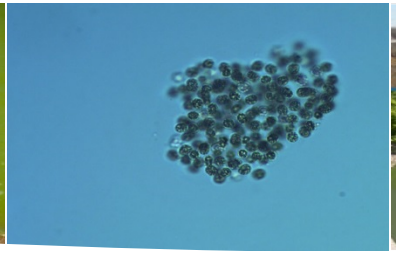
U.S. Environmental Protection Agency, Office of Research and Development

SAFE AND SUSTAINABLE WATER RESOURCES RESEARCH PROGRAM




Use of passive samplers for the detection of extra cellular algal toxins in stream mesocosms, lakes and streams

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SETAC – Sacramento CA November 5, 2018

Harmful Algal Blooms (HABs)

- HABs exact a cost of approximately $2.2B annually in the US, including costs associated with restricted use of recreational waters, declining waterfront real estate value, spending on recovery of biodiversity, and drinking water treatment.
- Typically detrimental to the aquatic system and can be harmful to humans and land animals (contact and consumption)
- Wide variety of taxa can produce blooms

  But… Not all algal blooms may produce toxins

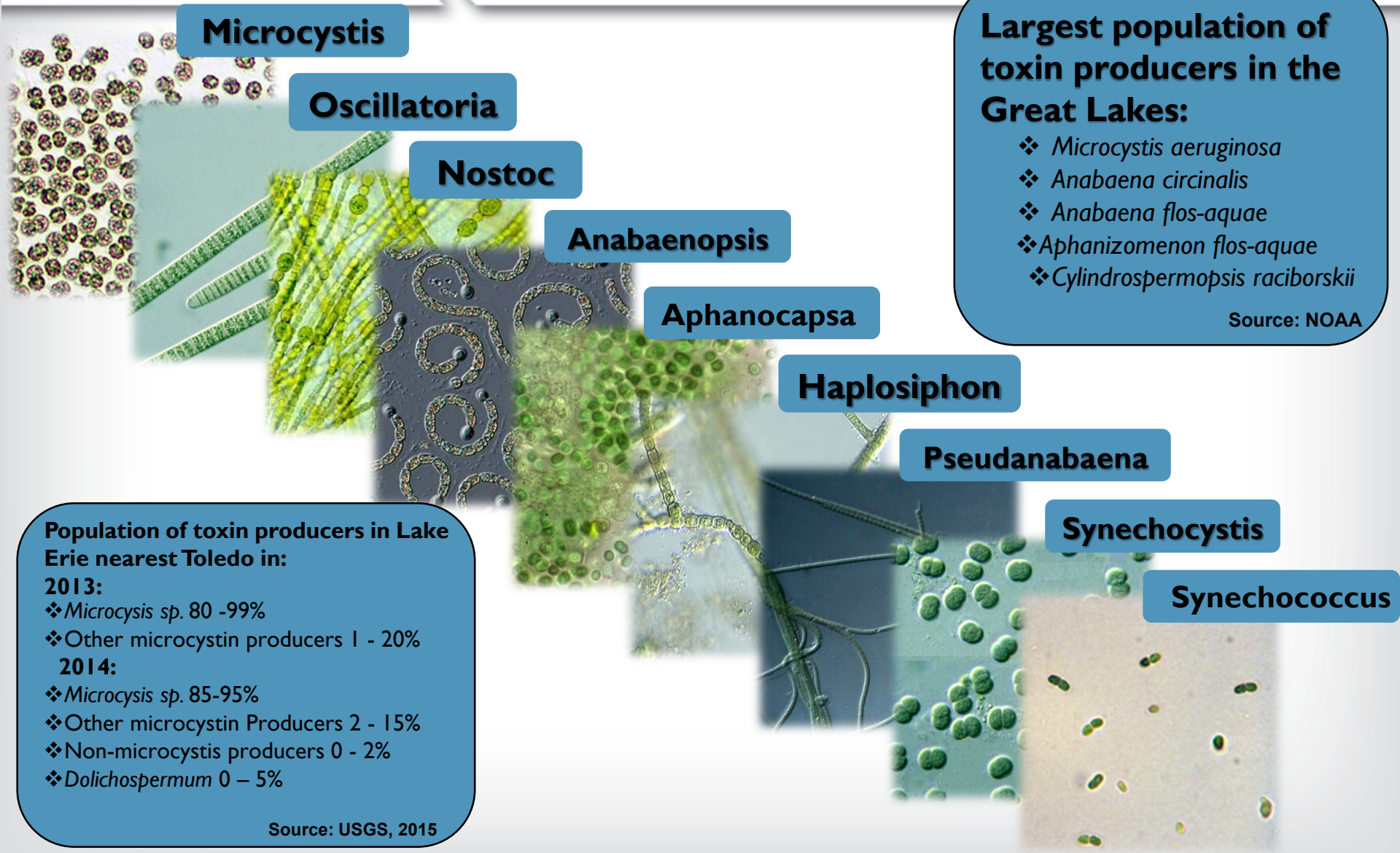
  However… Treatment is still impacted due to biofouling, taste and odor concerns, increasing disinfection by-product potential, etc.
- Blooms are dependent on numerous factors, including nutrient loading, temperature, water flow and weather patterns

