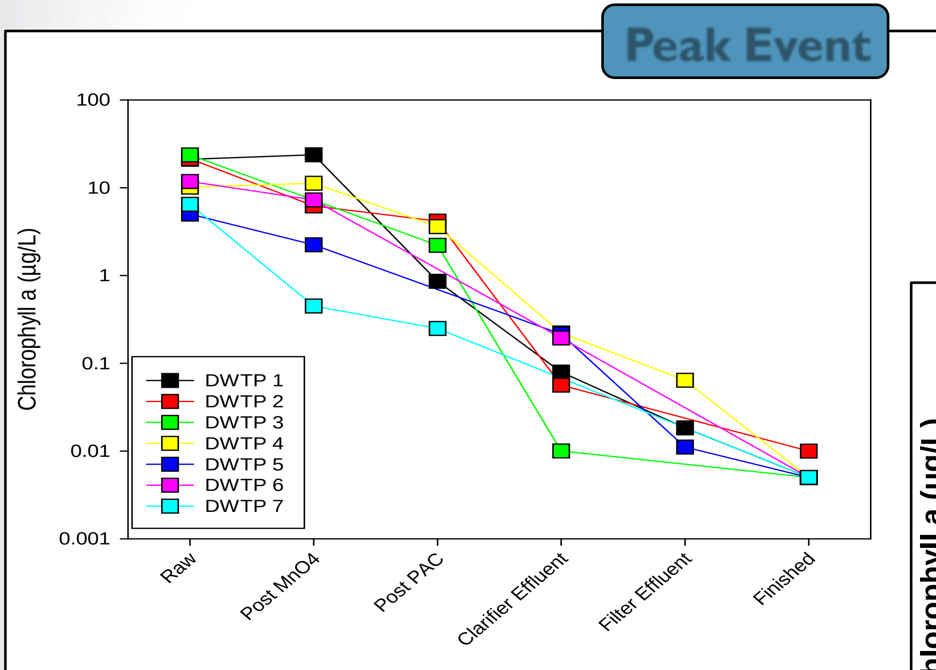


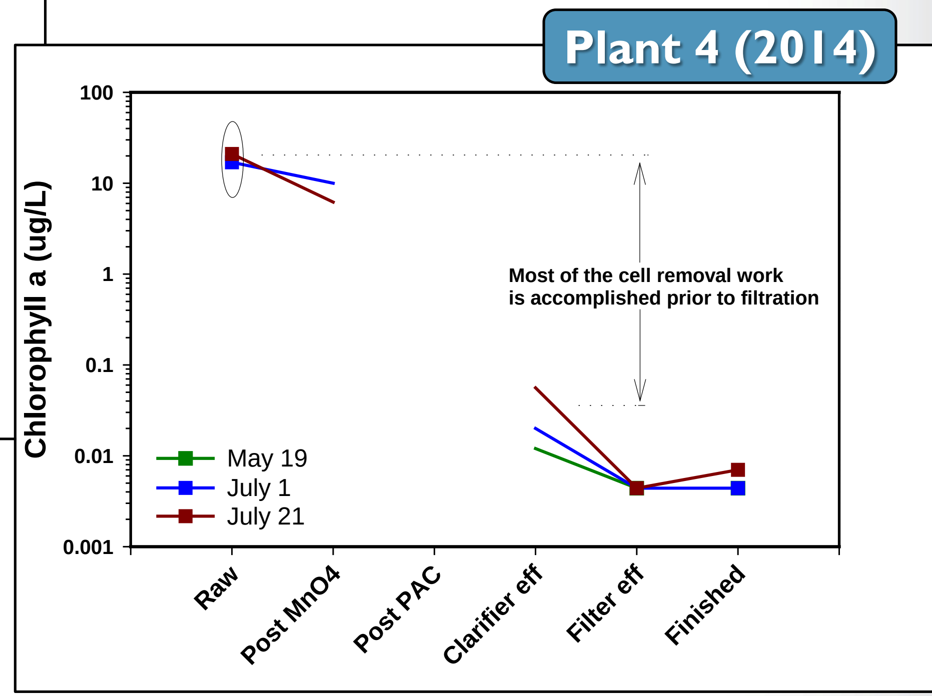
Similar concentration was observed at the peak of the bloom for each year



