2021 ODU Freshwater Summary

Kathryn Mogatas

Old Dominion University Phytoplankton Analysis Laboratory

VA HAB Taskforce Meeting
Gloucester Point, Virginia

02/25/2022

OLD DOMINION
UNIVERSITY

2021 Program Changes

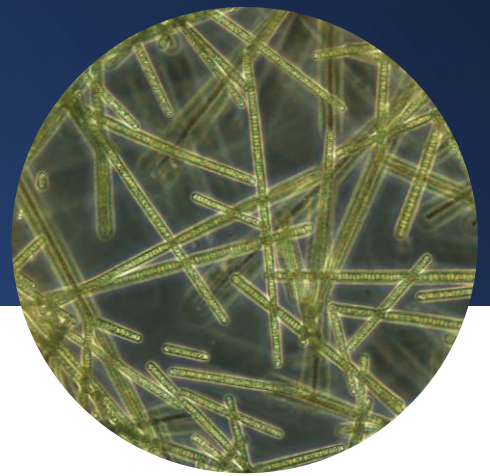
- HAB report numbers included in reporting form
- Staff changes
- Toxin sample batching
- Benthic sample processing

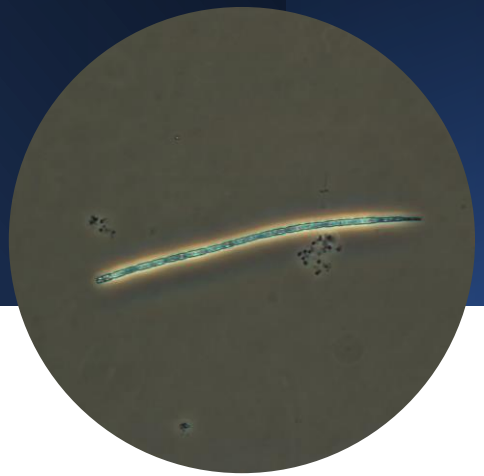
Overview

Samples Received through VDH contract
457 Shellfish monitoring
122 Rapid HAB response
- 9 samples from tidal waters
- **113** inland freshwater samples

Freshwater breakdown
Samples received 113
- **449** analyses conducted
- 113 Taxonomic enumeration
- 84 Microcystin (MC) by ELISA
- 84 Cylindrospermopsin (CYL) by ELISA
- 84 Anatoxin-a (ATX) by ELISA
- 84 Saxitoxin (STX) by ELISA

Freshwater Bloom Analysis



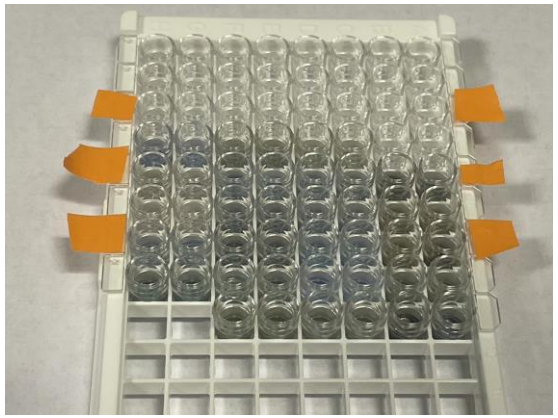


Taxonomic enumeration

- Scan is conducted to identify dominant species
- Individual cells of target species are counted to determine an estimate of density
- Results reported in cells/ml

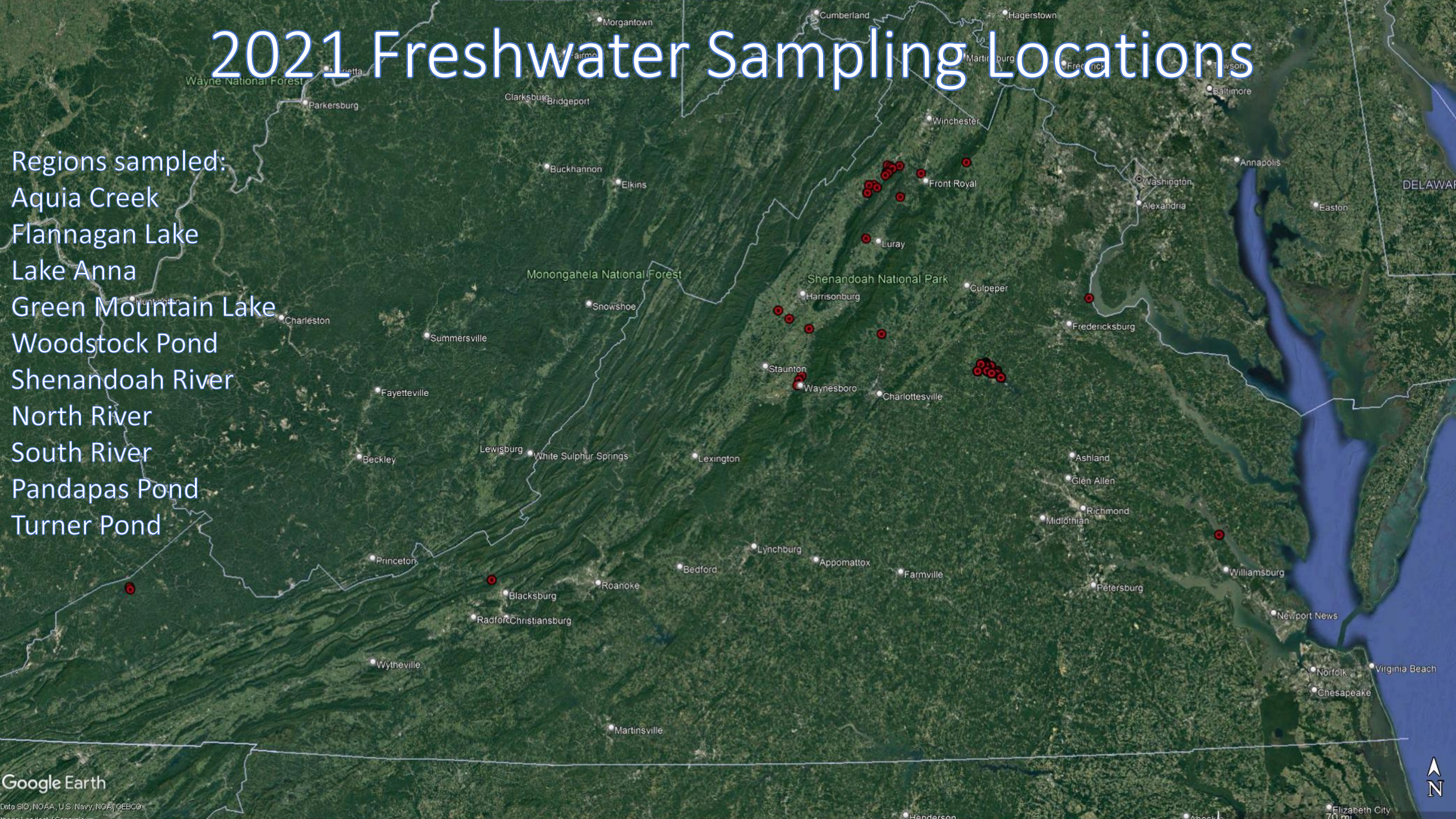
Freshwater Bloom Analysis

- Toxin assays
- Analyzed using Enzyme Linked Immunosorbent Assay (ELISA)



Assay	Range	Advisory Threshold
Eurofins Abraxis Microcystins (ADDA) ELISA	0.15ppb-5.0ppb	≥8.0 ppb
Eurofins Abraxis Cylindrospermopsin ELISA	0.05ppb-2.0ppb	≥15.0ppb
Eurofins Abraxis Anatoxin-a ELISA	0.15ppb-5.0ppb	≥8.0ppb
Eurofins Abraxis Saxitoxin ELISA	0.02ppb-0.4ppb	≥4.0ppb

2021 Freshwater Sampling Locations



Overview of maximum cell count of dominant PTOX for each Inland Freshwater HAB response region

Region	Cells/ml	Dominant PTOX
Aquia Creek	139,600	*Sphaerospermopsis spp.*
Flannagan Lake	0	none observed
Lake Anna	5,641,400	*Dolichospermum spp.*
Green Mountain Lake	0	none observed
Woodstock Pond	0	none observed
Shenandoah River	7,000	*Planktothrix sp.*
North River	1,900	*Jaaginema sp.*
South River	0	none observed
Panadapas Pond	5,296,900	*Planktothrix isothrix*
Turner Pond	800	*Microcystis spp.*

