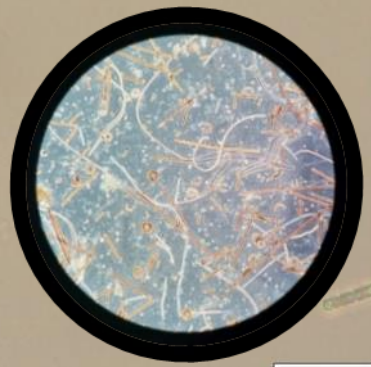
2019 ODU Freshwater Summary

Leah Anne Gibala-Smith, M.S.
Phytoplankton Analysis Laboratory
Old Dominion University

2019 Freshwater Overview

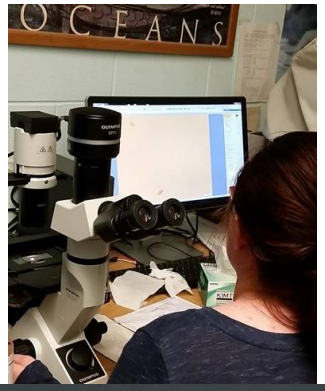
- **13 inland freshwater bloom investigations**
- **4/9/2019 for taxonomy (4/15/2019 for toxins) thru 10/14/2019**
- **141 freshwater bloom samples received**
- **465 analyses conducted**
  - **139 taxonomic enumerations**
  - **132 microcystins by ELISA**
  - **132 cylindrospermopsin by ELISA**
  - **28 anatoxin-a by ELISA**
  - **29 saxitoxin by ELISA**
  - **5 mc/cylindro by MBIO**

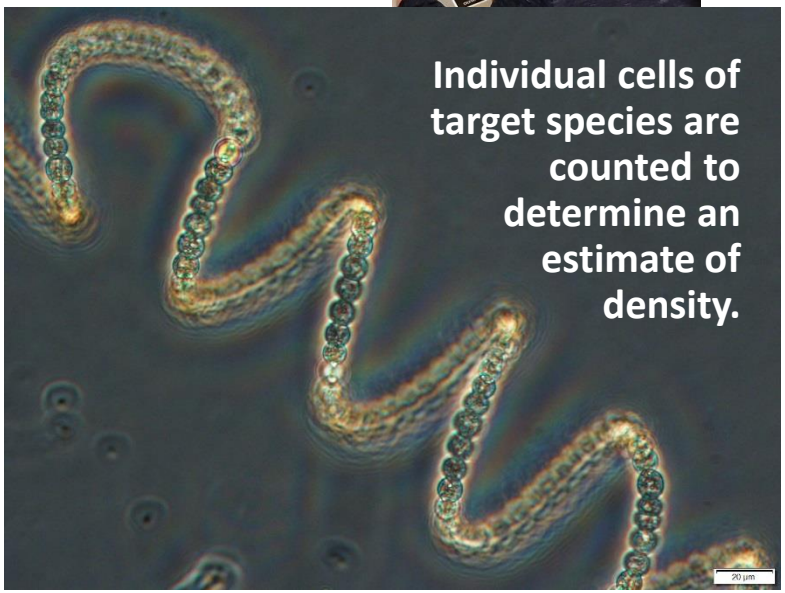


Freshwater Bloom Analysis

Taxonomic enumeration

Scan is conducted to identify dominant species.





Toxin assays

Abraxis microcystins (ADDA) ELISA

8.0 ppb 0.15ppb* - 5.0ppb (higher w/dilution)

*after 6/4/2019 ldl 0.30ppb

Abraxis cylindrospermopsin ELISA

15.0 ppb 0.05ppb - 2.0ppb (higher w/dilution)

Abraxis anatoxin-a ELISA

0.15ppb - 5.0ppb (higher w/dilution)

Abraxis saxitoxin ELISA

0.02ppb - 0.4ppb (higher w/dilution)

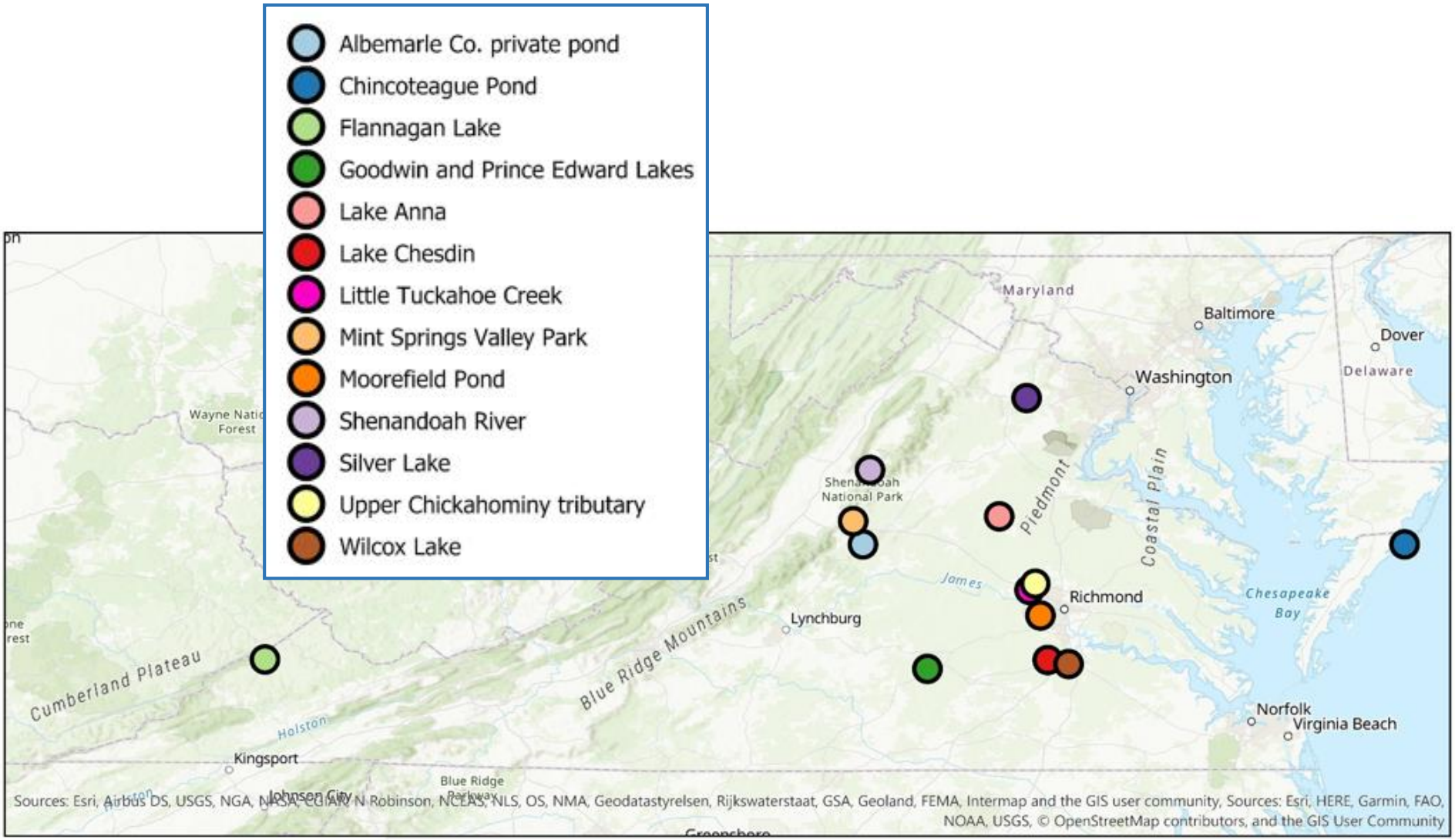
MBIO microcystins

0.4ppb - 3.1ppb (higher w/dilution)

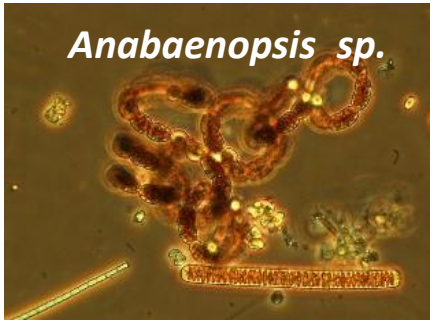
MBIO cylindrospermopsin

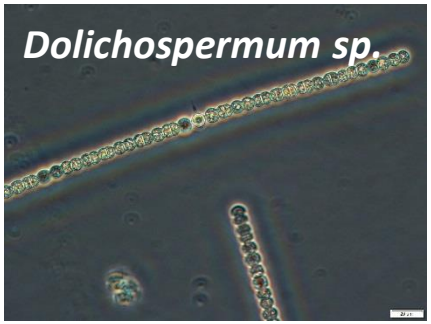
0.7ppb - 2.7ppb (higher w/dilution)

