ANNOUNCEMENT!

**CyanoSED: *A Workshop on Benthic Cyanobacteria and Cyanotoxins***
**August 6 & 7, 2018 (two-day workshop)**
**US EPA, Office of Research and Development**
**26 West Martin Luther King Dr., Cincinnati, OH 45220**

The US Army Corps of Engineers (USACE) in collaboration with Bowling Green State University (BGSU), the Cawthron Institute (New Zealand) and the US Environmental Protection Agency (US EPA) are organizing a workshop on benthic cyanobacteria and cyanotoxins.

**Objectives:**

- Identify knowledge gaps
- Prioritize research needs on issues surrounding benthic cyanobacteria

**Workshop Structure:**

- Invited presentations from experts in field
- Roundtable discussions on presented topics
- Audience – Federal, state and local government agencies, academics, non-profits, industry partners

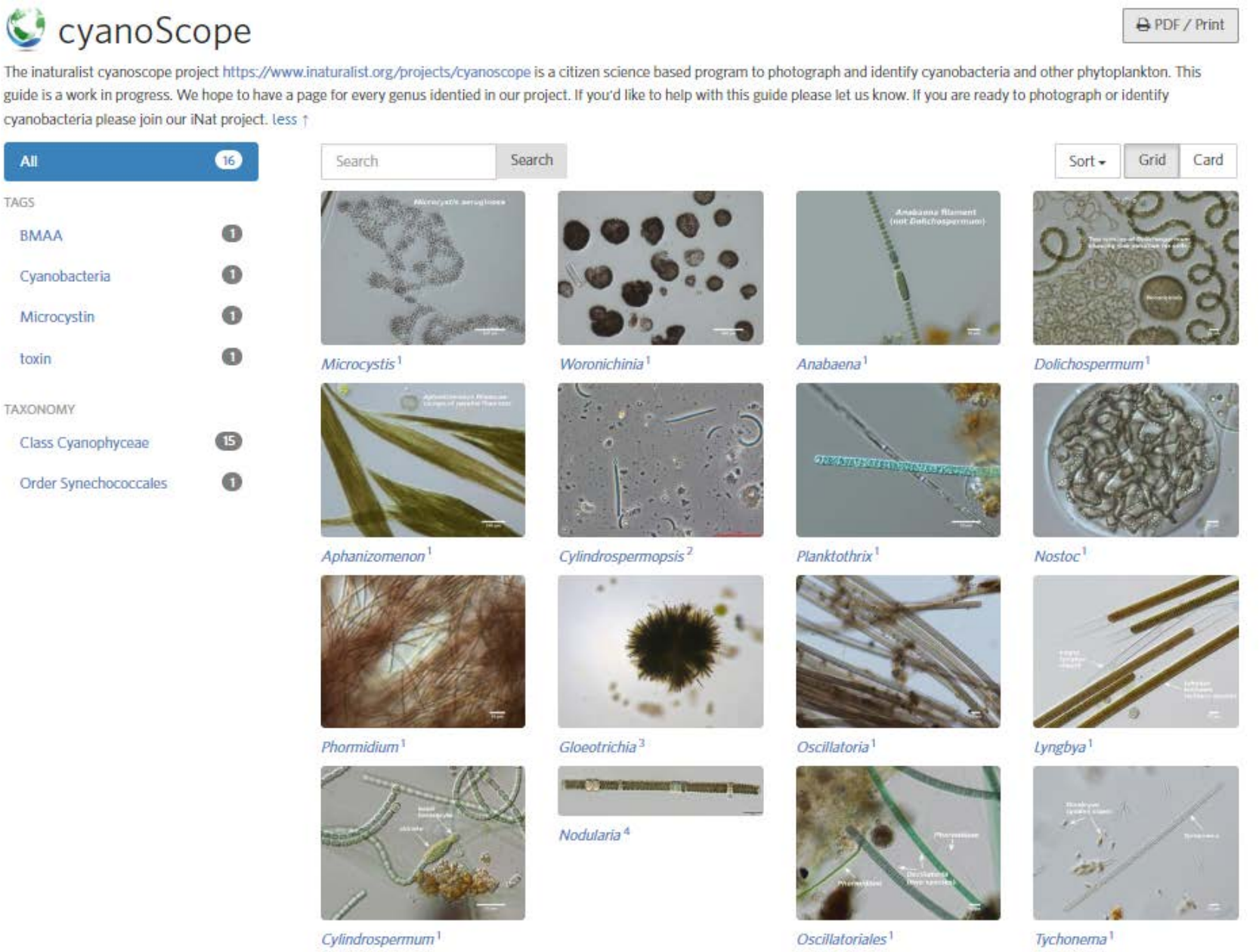
**For more information or to be added to the distribution list, contact:**


- Dr. Kaytee Pokrzywinski (USACE) – Kaytee.L.Pokrzywinski@erdc.dren.mil or (601) 634-3716
- Dr. Timothy Davis (BGSU) – timdavi@bgsu.edu or (419) 372-8553
- Dr. Jim Lazorchak (US EPA) – lazorchak.jim@epa.gov or (513) 569-7076

## iNaturalist CyanoScope

https://www.inaturalist.org/guides/6092

- Building a page for every cyanobacteria genus identified in the Guide
- Opportunity to contribute to guide for benthic cyanobacteria



## iNaturalist CyanoScope

https://www.inaturalist.org/guides/6092

- Contributes to our understanding of where benthic cyanobacteria are found
- Voluntary participation
- Contribute at own pace
- Provide photos, location and information to build library
- Contact Bryan Milstead at Milstead.Bryan@epa.gov

Guides / cyanoScope / Cylindrospermum

# *Cylindrospermum*

### Summary [7]

*Cylindrospermum* is a genus of filamentous cyanobacteria that is occasionally found in freshwater phytoplankton assemblages, but is not usually associated with toxic planktonic blooms. *Cylindrospermum* is more commonly associated with benthic environments, where it can form dense, slimy mats on damp soils or mixed in with shoreline vegetation of lakes and ponds.

