



UMBC

Using Environmental DNA to Study Brook Trout Populations in the Headwaters of the Chesapeake Bay

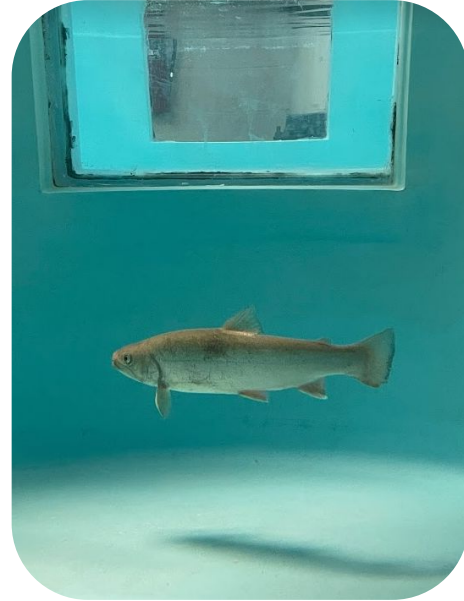
Aiman Raza
Thesis Defense
4/4/24





Outline

1. Introduction
2. Background
3. Research Objectives:
 - Temperature Trials
 - Distance Trials
 - Filter Pore Size Comparison
4. Applications
5. Outreach



Introduction: ICARE Program

- **Faculty Mentor:** UMBC - Tamra Mendelson
- **Partner mentors:** USGS Eastern Ecological Science Center - Aaron Aunins, Cheryl Morrison, Nathaniel Hitt
- **Community Stakeholder:** Gunpowder RIVERKEEPER® - Theaux Le Gardeur



UMBC



Background: Brook Trout

(Salvelinus fontinalis)

- Native to Eastern North America
- Bioindicators of cold, clean water
- MD DNR lists as “species of greatest need of conservation”



Photo Credit: Maryland Department of Natural Resources

Native Range of Brook Trout

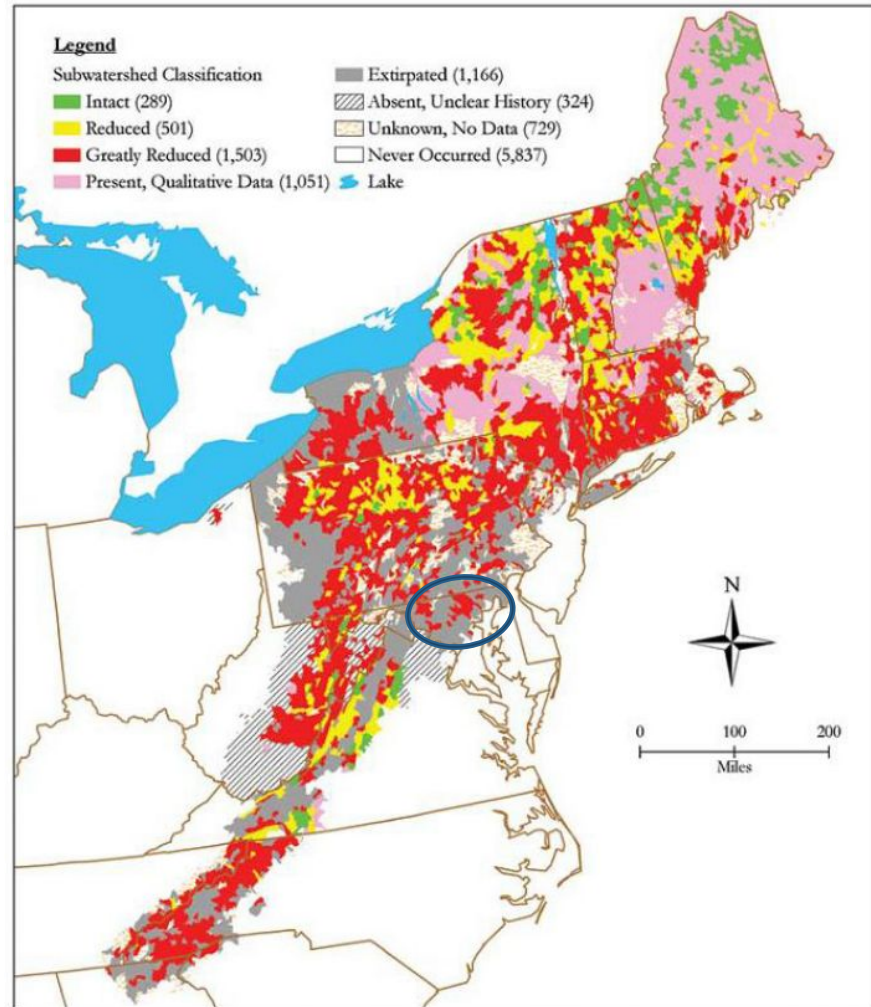


Photo Credit: Maryland Department of Natural Resources

Chesapeake Bay Program Goals for Brook Trout Conservation



CBP Watershed Agreement
set a goal for an **8%**
increase in Brook Trout
occupancy by 2025

Environmental DNA (eDNA)

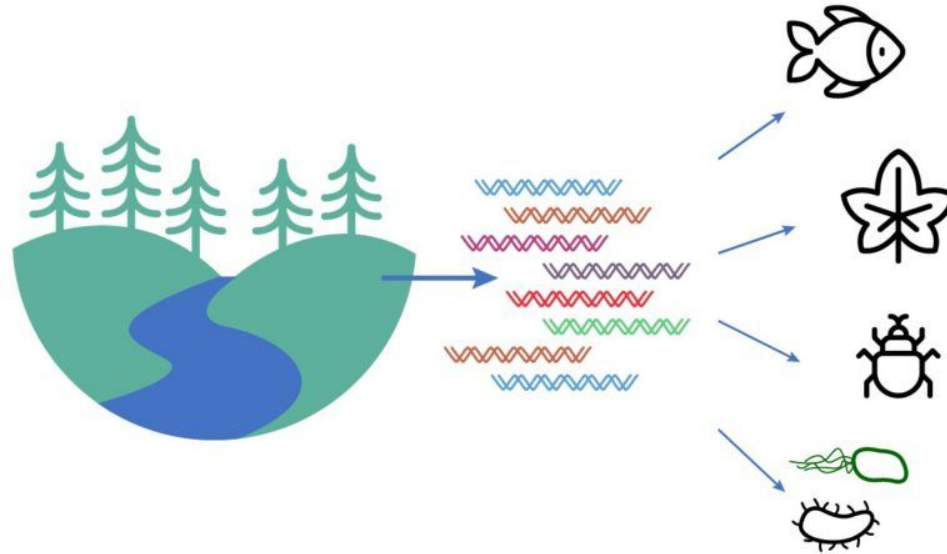


Photo Credit: miniPCR DNAdots

Background: eDNA

- Environmental DNA (eDNA) is genetic material left by an organism in its environment
- Revolutionizing how we survey aquatic systems
 - Management
 - Detect invasive species
- Methods to study eDNA
 - Single species
 - Multi-species