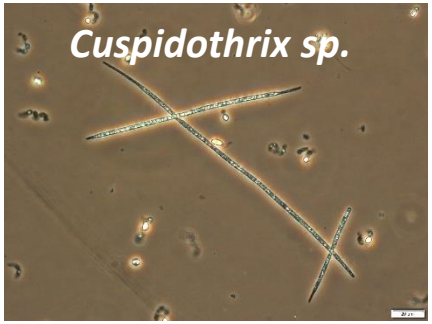
2019 Freshwater Blooms

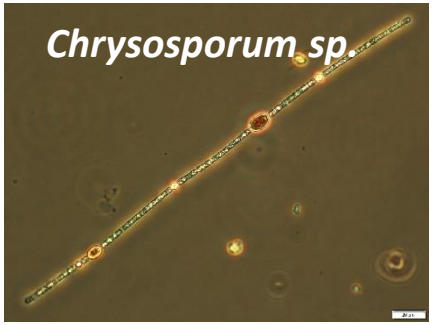


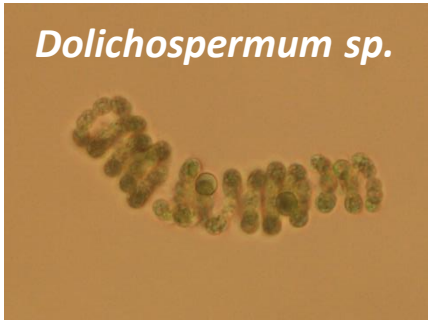
Cyanobacteria taxa groups

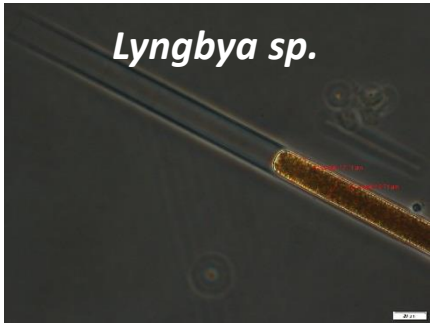










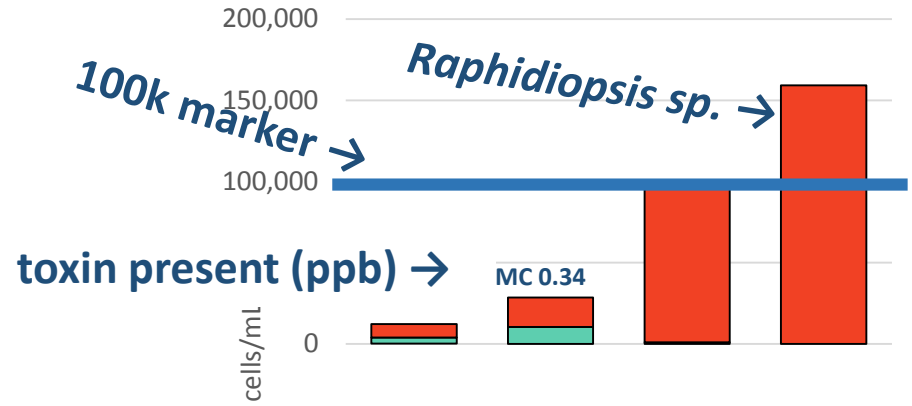


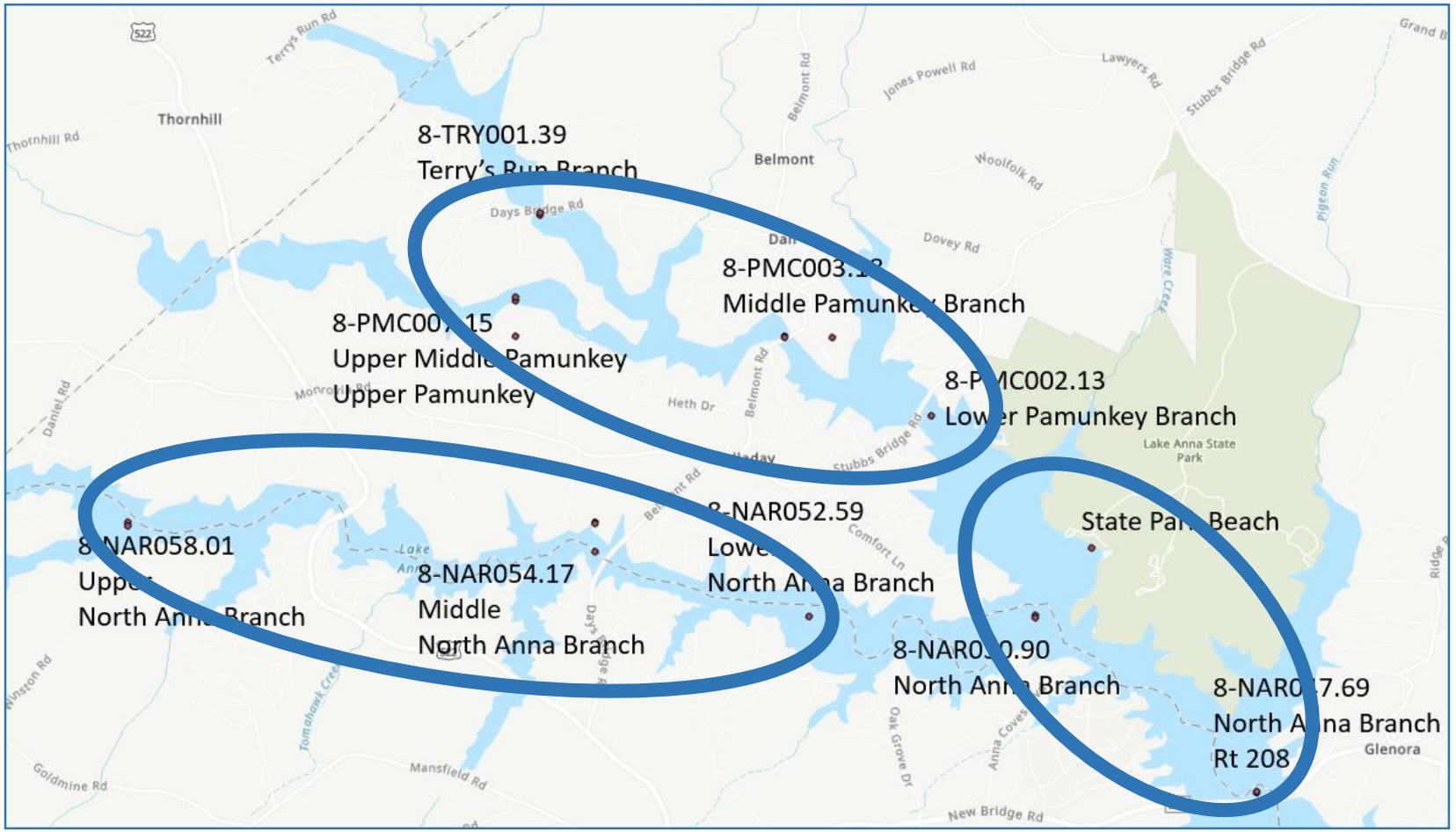
Cyanobacteria taxa groups

- Group 10 (Phormidium, Microcoleus, Arthrospira)
- Group 9 (Oscillatoria, Limnothrix)
- Group 8 (Woronichinia)
- Group 7 (Planktothrix)
- Group 6 (Microcystis)
- Group 5 (Microseira, Lyngbya)
- Group 3 (Raphidiopsis, Cylindrospermopsis)
- Group 2 (Aphanizomenon, Chrysosporum, Cuspidothrix)
- Group 1 (Anabaena, Anabaenopsis, Dolichospermum, Sphaerospermopsis)

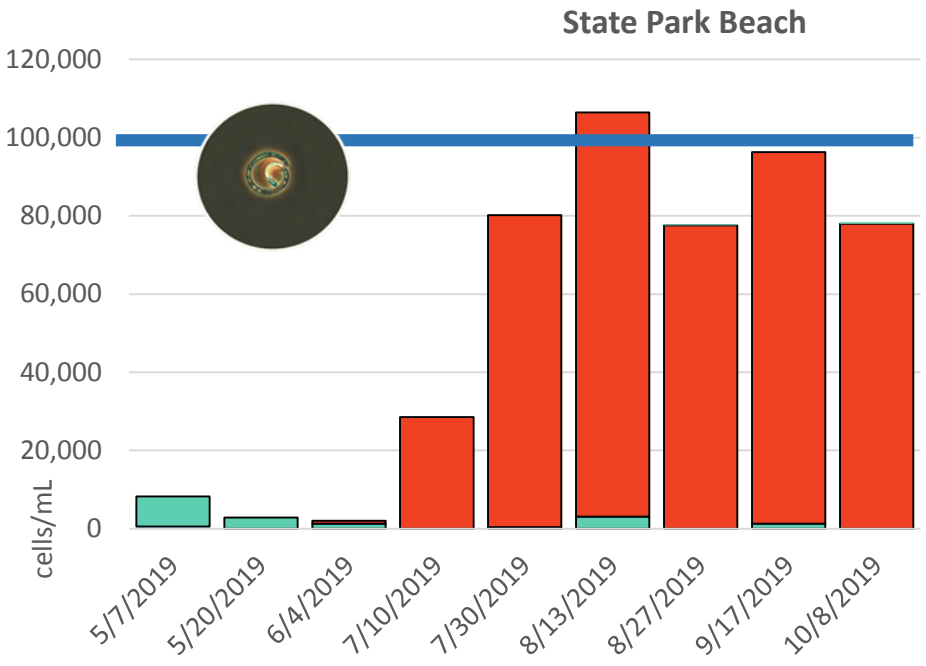
Taxa grouped by morphological similarities and clusters of adoptions of nomenclature changes