- Peak of a bloom season does not necessarily occur at the same time in a given year
- There may be more than one major bloom event

Chlorophyll-a Conc. Indicate Biomass is Effectively Removed Post-Clarification



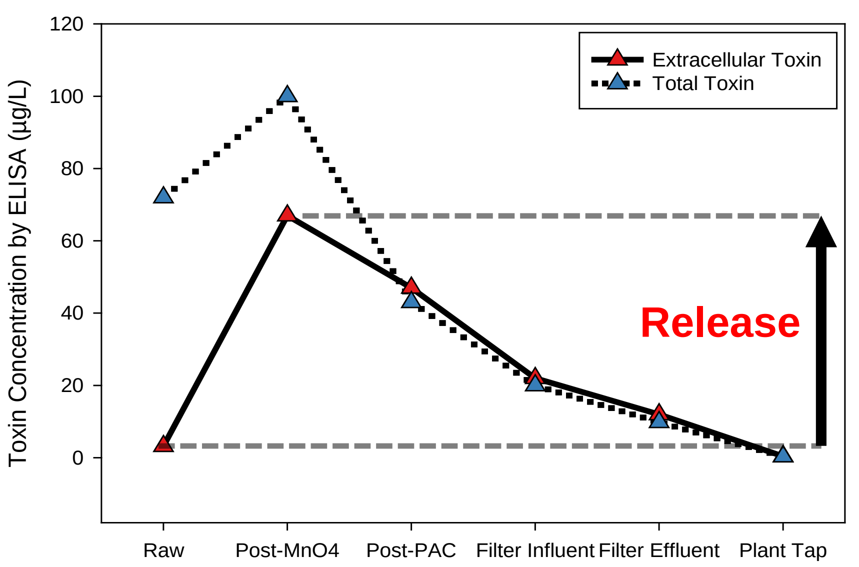


As an proxy measure of intact cells, chlorophyll-a concentrations decreases across the treatment train

- Most cell removal was accomplished prior to filtration

Toxin Propagation through DWTP - ELISA vs LC-MS/MS

ELISA



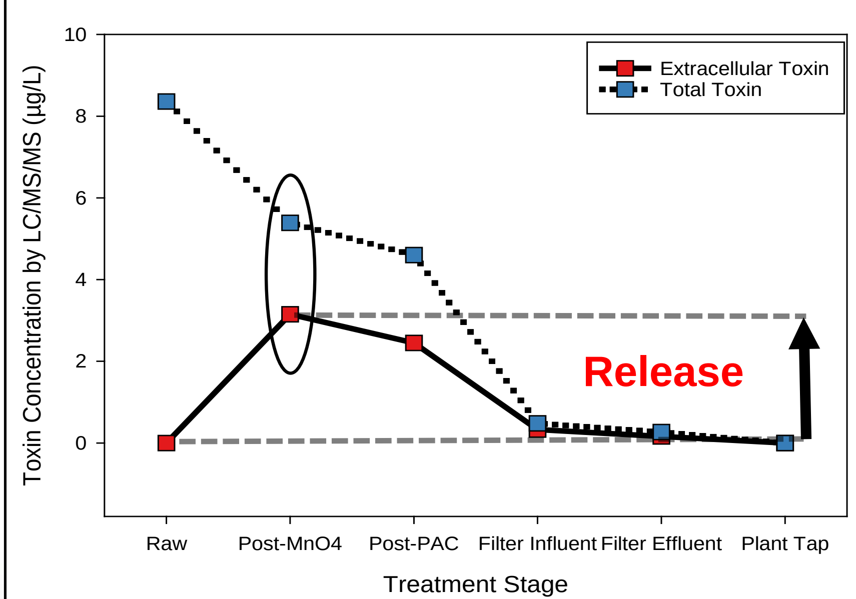
Credit: Nick Dugan USEPA

Intracellular toxin release following MnO_4 addition

Added at crib intake, some residency/ contact time occurs before entering at the head of the treatment train