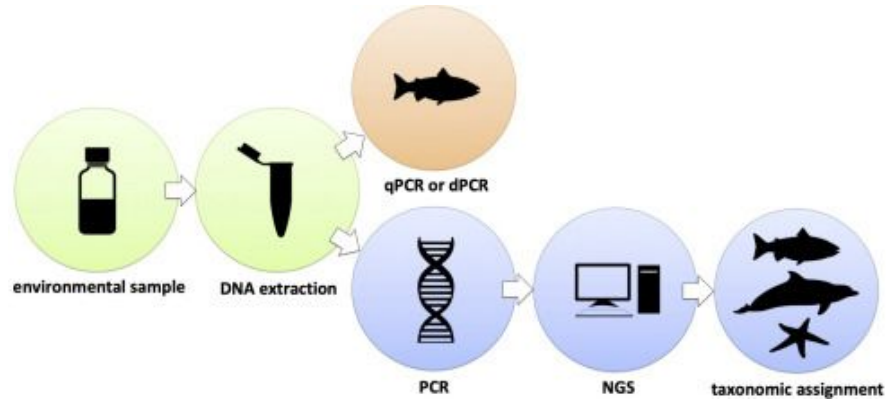


Photo Credit: Eble et al. 2020

Background: eDNA

- Environmental DNA (eDNA) is genetic material left by an organism in its environment
- Revolutionizing how we survey aquatic systems
 - Management
 - Detect invasive species
- Methods to study eDNA
 - Single species
 - Multi-species
- eDNA dynamics not well understood

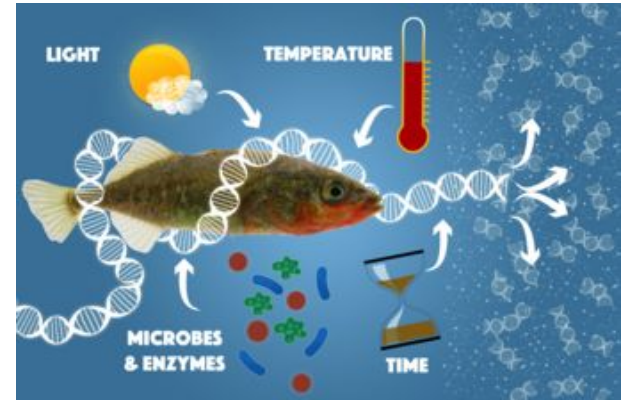


Photo Credit: USGS

Research Objectives

Study the effects of water temperature, distance, and filter pore size on eDNA detection to inform Brook Trout management



01

**Temperature
Trials**



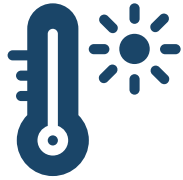
02

**Distance
Trials**



03

**Filter Pore Size
Comparison**



01

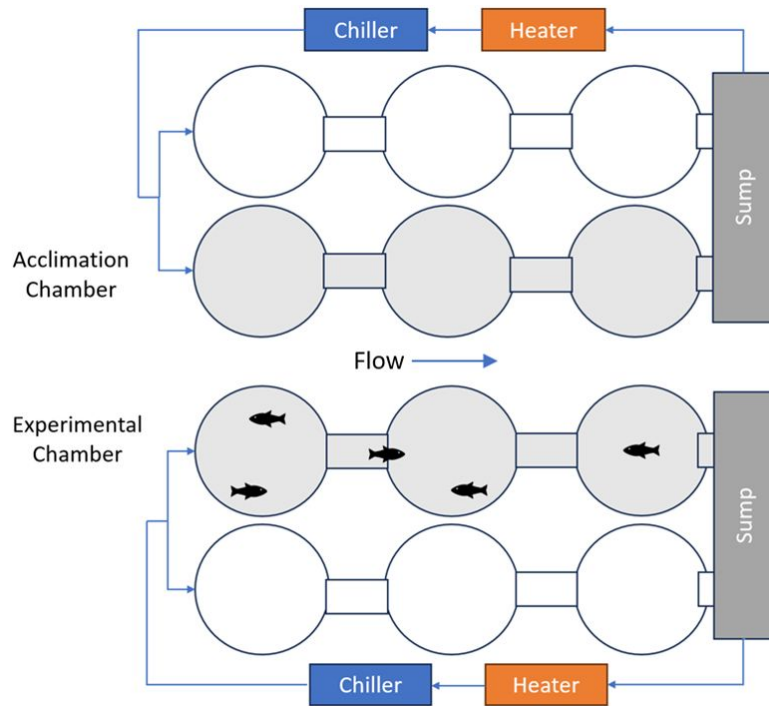
Temperature Trials

Does temperature affect Brook Trout
eDNA concentration?

(January 2023)



Temp Trials: Methods



- Recirculating stream tank setup
- Transfer 5 fish to acclimation chamber
- Set to target temp (10° or 20° C)
- Transfer to experimental chamber



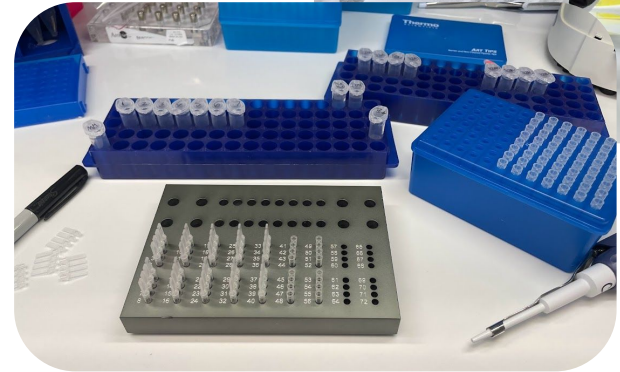
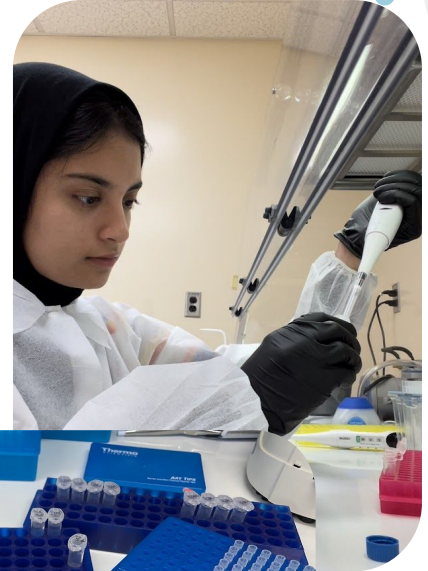
Temp Trials: Methods