### Description [8]

- *Cylindrospermum* cells are cylindrical or barrel-shaped, tiny (3-7 µm wide; for comparison, a strand of spider silk is about 5 µm wide), and slightly longer than wide.
- Under magnification the cells are blue-green or gray-green, and may appear granular.
- The cells are joined together end-to-end to form long, unbranched, untapered, straight or gently curved filaments.
  - The filaments are surrounded by clear, often transparent mucilage, and may slimy form mats on submerged vegetation or other surfaces.
- In addition to ordinary (vegetative) cells, one or both ends of the filament may contain pale blue heterocytes (=heterocysts).
- The filaments may also have one (occasionally two) large, blue-green or brown akinetes adjacent to the heterocytes.
  - Heterocytes are specialized cells that convert dissolved nitrogen gas into ammonium that can be used for cell growth.
  - Akinetes are resting cells that are resistant to cold temperatures and other unfavorable environmental conditions.
  - Akinetes are usually produced near the end of the growing cycle, and can overwinter in lake sediments.

### Ecology [8]

- *Cylindrospermum* is not a usually found in planktonic cyanobacteria blooms.
  - *Cylindrospermum* cells lack gas vesicles, which are common in bloom-forming planktonic taxa like *Dolichospermum* and *Microcystis*
- *Cylindrospermum* is usually found in shallow water associated with shoreline vegetation in nutrient-poor lakes and ponds, especially water that is slightly acidic, boggy, or peaty.
- *Cylindrospermum* is capable of fixing dissolved nitrogen gas, which helps provide nitrogen in boggy sites where inorganic nitrogen (ammonium, nitrate, and nitrite) is limiting to other types of algae.

### Toxicity [8]

Identifying which cyanobacteria species are producing toxins is more difficult that it sounds. Historically, cyanobacteria taxa were described as "potentially" toxic based on whether they were collected in a toxic bloom. With the advancement of culturing techniques and genetic analysis, toxicity information is becoming more exact. But this is an ongoing process, so the toxicity information on these pages should be considered a work in progress.

- *Cylindrospermum* cells may produce anatoxins (nerve toxin), lipopolysaccharides (skin irritants), and BMAA (beta-Methylamino-L-alanine; nerve toxin). These toxins are released into the ambient environment when the cell wall is disrupted (cell lysis).

### iNat Map

# New Publication

- Keith Bouma-Gregson, Raphael M. Kudela, and Mary E. Power
  "Widespread anatoxin-a detection in benthic cyanobacterial mats throughout a river network
- PLOS One http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0197669

PLOS ONE

## Widespread anatoxin-a detection in benthic cyanobacterial mats throughout a river network

Keith Bouma-Gregson , Raphael M. Kudela, Mary E. Power

Published: May 18, 2018 • https://doi.org/10.1371/journal.pone.0197669

### Abstract

Benthic algae fuel summer food webs in many sunlit rivers, and are hotspots for primary and secondary production and biogeochemical cycling. Concerningly, riverine benthic algal assemblages can become dominated by toxic cyanobacteria, threatening water quality and public health. In the Eel River in Northern California, over a dozen dog deaths have been attributed to cyanotoxin poisonings since 2000. During the summers of 2013–2015, we documented spatial and temporal patterns of cyanotoxin concentrations in the watershed, showing widespread distribution of anatoxin-a in benthic cyanobacterial mats. Solid phase adsorption toxin tracking (SPATT) samplers were deployed weekly to record dissolved microcystin and anatoxin-a levels at 10 sites throughout the watershed, and 187 *Anabaena*-dominated or *Phormidium*-dominated cyanobacterial mat samples were collected from 27 locations to measure intracellular anatoxin-a (ATX) and microcystins (MCY). Anatoxin-a levels were higher than microcystin for both SPATT (mean MCY = 0.8 and ATX = 4.8 ng g resin$^{-1}$ day$^{-1}$) and cyanobacterial mat samples (mean MCY = 0.074 and ATX = 1.89 μg g$^{-1}$ DW). Of the benthic mats sampled, 58.9% had detectable anatoxin-a (max = 70.93 μg g$^{-1}$ DW), while 37.6% had detectable microcystins (max = 2.29 μg g$^{-1}$ DW). SPATT cyanotoxin levels peaked in mid-summer in warm mainstem reaches of the watershed. This is one of the first documentations of widespread anatoxin-a occurrence in benthic cyanobacterial mats in a North American watershed.

## New Work Projects

- *Development of methods for Measuring Total Microcystins in Fish Tissue using the 2-methoxy-3-methyl-4-phenylbutyric acid (MMPB) procedure*
  - Authors: James Lazorchak, Toby Sanan, Devi Sundaravadivelu, Josh Kickish, Jenifer Jones, Raghuraman Venkatapathy
  - Presented at SETAC Rome
  - PowerPoint Presentation available

- Use of passive samplers for the detection of extra cellular algal toxins in Stream (and lake) mesocosms
  - Objective: To test out 2 types of passive sampler devices, SPATT and Large Format non-selective Passive Sampler Device (LF nsPSD) to determine their performance in measuring extracellular algal toxins in artificial stream mesocosms.
  - Project lake – Harsha Lake and downstream habitats of Harsha lake
  - Collaborators include: Jim Lazorchak (NERL), Chris Nietch (NRMRL), Heath Mash (NRMRL), Toby Sanan (NRMRL), Joel Allen (NRMRL), Allen Lindquist (NRMRL), Damian Shea (NCSU), Raphe Kudela (UC Santa Cruz), Meredith Howard (SCCWRP), Someone from the country or OEPA (Heather Raymond)

- Contact Jim Lazorchak for more information on these projects – Lazorchak.Jim@epa.gov

# Development of methods for Measuring Total Microcystins in Fish Tissue using the 2-methoxy-3-methyl-4-phenylbutyric acid (MMPB) procedure.