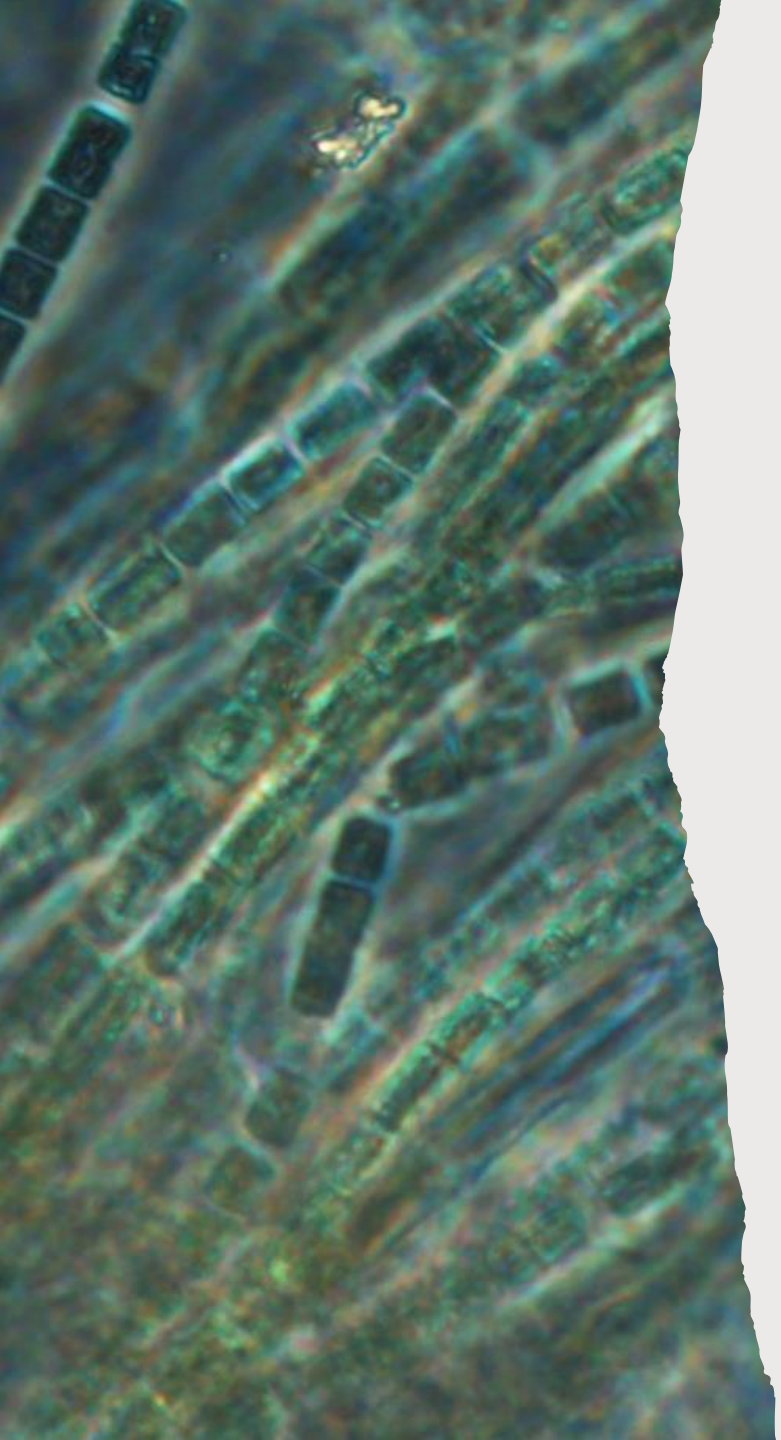
Highest cyanotoxin result in ambient recreation water in each sampled region excluding benthic samples (ppb)

Region	MC	CYL	ATX	STX
Aquia Creek	3.72	bdl	bdl	0.02
Flannagan Lake	not run	not run	not run	not run
Lake Anna	1.01	bdl	1.46	0.11
Green Mountain Lake	not run	not run	not run	not run
Woodstock Pond	not received	not received	not received	not received
Shenandoah River	bdl	bdl	1.77	bdl
North River	bdl	bdl	bdl	bdl
South River	bdl	bdl	bdl	bdl
Panadapas Pond	bdl	bdl	bdl	bdl
Turner Pond	bdl	bdl	bdl	bdl

Benthic samples collected on the Shenandoah River by site with cyanotoxins

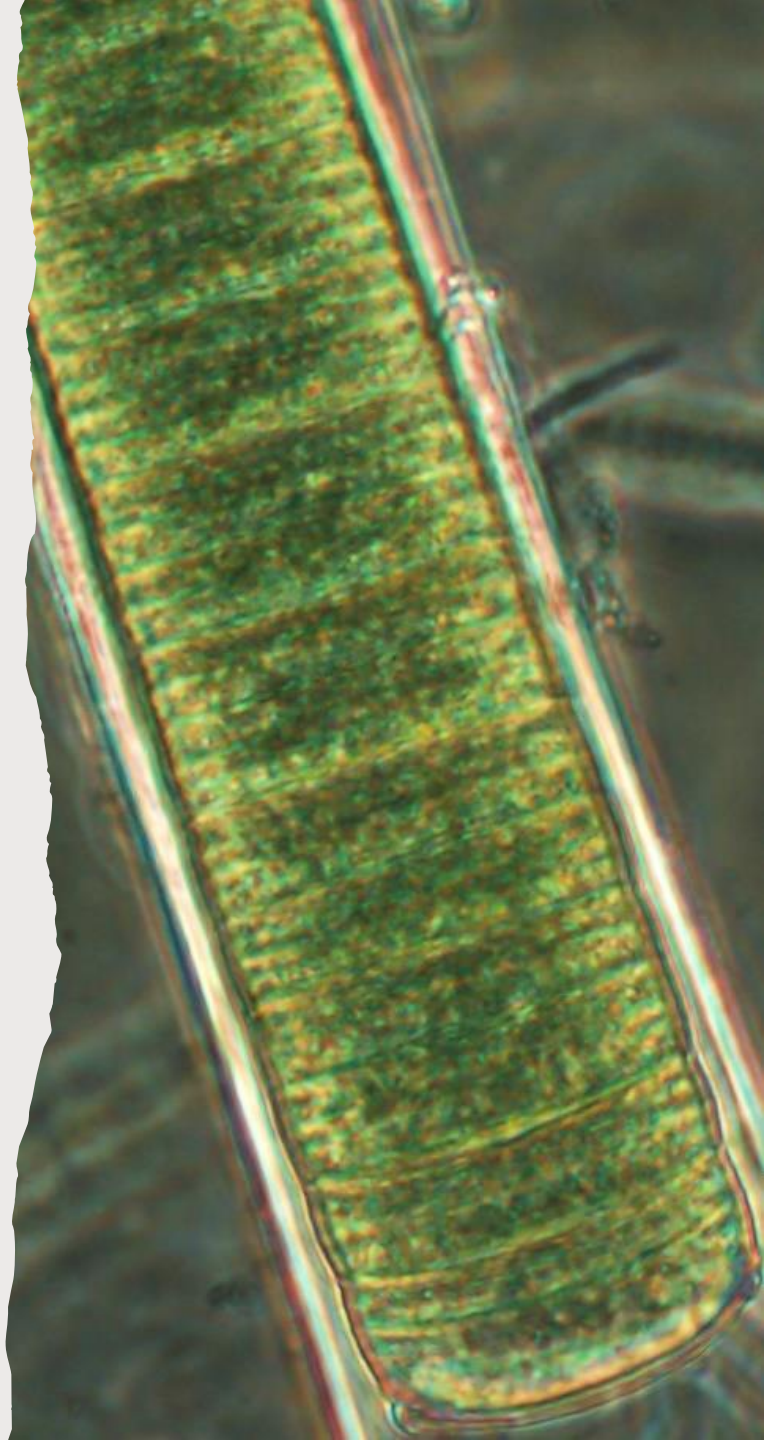
Toxin	Result Range ppb
MC	1.69-4.31
CYL	0.06-0.15
ATX	64.45-2804.00
STX	bdl-2.45

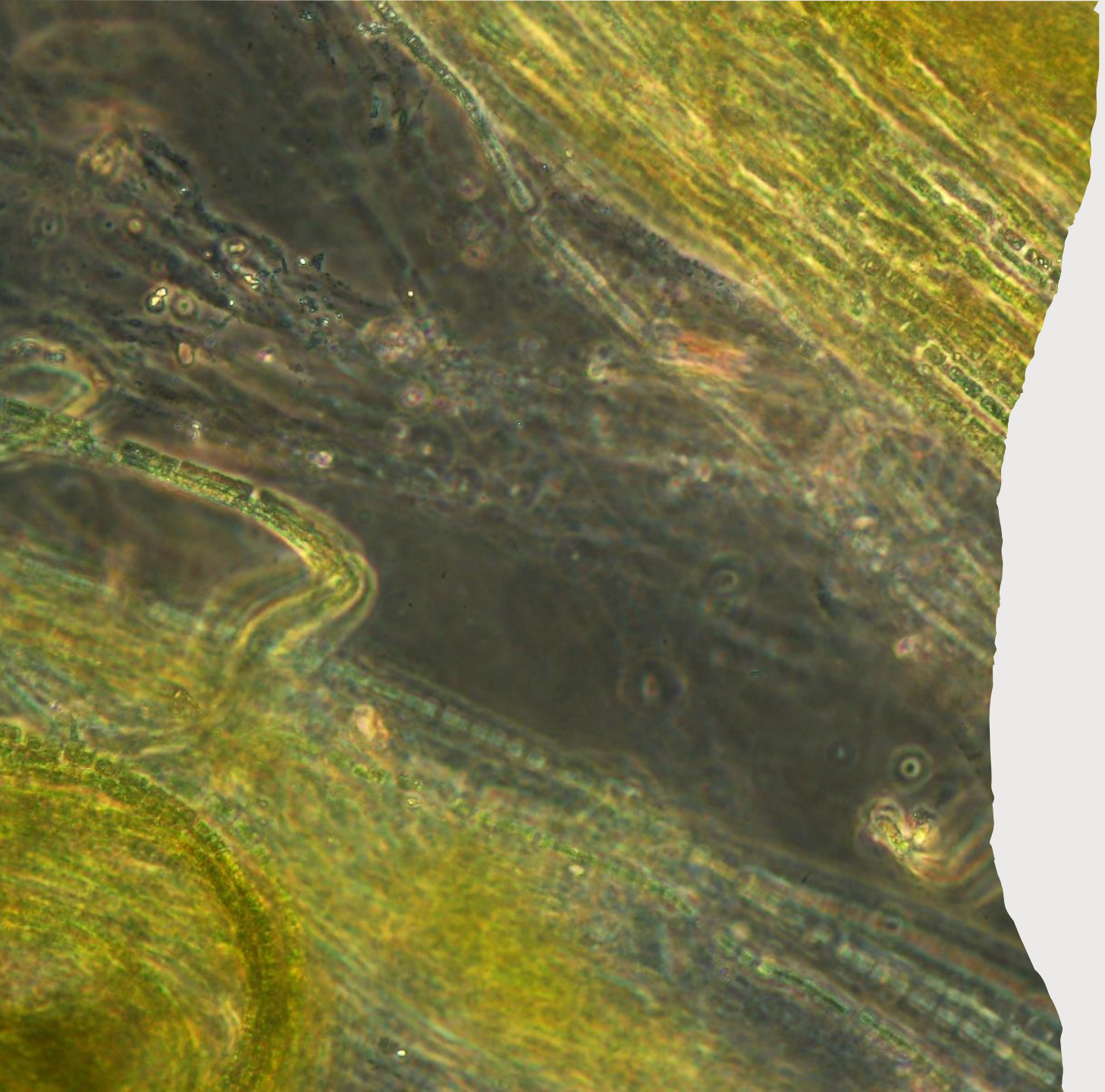
*These results are not intended to be compared with the advisory water thresholds; data are semi-quantitative only noting presence/absence of cyanobacteria community. Units in ppb.