- Take eDNA samples after 1 hour in experimental chamber
 - Smith-Root eDNA Sampler
 - 1 L triplicate samples
 - 1.2 micron filters
- Move fish back to acclimation chamber
- Clean and disinfect
- Repeat trial

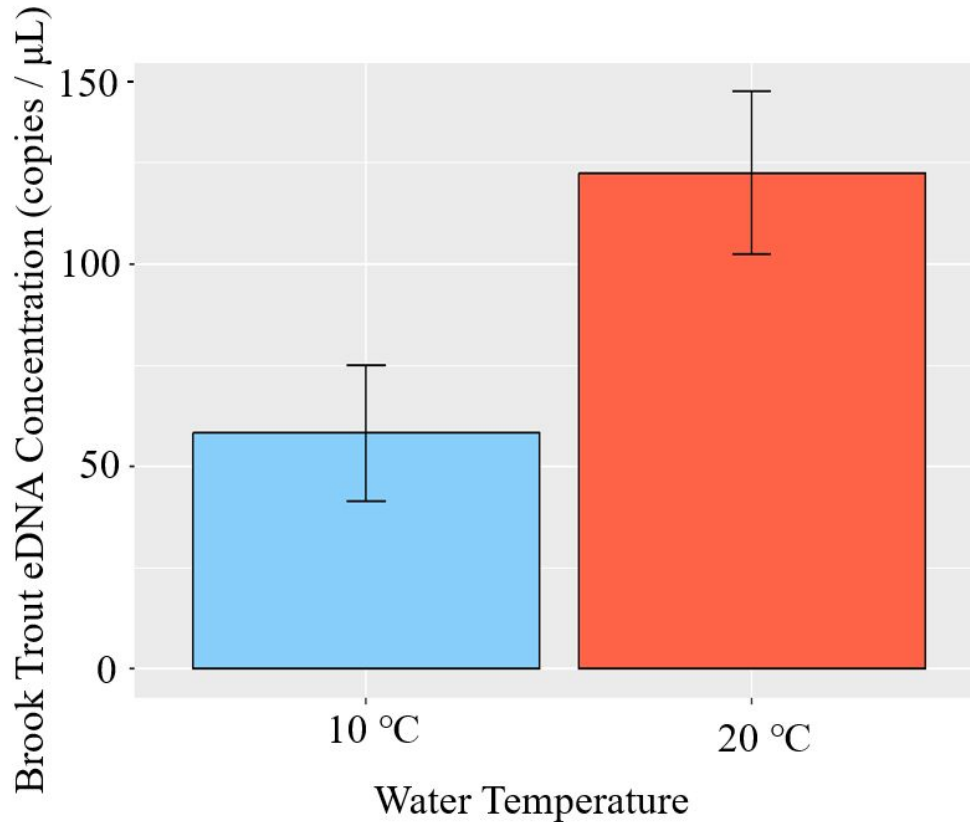


Temp Trials: Methods

- Extract DNA (Qiagen DNeasy PowerWater kit)
- Quantitative PCR (qPCR)
 - *BRK2* Taqman assay based on Wilcox et al. (2013)
 - Targets Brook Trout mitochondrial *cyt b* region

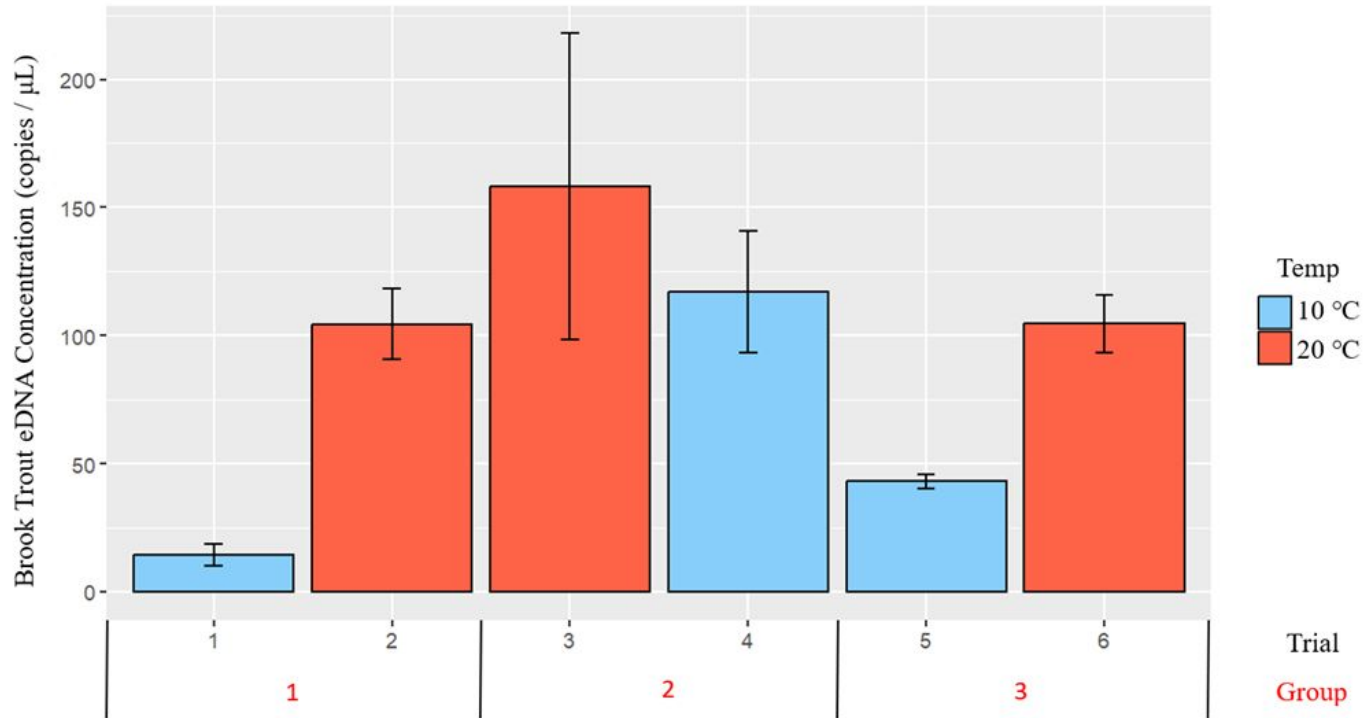


Results: Higher eDNA concentration at higher temperature



Repeated Measures ANOVA, **P-value = 0.0019**

Results: eDNA concentration per trial through time



Why was there a higher eDNA concentration at the higher temperature?