James Lazorchak[1], **Toby Sanan[2],** Devi Sundaravadivelu[3], Josh Kickish[3], Jenifer Jones[3], Raghuraman Venkatapathy[3]

[1]U.S. EPA ORD/NERL, Cincinnati, Ohio , [2]U.S. EPA ORD/NRMRL, Cincinnati, Ohio, [3]Pegasus Technical. Services, Inc. c/o U.S. EPA Cincinnati, Ohio. E-mail contact: lazorchak.jim@epa.gov

**sanan.toby@epa.gov**

SETAC Europe Annual Meeting Rome May 17, 2018




**Although this presentation has been cleared thru ORD review, nothing presented represents agency policy**

# Global Challenge of (HABs): treatment, detection, toxic effects, risk assessment and management.

Harmful Algal Blooms (HABs) are defined as an assemblage of eukaryotic or prokaryotic plankton which have the potential to cause negative health, ecological or economic impacts.

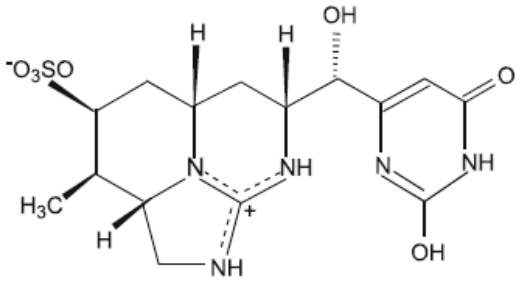
HABs have become a recurrent, increasing and widespread issue globally, with negative impacts that include, but are not limited to, public health and environmental risks from toxin(s) production, light attenuation, diurnal swings in pH and dissolved oxygen, offensive tastes and odors, and impaired visual aesthetics.

These blooms result in high cost to the water treatment and intoxication of the aquatic organisms and humans.

Studies have shown that several algal toxins can cause genotoxic effects, cellular damage and oxidative stress in fish tissues and can accumulate in the muscles, which gives the possibility of human exposure to these toxins through contaminated fish consumption.

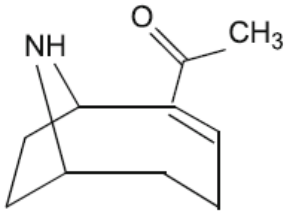
# Common Cyanotoxins Associated with HABs