2019 Lake Anna

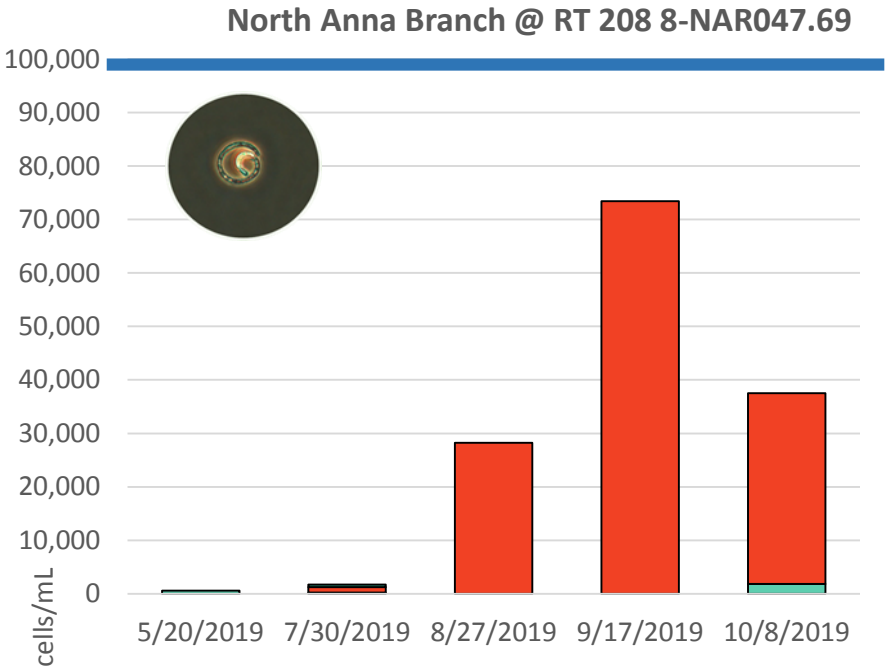


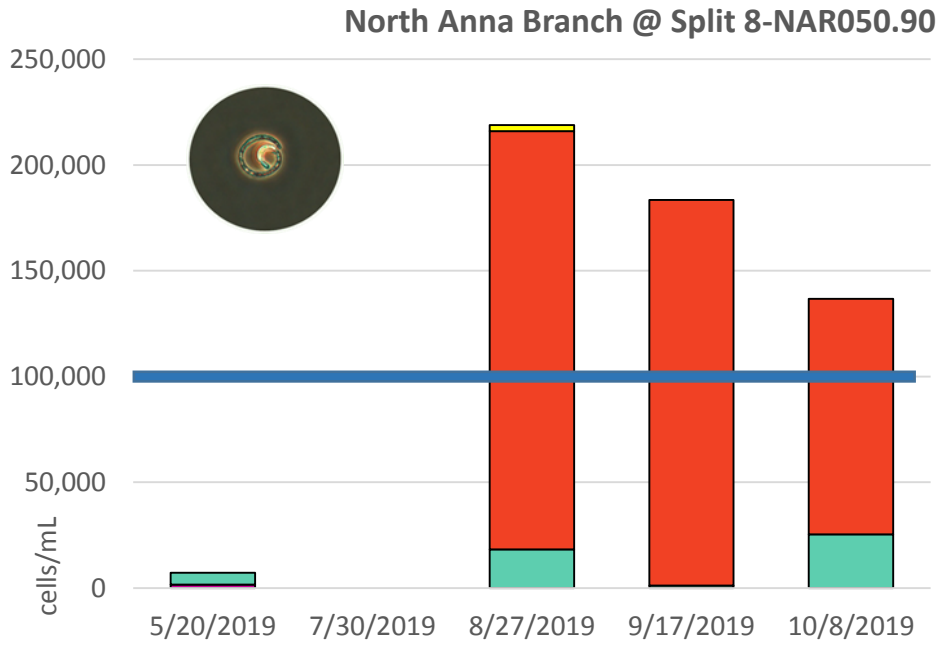


LOWER SECTION OF NORTH ANNA BRANCH



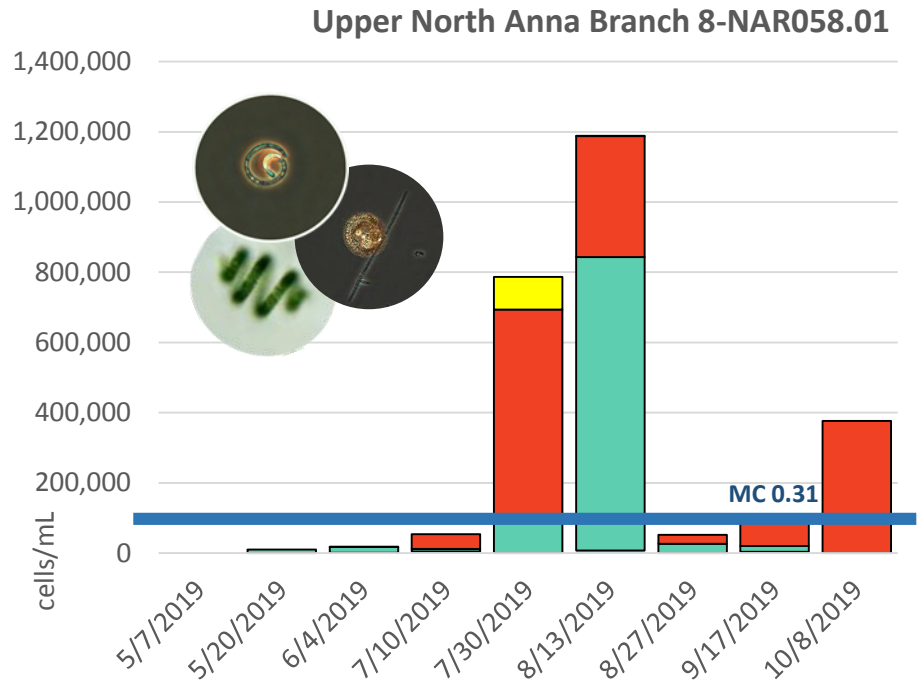
- ***Raphidiopsis spp.*** **dominant (<100 cells/mL – 198,000 cells/mL) July through October**
- **Small component of mixed-Group 2 taxa**
- **Only 21% of samples exceeded 100k T targeted PTOX**
- **No toxins detected above ldl of tests**

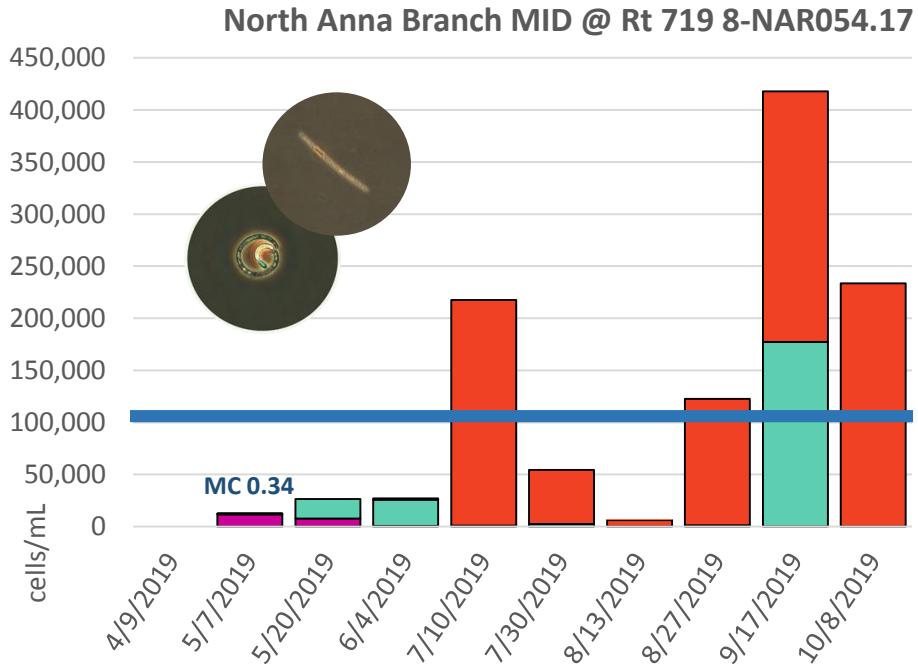


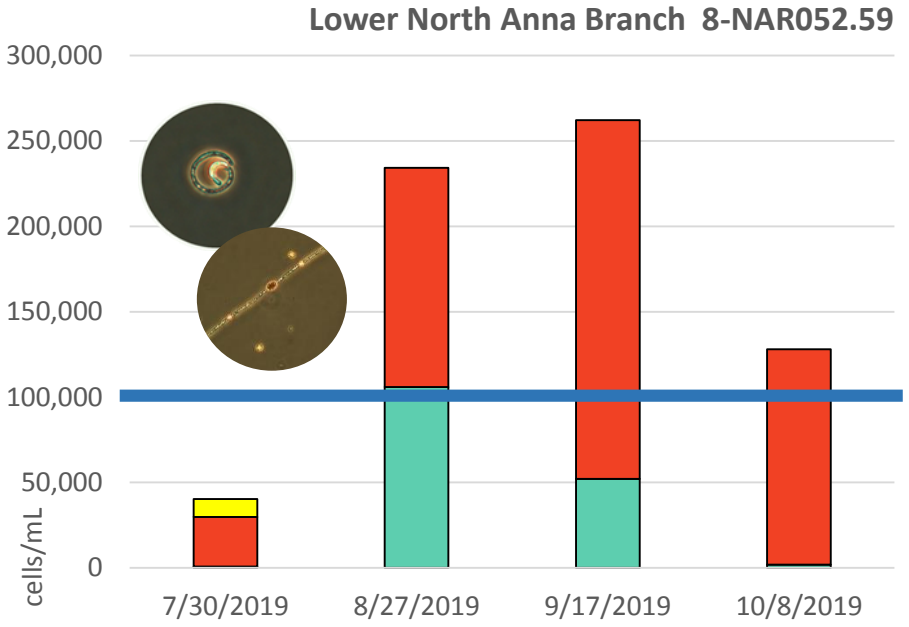


WESTERN SECTION OF NORTH ANNA BRANCH

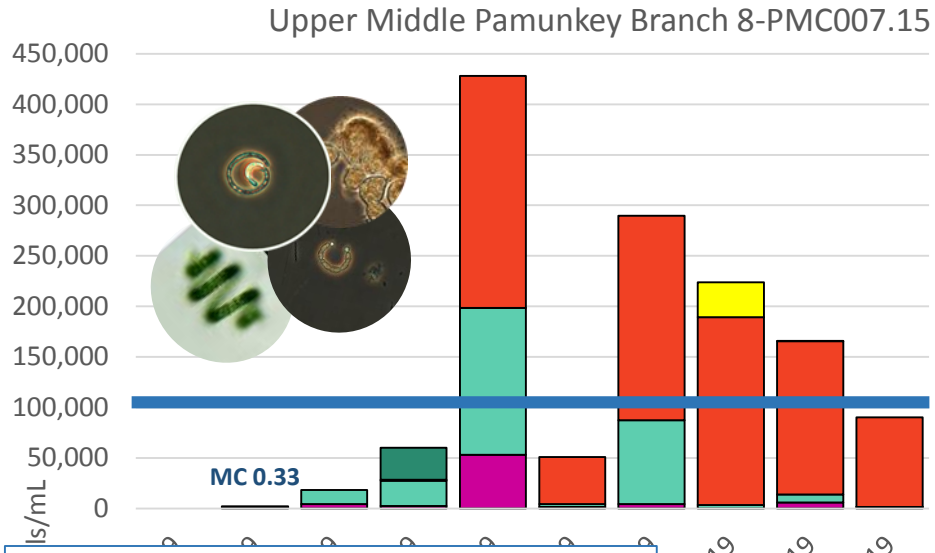
- ***Raphidiopsis spp.*** **dominant (<700 cells/mL – 590,000 cells/mL) July through October**
- **Occasionally co-dominant with mixed group 2 taxa (400 cells/mL – 836,000 cell/mL) May through September**
- **48% of samples exceeded 100k T targeted PTOX**
- **Microcystins (0.31 ppb & 0.34 ppb) detected at only 2 of 23 grabs, just above ldl (0.30 ppb) of test, and well below the advisory threshold (8.0 ppb)**