Increased shedding maybe due to:

- Stress: $>21^{\circ}\text{C}$ trigger stress response
- Metabolism: higher energy expenditure



02

Distance Trials

How does distance from a source of eDNA influence eDNA detection in a natural stream environment?

(March & June 2023)

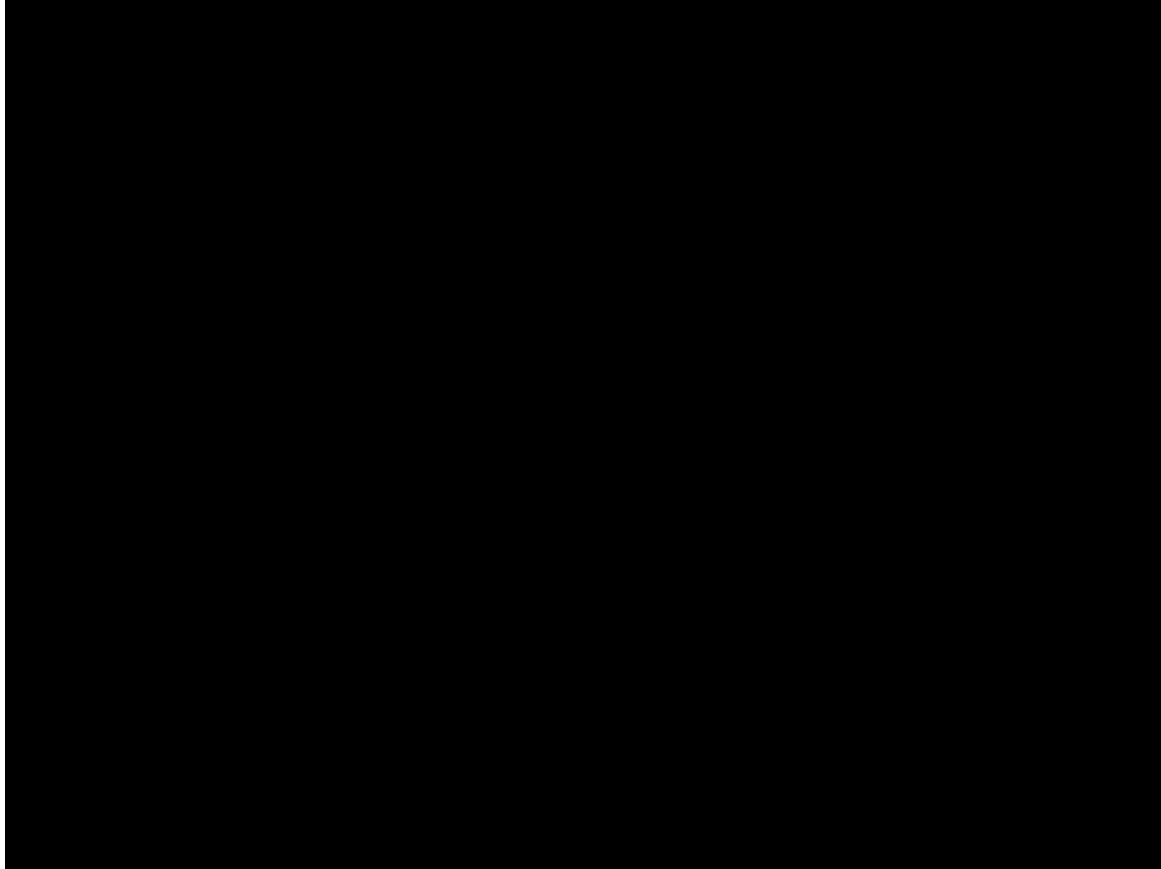


Distance Trials: Methods

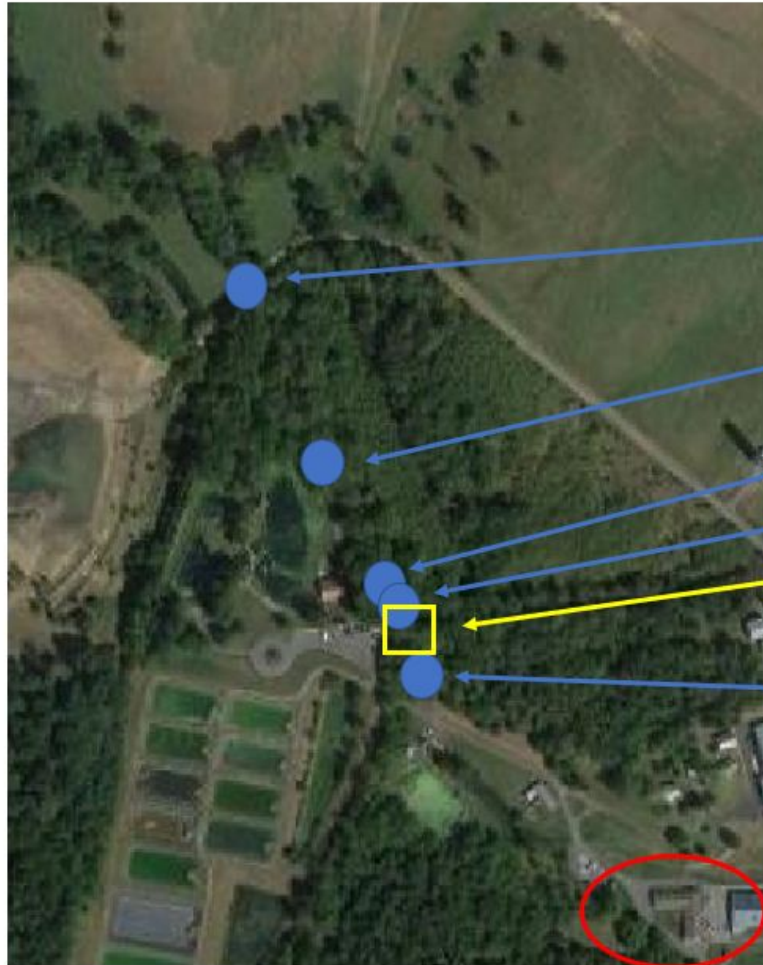


- Placed 3 fish in cage in troutless stream (Hopewell Run, WV)
- After 24 hours, sampled at 1m, 10m, 50m, 100m, and 2000m downstream of cage
- Also sampled 5m upstream of cage