Shenandoah River

- 34 Samples from the Shenandoah River received
  - 21 of which were ambient water samples while 13 were benthic
- Cell counts never exceeded 100,000 cells/ml for any PTOX cyanobacteria in ambient water samples
- No cells counts for benthic samples, only identification





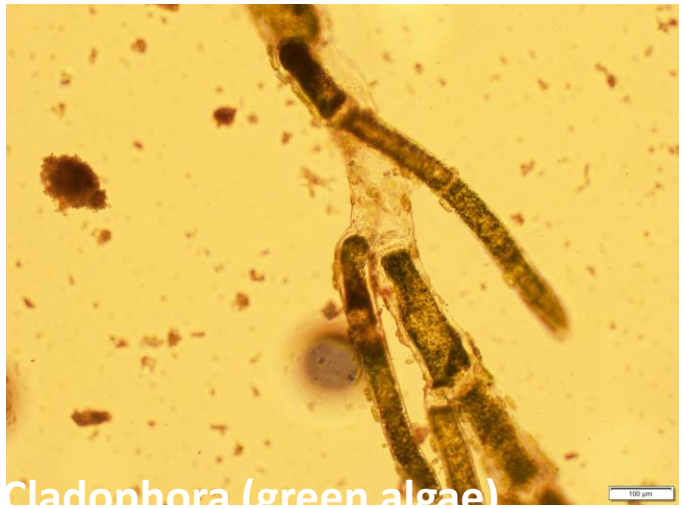
Shenandoah River

- *Microcoleus sp*. Bloom which formed visible benthic algal mats

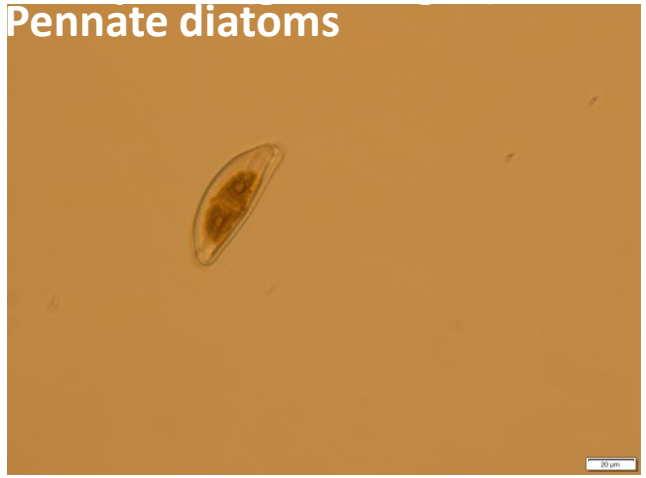
- With associated epiphytic taxa including:
  - *Oscillatoria spp.*
  - *Phormidium spp.*
  - *Lyngbya spp.*
  - *Planktothrix spp.*

- Water column samples composed of diatoms and Cladophora (a green algae)

June 2021

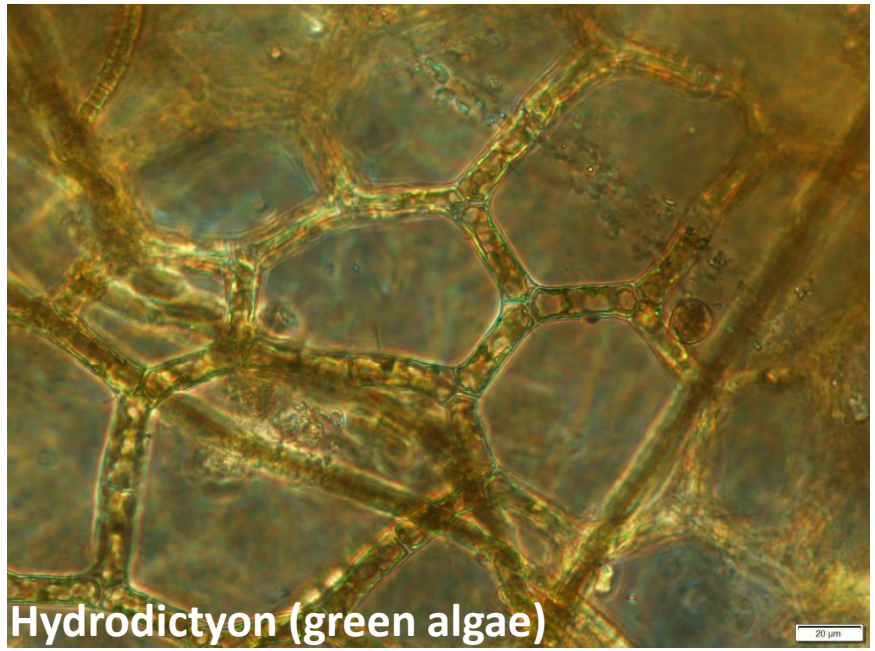


Cladophora (green algae)

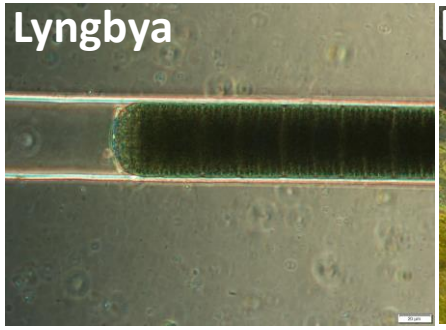


Pennate diatoms

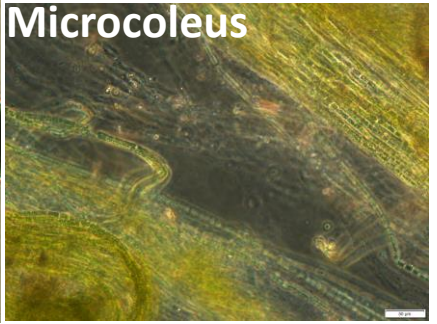
July 2021



Hydrodictyon (green algae)

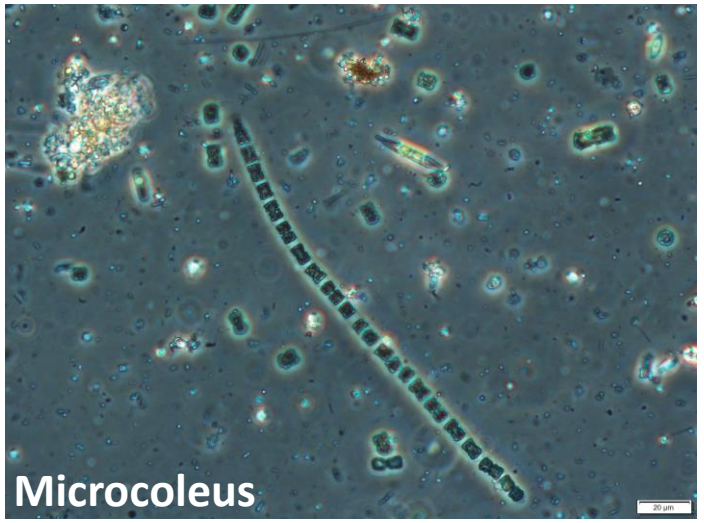


Lyngbya

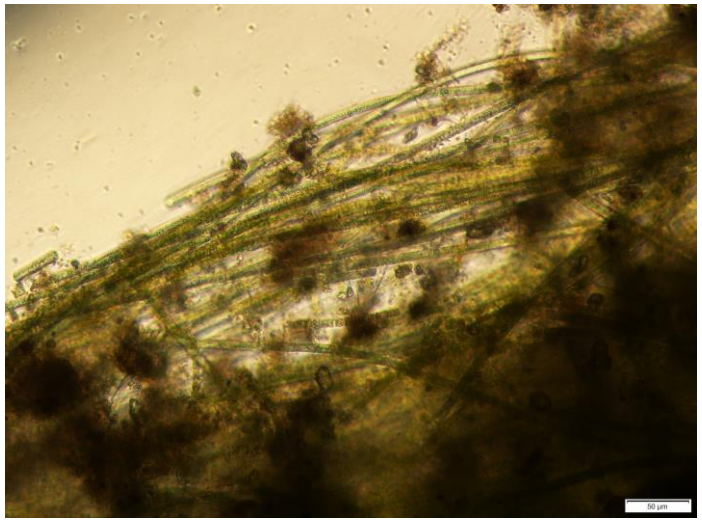


Microcoleus

August 2021



Microcoleus



Shenandoah By Month In Pictures

Benthic Protocol

Remove small portion for mat ID

Centrifuge excess water from algae mat

Weigh aliquot of mat

Freeze/lyse in -80 freezer

Thaw combine with Milli-Q water (1ml:1g of mat)

