

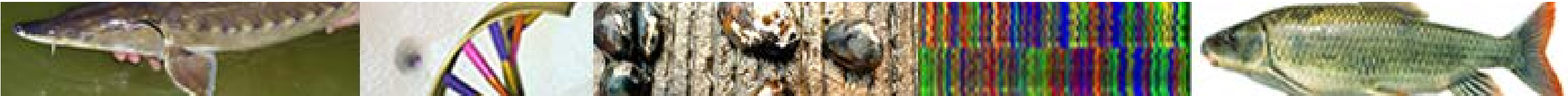


Applications of eDNA Methods for Inventory and Monitoring of Aquatic Species

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GOAL

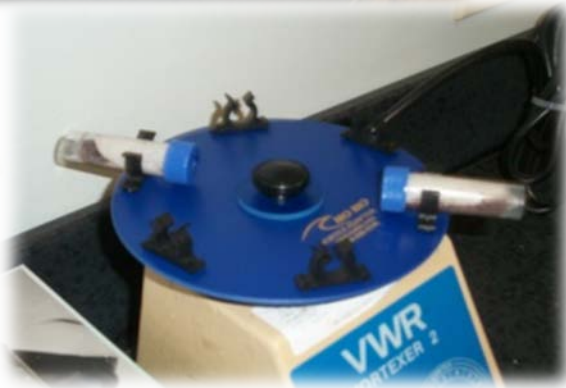
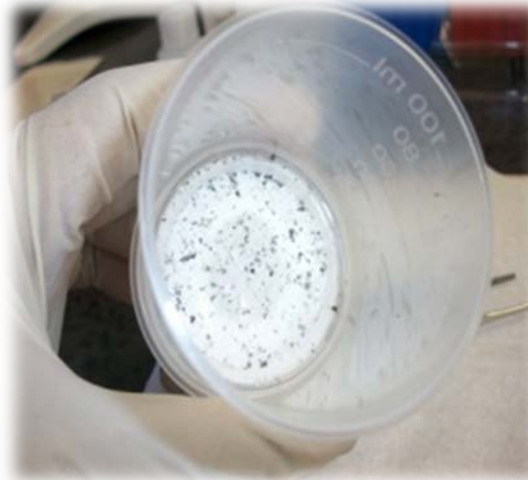
- Monitoring for *Didymosphenia geminata* – eDNA
- Influence of this funded project . . .
 - Quagga mussel detection
 - Swamp eel detection
 - American eel detection
 - eDNA applications for CEC





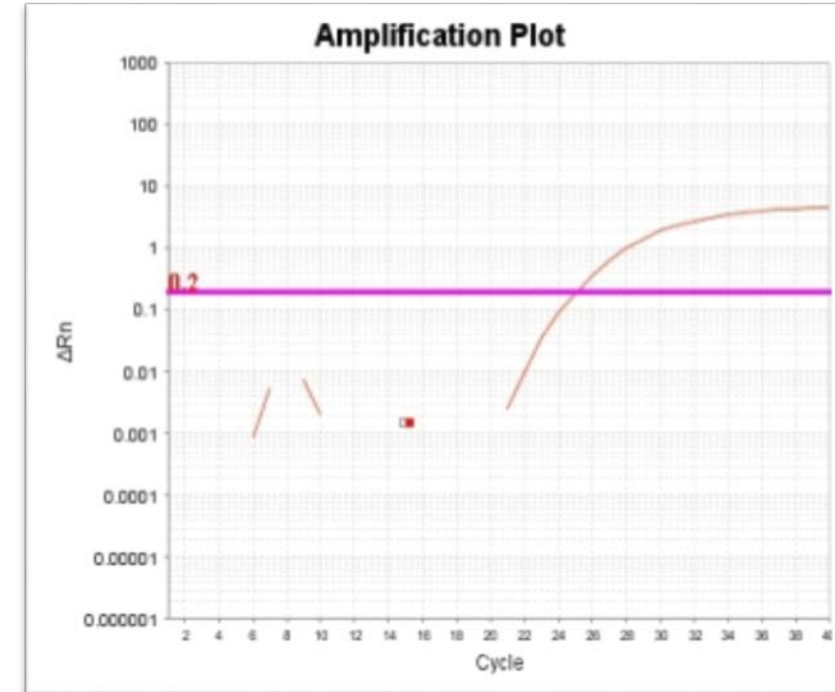
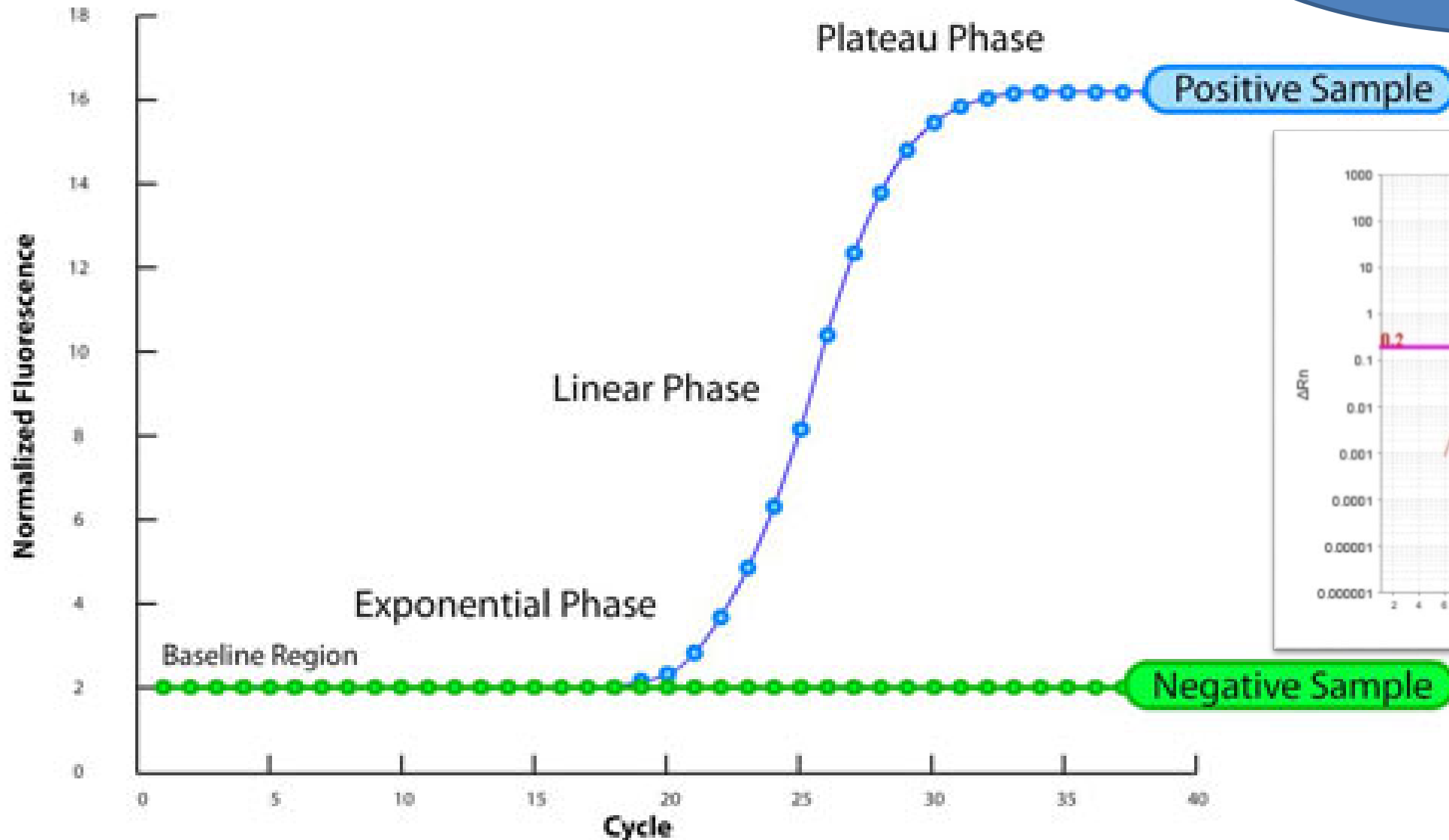