Transferring Fish to Cage



Sampling Sites



100 m from cage

50 m from cage

10 m from cage

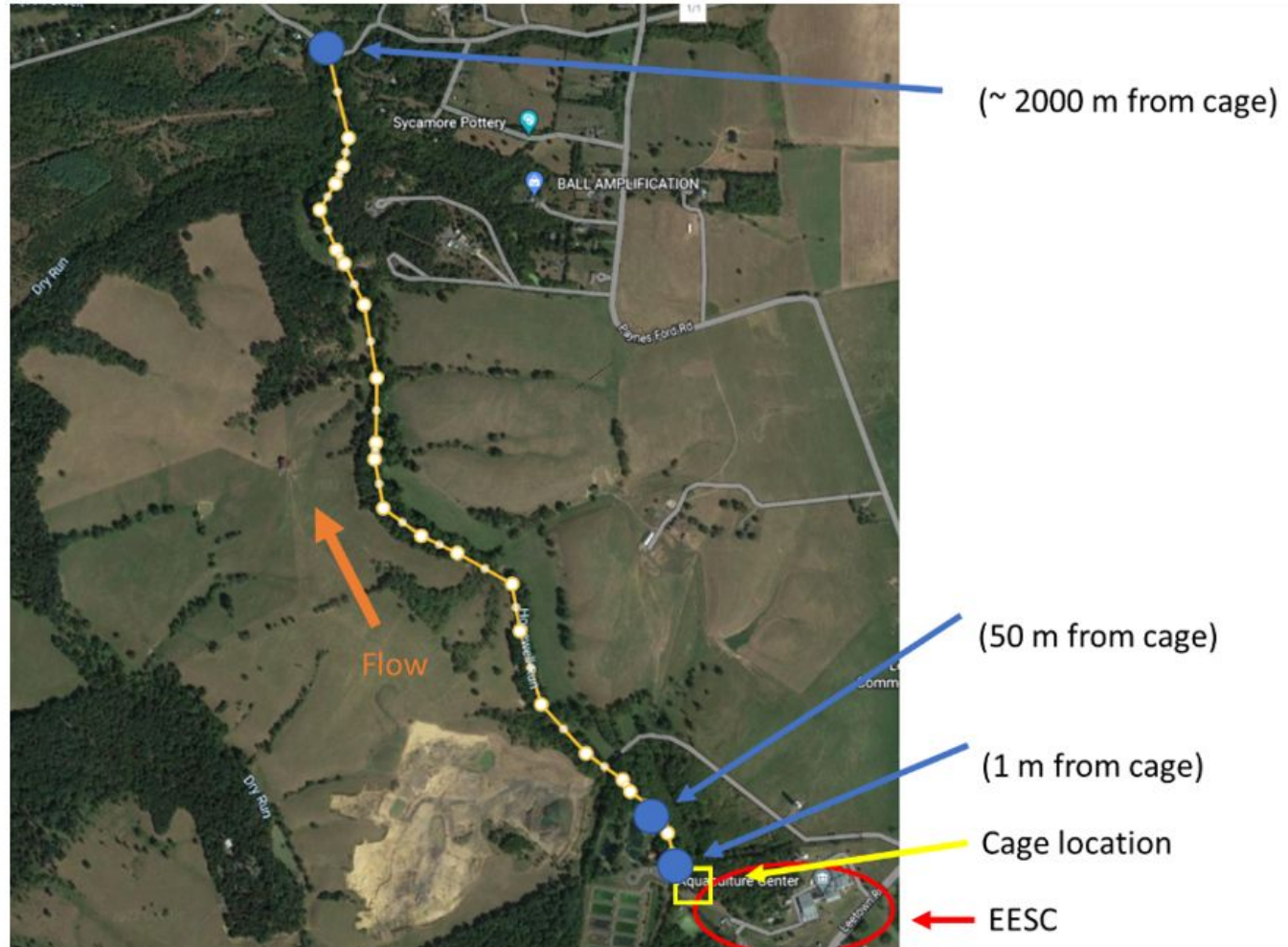
1 m from cage

Cage Location

5 m upstream
from cage

EESC Stream Lab

Sampling Sites

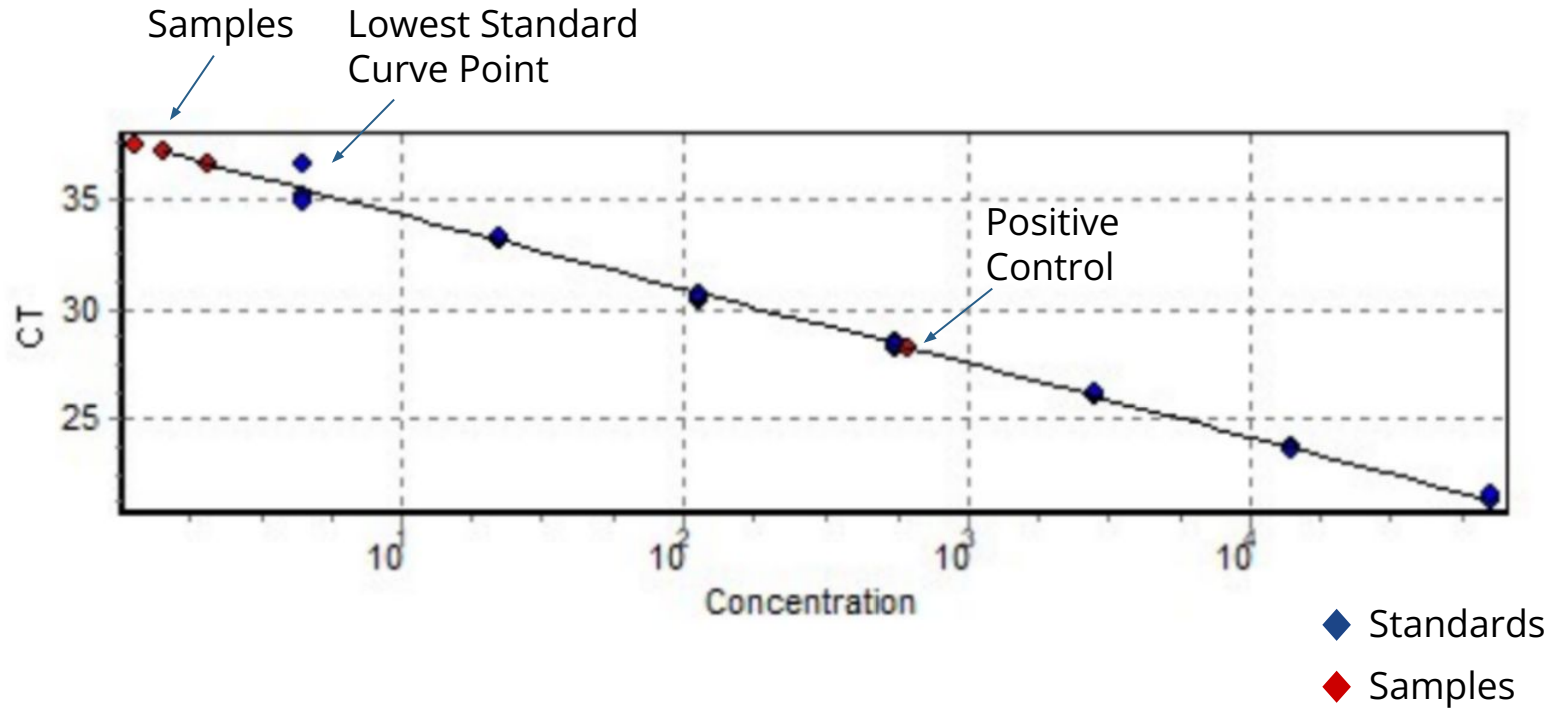


Distance Trials: Methods

- Smith-Root eDNA Sampler
 - 1 L triplicate samples
 - 1.2 micron filters
- Extracted DNA and qPCR
- Internal positive controls to test for inhibition



Results: Insufficient eDNA, cannot accurately quantify



Similar results to another experiment

- Similar results as Dr. Robert Hilderbrand at UMCES Appalachian Laboratory
- Only other Brook Trout distance trials in MD



Dr. Robert Hilderbrand

Why did we not collect enough eDNA?

- Not enough fish
- Insufficient water volume
- Filter pore size too small





03

Filter Pore Size Comparison

How does filter pore size influence eDNA collection
in streams with known Brook Trout occupancy?

(September - October 2023)



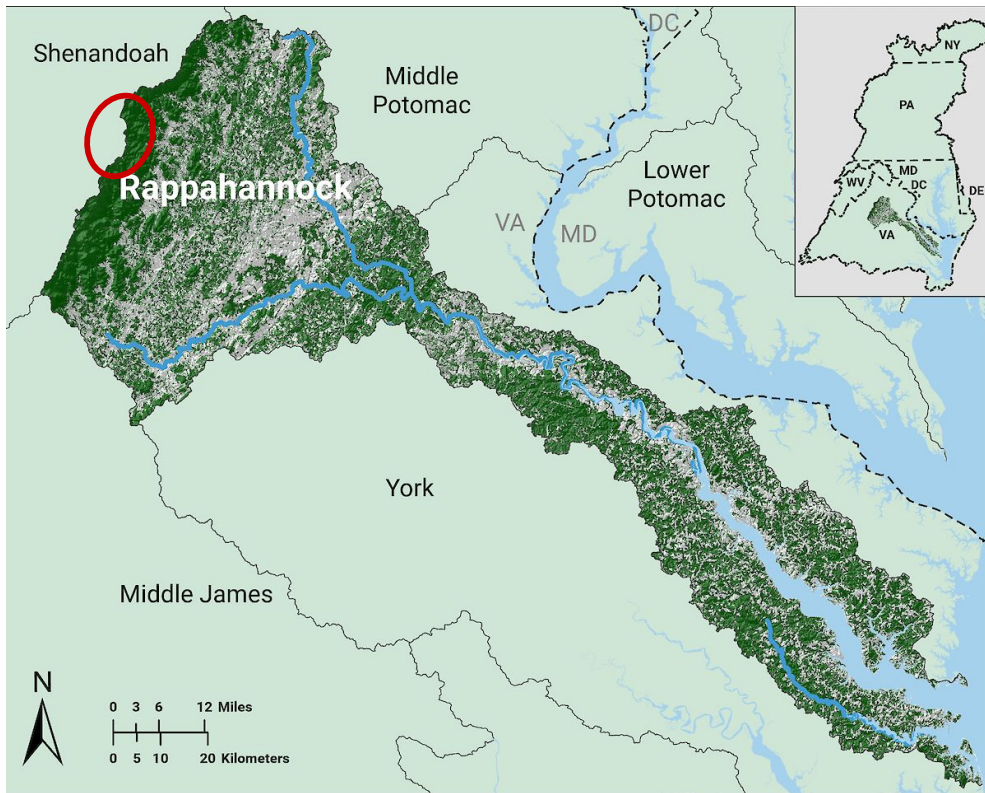
Methods: Filter Pore Size Comparison

- Compare 1.2-micron vs 5-micron filter pore size
- Pair with electrofishing data
- 3 sites in Gunpowder Falls watershed
- 3 sites in Rappahannock watershed