Homogenate mixture with mini-food processor

Freeze/thaw lyse -80

Second blend with mini-food processor

Freeze/thaw lyse -80 (3x total)

Centrifuge, run ELISA analyses on supernatant

Previously developed to handle *Microseira wollei* cyanobacterial mats on Lake Gaston, new opportunity to test it on *Microcoleus* mats

Found Anatoxin-a present in the mats from the Shenandoah River

*All work conducted in fume food with gloves, lab coat, and face shield

Lessons Learned

- Our method did detect anatoxin-a present in the mat samples, but we have room to improve our methods!

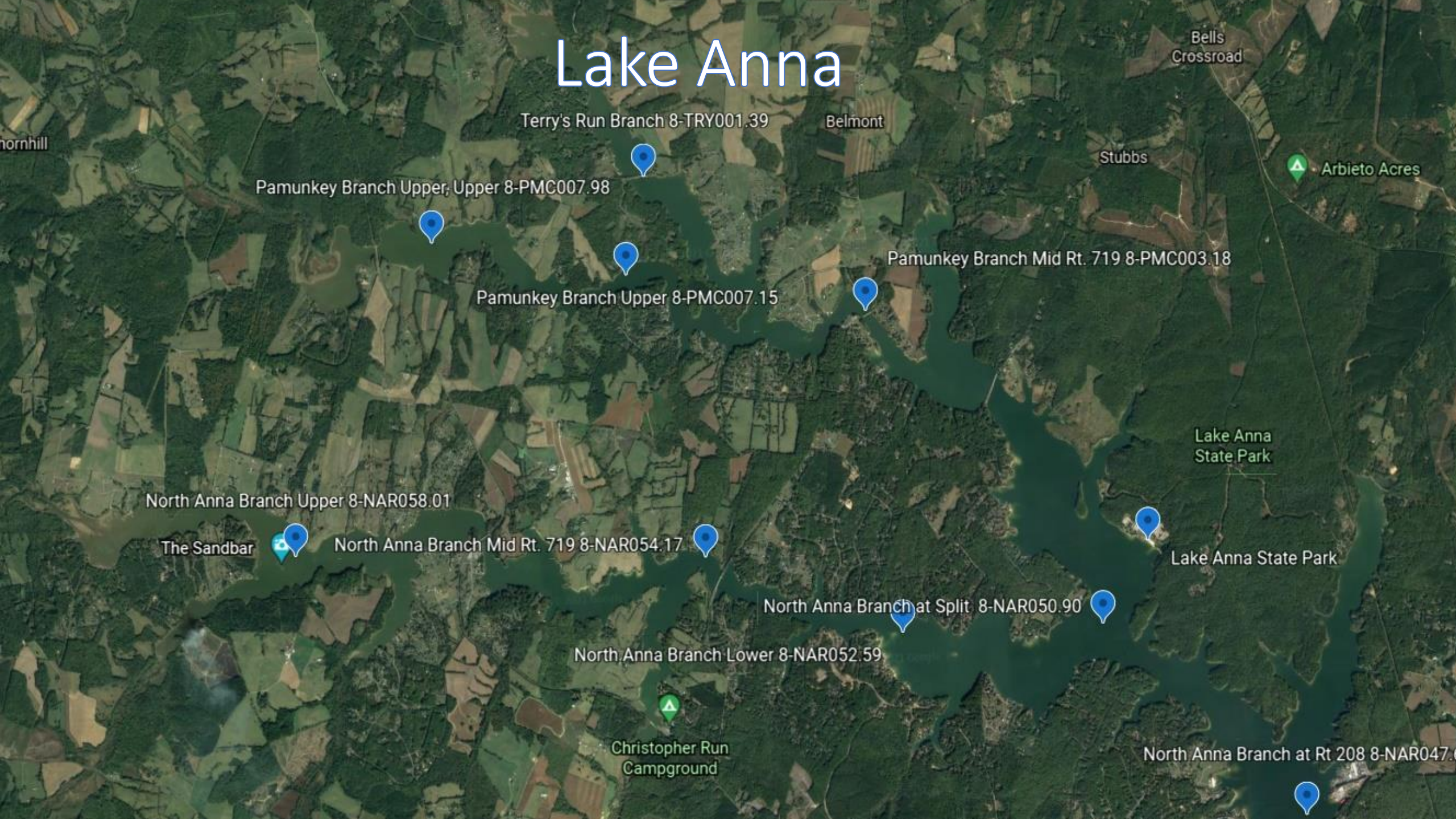
  Margaret Smigo presented the method at US EPA Benthic HAB workgroup meeting (Dec 2021), workgroup members had methodology feedback

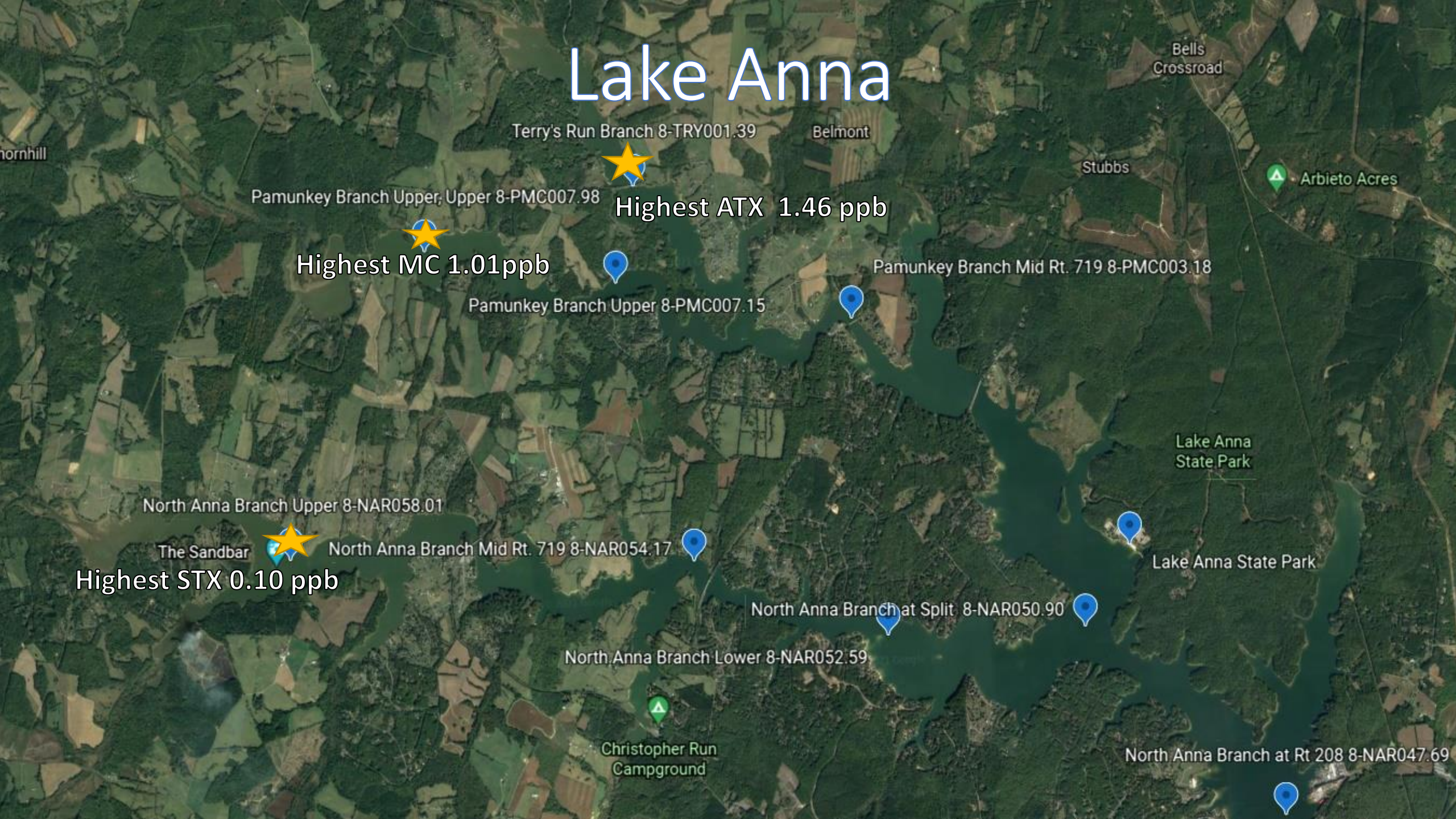
- Experienced some health symptoms after handling the material:

  Muscle weakness/headache/dizziness

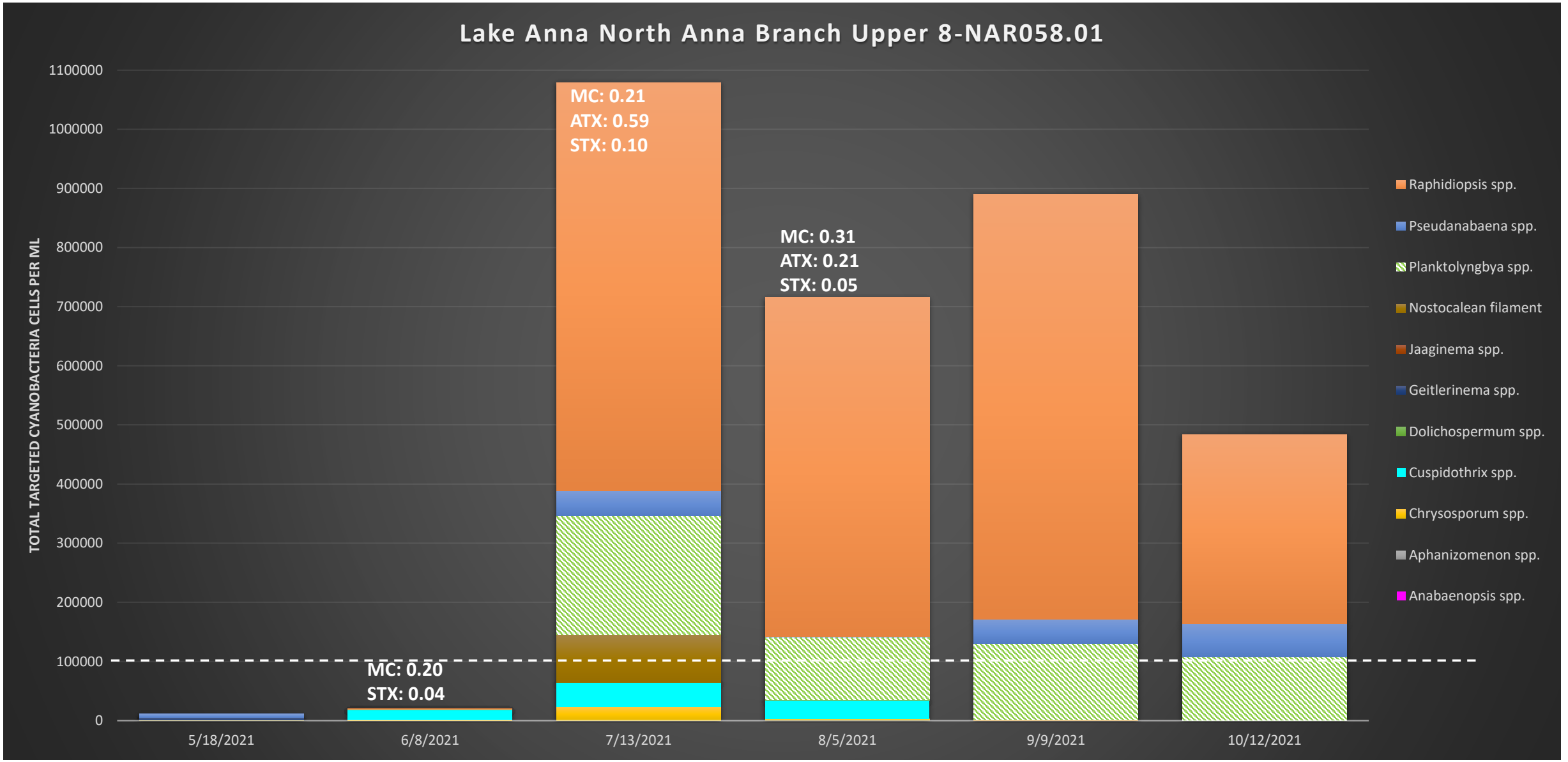
- We no longer will accept benthic mat material

Lake Anna


Bells Crossroad
Terry's Run Branch 8-TRY001.39
Belmont
hornhill
Stubbs
Arbieto Acres
Pamunkey Branch Upper, Upper 8-PMC007.98
Pamunkey Branch Mid Rt. 719 8-PMC003.18
Pamunkey Branch Upper 8-PMC007.15
Lake Anna State Park
North Anna Branch Upper 8-NAR058.01
The Sandbar
North Anna Branch Mid Rt. 719 8-NAR054.17
Lake Anna State Park
North Anna Branch at Split 8-NAR050.90
North Anna Branch Lower 8-NAR052.59
Christopher Run Campground
North Anna Branch at Rt 208 8-NAR047.

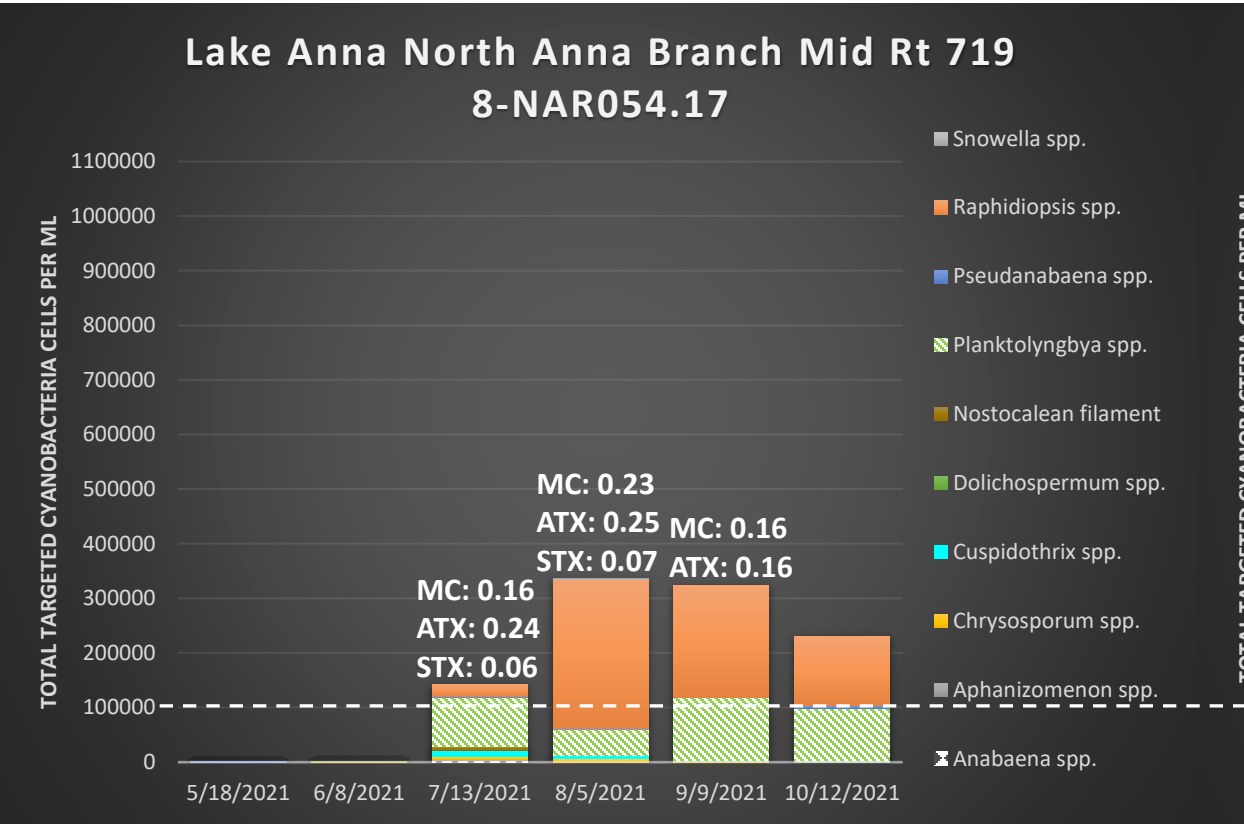

Lake Anna
Terry's Run Branch 8-TRY001.39
Belmont
Bells Crossroad
Stubbs
Arbieto Acres
Pamunkey Branch Upper, Upper 8-PMC007.98
Highest ATX 1.46 ppb
Highest MC 1.01ppb
Pamunkey Branch Mid Rt. 719 8-PMC003.18
Pamunkey Branch Upper 8-PMC007.15
Lake Anna State Park
North Anna Branch Upper 8-NAR058.01
The Sandbar
North Anna Branch Mid Rt. 719 8-NAR054.17
Highest STX 0.10 ppb
Lake Anna State Park
North Anna Branch at Split 8-NAR050.90
North Anna Branch Lower 8-NAR052.59
Christopher Run Campground
North Anna Branch at Rt 208 8-NAR047.69