United States Environmental Protection Agency



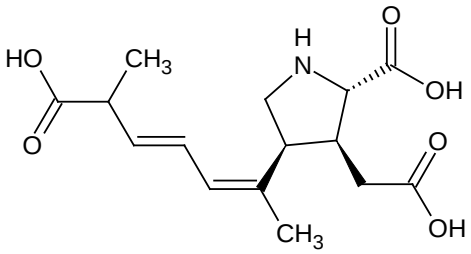
**Cylindrospermopsin**

**Target organs: Kidney, liver**



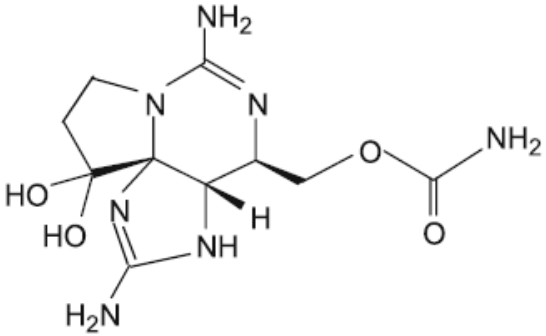
**Anatoxin-A**

**Targets CNS**



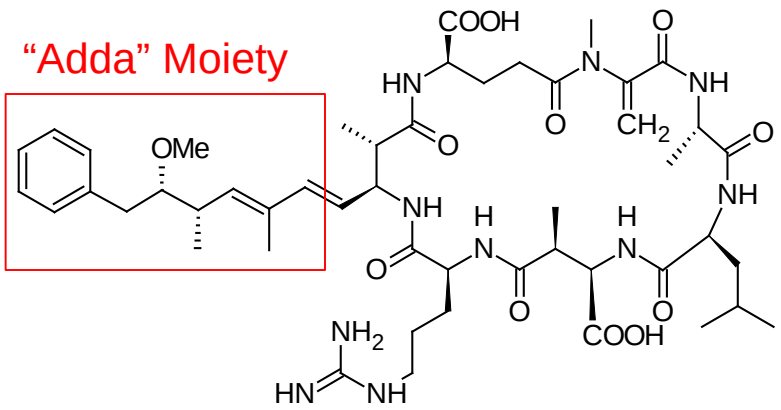
**Domoic Acid**

**Neurotoxin/Amnesic Shellfish Poisoning**



**Saxitoxin**

**(+ Gonyautoxin, other related paralytic shellfish poisons)**




**Microcystin-LR, and Hepatotoxic, probable carcinogen. over 160 other congeners.**

**0.3 ug/L health advisory level**

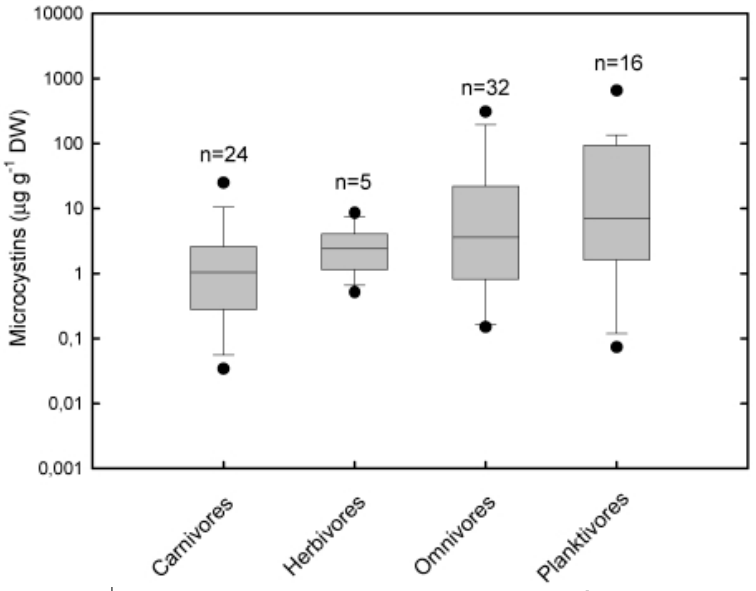
# Bioaccumulation/Biomagnification risks of Cyanotoxins

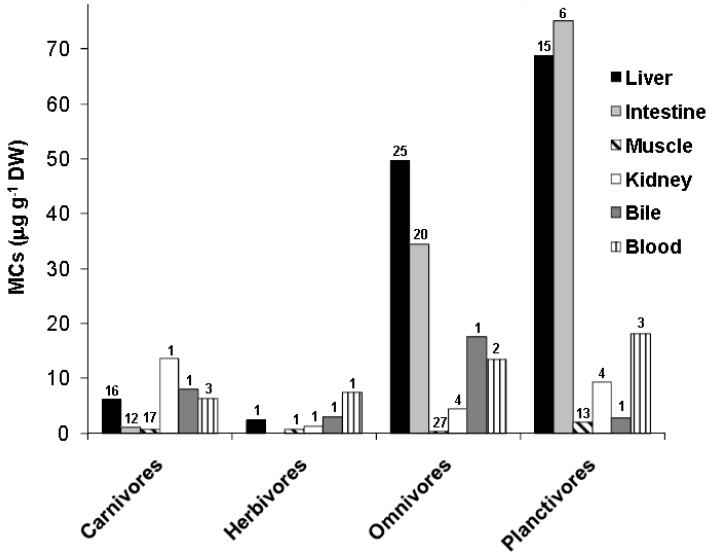
Potential for bioaccumulation or biomagnification of cyanotoxins in food web

- Bioaccumulation from consuming cyanobacteria or toxins in environment
- Biomagnification from persistence in prey species

Shellfish/Clams are known to bioaccumulate saxitoxins and other PSPs

Human health risks from consumption – where do toxins accumulate in tissue? “Are the fish safe to eat” Post-Bloom?






Ferrao-Filho *et al*. *Marine Drugs* **2011**, 9,2729-2772

# Microcystins – An analytical challenge

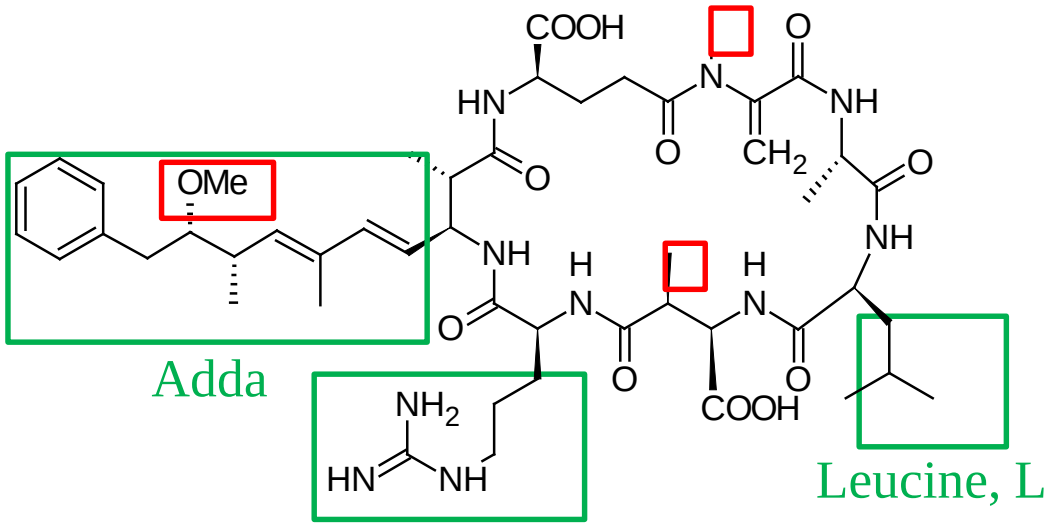
United States Environmental Protection Agency

> 160 microcystin (MC) congeners have been found in the environment

- Most common cyanotoxins in inland lakes
- Only ~ 15 are available as analytical standards

Variations include **amino acid substitutions** (including non-standard amino acids), methylation and desmethylation

Chemical properties (hydrophobicity/hydrophilicity, susceptibility to treatment) can vary significantly by congener, and the congeners produced vary by species and geography




Microcystin-LR

# Recovery of MC congeners from tissue

Even for "known" MC congeners there is considerable variation in recovery from tissue matrices. Unknown congeners provide an additional challenge.