Credit: Smith-Root

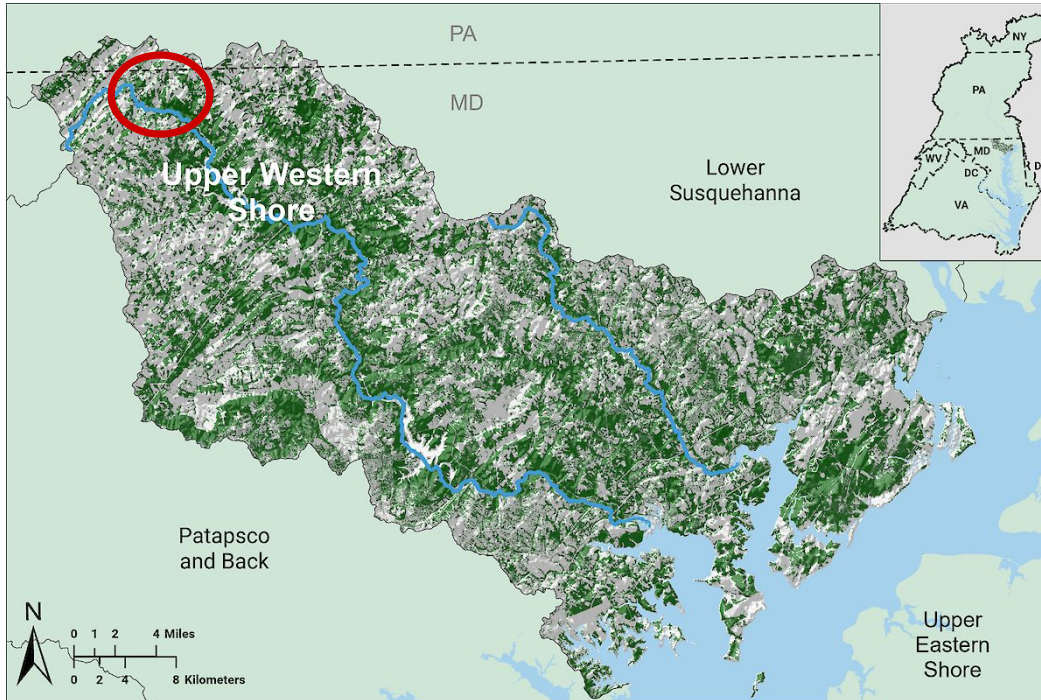
Sampling Locations



Rappahannock Watershed, VA (Shenandoah National Park)