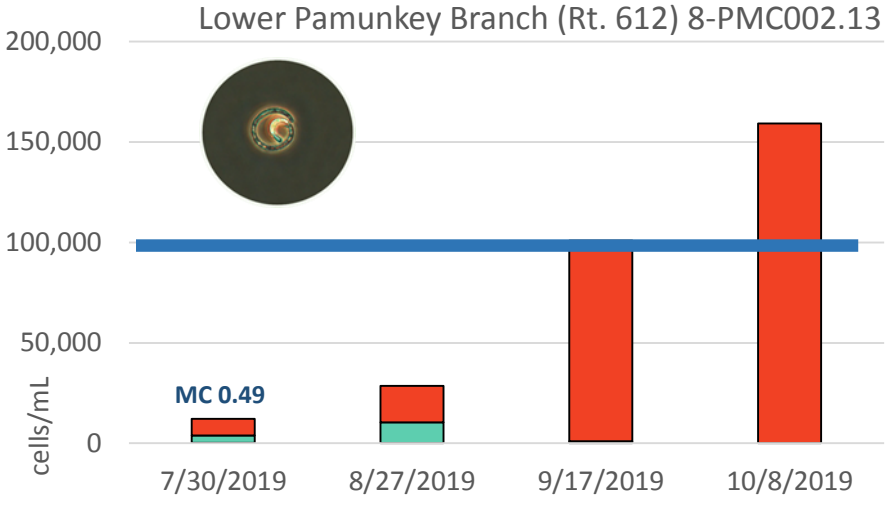




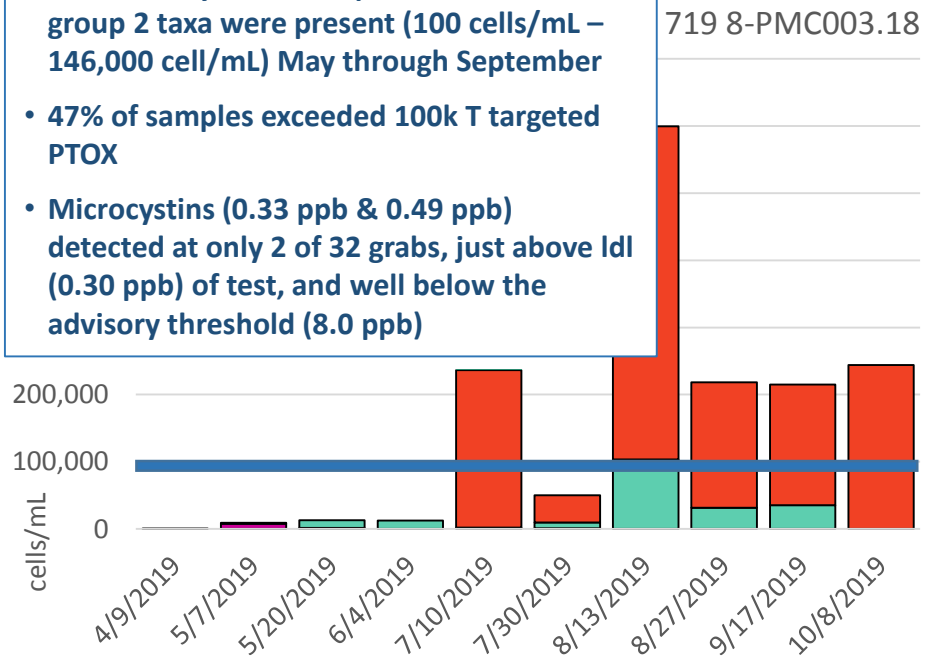


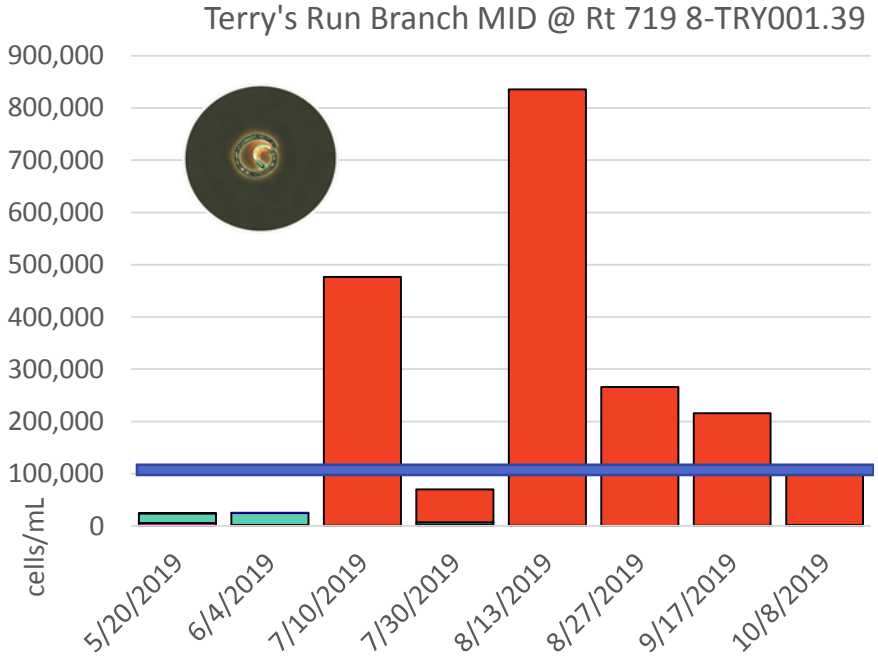
PAMUNKEY BRANCH





- ***Raphidiopsis spp.*** **dominant (<100 cells/mL – 834,000 cells/mL) July through October**
- **Occasionally small components of mixed group 2 taxa were present (100 cells/mL – 146,000 cell/mL) May through September**
- **47% of samples exceeded 100k T targeted PTOX**
- **Microcystins (0.33 ppb & 0.49 ppb) detected at only 2 of 32 grabs, just above ldl (0.30 ppb) of test, and well below the advisory threshold (8.0 ppb)**





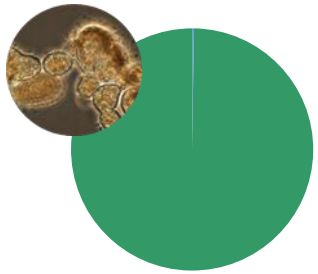
2019 Upper Chickahominy Trib

Chickahominy Trib Microcystins Conc.						
	Trib Site 1 scum	Trib Site 1 subsurface	Trib Site 2 scum	Trib Up Station subsurface	Trib Mid Station subsurface	Trib Down Station subsurface
8/27/2019	bdl*		5.84*			
8/30/2018		bdl*	5.31*			
9/3/2019				bdl	0.53	0.41
10/1/2019					bdl	bdl

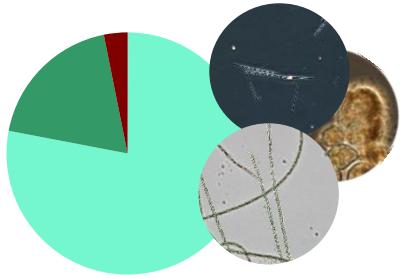
Chickahominy Trib Cylindrospermosin Conc.						
	Trib Site 1 scum	Trib Site 1 subsurface	Trib Site 2 scum	Trib Up Station subsurface	Trib Mid Station subsurface	Trib Down Station subsurface
8/27/2019	bdl*		0.06*			
8/30/2018		bdl*	0.14*			
9/3/2019				bdl	bdl	bdl
10/1/2019					bdl	bdl

*no taxonomy collected

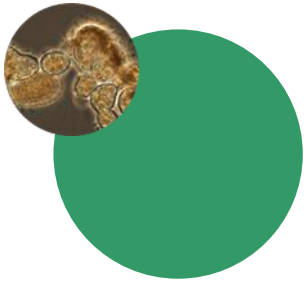
9/3/2019
Downstream Station



10/1/2019
Downstream Station



9/3/2019
Middle Station



- **Microcystins (5.84 ppb and 5.31 ppb) detected at single location at start of bloom, although higher than normally seen in state, concentrations well below advisory threshold of 8.0 ppb**
- **Low cylindrospermopsin (0.6 ppb and 0.14 ppb) also detected**
- ***Microcystis virdis* dominant (4.7 and 12.6 million cells/mL) in middle of bloom, both grabs had low microcystins (0.53 ppb & 0.41 ppb)**
- **End of bloom dominated by *Cuspidothrix issatchenkoi* with small component of *Microcystis virdis, and Planktothrix isothrix* , no toxins detected**