| Analyte | Method 1: original QuEChERS ($n$=5) | Method 2: MeCN ($n$=5) | Method 3: MeOH ($n$=15) | Method 3: MeOH with filtration ($n$=15) | Method 4: MeCN ($n$=42) |
|---|---|---|---|---|---|
| MC-RR | *59*±12 | *58*±1 | 94±*33* | 86±*50* | *130*±16 |
| Nod-R | *67*±16 | *61*±13 | 72±18 | 91±17 | 94±10 |
| MC-YR | 82±9 | 74±7 | *66*±17 | 94±19 | 97±17 |
| MC-LR | 90±6 | *69*±14 | *66*±17 | 89±*34* | 107±15 |
| MC-WR | 79±13 | *63*±8 | 70±17 | *66*±17 | 115±13 |
| MC-LA | *42*±14 | 84±12 | *57*±9 | *67*±7 | 90±11 |
| MC-LY | *48*±18 | 84±8 | *51*±13 | *63*±13 | 91±16 |
| MC-LW | *62*±9 | *60*±10 | *51*±19 | *68*±*21* | 107±20 |
| MC-LF | *32*±17 | *66*±16 | *51*±16 | *62*±*21* | 104±*26* |

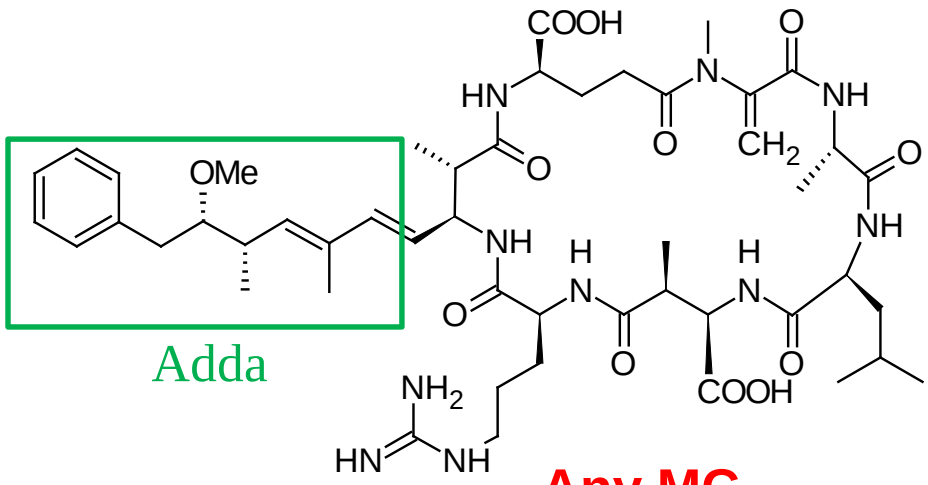
Spike levels in catfish tissue are: method 1=100 ng/g; method 2=100 ng/g; method 3=10, 25, and 100 ng/g; and method 4=10, 25, 50, and 100 ng/g. Values in italics refer to <70% or >120% recovery and >20% SD

(Hydrophobicity generally increasing going down the series, from fillets only)



Geis-Asteggiante, L., Lehotay, S. J., Fortis, L. L., Paoli, G., Wijey, C., & Heinzen, H. (2011). Development and validation of a rapid method for microcystins in fish and comparing LC-MS/MS results with ELISA. *Analytical and bioanalytical chemistry*, *401*(8), 2617-2630.

# “Total MCs” via MMPB Technique

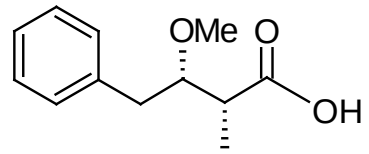



Adda

Any MC congener

$KMnO_4/NaIO_4$

Or Ozone, $OsO_4$




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

Application of the Lemieux Oxidation to convert the Adda moiety in all MCs present to MMPB, which is measured as a surrogate of total toxin concentration

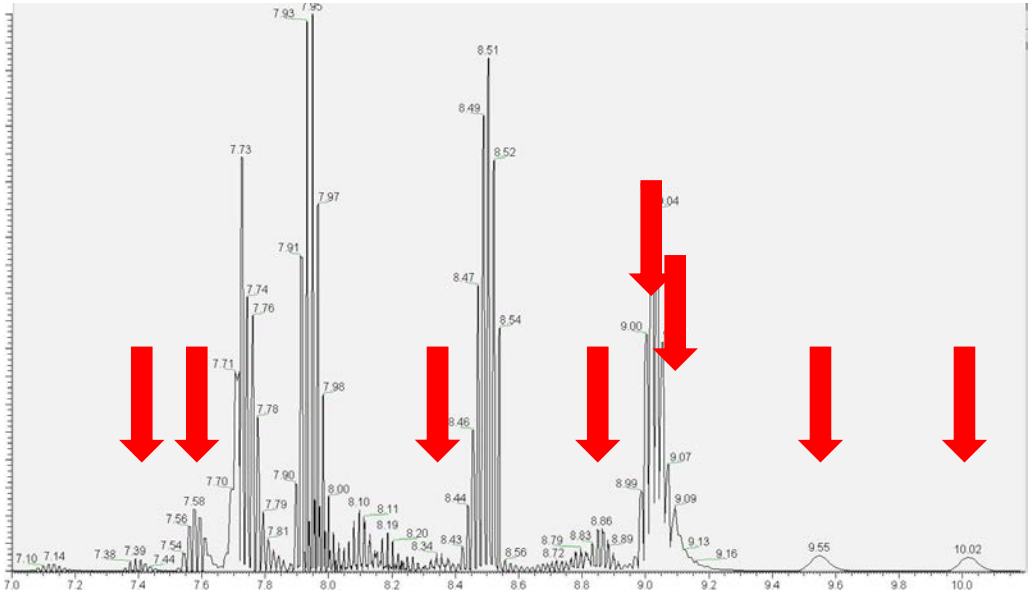
- Simplifies analysis, many congeners to one measurable product
- Cross-reactive with all microcystins containing Adda
- Simplifies extraction from complex matrices (surface water, tissue)


Lemieux, *et. al.* “Periodate-Permanganate Oxidations: I. Oxidation of Olefins”, 1955, Canadian Journal of Chemistry.