Credit: Eco Health Report Cards

Sampling Locations



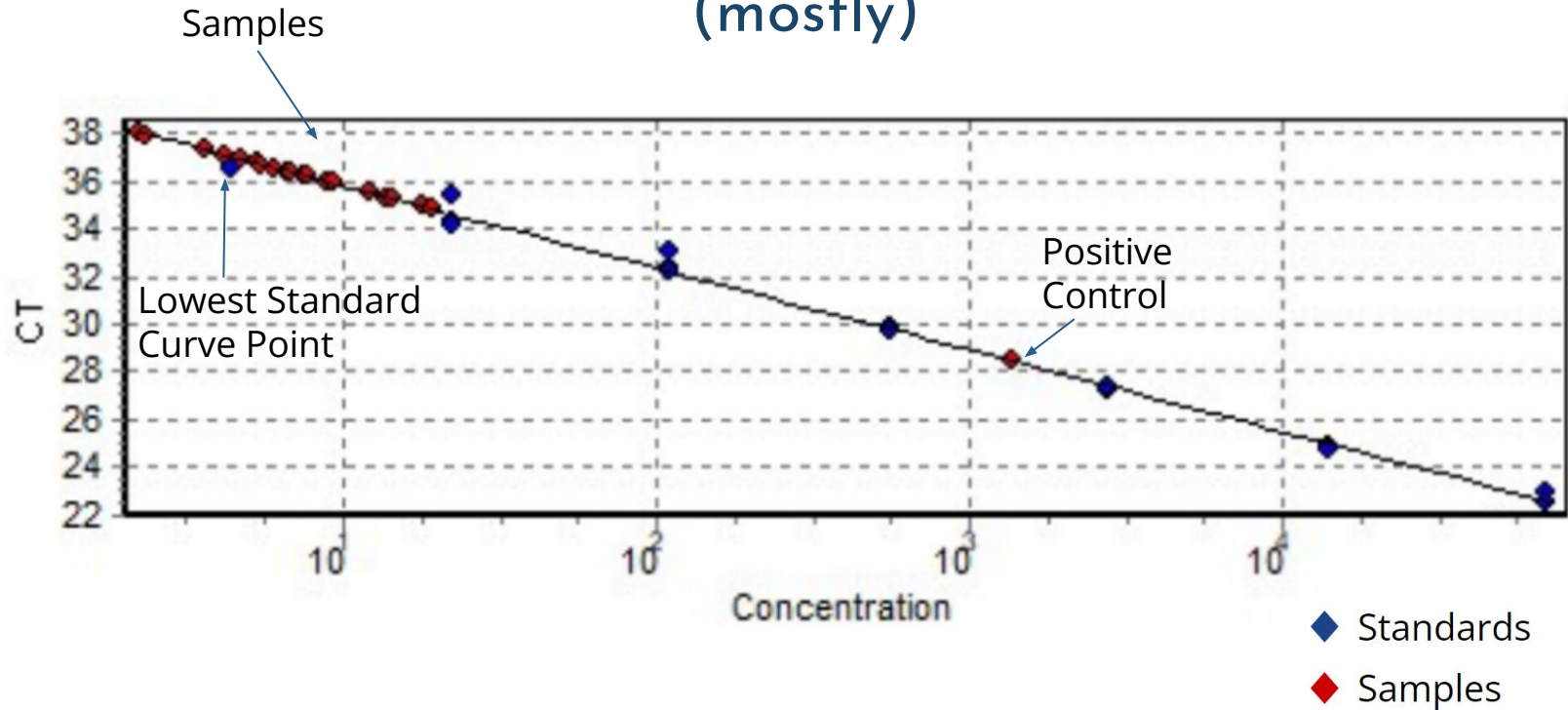
Gunpowder Watershed, MD

Methods: Filter Pore Size Comparison

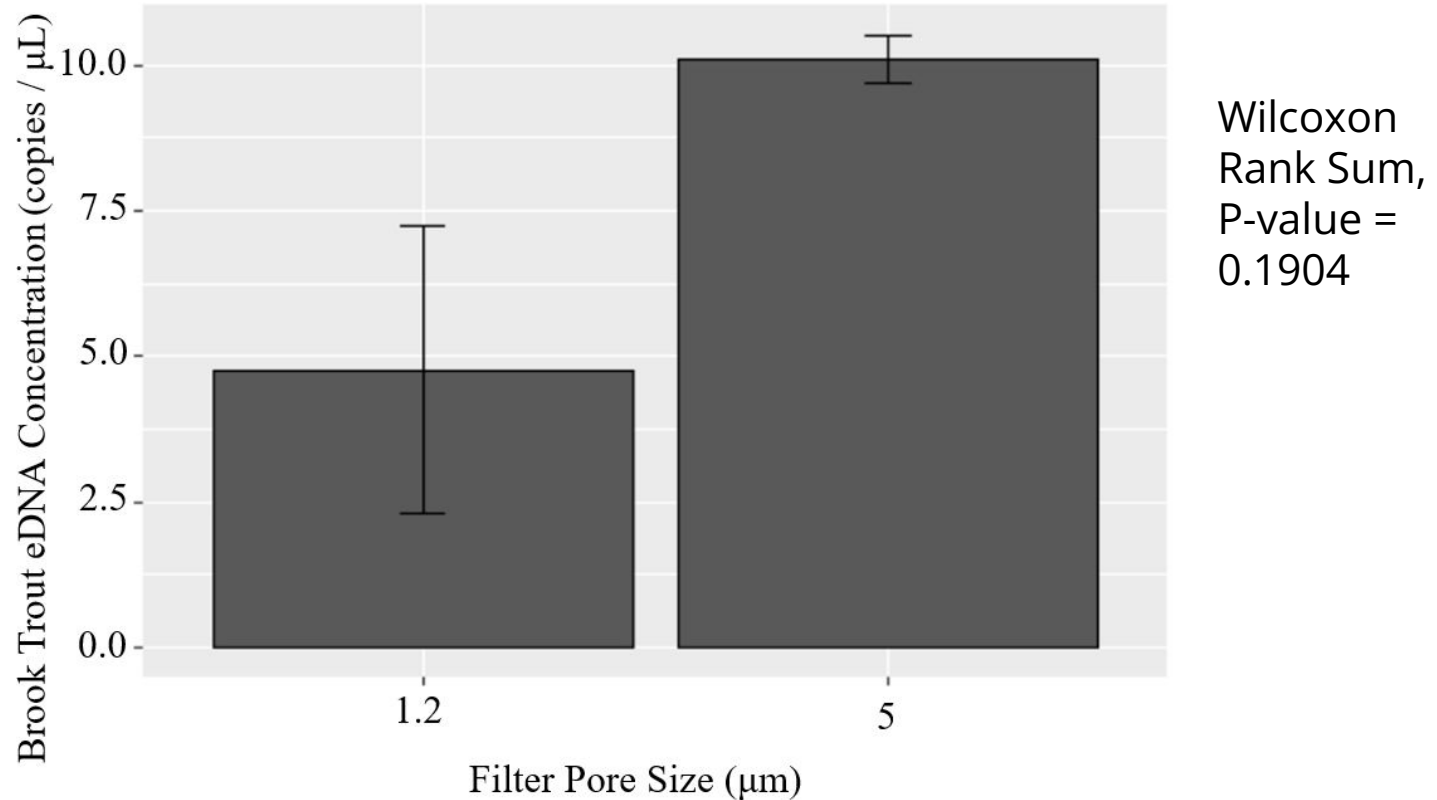
- Smith-Root eDNA Sampler
 - 9 L triplicate samples
 - 1.2 and 5 micron filters
- Extracted DNA and qPCR
- Internal positive controls to test for inhibition



Results: Insufficient eDNA, cannot accurately quantify (mostly)

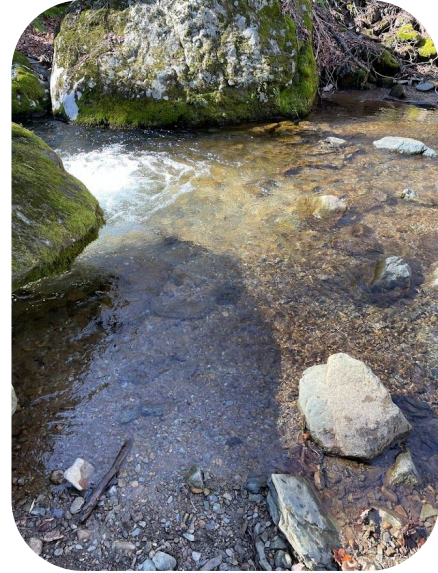


Hogcamp Branch: No significant difference in eDNA concentration collected between filter pore sizes



Why did we not collect enough eDNA (again)?

- Insufficient water volume
- Inappropriate filter material
- Inappropriate collection method
- Hogcamp Branch was larger



Hogcamp Branch,
Shenandoah National Park



Follow-up: Water Volume & Filter Type

Will increased water sample volume or different filter material allow for increased eDNA collection?

(February 2024)



Methods: Water Volume & Filter Material

- Compare Smith-Root self-preserving PES filter vs glass fiber filter
- Compare 9 L (3 L per filter) vs 18 L (6 L per filter)
- Hogcamp Branch in Shenandoah National Park



PES Filter