2019 Pilot Neurotoxin Investigation

ODUID	18-1	18-2	18-3	18-171	18-243	18-335	18-429	19-307	19-315	19-373	19-374	19-397	19-398	19-461	19-476	19-477
Anabaena spp.				2,980						335	5,778					
Anabaenopsis spp.															2,304,120	758,330
Aphanizomenon spp.															118,497	
Arthrospira fusiformis															3,291	4,680
Chrysosporum ovalisprum										113,940					455	11,440
Cuspidothrix issatschenkoi						1,550				2,012,940	1,958,100	6,322,929	3,229,260	260,010		
Dolichospermum spp.					8,736	17,570	141,561			481,080	17,013					
Limnothrix spp.				360												
Lygnbya spp.						58,800		5,080,000								
Microcystis spp.					437,112		378,780			600	3,725					784
Oscillatoria spp.						8,720	3,053,592		252,640						19,660,980	16,265,570
Phormidium spp.						3,080			151,400						10,634	24,390
Planktothrix spp.	8,225,409	1,042,730	732,822	357,012				42,000							6,709,800	143,050
Raphidiopsis spp.						246,540					1,734					
Woronichinia sp.							33,880									
T targeted PTOX	8,225,409	1,042,730	732,822	360,352	445,848	336,260	3,607,813	5,122,000	404,040	2,608,895	1,986,350	6,322,929	3,229,260	260,010	28,807,777	17,208,244
Microcystins	20	2.272	2.6725	0.038	1.1175	0.0475	0.332	0.435	0.123	0.045		0.02	0.166	0.041	8.189	1.694
Cylindrospermopsin	0.484	0.4855	0.275			0.013	0.0165	0.034							0.119	
Anatoxin-A		0.082		0.2055		0.3455			0.2715	1.343	1.2665	1.8305	0.8475	0.6645	0.759	
Saxitoxin							0.081								0.0045	0.0215

Anatoxin-a

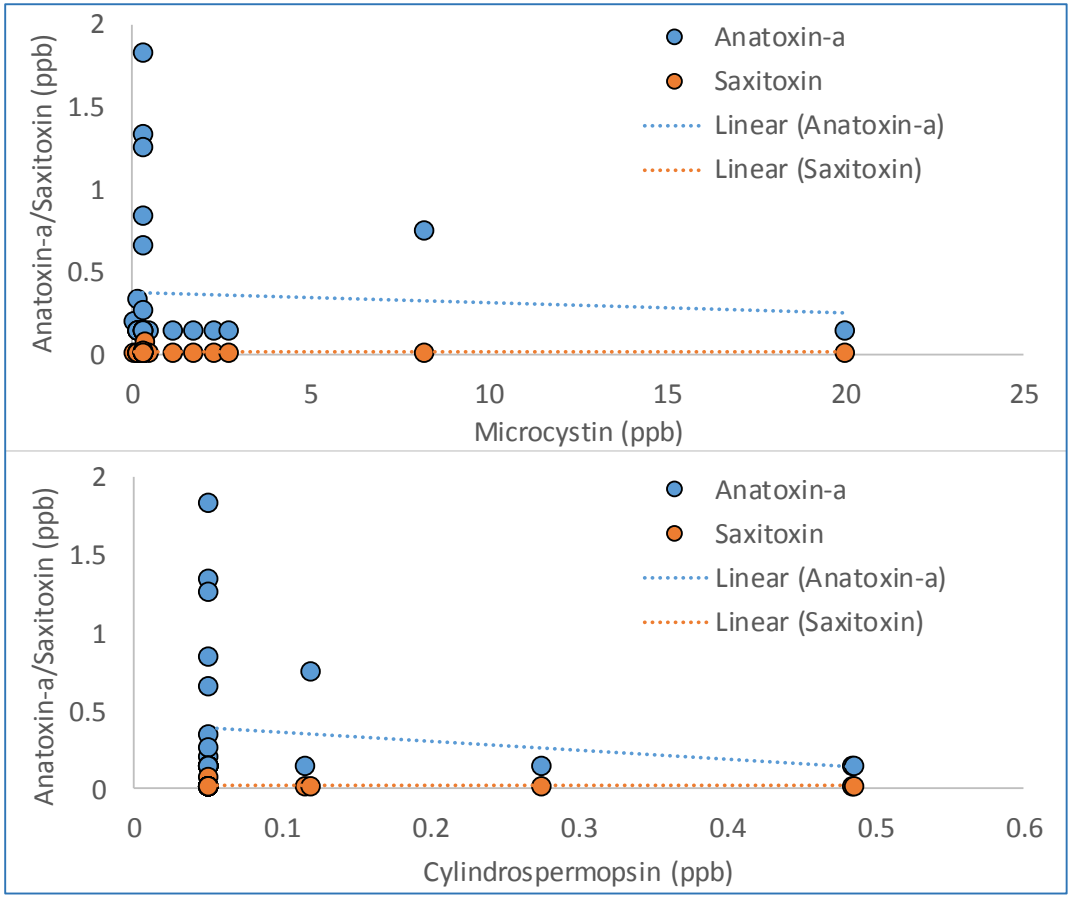
- Abraxis ELISA
- 0.15ppb lower quantitative limit
- Maximum: 1.83 ppb
- 9/4/19 Wilcox Lake
- *Cuspidothrix issatschenkoi*
  (>6 million cells/mL)

Saxitoxin

Subset (16) of the 28 samples drawn from the 248 samples collected and analyzed in 2018 & 2019

- Abraxis ELISA
- 0.02ppb lower quantitative limit
- Maximum 0.081ppb
- 9/27/2018 Williamsburg
- Mixed assemblage
  Microcystis, Dolichospermum, Oscillatoria

2019 Pilot Neurotoxin Investigation

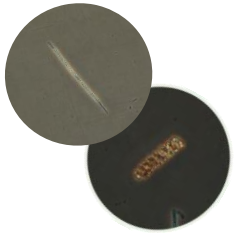


Little to no relationship between the presence, absence, or concentration of microcystins or cylindrospermopsin on the presence, absence, or concentration of neurotoxins.

Abraxis microcystins (ADDA) ELISA
0.15ppb* - 5.0ppb (higher w/dilution)
Abraxis cylindrospermopsin ELISA
0.05ppb - 2.0ppb (higher w/dilution)
Abraxis anatoxin-a ELISA
0.15ppb - 5.0ppb (higher w/dilution)
Abraxis saxitoxin ELISA
0.02ppb - 0.4ppb (higher w/dilution)