- Zebra mussel and taste & odor control

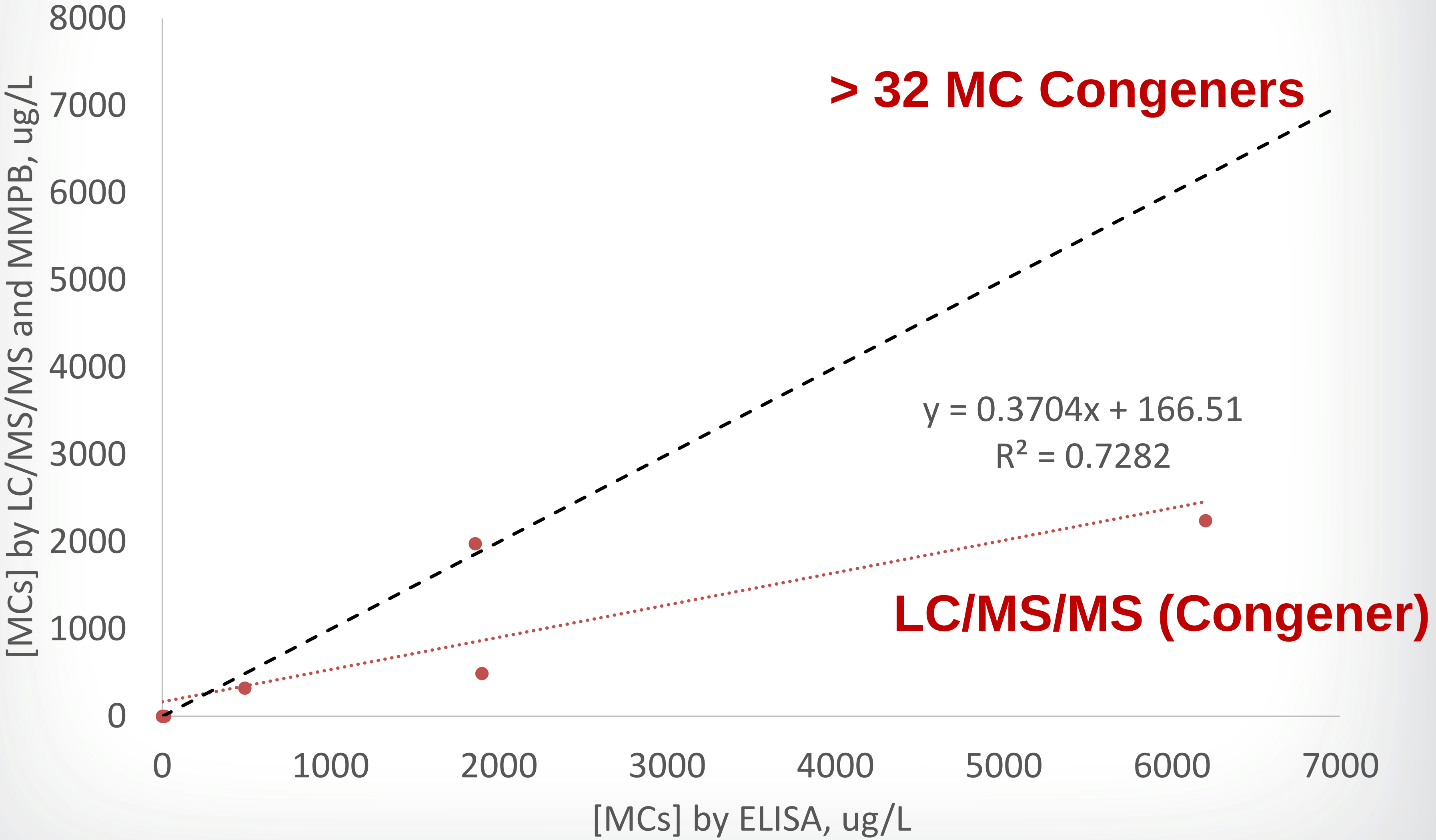
LC-MS/MS



Unlike ELISA, total MYC measured by LC-MS/MS decreases after MnO_4 addition

- However, only 8 MYC (and NOD) variants were measured