  Forecasting is difficult because algal/cyanobacteria strains bloom under different conditions at different times

  Excessive nitrogen and phosphorous levels can cause harmful algal blooms

  Agriculture (non-point source) is often the largest contributor of nitrogen load into waterways

Microcystin and other Cyanotoxin Toxin Producers



Largest population of toxin producers in the Great Lakes:

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

Source: NOAA

Population of toxin producers in Lake Erie nearest Toledo in:

2013:

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

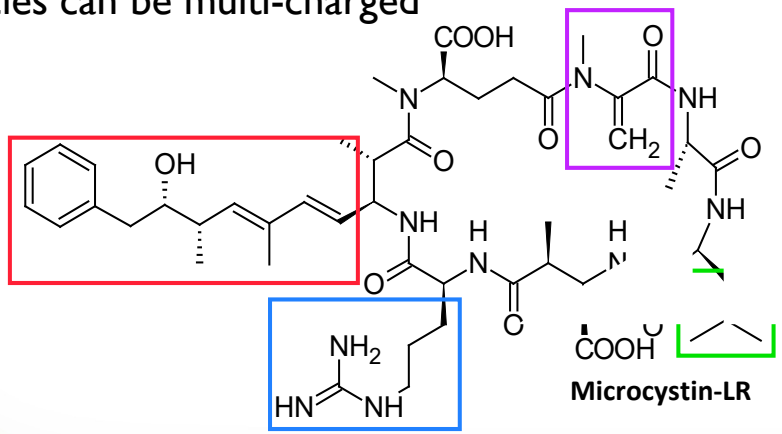
2014:

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

Source: USGS, 2015

Microcystin Toxin Variants

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



Microcystin-LR

All MCs 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.

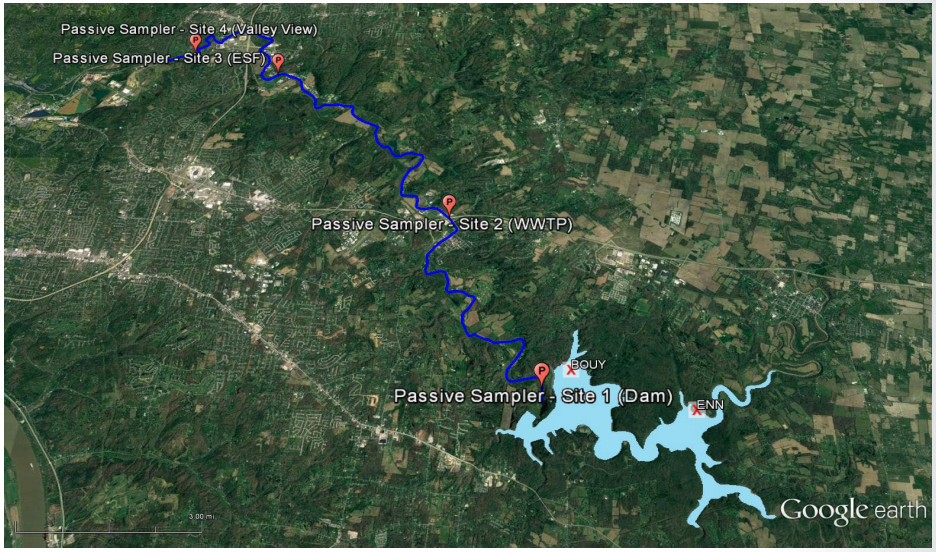
Study Sites – 2018 Passive Sampler Deployment

Lake Harsha (East Fork State Park)