Harada, *et. al.* “Mass spectrometric screening method for microcystins in cyanobacteria,” *1996, Toxicon.*

Foss, *et. al.* “Using the MMPB technique to confirm microcystin concentrations in water measured by ELISA and HPLC (UV, MS, MS/MS)”, Toxicon, 2015.

# Why we want a “total” MC method:




- **Surface water sample, > 32 MC Congeners observed**
  - **Concentrations: 6 mg/L by ELISA, 5 mg/L by MMPB, 2 mg/L by LC/MS/MS counting 15 congeners**
  - **Peaks in red have no analytical standards.**
- **Tissue extraction requires solvents incompatible with ELISA without solvent exchange processes, potential matrix interferences, and potential loss of analytes during each sample processing step**

# Study Goals

- Evaluate analyte recovery in fish tissue
  - Can we reproducibly recover MMPB from spiked samples?
  - Effects of lipid, species
- Microcystin Spike-recovery studies in fish tissue
  - Method performance with various congeners
- Application to fish in HAB-impacted water bodies
  - Sequestration of toxins to certain organs/intracellular?
- Expand study to non-microcystin cyanotoxins, where direct extraction is more feasible

# General MMPB Method Workflow

United States Environmental Protection Agency

**Sample Preparation**
- 10-200 mg of lyophilized fish material
- Standard addition of MC-LR CRM to exceed the expected MC concentration

**Oxidation**
- 0.05 M $KMnO_4/NaIO_4$ in 100 mM sodium bicarbonate, pH 9.0 in the dark
- Monitor coloration and add additional oxidant as necessary

**Quench and Workup**
- Add saturated sodium bisulfite to quench
- Filter sample with 0.7 µm Whatman GF/F filter
- Add 10% sulfuric acid to pH < 2 and MMPB-D3 surrogate

**Extraction of Tissue**
- Liquid/liquid extraction with ethyl acetate
- High fat content samples require additional settling time
- Use of hexanes or other solvents for cleanup reduced effective yield

**Evaporation, Reconstitution**
- Evaporation under gentle N2 stream using N-Evap; bath temp. must not exceed 35°C (Volatile loss)
- Internal standard (4-PB) included in reconstitution mixture (90:10 $H_2O$:MeOH)

**Analysis**
- Centrifuge prior to analysis to remove salts
- DI-LC/MS/MS, negative ESI
- Plot [MMPB] vs [MC-LR] spike to obtain yield and response ratio if desired

(Recovery of individual MC congeners would use a similar workflow, but omit the oxidation/quenching steps)



Liquid/liquid conditions similar to Sauve, *et. al. Analytical Chimica Acta, 2014.*

# MMPB Application to Fish Tissue – MMPB Spike/Recovery Studies

Spikes at high (40 ng) and low (4 ng) MMPB and MMPB-$D_3$ were performed to evaluate extraction performance.

Consistent recovery with low and high fish samples, and for 4 and 14% lipid samples.

Recovery of MMPB in 'blank' samples (9, 10) shows stability under derivatization conditions even in low background matrix.

| Sample # | MMPB Spike (ng): | MMPB-D3 Spike (ng) | Fish (mg) | Lipid % | MMPB % Recovery |
|---|---|---|---|---|---|
| 1 | 40 | 40 | 10 | 4 | 85 |
| 2 | 40 | 40 | 100 | 4 | 102 |
| 3 | 40 | 40 | 10 | 14 | 84 |
| 4 | 40 | 40 | 100 | 14 | 73 |
| 5 | 4 | 4 | 10 | 4 | 81 |
| 6 | 4 | 4 | 100 | 4 | 61 |
| 7 | 4 | 4 | 10 | 14 | 87 |
| 8 | 4 | 4 | 100 | 14 | 79 |
| 9 | 40 | 40 | 0 | na | 102 |
| 10 | 4 | 4 | 0 | na | 83 |

# MMPB Application to Fish Tissue – MC Mixture Spike/Recovery Studies

- Mixtures of microcystins were also tested to see if hydrophobicity/hydrophilicity would influence recovery from tissues
- MMPB yields for MC-LA and MC-RR were not significantly different from 1 to 14% lipid content in the spiked tissue.
- Overall yields were typically 30-40% MMPB based on spike amounts
- Some discrepancies in standard concentration complicate 'absolute' MMPB yield (MC standards were ~50% of certified reference standards upon comparison – this is a common issue in cyanotoxin studies)

| Sample: | MC-LA | MC-RR | Lipid % | Normalized MMPB Yield: |
|---|---|---|---|---|
| 1 | 20 | 20 | 0 | 35% |
| 2 | 30 | 10 | 0 | 39% |
| 3 | 10 | 30 | 0 | 31% |
| 4 | 20 | 20 | 1 | 32% |
| 5 | 30 | 10 | 1 | 34% |
| 6 | 10 | 30 | 1 | 33% |
| 7 | 20 | 20 | 1 | 29% |
| 8 | 30 | 10 | 1 | 32% |
| 9 | 10 | 30 | 1 | 33% |
| 10 | 20 | 20 | 14 | 37% |
| 11 | 30 | 10 | 14 | 33% |
| 12 | 10 | 30 | 14 | 32% |
| 13 | 20 | 20 | 14 | 32% |

# MMPB Application to Fish Tissue – Field Studies

Presently applying the method to field studies

- Spiking tissue may not adequately represent state of bioaccumulated toxins, particularly concentration in organs (esp. liver) or fats