What is environmental DNA?



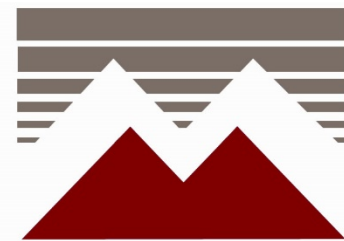
qPCR detection?

How do we measure when they are so hard to find?





MANSFIELD
UNIVERSITY

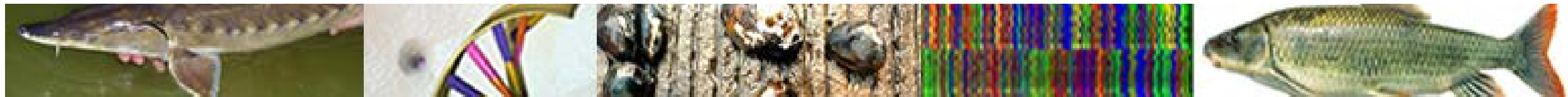


Monitoring for didymo: an environmental DNA approach

Gregory R. Moyer¹, Andrea E. Bowman², and Justin Murdock²

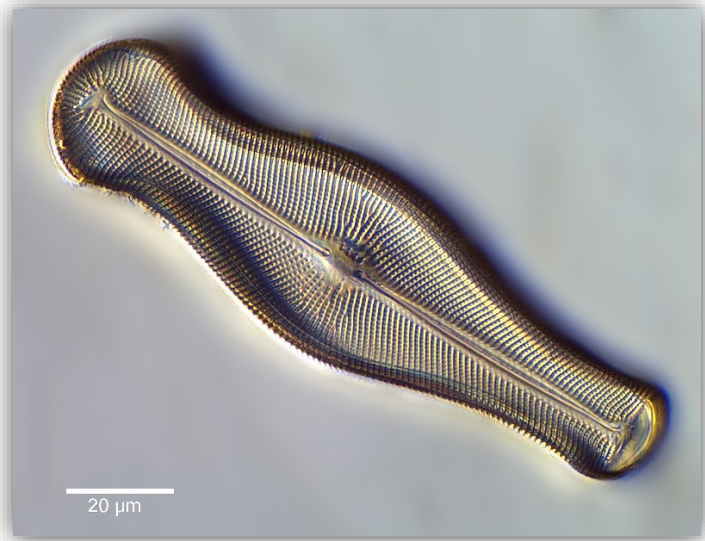
¹Fisheries and Aquatic Sciences; Department of Biology; Mansfield University; Mansfield, PA

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Snot rock

- *Didymosphenia geminate* (didymo)
 - extracellular polysaccharide stalk



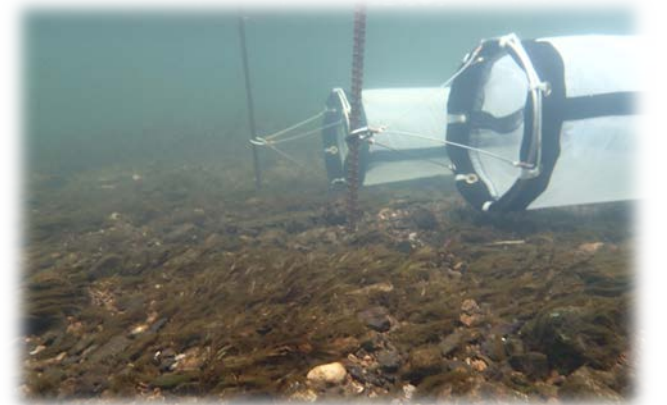
Objectives

- Detection of didymo eDNA?
- Association between eDNA and microscopy?