2 Sites – Depth Sampling

- Inlet (ENN)
  - 3', 12', 21'
- Buoy near drinking water intake (BOUY)
  - 3', 15', 27', 39', 51'

Study Site Little Miami River
(downstream of L. Harsha Release)



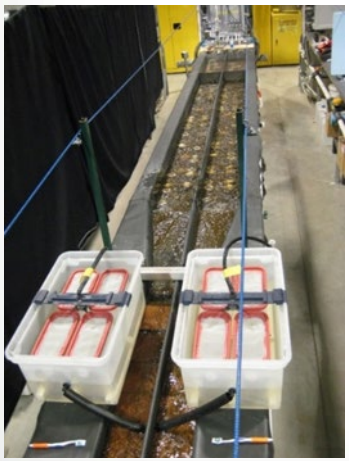
Site	Site Name	Latitude	Longitude	Distance Downstream (miles)
Site 1 (Dam)	Dam Site	39.029398°	-84.147675°	0.4
Site 2 (WWTP)	Clermont Co WWTP	39.088830°	-84.187297°	7.2
Site 3 (ESF)	Milford Water WTP	39.145331°	-84.252146°	15.1
Site 4 (V V)	Valley View Preserve	39.155361°	-84.288726°	19.8

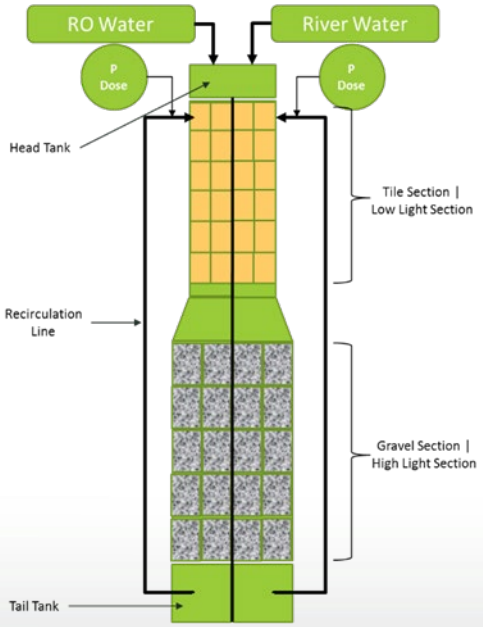
Study Sites – 2018 Passive Sampler Deployment

Experimental Stream Facility - Small Stream Ecosystems (Mesocosms)

Sixteen mesocosms

- Two N:P ratios
  - 6.0, 8.3
- Variable N and P Concentrations
  - N, 240 to 3300 ppb
  - P, 40 to 400 ppb





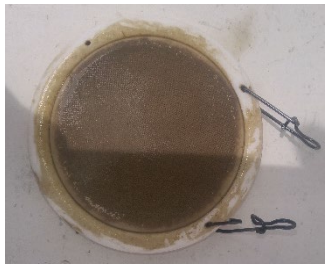
Mesocosm	P as Phosphate Target (ppb)	N as Nitrate Target (ppb)	IngN:P
7.1	40	240	**6.0**
1.1	40	240	**6.0**
3.2	40	240	**6.0**
3.1	60	500	**8.3**
4.1	60	500	**8.3**
7.2	60	500	**8.3**
5.2	90	750	**8.3**
8.1	90	750	**8.3**
8.2	90	750	**8.3**
6.2	180	1500	**8.3**
4.2	180	1500	**8.3**
2.2	180	1500	**8.3**
6.1	300	2500	**8.3**
5.1	300	2500	**8.3**
1.2	400	3300	**8.3**
2.1	**400**	**3300**	**8.3**

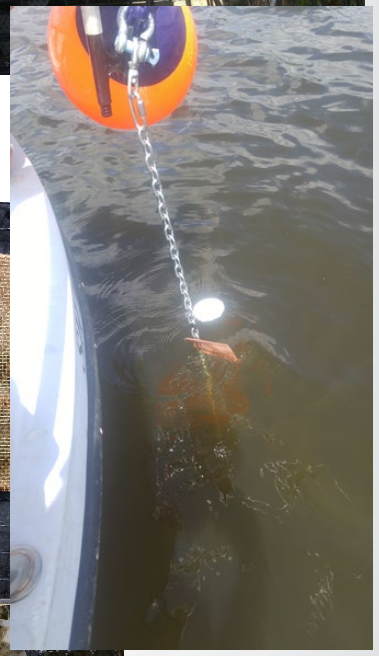
Sample Deployment and Handling

Solid Phase Adsorption Toxin Tracking (SPATT)

