Cornell University
College of Veterinary Medicine

Sea Grant
NewYork

# WNY PRISM Partners Meeting: Using eDNA to Detect and Monitor Invasive Species in New York State

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USDA NIFA
United States Department of Agriculture
National Institute of Food and Agriculture

SEPA SCIENCE EDUCATION PARTNERSHIP AWARD
Supported by the National Institutes of Health

# Environmental DNA (eDNA) What is it?

- Environmental DNA refers to DNA that can be extracted from air, water, or soil without isolating any specific type of organism beforehand.
- Two types:
  - Intracellular eDNA
  - Extracellular eDNA
- Intracellular eDNA is commonly used by microbiologists and is derived from cells that are collected.
- Extracellular eDNA is generally assumed to pass through filtration, despite the high concentration of naked DNA in the aquatic environment.

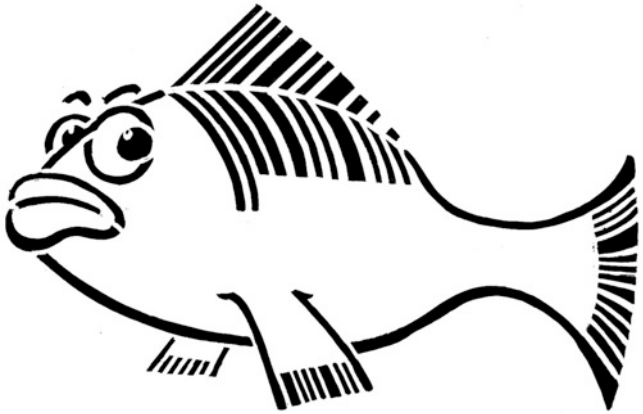
# eDNA Capture and Extraction

Shed cells are collected by filtering the suspect water sample and then extracting DNA from the filter using MoBio PowerWater® DNA Kit.

# eDNA is a viable tool to quantitatively monitor invasive species

The eDNA methods rely on the combination of three complimentary technologies:

1. The Barcode of Life.
2. Allelic discrimination and SNP analysis.
3. qPCR



NIFA

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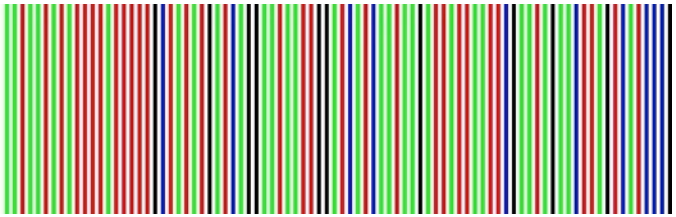
# Barcode of Life

- In 2003, Paul Hebert proposed “DNA barcoding” as a way to identify species.
- Barcoding uses the sequence from a 658-base pair region in the mitochondrial cytochrome c oxidase 1 gene (CO1) the way a supermarket scanner distinguishes products using the black stripes of the Universal Product Code.