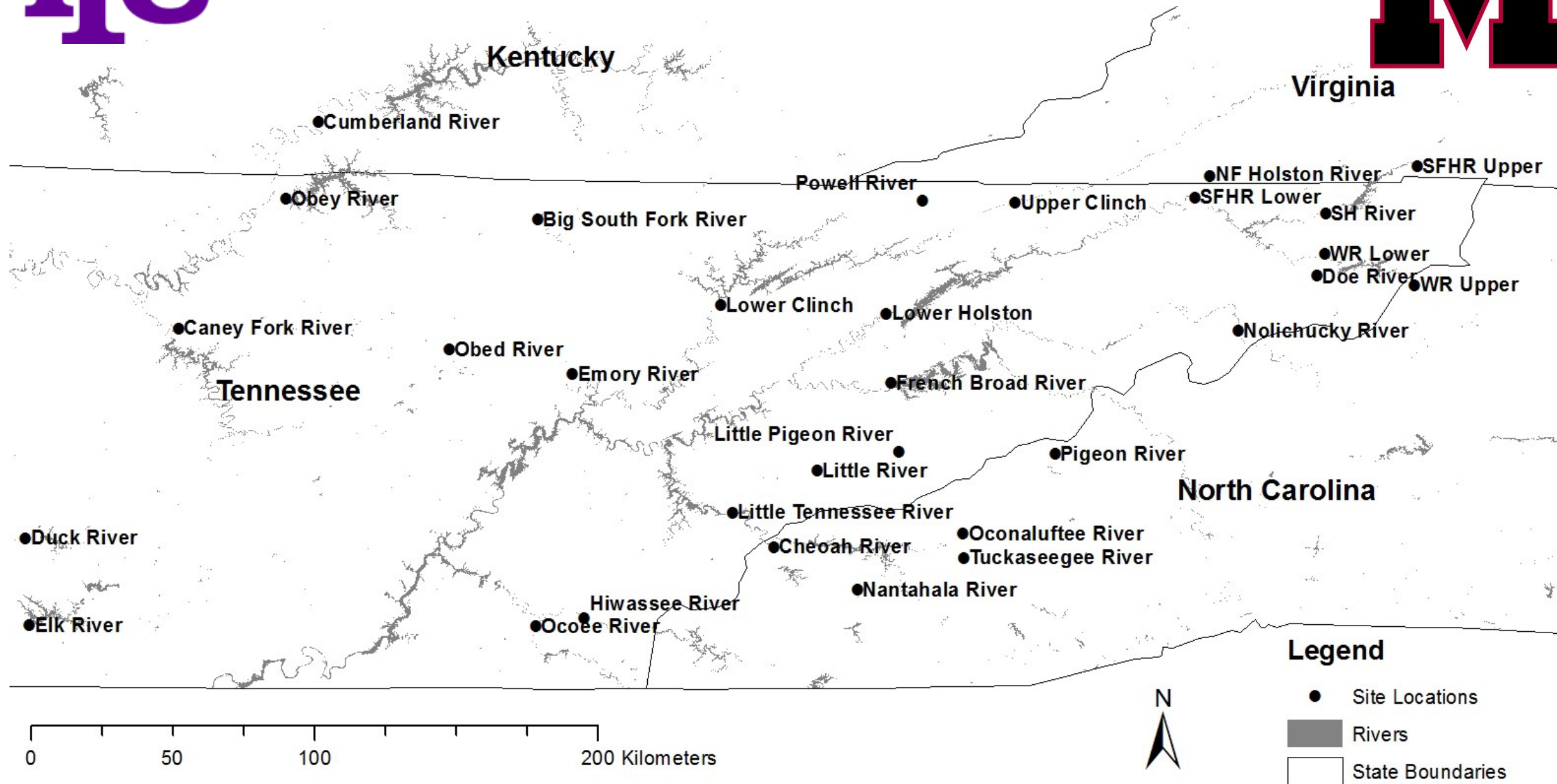
Methods

- Filter stream water ($n = 31$ streams)





Survey of TN streams for snot rock



Methods

- Filter stream water ($n = 31$ streams)
 - 40- μm mesh using a drift net
 - 240 mm in diameter
 - 2 min soak ($\sim 10,000$ L)
 - 5ml taken for microscope analysis
 - Concentrated to 50ml
 - Frozen
 - Duplicate samples