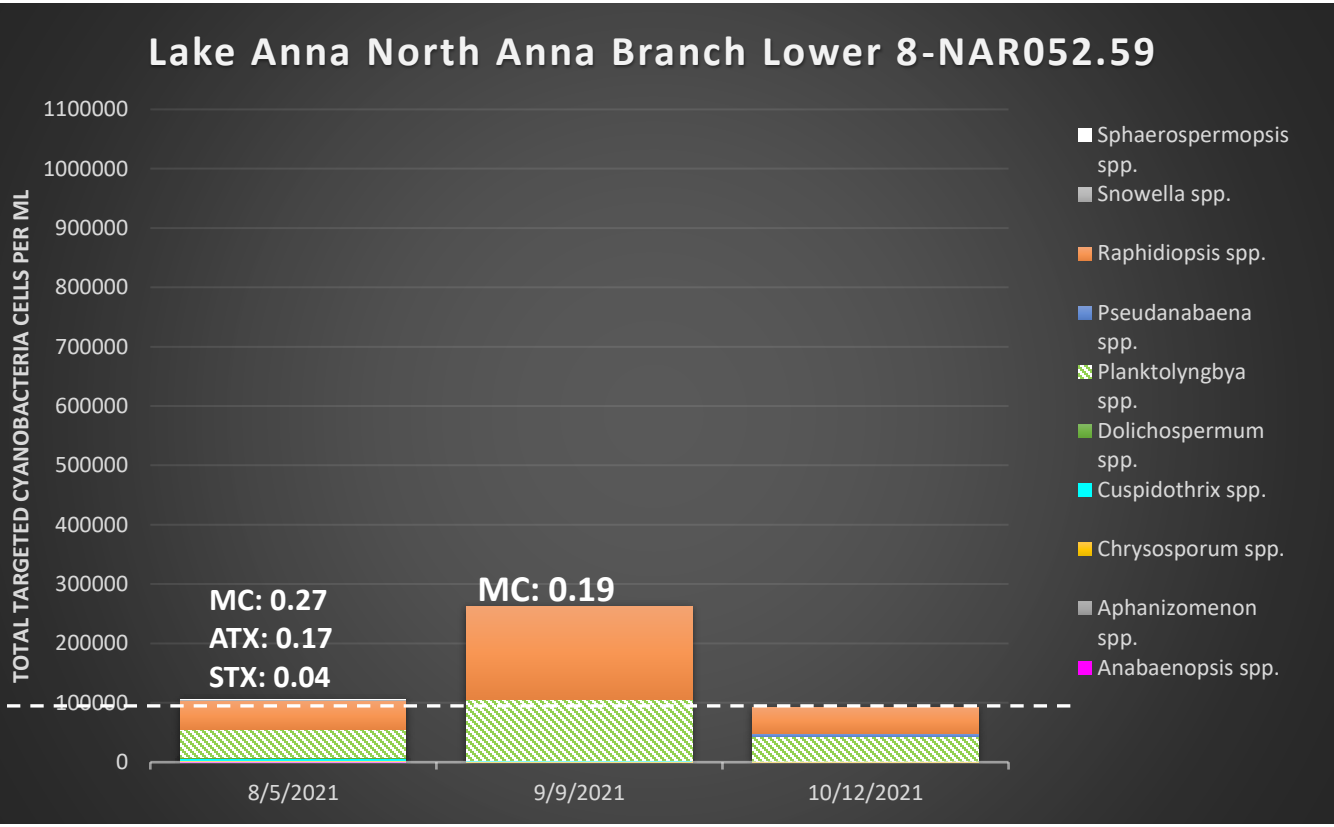
Lake Anna North Anna Branch



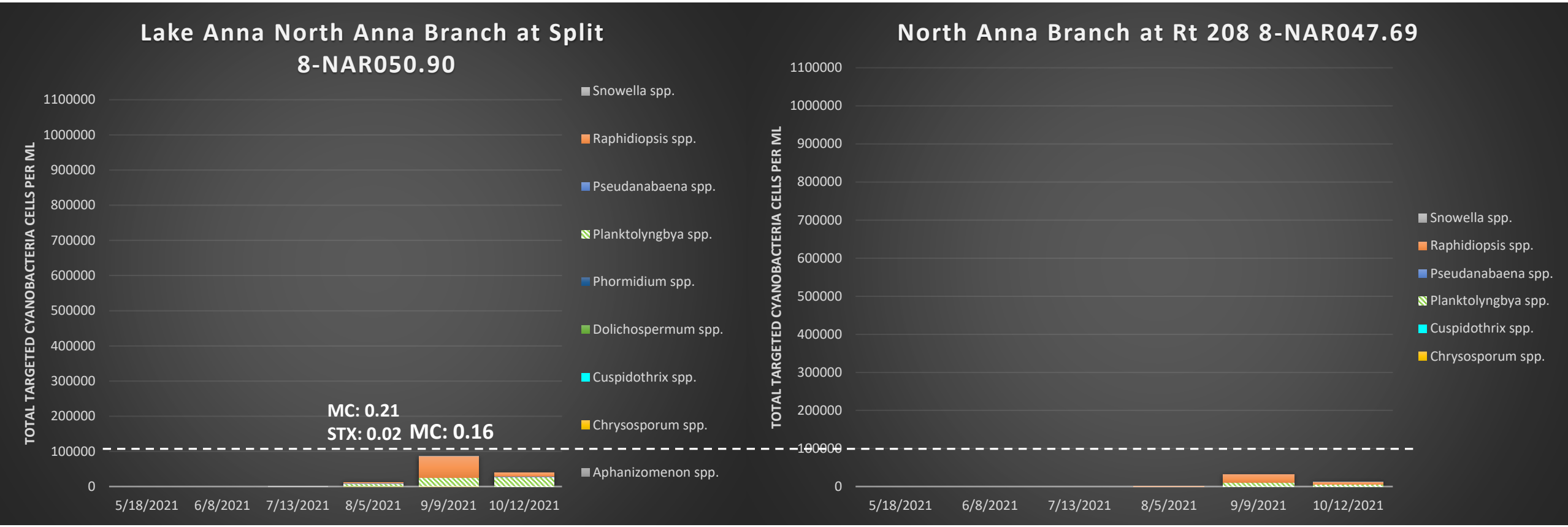
*All toxin results reported in ppb

Lake Anna North Anna Branch



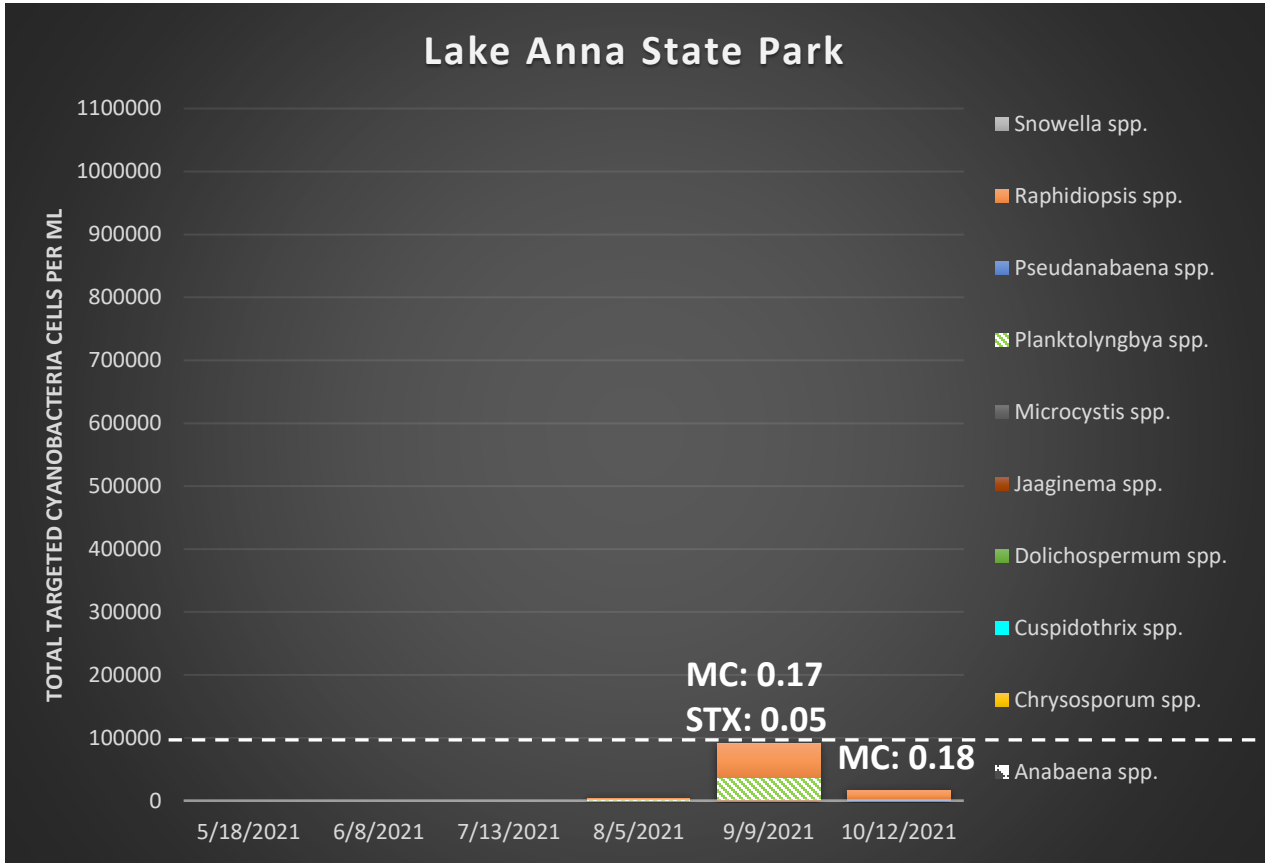