Glass Fiber Filter

Results TBD, Analysis Ongoing



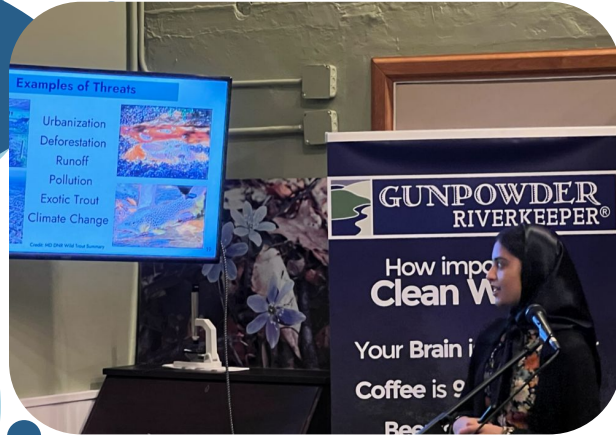
Study Conclusions

1. **Temp Trials:** Higher eDNA concentrations at higher temperatures
2. **Distance Trials:** Insufficient eDNA collection
3. **Filter Pore Size Comparison:** No significant difference in 1.2- and 5-micron pore size (lack statistical power)
4. **Water Volume Collection:** TBD
5. **Filter Material:** TBD

Applications

- Consider temperature when estimating Brook Trout abundance from eDNA
- Consider water volume filtered
- Optimize methods, eDNA protocols are not “one-size-fits-all”
- Electrofishing surveys are still needed

Outreach

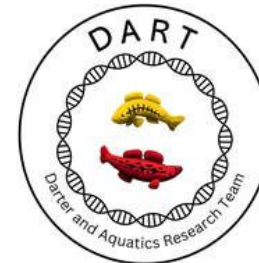


- Talk at Sparks Bank Nature Center on 9/28/23
- Public survey: "Are You Smarter than a Brook Trout?"



Acknowledgements

- Faculty Mentor (UMBC):
Tamra Mendelson
- Partner Mentors (USGS - EESC)
 - Aaron Aunins
 - Cheryl Morrison
 - Than Hitt
 - Stephen Faulkner (retired)
- Community Stakeholder (Gunpowder RIVERKEEPER®):
Theaux Le Gardeur
- SNP - Evan Childress
- MD DNR - Mark Staley
- DART Lab



**Thank you
for listening!**

Questions?

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