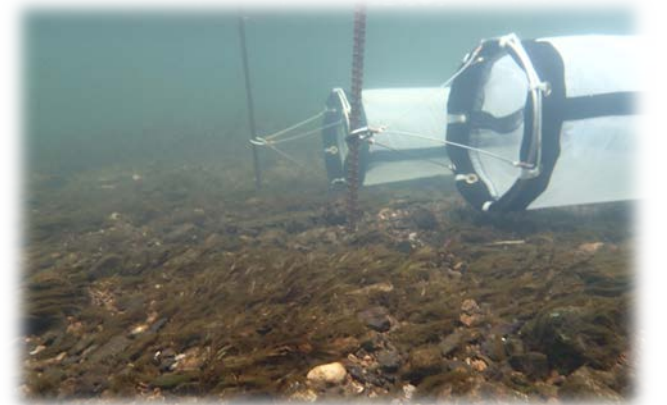
Methods

- Microscope analysis
 - 1 mL Sedgewick-Rafter chamber
 - 3x
 - + if viable cells found

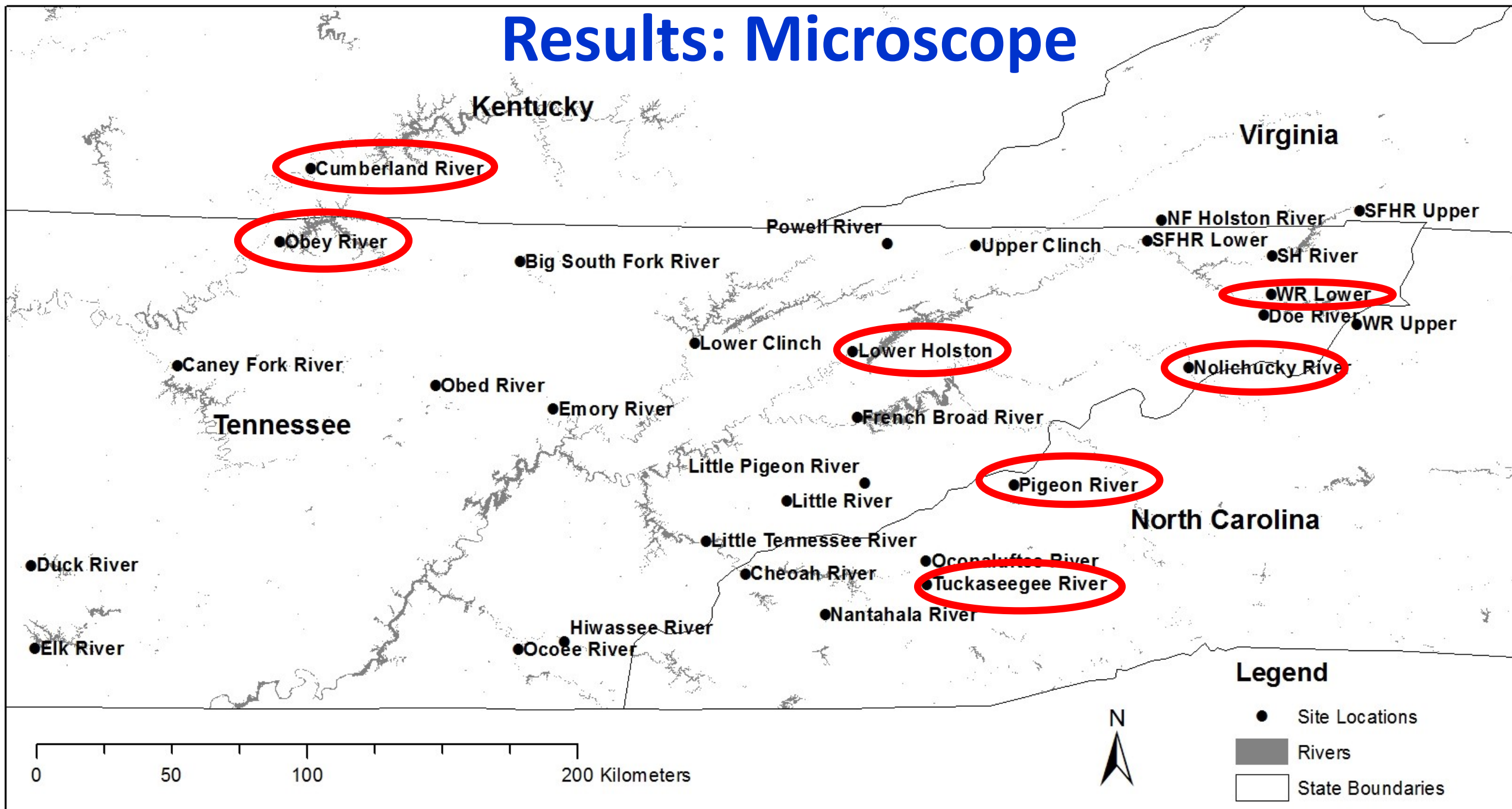


Methods

- eDNA analysis
 - Filtrate frozen
 - Developed assay (Cary et al. 2007)
 - mtDNA CI1 gene
 - Negative and positive controls
 - Internal positive control