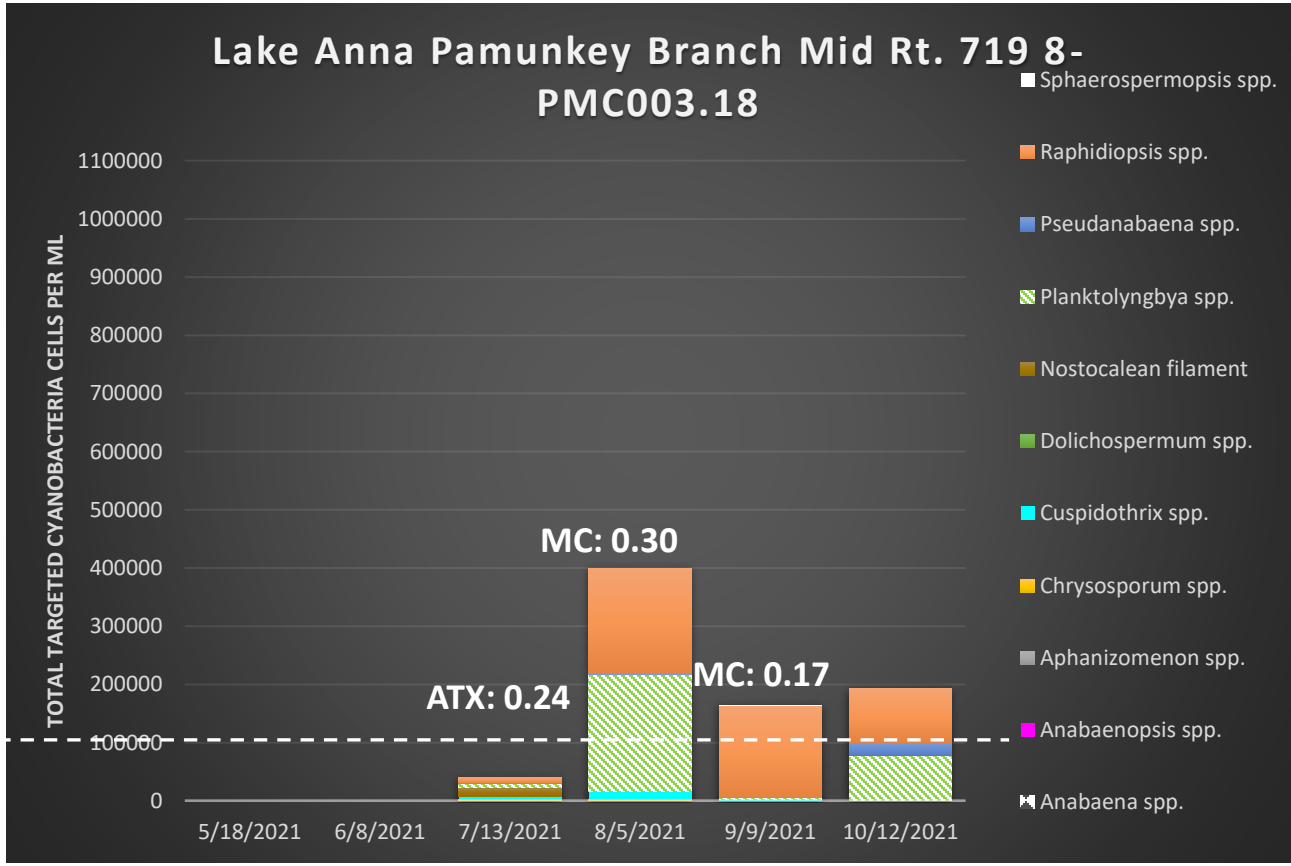
Lake Anna North Anna Branch



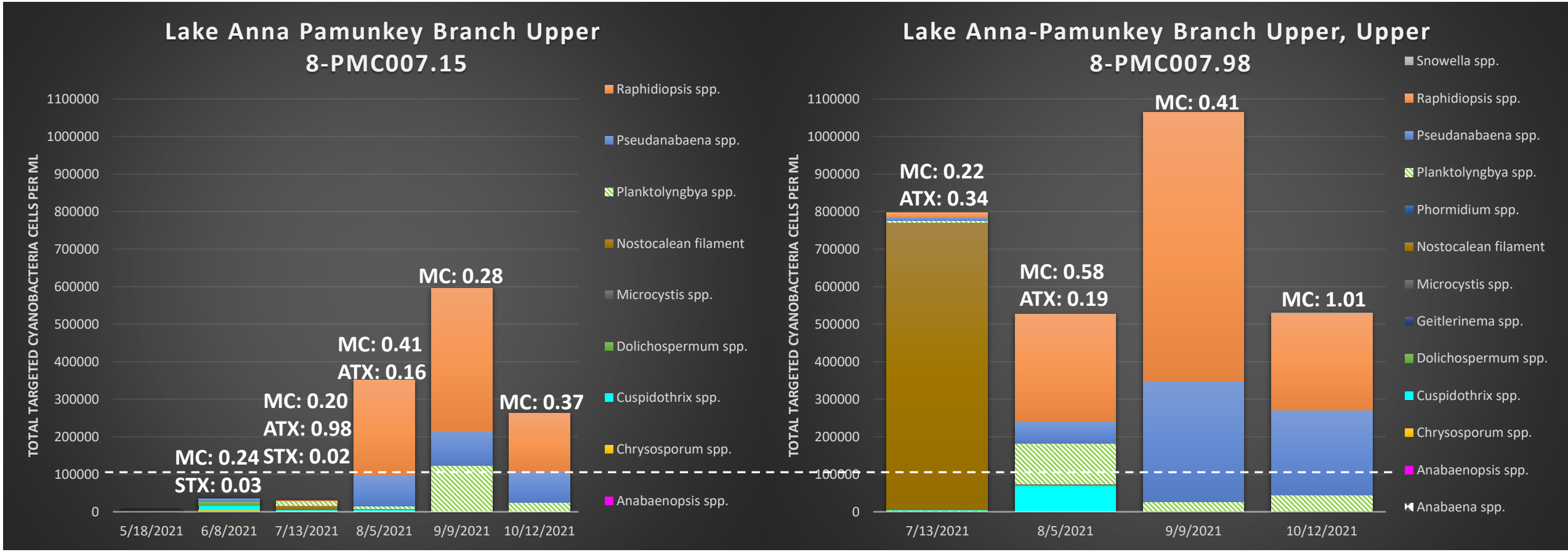
North Anna Branch at Split 8-NAR050.90 and North Anna Branch at Rt 208 8-NAR947.69 all samples below 100,000 cells/ml PTOX