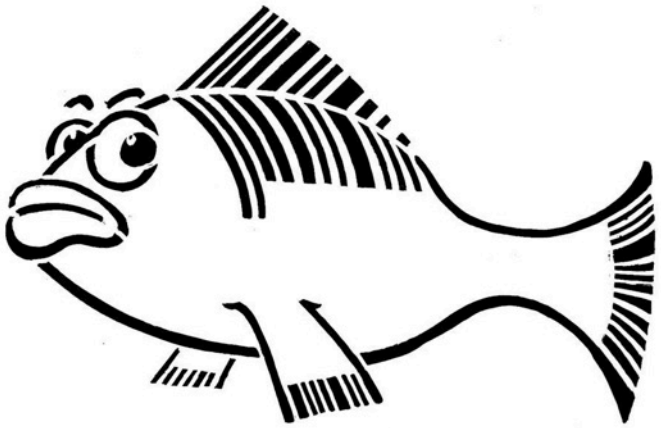



Color DNA sequence barcode for a bee

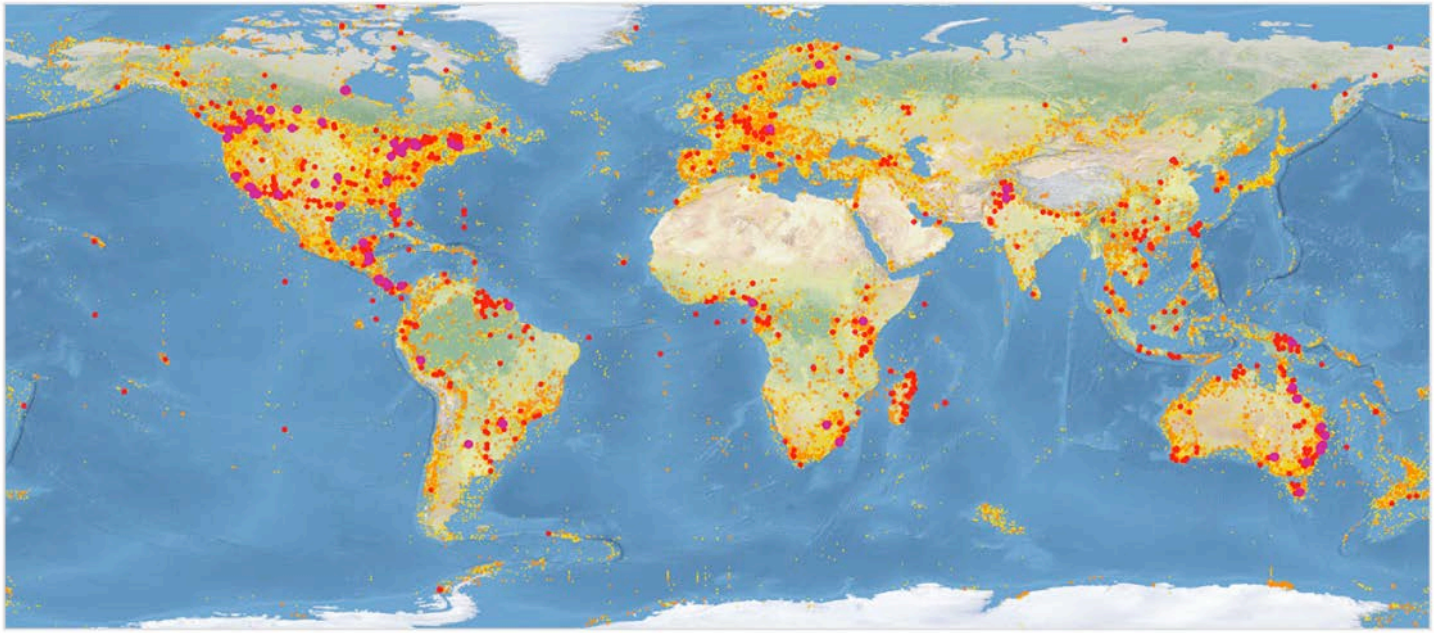
Hebert, P.D.N., Cywinska, A., Ball, S.L., and J.R. deWaard. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society London B. 270:313-321. doi: 10.1098/rspb.2002.2218

# Barcode of Life

- The Consortium for the Barcode of Life (CBOL) is an international initiative dedicated to supporting the development of DNA barcoding.
- More than 4.63 million records are in the public data portal.
- Importantly, most of the fish species in the Great Lakes have been Barcoded.



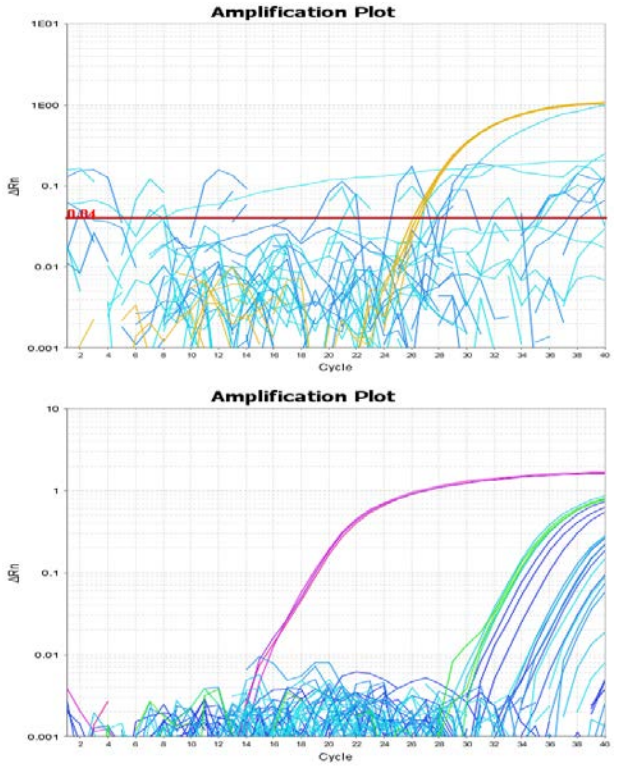
# Barcode of Life



**Project Overview**

**Title:** International Barcode of Life
**CBG Units:** All
**Scope:** International consortium and research alliance with the mission to DNA barcode 500K species
**Project Duration:** Phase I – 2010-2015; Phase II – 2016-2020
**Stage:** In Progress
**Reach:** 26 Nodes (partner nations)
**Sequences:** 4.63M+ DNA barcodes; 500K+ species