Two Resins

- HP20 - Diaion Resin, Styrenic Adsorbent
  - 3 g
- SP700 - Sepabeads Resin Styrenic Adsorbent





Large Format non-selective Passive Sampler Device (LF nsPSD)

Flat-Sheet membrane

14-day Exposure between June-September

Sample Handling and Analytical Methods

SPATTs

Extraction
10 mL 50% MeOH in MQ
20 mL 50% MeOH in MQ
20 mL 50% MeOH in MQ
Combined and Evaporated
Reconstituted in 5% MeOH with Internal Standards

LC/MS

- IT/HRMS (Thermo Discovery Orbitrap)
  - On-line SPE for MCs
- Triple Quadrupole (Thermo Vantage)
  - Anatoxin-A and Cylindrospermopsin

Microcystins Targeted for Mass Spectroscopy Analysis

Algal toxin
Cylindrospermopsin
Anatoxin
Deoxy-cylindrospermopsin
Homo-anatoxin
Microcystin LF
Microcystin LR
Microcystin LW
Microcystin LY
Microcystin RR
Microcystin WR
Microcystin YR
Nodularin
[D-Asp3-(E)-Hhb7] Microcystin-HphR
[D-Asp3-(E)-Dhb7] Microcystin-RR
Microcystin-HilR
Microcystin-HtyR
[D-Asp3] Microcystin-RR
[D-Asp3] Microcystin-LR

LF nsPSD

Extraction
90% MeOH in MQ

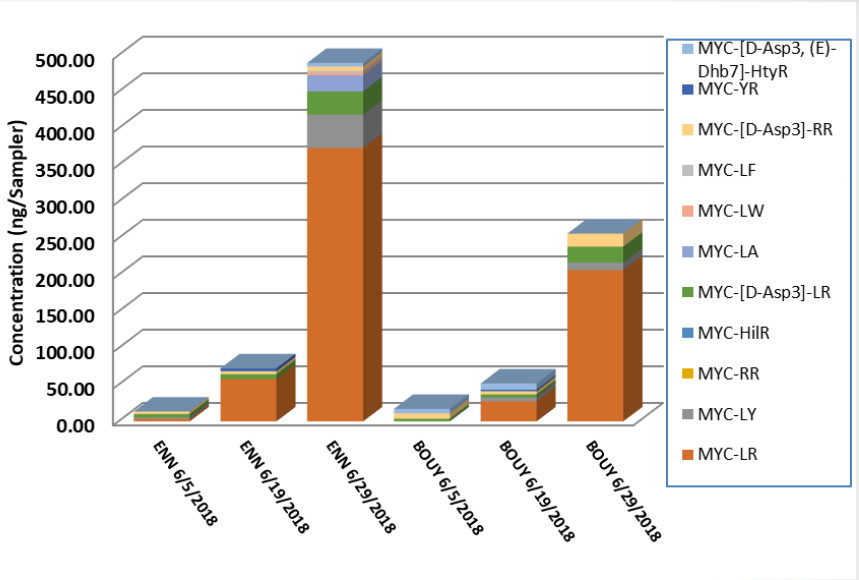
LC/MS

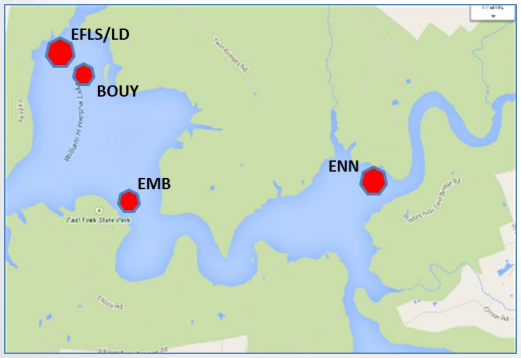
- HRMS (Thermo Q Exactive Plus)
  - Recovery surrogate standard
    - C_2D_5-Microcystin-LR
  - Quantitation internal standard
    - Cyclosporin-A $^{13}C_2$, d_4