To-date have tested carp from a HABs fish kill in an Ohio lake (negative, possibly rotten) and fathead minnows from an on-site Phosphate dosing study (positive, but no clear trend with phosphate concentration)

Sample collection associated with multiple ongoing research efforts on lakes with endemic HAB activity

| Sample: | Measured MMPB, ug/L | Surrogate Recovery: | Estimated Microcystins, ug/kg |
|---|---|---|---|
| Carp, 100 mg tissue | nd | 86% | nd |
| Carp, 200 mg tissue | nd | 95% | nd |
| Fathead Minnow, 100 mg tissue | < MRL | 80% | < MRL |
| Fathead Minnow, 200 mg tissue | 0.12 | 75% | 15 |

**Fathead minnows after 9 weeks Phosphate dosing**

| Dose mg/L | Stream/Gender | ug/Kg MCs | | Stream/Gender | ug/Kg MCs |
|---|---|---|---|---|---|
| 28 | Stream-03.2_Female | <MRL | | Stream-03.2_Male | <MRL |
| 28 | Stream-07.1_Female | <MRL | | Stream-07.1_Male | <MRL |
| 28 | Stream-08.1_Female | | | Stream-08.1_Male | <MRL |
| 60 | Stream-01.2_Female | <MRL | | Stream-01.2_Male | <MRL |
| **60** | **Stream-03.1_Female** | **19.3** | | **Stream-03.1_Male** | **26.3** |
| 60 | Stream-04.2_Female | <MRL | | Stream-04.2_Male | <MRL |
| 100 | Stream-02.2_Female | <MRL | | Stream-02.2_Male | <MRL |
| 100 | Stream-05.1_Female | <MRL | | Stream-05.1_Male | <MRL |
| 100 | Stream-08.2_Female | <MRL | | Stream-08.2_Male | <MRL |
| 300 | Stream-05.2_Female | <MRL | | Stream-05.2_Male | <MRL |
| **300** | **Stream-06.1_Female** | **60.4** | | Stream-06.2_Male | <MRL |
| 300 | Stream-07.2_Female | <MRL | | Stream-07.2_Male | <MRL |
| **600** | **Stream-01.1_Female** | **10.0** | | Stream-01.1_Male | <MRL |
| **600** | **Stream-04.1_Female** | **31.4** | | **Stream-04.1_Male** | **13.5** |
| 1200 | Stream-02.1_Female | <MRL | | Stream-02.1_Male | <MRL |
| **1200** | **Stream-06.2_Female** | **10.7** | | **Stream-06.1_Male** | **<MRL** |

# Conclusions

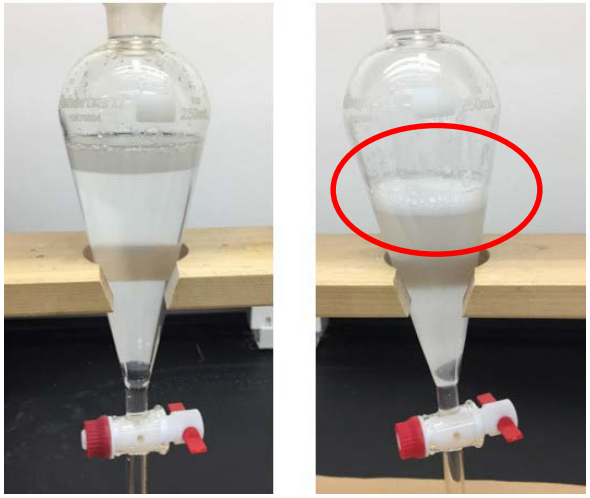
The MMPB technique can be reliably employed for microcystin quantification in fish tissue and appears to perform well with even high lipid content

MMPB method quantitation limits of 0.1 to 100 ug/L MMPB correspond to roughly 1 to 1000 ug/kg MCs, depending on dilution factors/mass balance

> For higher lipid fish samples significant impacts on sample quality are observed – primarily oils and fatty residues following sample processing

On a per-sample basis the labor requirement is significantly higher than for ELISA or conventional LC/MS/MS analysis, as is the initial training requirement

Fathead MC tissue analyses didn't correlate well with the phosphate dosing. Higher concentrations of Phosphate resulted high cyanobacteria periphyton, but MMPB/total MC concentrations were inconclusive. This year we will be measuring ambient concentrations of microcystin via passive samplers to improve the comparison with tissue.



Extraction of a 10 mg fish sample (left) and 100 mg fish sample (right) with "soap" emulsion

Experimental Stream Facility

# Remember What's Good for Wildlife is

# Good for us Also

In memory of a toxicologist
& devoted Fisherman
Mark Smith

Photography by
Jamie Mac Arthur

**Use of passive samplers for the detection of extra cellular algal toxins in Stream mesocosms, lakes and streams** [Contact Dr. Jim Lazorchak (US EPA) for more information: Lazorchak.Jim@epa.gov ]

**Collaborators for Stream Mesocosms**
Jim Lazorchak - NERL
Chris Nietch -NRMRL
Heath Mash – NRMRL
Toby Sanan - NRMRL
Damian Shea – NCSU
Raphe Kudela – UC Santa Cruz
Meredith Howard – SCCWRP

**Collaborators for Lakes & Streams**
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Joel Allen – NRMRL
Chris Nietch - NRMRL
Allen Lindquist – NRMRL
Toby Sanan – NRMRL
Jim Lazorchak – NERL
Damian Shea – NCSU
Raphe Kudela – UC Santa Cruz
Meredith Howard – SCCWRP
Someone from the country or OEPA (Heather Raymond)

**Objective of Project**

To test out 2 types of passive sampler devices, Solid Phase Adsorption Toxin Testing (SPATT bags) and Large Format non selective Passive Sampler Device (LF nsPSD) to determine their performance in measuring extracellular algal toxins in artificial stream mesocosms, a project lake that is experiencing annual algal blooms Harsha Lake, and in downstream habitats of Harsha lake.