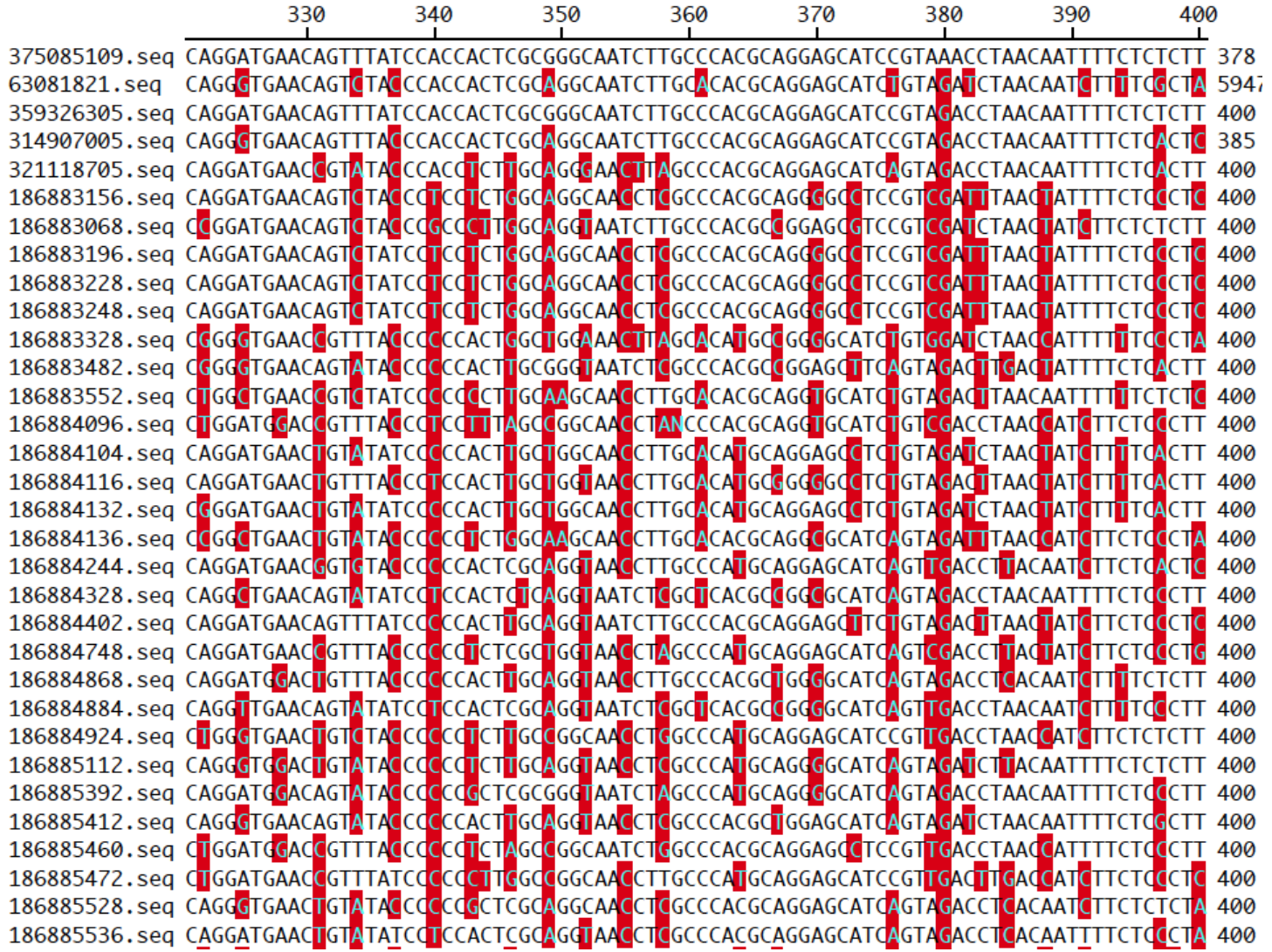
# First Challenge: Asian Carp

# In silico Assessment of qPCR Specificity

CO1 