*after 6/4/2019 ldl 0.30ppb

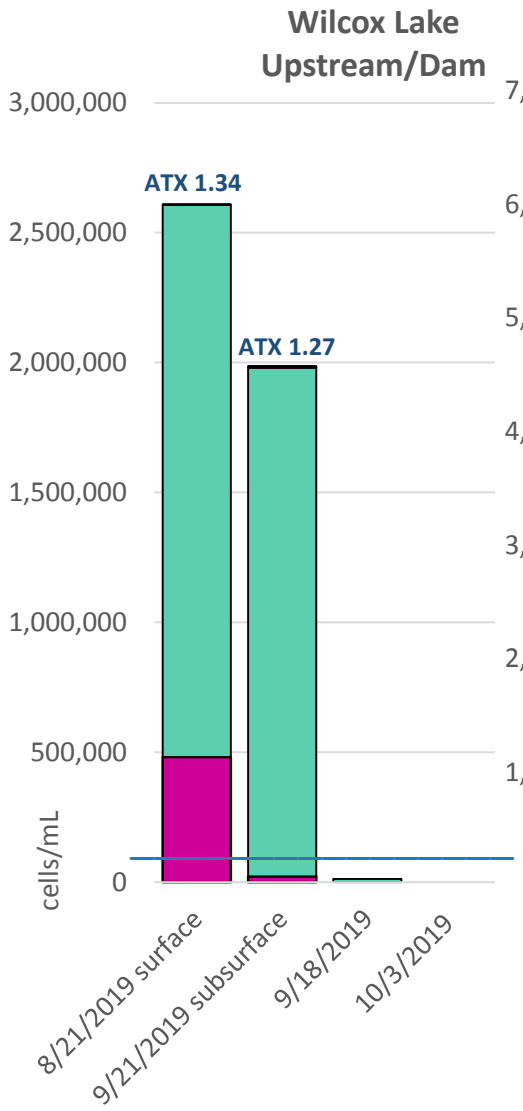
2019 Wilcox Lake

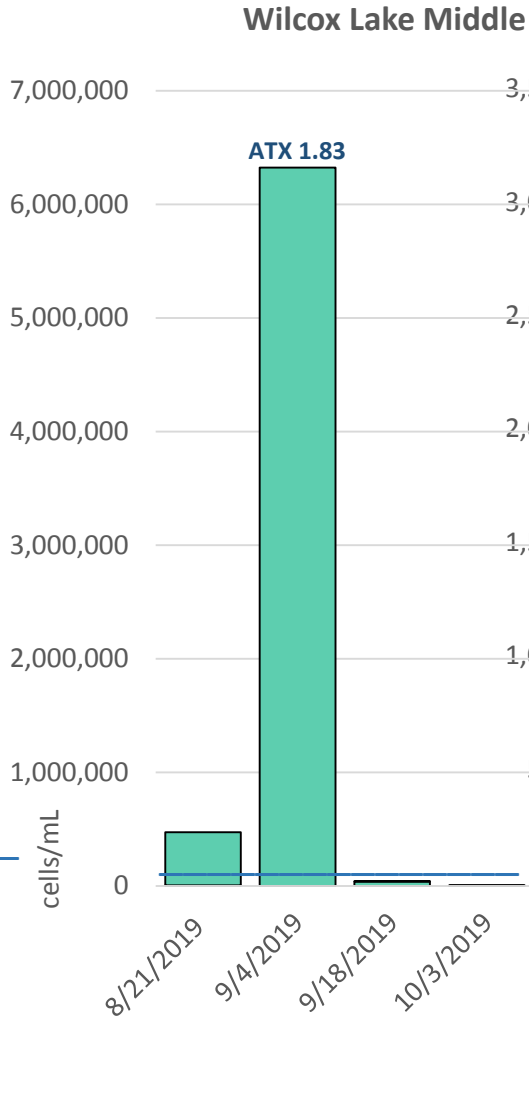


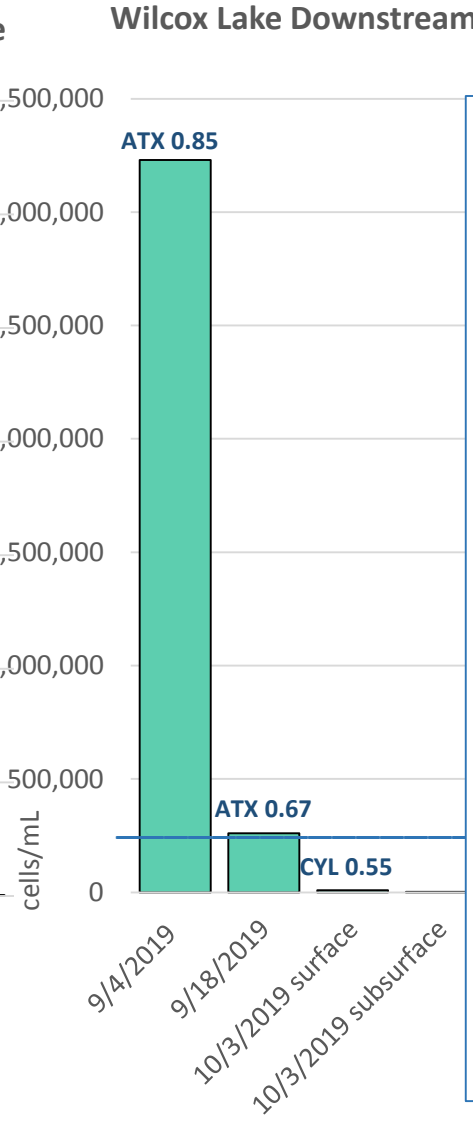
100k marker →

Cuspidothrix issatschenkoi

Dolichospermum spp.

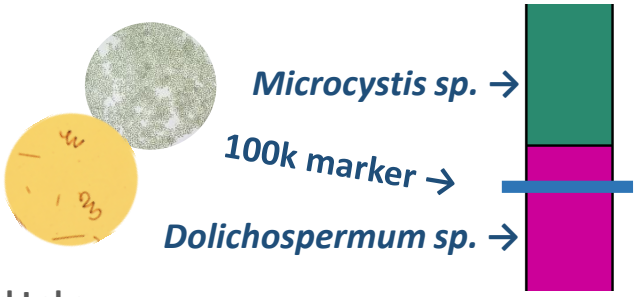


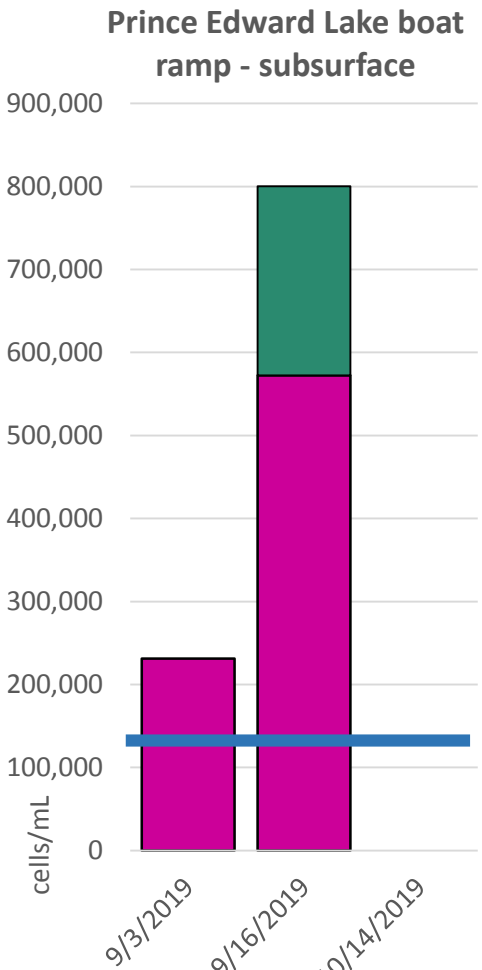


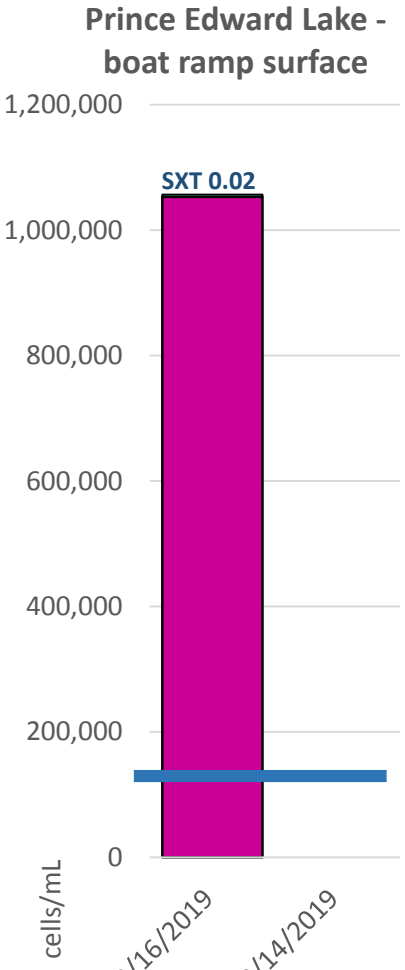


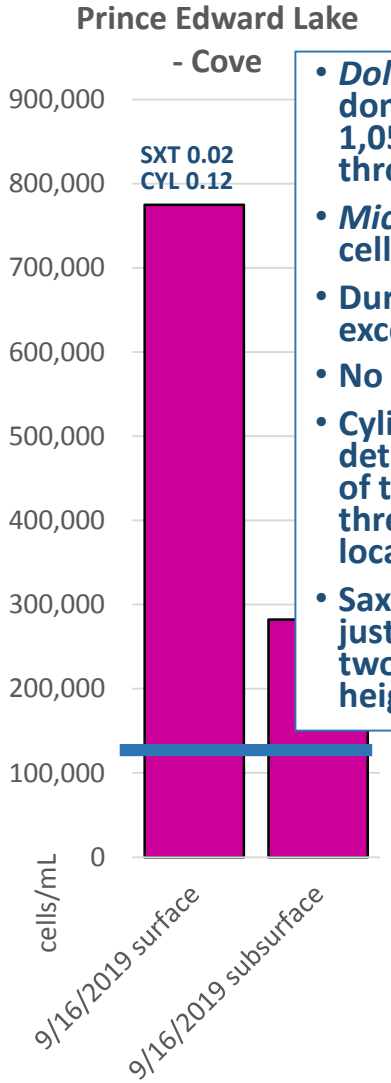
- ***Cuspidothrix issatschenkoi*** **dominant throughout season (260,000 cells/mL - > 3million cells/mL) August through October**
- **Small component of *Dolichospermum spp.* (>480,000 cells/mL) present at start of bloom near dam**
- **T targeted PTOX taxa exceeded 6 million cells at height of bloom**
- **No microcystins detected**
- **Cylindrospermopsin (0.55 ppb), well below advisory threshold (15.0 ppb) at single location at end of bloom**
- **Anatoxin-a (0.67 ppb - 1.83 ppb) detected throughout lake over course of a month**

2019 Twin Lakes State Park



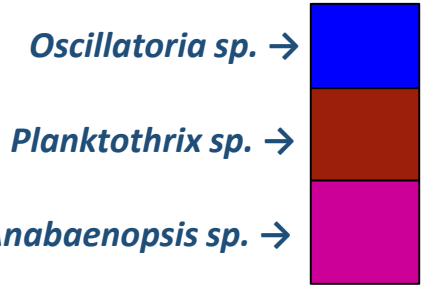


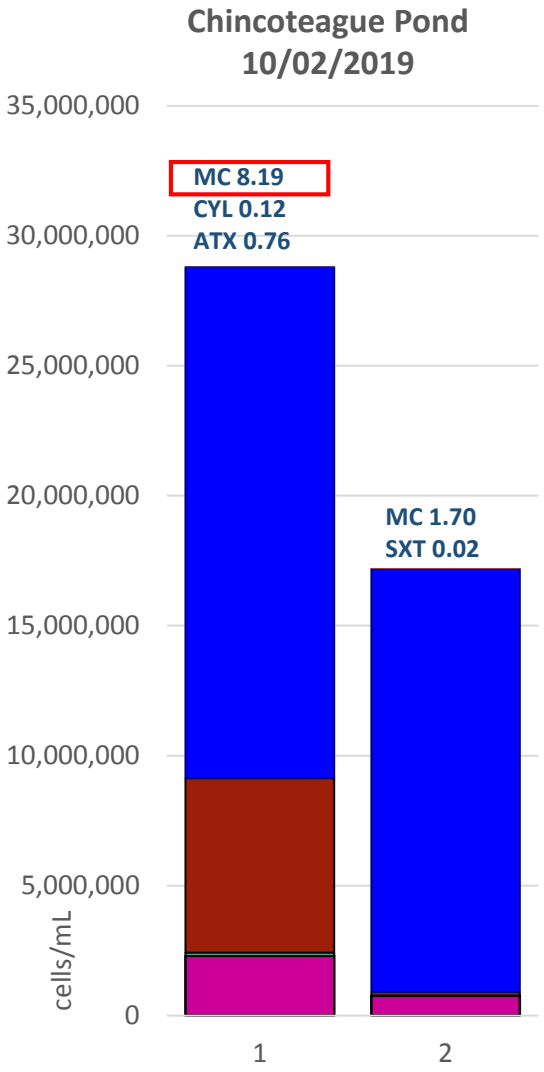




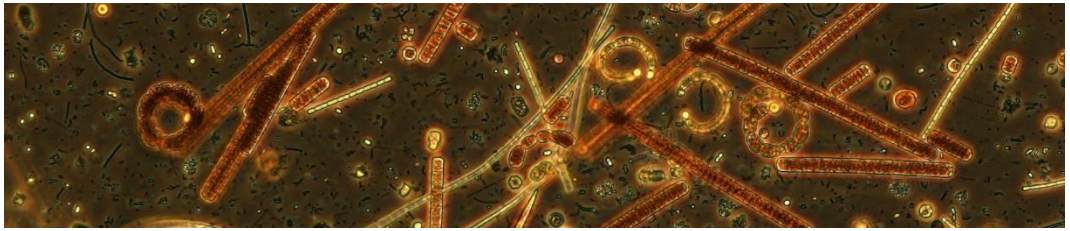
- ***Dolichospermum spp.*** **dominant (231,000 cells/mL – 1,053,000 cells/mL) September through October**
- ***Microcystis spp*****. present (230,000 cells/mL) in September**
- **During the bloom, T targeted PTOX exceeded 1,057,000 cells/mL**
- **No microcystins detected**
- **Cylindrospermopsin (0.12 ppb) was detected slightly above ldl (0.05 ppb) of test, and well below the advisory threshold (15.0 ppb) at single location at end of bloom**
- **Saxitoxin (>0.02 ppb) was detected just above ldl (0.02 ppb) of test at two locations on September 16th, the height of the bloom**