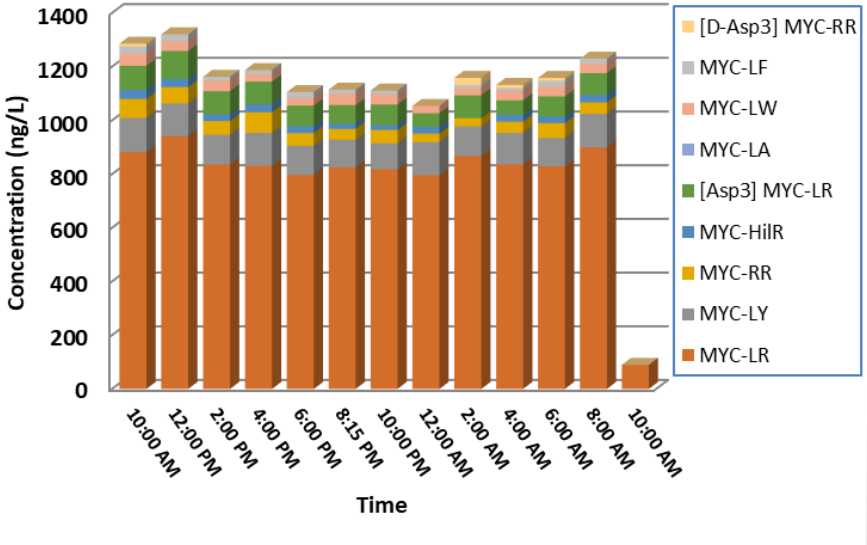
Target Microcystins – Previous Direct Samples vs Passive Samples (Lake Harsha)

LF nsPSD

- MYC-LR observed to be dominant
- MYC-RR may be underestimated by passive sampling



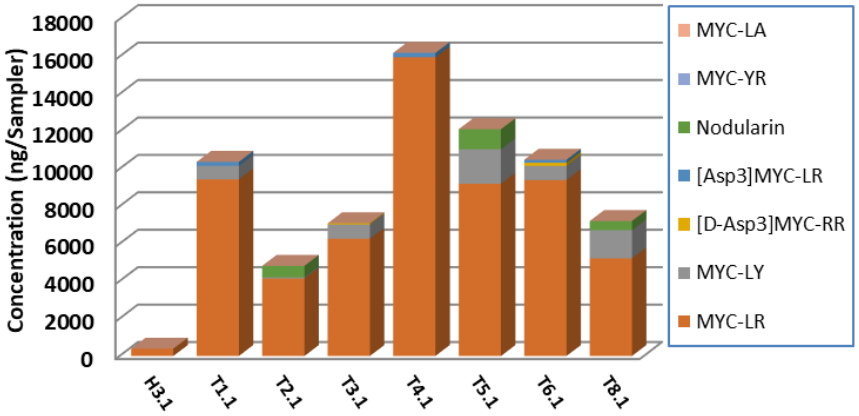




2017 Study Season – Target MC Concentrations

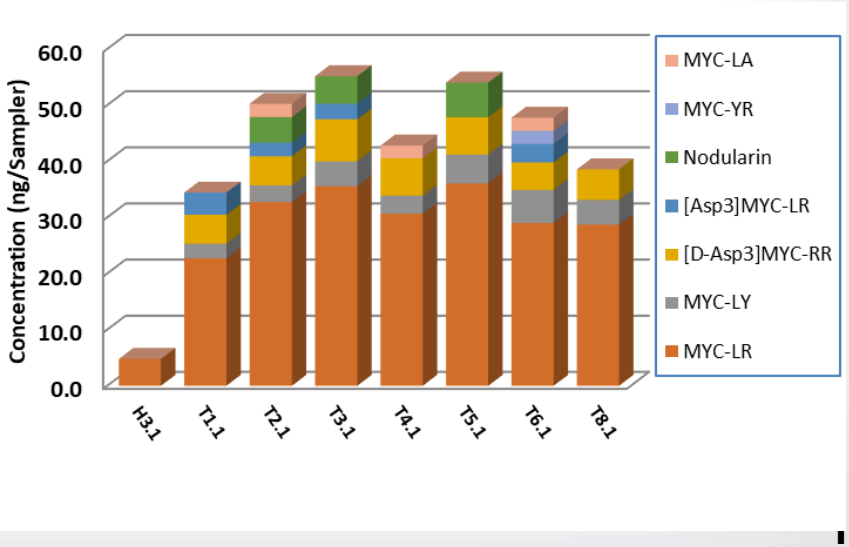
Mesocosms – Comparison of Passive Samplers