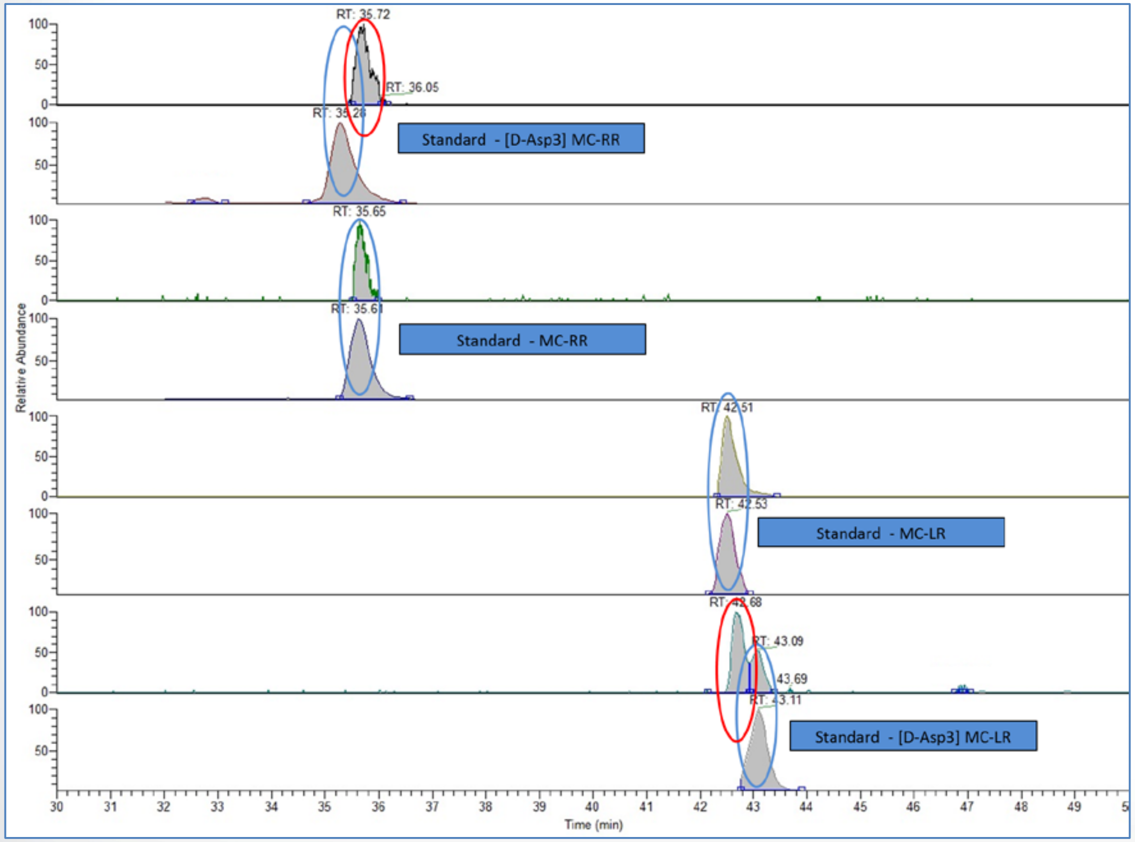
Comparison of Total MCs by ELISA and LC/MS/MS (Lake Harsha)



Courtesy of Toby Sanan

Comparison of Total MCs by ELISA and LC/MS/MS (Lake Harsha)