2019 Chincoteague Island





- ***Oscillatoria spp.*** **dominant (16.3 million cells/mL – 19.7 million cells/mL) on October 2nd**
- **Co-dominant with *Planktothrix spp*. (6.7 million cells/mL – 143,000 cells/mL) and *Anabaenopsis spp*. (>750,000 cells/mL - >2.3 million cells/mL)**
- **Cell counts for T targeted PTOX taxa were 280 x's greater than the state's advisory threshold of 100k at the cove location**
- **Microcystins (8.19 ppb and 1.70 ppb) were detected, the concentration at the cove location exceeded the state's advisory threshold of 8.0 ppb - the only sample to do so this year**
- **Cylindrospermopsin (0.12 ppb), Anatoxin-a (0.76 ppb), and Saxitoxin (0.02 ppb) were also detected in the pond**



2019 Taxa and Toxins

VIRGINIA 2019

Species	Date Range	Max conc in VA water cells/mL	Toxin Potential	Toxins confirmed in monospecific blooms with taxa occuring at **>100k cells/mL w/out other taxa occuring at >30k cells/mL** in VA by ELISA	Toxins confirmed in blooms **dominated by taxa at >100k cells/mL w/ other taxa occuring at >100k/cells/mL** in VA by ELISA	Toxins confirmed in blooms w/ **co-dominant taxa between 30k - 100k cells/mL** in VA by ELISA	Toxins confirmed in blooms **co-dominant taxa > 30k cells/mL w/ > 40k mL of microcystis spp.** in VA by ELISA	Species ID in VA waters
Anabaena spp.	may - aug	3,976	✓					
Anabaena/Sphaerospermopsis - like filamant	july - oct	7,807	✓					
Anabaenopsis spp.	may - oct	1,304,120	✓		MC, ATX, STX			
Aphanizomenon spp.	may - oct	118,497	✓		MC, ATX			*gracile, flos-aqua*
Arthrospira spp.	aug - oct	34,780	✓					*fusiformis*
Chrysosporum - like / Sphaerospermopsis - like filament	may - july	145,590	✓					
Chrysosporum spp.	july - oct	113,940	✓		ATX	CYL		*ovalisporum*
Cuspidothrix spp.	may - oct	6,322,929	✓	ATX, CYL	ATX			*issatschenkoi*
Cylidrospermposis spp.	x	x	✓					
Dolichospermum spp.	may - oct	1,053,312	✓	CYL, STX	ATX			*circinale/crassum, compactum, sigmoidum*
Jaaginema spp.	april - oct	4,220,000	✓			MC, CYL		
Limnothrix spp.	may* - oct	440	✓					
Lygnbya spp.	july	5,080,000	✓		MC			
Microcystis spp.	may - oct	12,604,296	✓	MC				*aeuriginosa, virdis, wesenbergii*
Nostocalean filament	sept	2,152	✓					
Nostoc spp..	x	x	✓					
Oscillatoria spp.	aug - oct	19,660,980	✓		MC, ATX, STX			
Phormidium spp.	july - oct	> 1,000,000	✓	MC	ATX			
Planktolyngbya spp.	june - oct	367,140	?		ATX			*contorta, contorta var. circumcreta, limnetica*
Planktothrix spp.	july - oct	6,709,800	✓		MC, ATX, STX	MC		*isothrix*
Pseudanabaena spp.	april - oct	856,132	✓		MC, ATX	MC		*limnetica*
Raphidiopsis spp.	april - oct	589,956	✓		CYL	MC, CYL		*raciborskii*
Sphaerospermopsis spp.	sept	1,160	✓					
Woronichinia sp.	x	x	✓					*naegeliana*

VIRGINIA 2017

Species	Date Range	Max conc in VA water cells/mL	Toxin Potential	Toxins confirmed in monospecific blooms with taxa occuring at **>100k cells/mL w/out other taxa occuring at >30k cells/mL** in VA by ELISA	Toxins confirmed in blooms **dominated by taxa at >100k cells/mL w/ other taxa occuring at >100k/cells/mL** in VA by ELISA	Toxins confirmed in blooms w/ **co-dominant taxa between 30k - 100k cells/mL** in VA by ELISA	Toxins confirmed in blooms **co-dominant taxa > 30k cells/mL w/ > 40k mL of microcystis spp.** in VA by ELISA	Species ID in VA waters
Anabaena spp.	jun-nov	511,948	✓	MC		MC	MC	
Anabaena/Sphaerospermopsis - like filamant	x	x	✓					
Anabaenopsis spp.	x	x	✓					
Aphanizomenon spp.	feb-oct	7280274.4	✓					*gracile, flos-aqua*
	x	x	✓					*fusiformis*
	x	x	✓					

VIRGINIA 2018

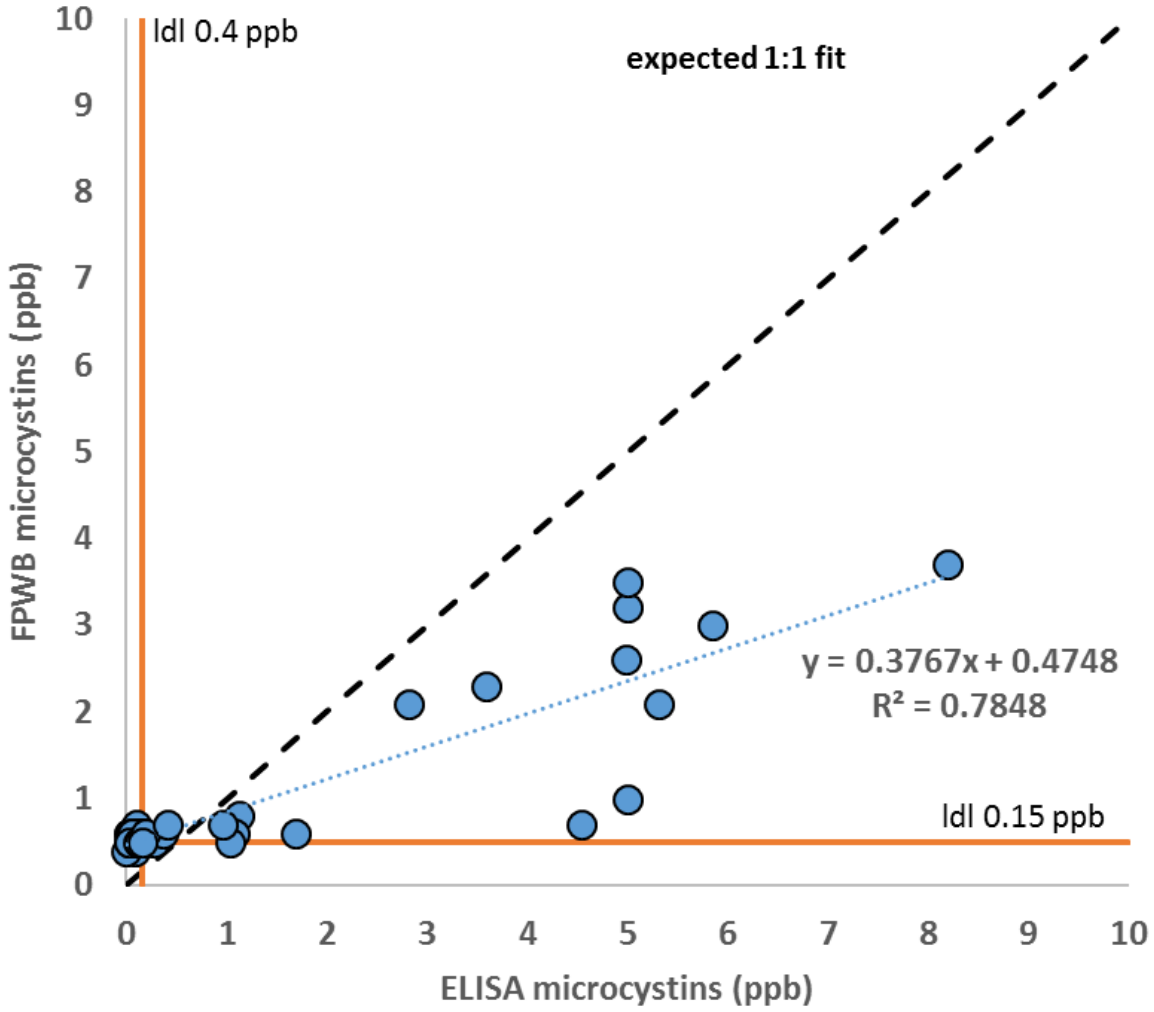
Species	Date Range	Max conc in VA water cells/mL	Toxin Potential	Toxins confirmed in monospecific blooms with taxa occuring at **>100k cells/mL w/out other taxa occuring at >30k cells/mL** in VA by ELISA	Toxins confirmed in blooms **dominated by taxa at >100k cells/mL w/ other taxa occuring at >100k/cells/mL** in VA by ELISA	Toxins confirmed in blooms w/ **co-dominant taxa between 30k - 100k cells/mL** in VA by ELISA	Toxins confirmed in blooms **co-dominant taxa > 30k cells/mL w/ > 40k mL of microcystis spp.** in VA by ELISA	Species ID in VA waters
Anabaena spp.	june-sept	2,980	✓					
Anabaena/Sphaerospermopsis - like filamant	x	x	✓					
Anabaenopsis spp.	x	x	✓					
Aphanizomenon spp.	aug-oct	640	✓					*gracile, flos-aqua*
Arthrospira spp.	x	x	✓					*fusiformis*
Chrysosporum - like / Sphaerospermopsis - like filament	x	x	✓					
Chrysosporum spp.	x	x	✓					*ovalisporum*
Cuspidothrix spp.	july-oct	45,760	✓			MC		*issatschenkoi*
Cylidrospermposis spp.	july-oct	1,799,526	✓	MC	MC, ATX			
Dolichospermum spp.	june-oct	141,561	✓		MC, STX	MC	MC, STX	*circinale/crassum, compactum, sigmoidum*
Jaaginema spp.	x	x	✓					
Limnothrix spp.	june	360	✓					
Lygnbya spp.	aug	58,800	✓			ATX		
Microcystis spp.	july-oct	10,738,212	✓	MC	MC, STX		MC, STX	*aeuriginosa, virdis, wesenbergii*
Nostocalean filament	x	x	✓					
Nostoc spp..	sept	1,110	✓					
Oscillatoria spp.	aug-sept	3,053,592	✓		MC, STX		MC, STX	
Phormidium spp.	aug-sept	5,280	✓					
Planktolyngbya spp.	aug-sept	125,511	?					*contorta, contorta var. circumcreta, limnetica*
Planktothrix spp.	jan-oct	8,225,409	✓	MC, CYL, ATX				*isothrix*
Pseudanabaena spp.	aug-oct	288,579	✓	MC	MC			*limnetica*
Raphidiopsis spp.	aug-oct	417,300	✓		MC			*raciborskii*
Sphaerospermopsis spp.	x	x	✓					
Woronichinia sp.	sept-oct	39,500	✓			MC, STX	MC, STX	*naegeliana*