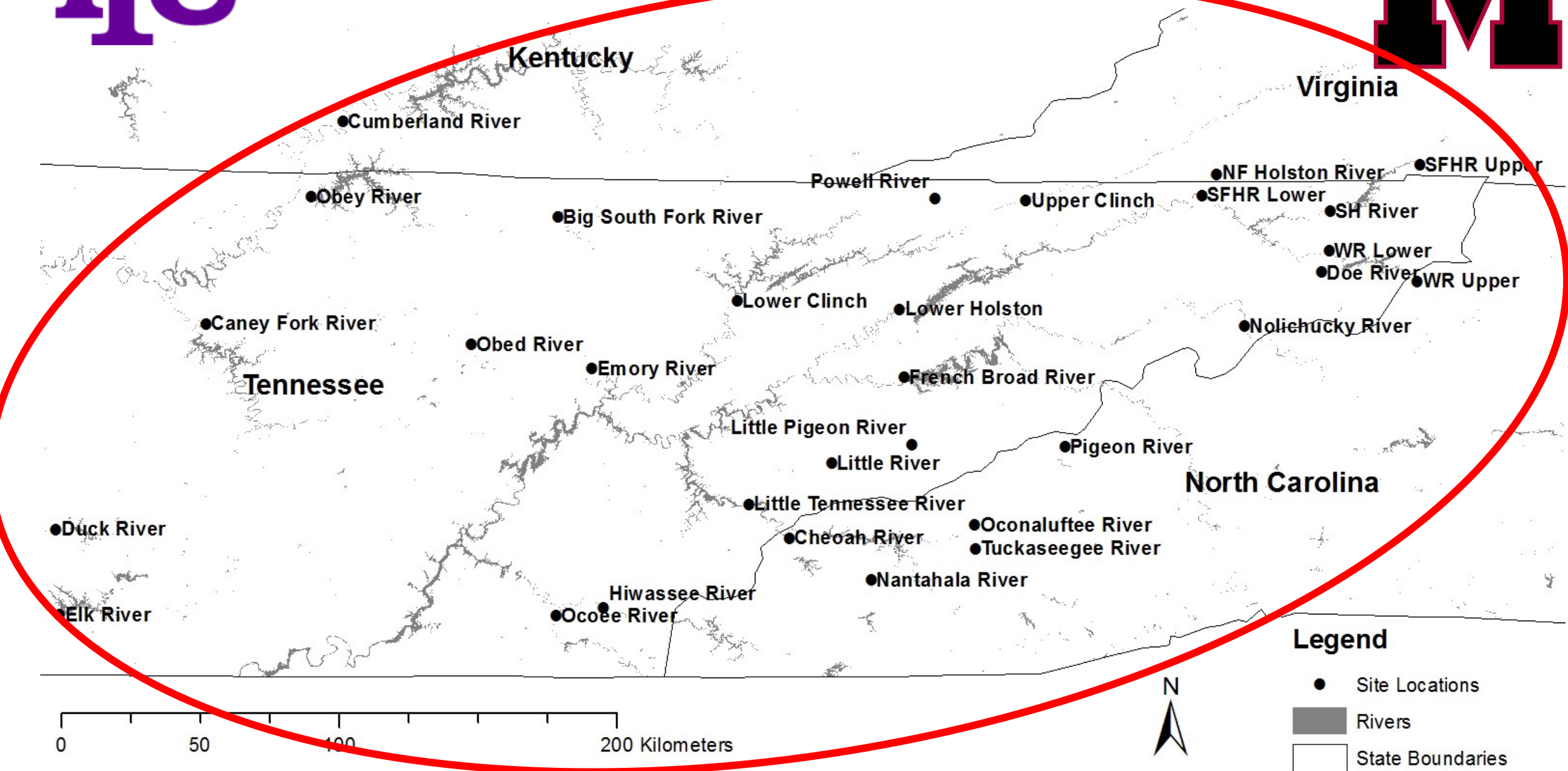


Results: Microscope



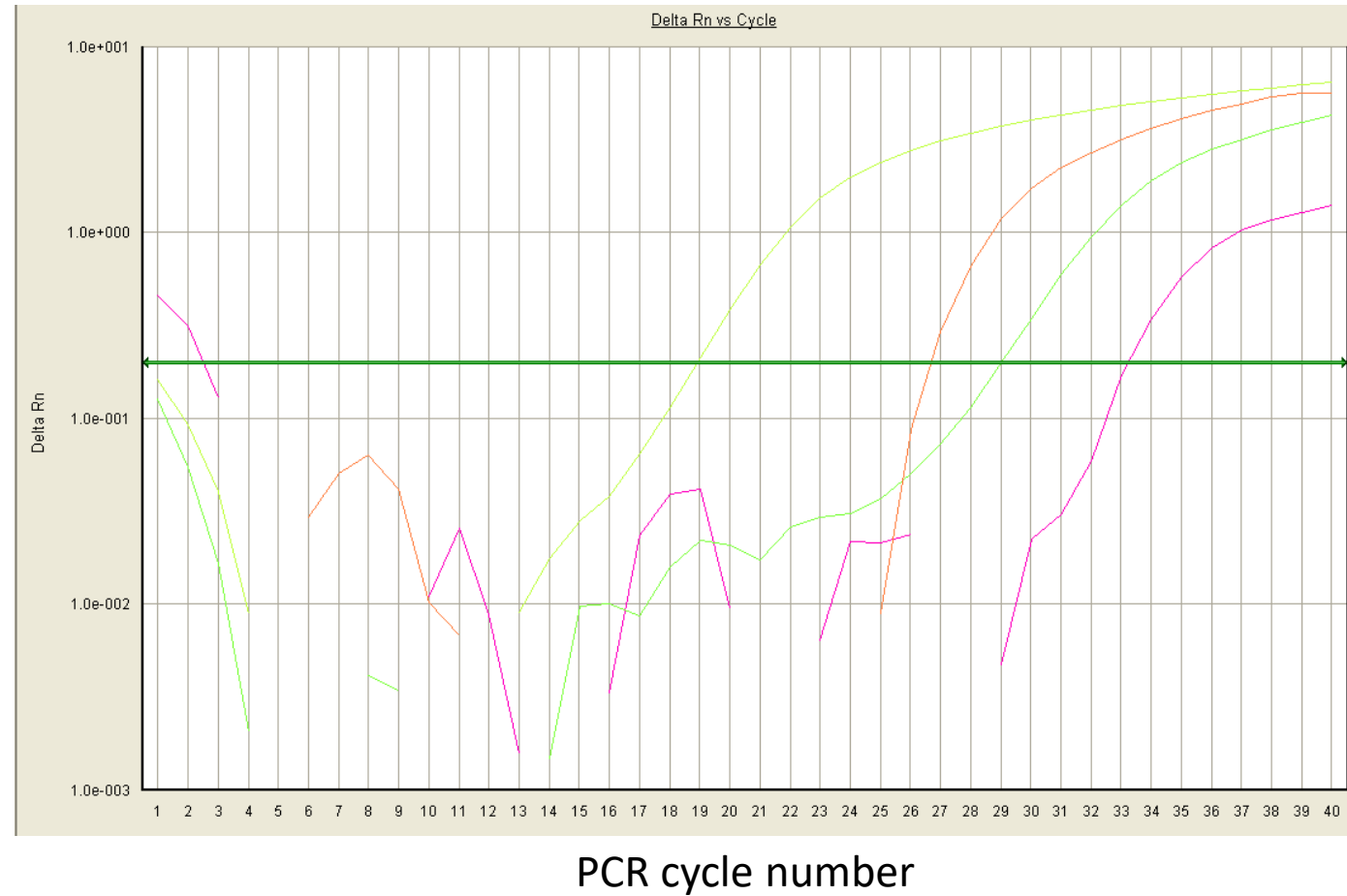


Results – eDNA



Results eDNA

- All positive rxns worked

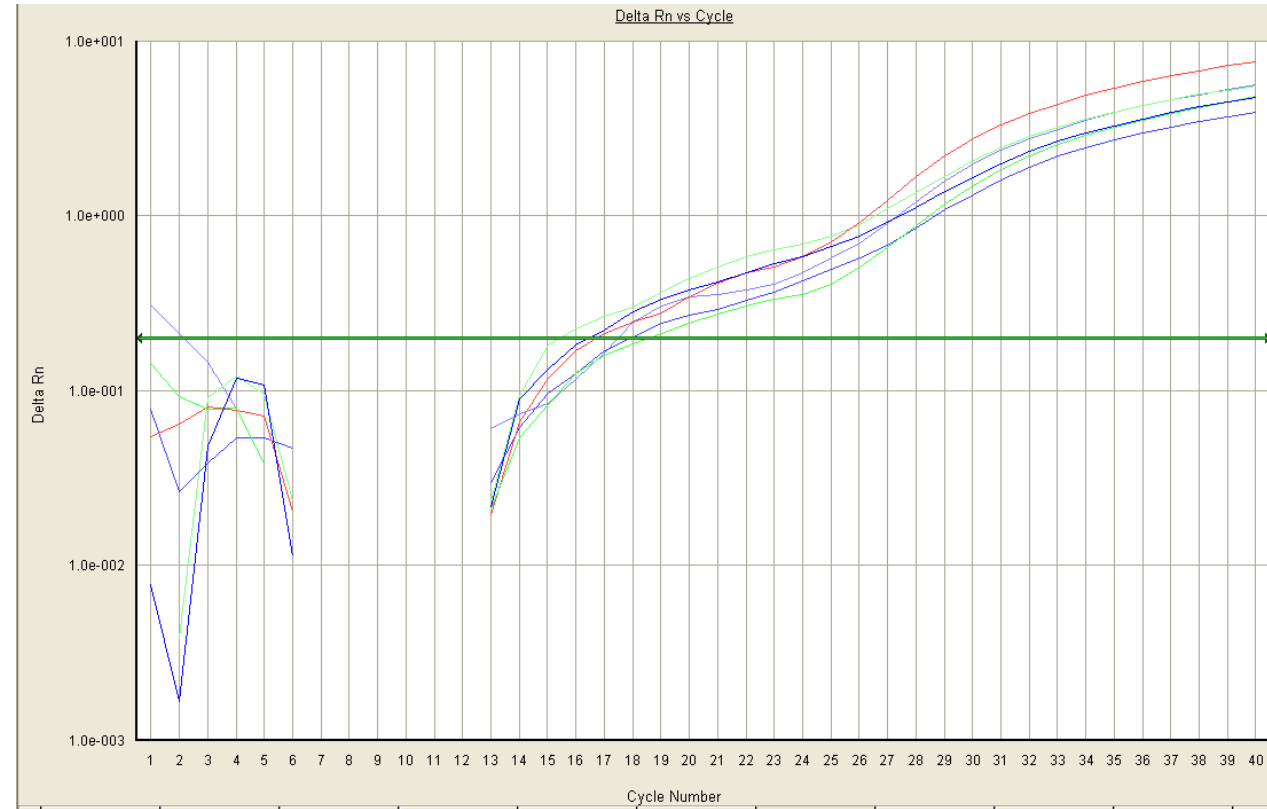


Results eDNA

- All positive rxns worked
- No contamination in negatives

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 - Nantahala
 - Cheoah
 - NF Holston



Results eDNA

- All positive rxns worked
- No contamination in negatives
- No PCR inhibition
 - Nantahala
 - Cheoah
 - NF Holston
- Field/lab contamination?
- Amplification of another species?



Results eDNA?