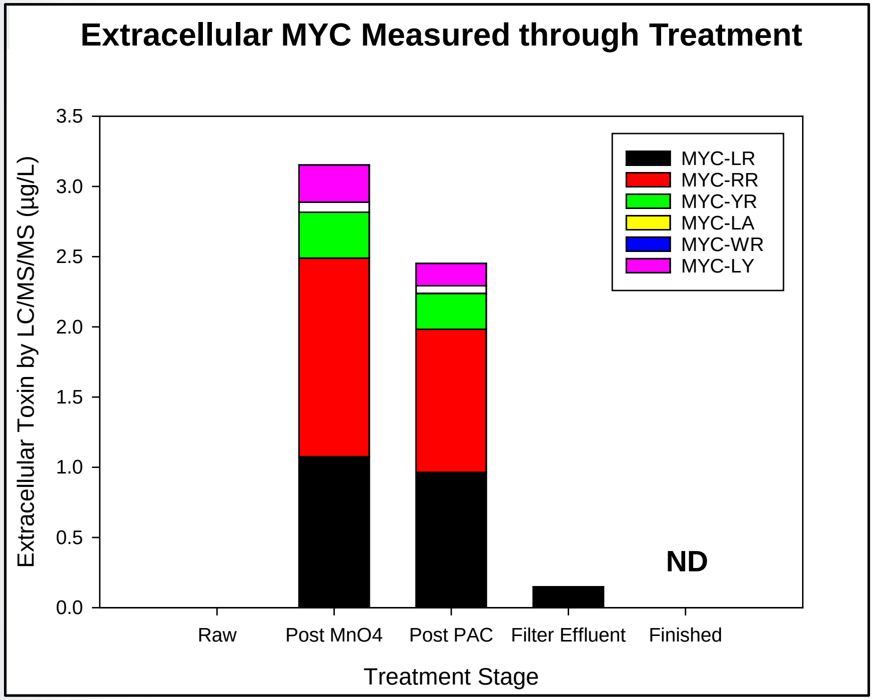
Lake Harsha Sample SRM Chromatograph

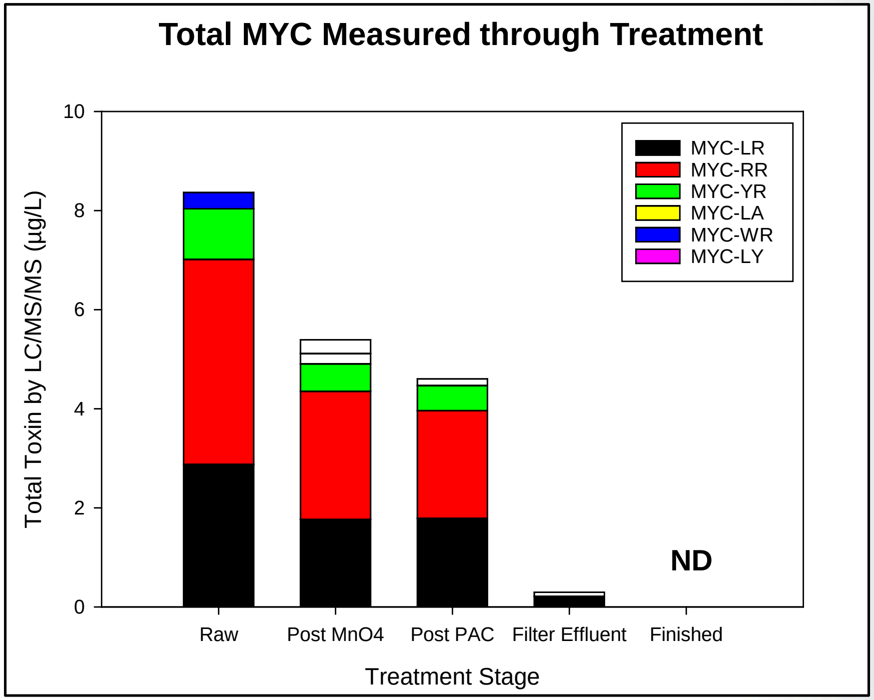


Why the difference?

- Non-targeted MC variants
- Need to ensure similarly massed variants are not misidentified
  - chromatography and confirmation ions are important