Preliminary look at occurrence of toxins in samples separated by taxa, taxa cell counts, taxa dominance, and associations with *Microcystis spp.* in Virginia

MBIO

Abraxis microcystins (ADDA) ELISA
0.15ppb* - 5.0ppb (higher w/dilution)
Abraxis cylindrospermopsin ELISA
0.05ppb - 2.0ppb (higher w/dilution)
MBIO microcystins
0.4ppb - 3.1ppb (higher w/dilution)
MBIO cylindrospermopsin
0.7ppb - 2.7ppb (higher w/dilution)

*after 6/4/2019 ldl 0.30ppb



Comparison of microcystins concentrations using enzyme linked immunosorbent assay (ELISA) and fluorescence planar waveguide biosensor immunoassay (FPWB) found that MBIO results are significantly lower than what is being measured with the Abraxis ELISA.

***Cylindrospermopsin was predominantly below detection limits for both methods**

PHYTOPLANKTON ANALYSIS SAMPLE CHAIN OF CUSTODY REPORT

ORGANIZATION _____
DATE _____
LOCATION NAME _____
COLLECTED BY _____

SAMPLE LOCATION	Latitude	Longitude	SAMPLE TIME	SAMPLE DEPTH m	pH	DO mg/L	Salinity ppm	Cond. uS/cm	Temp °C	Secchi depth m	Fish kill/ bloom?	preserved taxonomy bottle (Lugol's)	live taxonomy bottle	toxin assay bottle

Water and atmospheric conditions _____

remit to: Phytoplankton Analysis Laboratory
Old Dominion University
244 Oceanography and Physical Sciences Building
4600 Elkhorn Ave, Norfolk, VA 23529
Lab: 757-683-4994

PHYTOPLANKTON IDENTIFICATION AND ENUMERATION SAMPLE COLLECTION

- For the preserved sample, collect 500 mL in a plastic bottle or cubitainer and administer Lugol's iodine solution at a ratio of 1:100. To achieve a ratio of 1:100, add approximately 1 mL of Lugol's to 100 mL of sample (3-5 mL for a 500 mL bottle) so that the final preserved sample color resembles weak tea.
- For the live sample, collect 500 mL in a plastic bottle or cubitainer. Do *not* add preservative.

Preserved sample in cubitainer

Live sample in cubitainer

Preserved samples should resemble weak tea

Virginia Harmful Algae Bloom Collection Protocol

****Contact the ODU Phytoplankton lab prior to collecting or shipping samples to make arrangements for analysis to ensure proper turn around time and scheduling.****

SITE INSPECTION:

- ☐ Record observations for each sample taken (location in body of water, color of bloom, presence of odor, scum, dead fish, proximity to culverts, docks, recreational beaches, ect. Photos of conditions at the site are useful.
- ☐ Record environmental parameters including water temperature, salinity, pH, dissolved oxygen, conductivity, and turbidity (secchi depth).
- ☐ Record site name, latitude and longitude of sample location, and position of sample taken (ie: scum layer, sub surface layer (-.5 m), bottom (+.5 m), ect…).
- ☐ For each event, take two sub surface (-.5 m) samples (one live and one preserved) from the center of the bloom. When there is a scum present, take a second collection at the scum-water interface. The cells present at the surface exposed to air and sun are often degraded, dying, or dead and not well suited for taxonomic enumeration.

PHYTOPLANKTON IDENTIFICATION AND ENUMERATION SAMPLE COLLECTION:

Preserved samples are for biomass quantification and multi species identification. Live samples are useful for determining color and motility in most phytoplankton or sheath formation in cyanobacteria.

- ☐ For the preserved sample, collect 500 mL in a plastic bottle or cubitainer and administer Lugol's iodine solution at a ratio of 1:100. To achieve a ratio of 1:100, add approximately 1 mL of Lugol's to 100 mL of sample (3-5 mL for a 500 mL bottle) so that the final preserved sample color resembles weak tea.
- ☐ For the live sample, collect 500 mL in a plastic bottle or cubitainer. *Do not* add preservative.
- ☐ Label each bottle clearly with location name, sampling site (ie: boat landing, scum or dock, -.5m), date, treatment (ie: preserved or live), and analysis (ie: taxonomy or microcystis).

TOXIN SAMPLE COLLECTION:

The purpose in collecting for toxins is to determine if there is an exposure risk to the public. Toxin samples should be taken when there is an obvious scum or fish kill present. Microcystins and cylidrospermopsin assays conducted, make sure to contact the lab prior to taking samples if the need for anatoxin-a or saxitoxin analysis is anticipated.

- ☐ For microcystin and/or cylindrospermopsin assays, collect 250 mL of sample in an amber glass bottle. The same bottle can be used for both assays.
- ☐ For saxitoxin and/or anatoxin-a assays, collect 250 mL of sample in an amber glass bottle. In freshwater systems, add 10x Concentrated Sample Diluent to prevent absorptive loss of toxin. Saltwater samples *do not* need the addition of this reagent. The same bottle can be used for both assays.
- ☐ Label each bottle clearly with location, date, treatment (ie: live or Lugol's), and analysis (ie: microcystin).

SHIPPING:

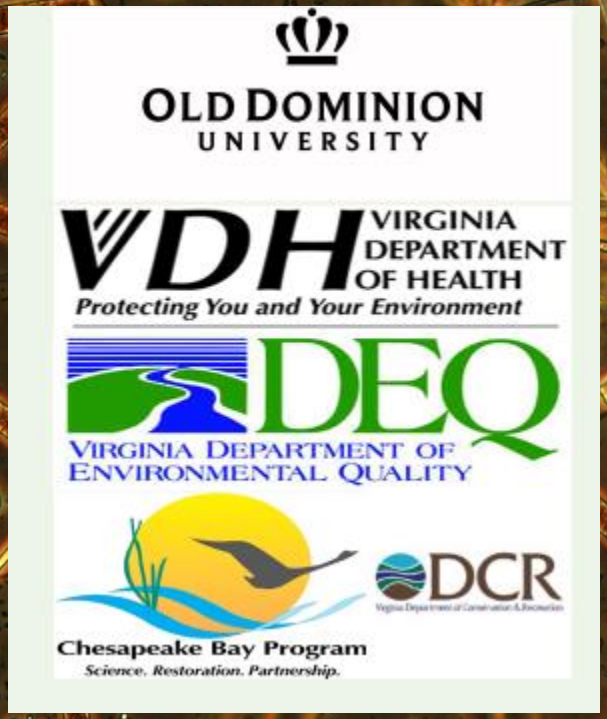
Shipping containers should be packed to prevent leakage or breakage as the shipping company will remove leaking coolers from their trucks, delaying or cancelling delivery. Shipping should be expedited overnight, and delivery within 24 hours of sampling.

- ☐ Close sample bottle tightly. Include the ODU COC report with the shipment.
- ☐ Samples should be kept cool with freezer packs (preferred) or ice sealed in airtight bags, and the **bottles protected from freezing**.
- ☐ All shipping containers should be lined with a garbage bag to avoid leakage.
- ☐ All shipping containers should be firmly packed with Styrofoam, paper, or bubble wrap to avoid breakage.
- ☐ Include a prepaid shipping label for the return of your shipping container.

3-5 mL of 5% acidified Lugol's solution from ODU YES!

7 or 10 *drops* of 2% Lugol's solution from the internet NO!

Thankyou



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Rachel Cerjan
Alyiah Downing

lgibalas@odu.edu

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