- Field contamination?
 - Stringent field protocols
 - 15 min soak; 5% bleach

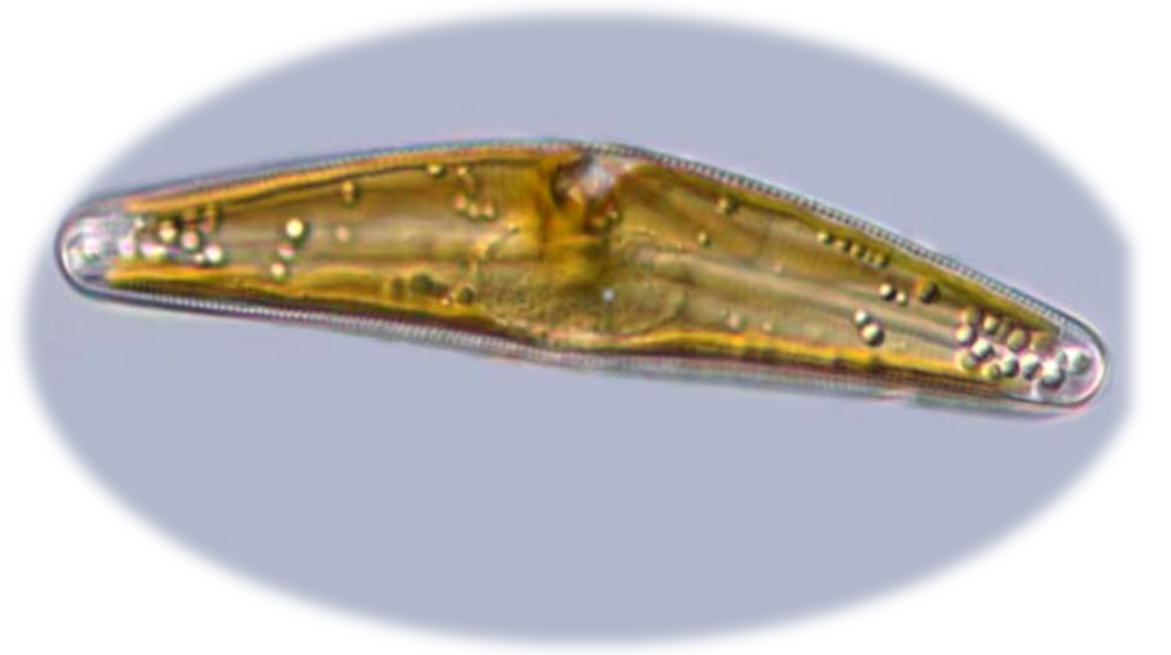


Results eDNA?

- Lab contamination?
 - Stringent lab protocols
 - DNA extracted in another room
 - UV sterilization
 - Multiple DNA extraction attempts
 - New kits
 - Multiple PCR runs
 - No positive rxns in negative controls
 - New chemicals

Results eDNA?

- Amplification of another species?
 - *Cymbella* sp.
 - Primers appear specific to didymo
 - Something else?
 - Sequencing PCR product



Conclusions

- Standard microscope evaluation
 - Fast
 - Limited training





Detection of quagga mussels using eDNA



- Quagga mussel (*Dreissena bugensis*)
 - Environmental impacts
 - Human impacts





Detection of quagga mussels using eDNA



- **GOAL:** Confirm presence of Quagga in a quarry linked to Susquehanna River





West 1

West 2

West 3

Outflow

East 1

East 2

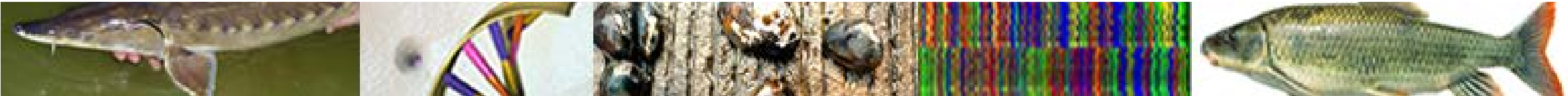
East 3

Small Quarry



eDNA detection of aquatic species from the Susquehanna and St. Lawrence basins

Gregory R. Moyer (Mansfield University),
Meredith Bartron (USFWS), Daniel Gefell (USFWS), Chris Reese (USFWS),
James Casey (Cornell University), Rodman Getchell (Cornell University)





qPCR Primer Development



- Four taxa
 - *Lepomis gibbosus*
 - *Pimephales notatus*
 - *Rhinichthys atratulus*
 - *Etheostoma olmstedii*

Species	St. Lawrence*			Susquehanna*	
	OSWE	RAQU	SREG	OTSL	CHEN
<i>L. gibbosus</i>	XX	XX	XX	XX	XX
<i>P. notatus</i>	O	O	O	X	X
<i>R. atratulus</i>	O	O	O	XX	XX
<i>E. olmstedii</i>	X	X	O	XX	XX

*Based on visual inspection of Carlson et al 2016 *Atlas of Inland Fishes of NY*

XX = Densely and widely populated;

X = Widely populate;

O = Limited range;



Research questions

- 1) Between basin?
 - Difference in eDNA detection bw drainages?
 - Expectation?