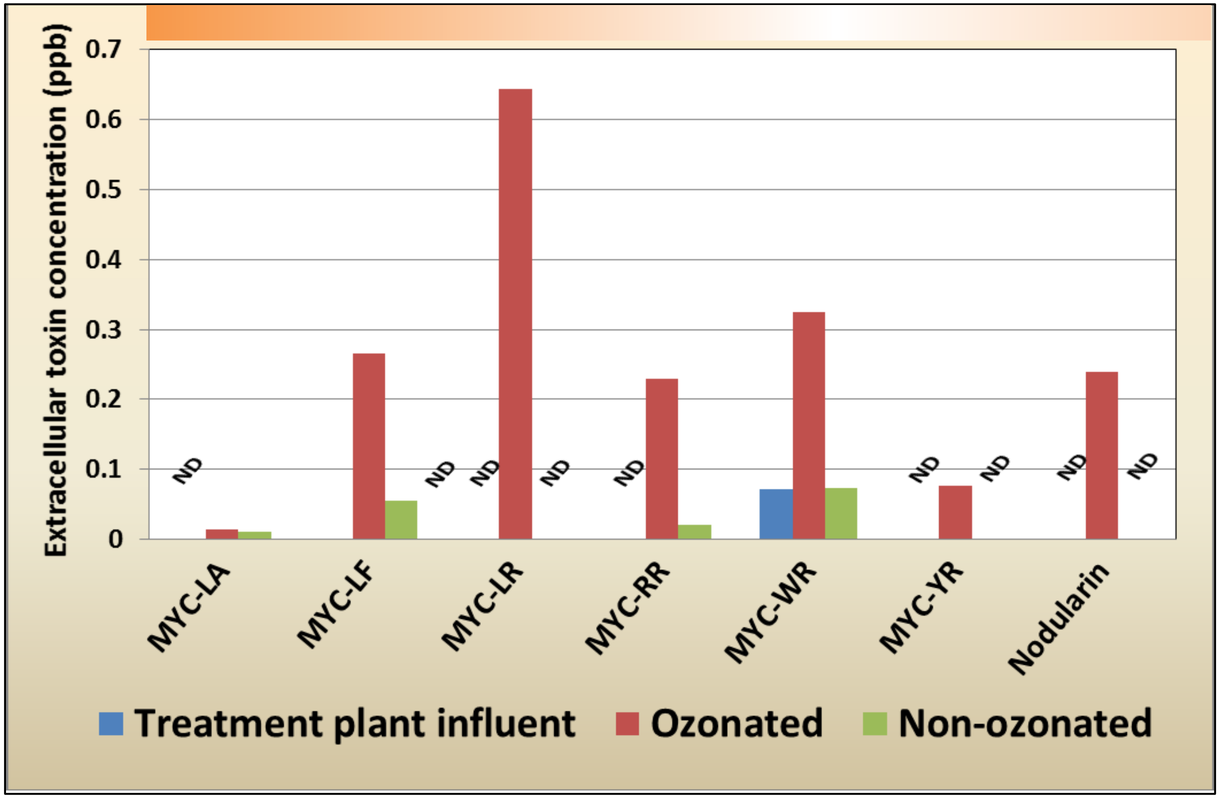
Toxin Speciation and Propagation - LC-MS/MS





- Significant diversity in toxin variants
- Both extracellular and total MYC concentrations decrease through the treatment train; no MYC observed in finished water

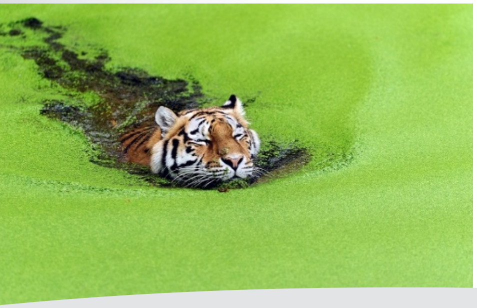
Ozonation and Intracellular Toxin Release



- Ozone effectively lysed cyanobacteria
- However, the applied ozone dose was not sufficient to further remove toxins from post-ozonation water

Conclusions

- Lake Erie water quality was significantly degraded in the western basin as compared to the eastern basin
- The bulk of toxin in treatment facility influents was intracellular
- Therefore, if this holds true, a facility originally designed for particulate control (conventional particulate removal strategies - coagulation, flocculation, sedimentation, filtration) are effective in removing biomass, hence intracellular toxins trapped within intact cells, and can serve as an effective barrier against exposure
- Powdered activated carbon reduces the extracellular toxin
  - Treatment facilities are currently adding oxidants and powdered activated carbon (PAC) at significant expense and uncertain effectiveness
- Preliminary evidence indicates that common doses of oxidants (i.e. permanganate) are sufficiently high to damage cells and release toxins, yet may be too low to completely degrade the released toxin
- More must be known about the formation and control of cyanobacteria and their toxins to assure safe drinking water



Special Thanks to:

- *The various Lake Erie DTWPs*
- *Ohio EPA*
- *Christy Muhlen, Maily Pham, Joel Allen, Kit Daniels, Dana Macke, Toby Sanan*

EPA's informational webpage

http://www2.epa.gov/nutrient-policy-data/cyanobacterial-harmful-algal-blooms-cyanohabs