Alignment Great Lakes Fish

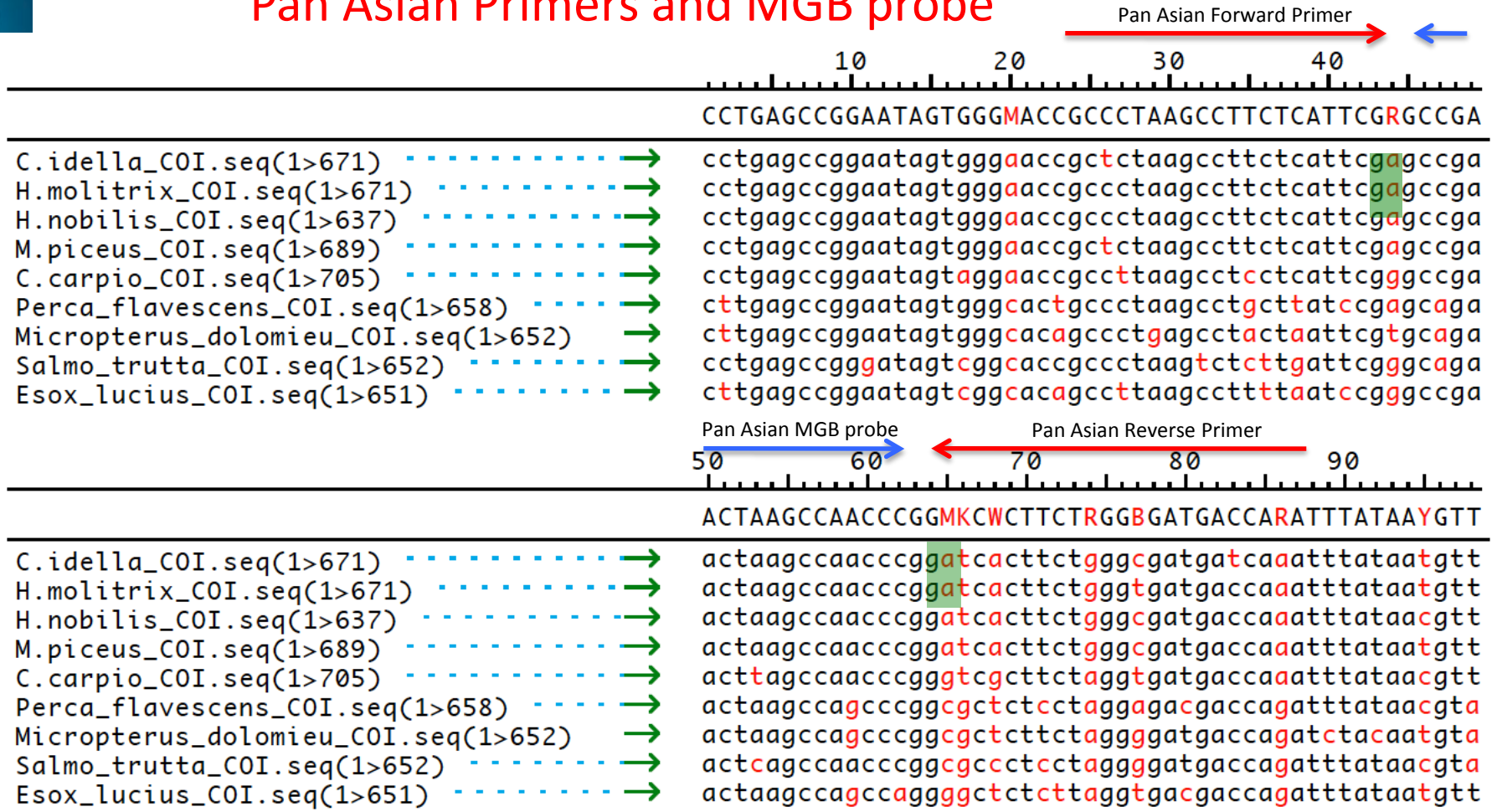
Visually select qPCR targets with maximal mismatch.