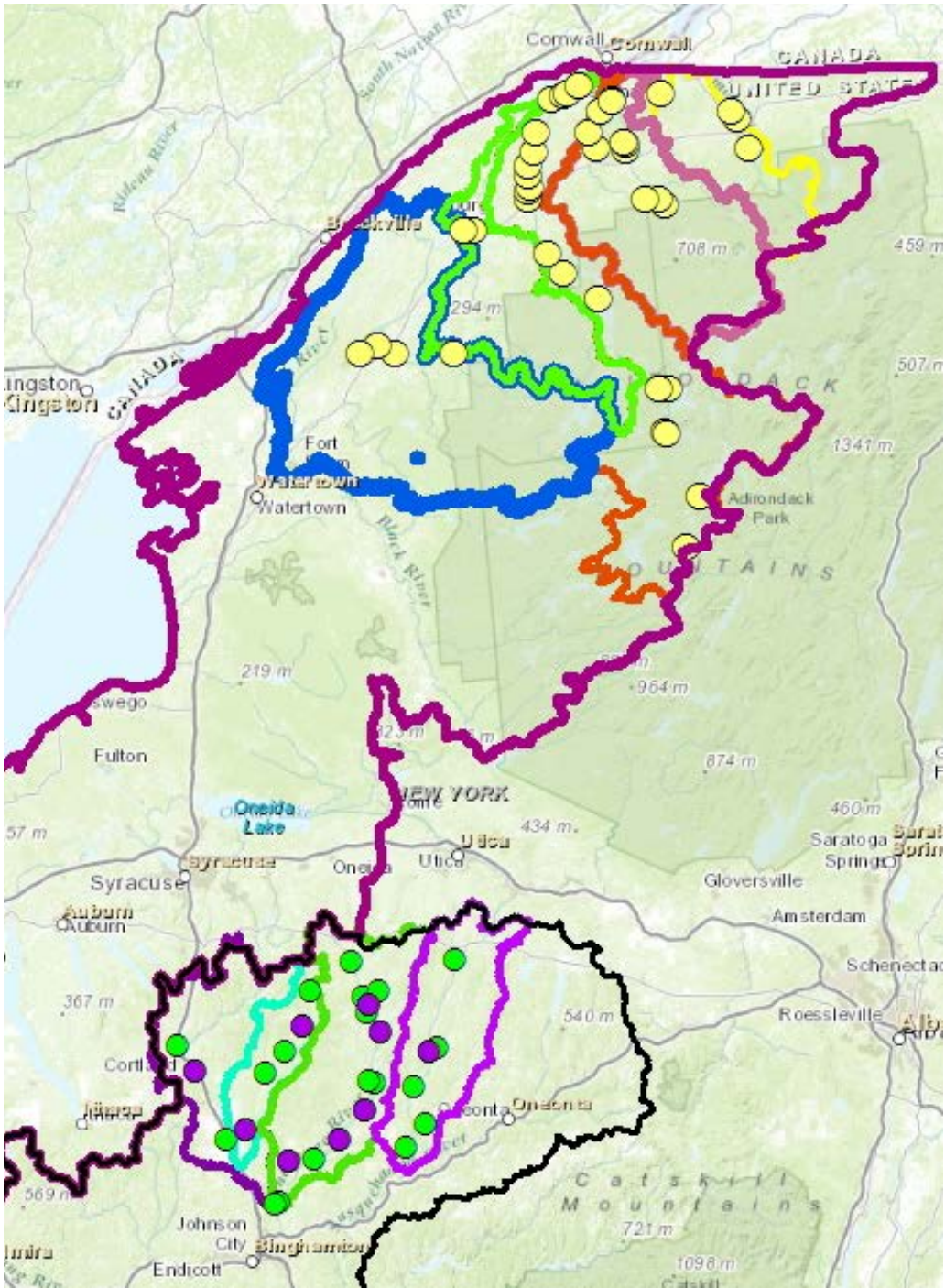
	St. Lawrence			Susquehanna	
Species	OSWE	RAQU	SREG	OTSL	CHEN
<i>L. gibbosus</i>	XX	XX	XX	XX	XX
<i>P. notatus</i>	O	O	O	X	X
<i>R. atratulus</i>	O	O	O	XX	XX
<i>E. olmstedii</i>	X	X	O	XX	XX

*Based on visual inspection of Carlson et al 2016 *Atlas of Inland Fishes of NY*

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Research questions

1) Between basin - results

Species	Coefficients	Estimate	SE	z value	Pr(> z)	odds ratio
<i>L. gibbosus</i>	Susquehanna	-0.539	0.475	-1.132	0.257	0.5
<i>P. notatus</i>	Susquehanna	2.773	1.092	2.538	0.011	16
<i>R. atratulus</i>	Susquehanna	2.023	0.533	3.796	>0.001	7.56
<i>E. olmstedii</i>	Susquehanna	4.836	0.852	5.670	>0.001	126

	St. Lawrence			Susquehanna	
Species	OSWE	RAQU	SREG	OTSL	CHEN
<i>L. gibbosus</i>	XX	XX	XX	XX	XX
<i>P. notatus</i>	O	O	O	X	X
<i>R. atratulus</i>	O	O	O	XX	XX
<i>E. olmstedii</i>	X	X	O	XX	XX

eDNA metabarcoding



Results: Relative abundance

Common name	eFish % abundance	eDNA % abundance	eFishing Rank	eDNA rank
Safron shiner	0	0	2	2
Tennessee shiner	0	0	2	2
Northern hogsucker	0	0	2	2
River chub	0.0219	0.0691	8	8
Warpaint shiner	0.011	0.0023	6.5	4
Creek chub	0.2326	0.0318	10	6
Stoneroller	0.011	0.1022	6.5	9
Rosyside dace	0.0037	0.0387	4	7
Tennessee snubnose darter	0.0055	0.008	5	5
Blacknose dace	0.425	0.148	12	11
White sucker	0.0348	0.1292	9	10
Rainbow trout	0.2545	0.4685	11	12

Spearman's $\rho = 0.86$; p -value = 0.0004

Questions?