Lake Anna State Park into Pamunkey Branch

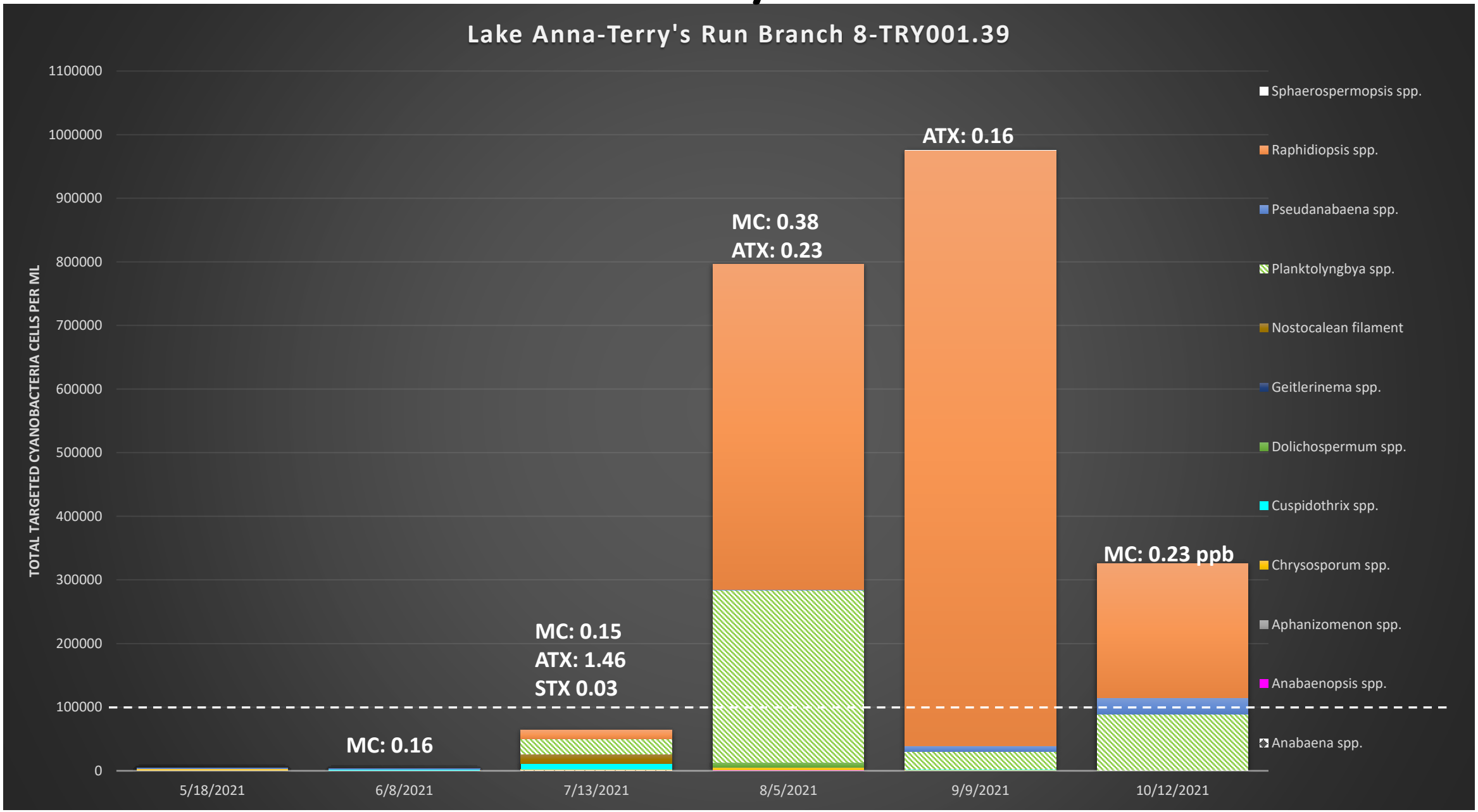




Lake Anna Pamunkey Branch



Lake Anna Terry's Run Branch



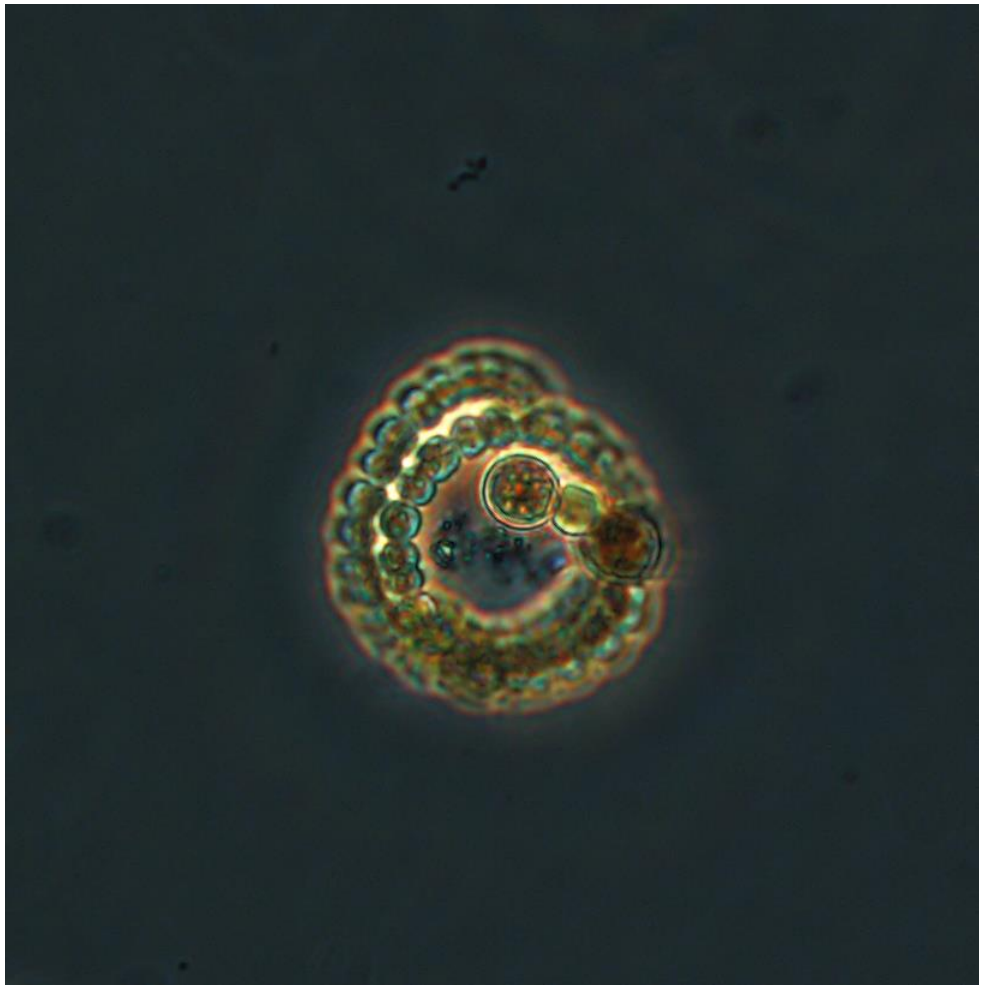
Aquia Creek Widewater

7/14/2021

Sphaerospermopsis spp.

139,600 cells/ml

MC: 3.72 ppb
STX: 0.02 ppb



Follow up 9/7/2021

Total PTOX below 100,000
1,300 cells/ml total; highest taxa *Phormidium* spp. 400 cells/ml

MC: 0.49 ppb
STX: 0.03 ppb

Pandapas Pond

8/10/2021

Planktothrix isothrix bloom

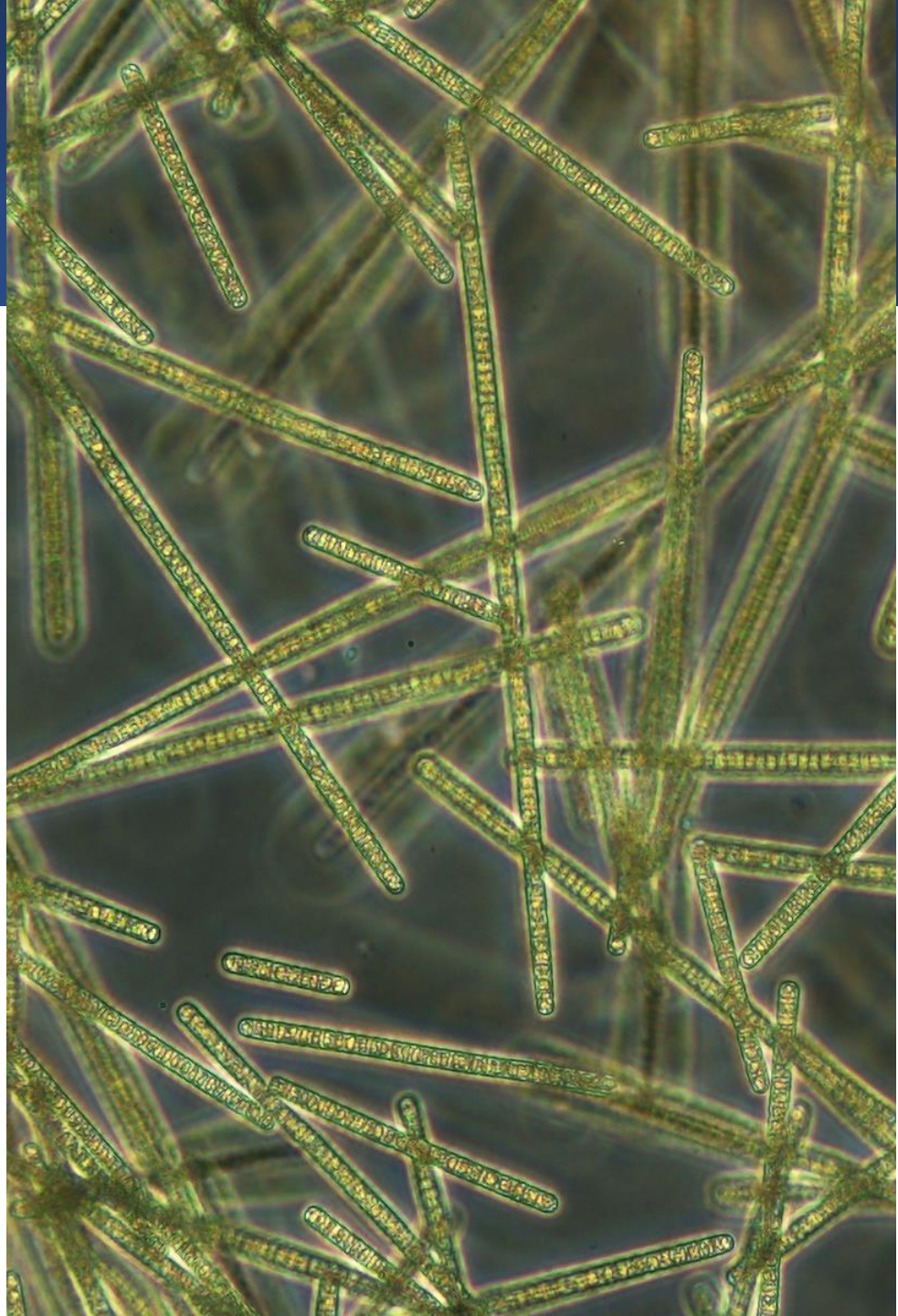
~5,000,000 cells/ml

All toxins below detection

Follow up 9/2/021

Planktothrix isothrix bloom gone

All toxins below detection



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Todd Egerton

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Contact:
kwiesner@odu.edu
phytolab@odu.edu