**Approach**

**Stream Mesocosms**

Deploy both PSDs in 3 of the head and all 16 of the tail tanks during the 2018 nitrate/phosphate dosing study. See if both samplers can include an internal standard. The 16 streams will be divided up into replicates for control (undosed) and treatment streams. That will mean 2-3 replicate streams per treatment. Both PSDs will be deployed on a biweekly basis throughout the study period. Starting 2 weeks prior to dosing during the colonization period and then during an 8 week dosing period. That would come out to 5 biweekly sampling events. 16 biweekly with a duplicate for each treatment (usually 3 doses plus control). During each deployment a water sample will be collected from each stream weekly for a grab sample Microcystin analyses Total Microcystin using MMPB and specific congener analyses.

Fathead minnows will be deployed as in previous studies. At the end of the experiment the surviving fatheads will be retrieved and frozen for total Microcystin analyses. Fatheads will be analyzed with and without gut contents in order to determine if fish muscle has accumulated microcystin vs gut/liver tissues.

The purpose of this portion of the study will be to evaluate PSD performance by relating PSD concentration to grab sample concentration and to determine a bioconcentration factor (BCF) for microcystin by comparing fathead minnow tissue concentrations to PSD and grab sample concentrations.

**Anticipated sample #s**

**Mesocom Study**

Biweekly

20 head tank SPATTs (5 sampling events for 3 streams with 1 duplicate)
20 head tank LS nsPSD (5 sampling events for 3 streams with 1 duplicate)
100 tail tank SPATTs (5 sampling events for 16 streams with 4 duplicates)
100 tail tank LS nsPSD (5 sampling events for 16 streams with 4 duplicates)

240 analyses (120 SPATTs & 120 LS nsPSDs)

200 grab water samples from tail tank (10 sampling events 16 streams with 4 duplicates)
40 grab water samples from head tank 5 sampling events 3 streams with 1 duplicate)

240 analyses
Total analyses for both PSDs and water = 480 analyses

Roles of Collaborators

Jim and Heath will coordinate deployment and retrievals of PSDs and water sampling

Jim will be responsible for collection of fathead minnows at the end of the study for tissue analyses

Raphe and Meredith participate in study by construction of SPATTS needed for study
Damian participate in study by construction of a smaller LF nsPSD

Heath and Toby responsible for chemical analyses of water, passive samplers and tissues.

**Harsha Lake and River sites East Fork Downstream of Harsha Lake**

Starting in May 2018, SPATT and LF-nsPSDs will be deployed at the Harsha Lake Buoy site at 1-meter and 10-meter depths from the surface. SPATTs will also be deployed at other various depths from the surface to just above the lake bottom. Water samples will be collected at each of the passive samplers' deployments at the time of deployment and time of retrieval. Deployment durations will likely be for two week intervals, except during blooming conditions when deployments will likely be at one week intervals.

| May-16 | May-30 | June-13 | June-27 | July-11 | July-25 | Aug-8 | Aug-22 | Sept-5 | Sept-19 |
|---|---|---|---|---|---|---|---|---|---|
| 0/0/2 | 2/2/2 | 4/4/4 | 4/4/4 | 4/4/4 | 4/4/4 | 2/2/2 | 2/2/2 | 2/2/2 | 2/2/2 |

| | |
|---|---|
| | Deployment and Water Sampling |
| | Retrieval, Water Sampling, Deployment (Weekly Sampling) |
| | Retrieval, Water Sampling, Deployment (Bi-weekly Sampling) |
| | Retrieval and Water Sampling |

Collect water samples at deployments and retrievals June, July, August, September = 16 weeks

Anticipated # of samples

26 SPATT samples
26 LF-nsPSDs

28 water samples

Total 80 samples

**River sites to be sampled biweekly**

Site 1 Tailwater site (ID = DAMM) historical site that has been sampled biweekly
Site 2 A site off of 222 that is easily accessible downstream of the Middle East Fork Waste Water Treatment Plant
Site 3 A location at the South Milford Rd. Bridge site (ID=EFC), which is essentially the mouth of the EFLMR but it far enough upstream of the mouth to not experience backwater flows from the LMR (we sample there every other week).
Site 4 ESF Main River inflow monitoring station (include duplicates)

Conduct bi-weekly deployments at 4 sites starting in mid-May 2018 (Ex. Wednesdays, bi-weekly).

# of analyses if biweekly
25 20 SPATTs + 5 duplicates
25 20 LF nsPSDs + 5 duplicates
40 water samples plus 5 duplicates

95 total analyses

Roles of Collaborators

Heath and Joel will coordinate sample locations and deployments/retrievals of lake PSDs and water samples

Jim will be responsible for deployments and retrievals of downstream PSDs and collection of water samples

Raphe and Meredith participate in study by construction of SPATTS for downstream SPATTs
Damian participate in study by construction of a smaller LF nsPSD for lake and downstream

Summary of samples across the 3 study sites (Mesocosms, Lake, & River)

**Mesocosm = PSDs plus water = 480**

120 SPATTs + 120 LF nsPSD = 240
Water samples = 240

**Lake sites = PSDs plus water = 80**

26 SPATTs + 26 LF nsPSDs
Water Samples = 28

**River Sites = 95**

25 SPATTs + 25 LF nsPSDs
Water samples = 45

**Total = 660**

176 SPATTs
176 LF nsPSDs

308 water samples