SPATT – HP20



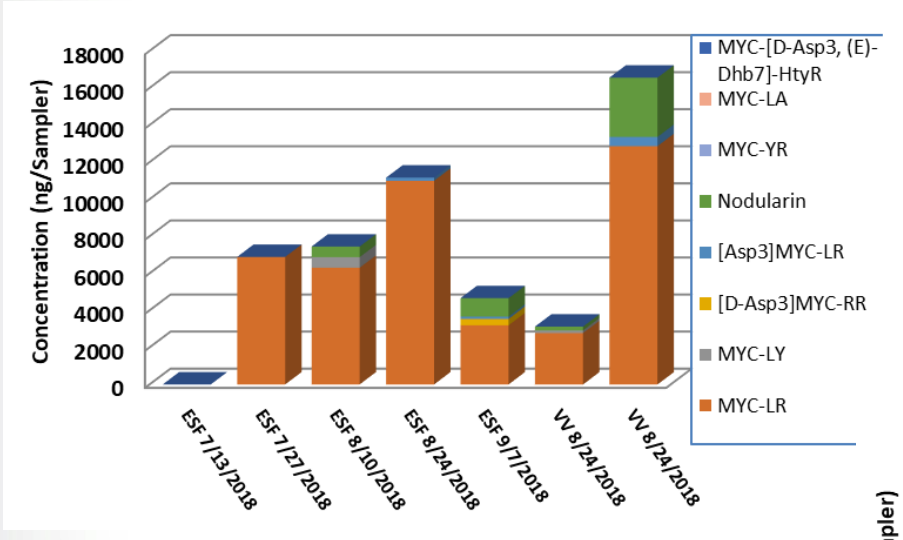
- MYC-LR is the dominant variant measured by both
- [D-Asp3]MYC-RR appears to be underestimated by SPATT

LF nsPSD



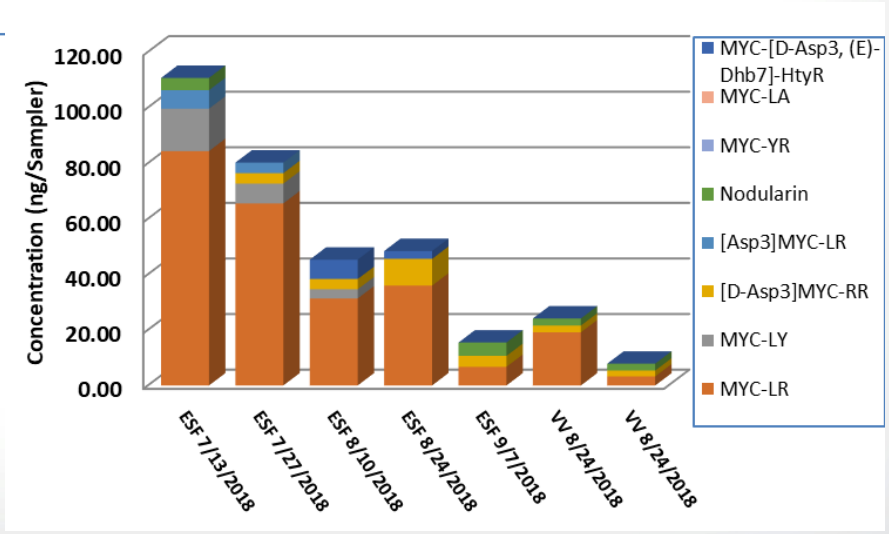
River Samples – Comparison of Passive Samplers

SPATT – HP20



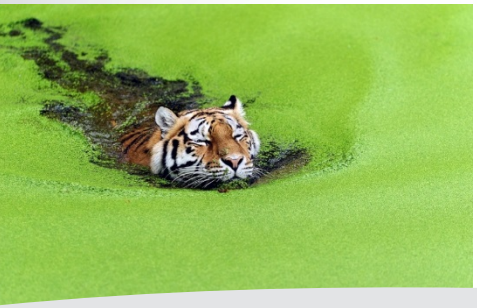
- MYC-LR is the dominant variant measured by both
- As seen previously, [D-Asp3]MYC-RR appears to be underestimated by SPATT

LF nsPSD



Conclusions

- Initial results indicate that both types of passive samplers can uptake several variants of microcystins
- The charge status of the microcystin appears to be important
- Little is understood how background organic matter interferes with uptake or the performance of the passive samplers



Special Thanks to:

- *Clermont County WWTP*
- *Clermont County WTP*
- *Kit Daniels, Dana Macke*

Questions?

EPA's informational webpage

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