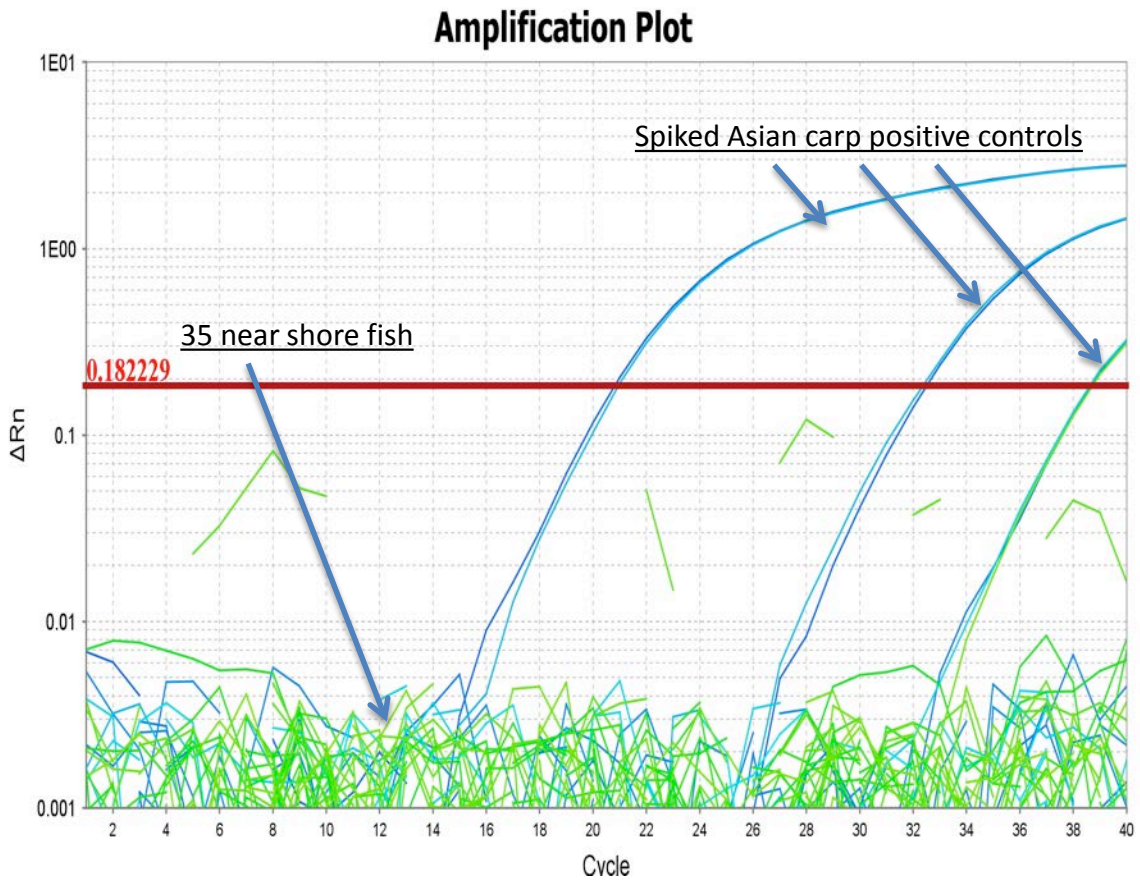
# qPCR Specificity

CO1 Alignment of Asian Carp & Selected Great Lakes Fish
Also design primers and probe for maximal mismatch.

## Pan Asian Primers and MGB probe



# *In vitro* Assessment of qPCR Specificity




| | |
|---|---|
| 1433 | Emmerald Shiner |
| 1568 | Freshwater drum |
| 1650 | White perch |
| 1697 | Goldfish |
| 1920 | Goby |
| 2048 | Black crappie |
| 2441 | Pugnose minnow |
| 2580 | Spottail shiner |
| 2592 | Fall fish |
| 2989 | Brown bullhead |
| 3036 | Yellow perch |
| 3046 | Bluntnose minnow |
| 3057 | Largemouth bass |
| 3061 | Bluegill |
| 3081 | Smallmouth bass |
| 3100 | Pumkinseed |
| 3120 | Goby |
| 3134 | Rock bass |
| 3230 | Golden shiner |
| 3316 | Green sunfish |
| 3558 | Brown trout |
| 3640 | Golden redhorse sucker |
| 3824 | Alewife |
| 3860 | Longnose dace |
| 4072 | Common shiner |
| 4100 | White sucker |
| 4387 | Spoonhead sculpin |
| 4401 | Lake trout |
| 4407 | Eastern brook trout |
| 4424 | Rainbow trout |
| 4431 | Longnose sucker |
| 4752 | Ruffe |
| 4846 | Ninespine stickleback |
| 4882 | Coho salmon |
| 4944 | Mimic shiner |

# Other Invasive Species

## Round Goby

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## Sea Lamprey

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NOAA Great Lakes Environmental Research Laboratory
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"Boca de lamprea. Licensed under CC BY-SA 3.0 via Wikimedia Commons

*** Snakeheads being added in at-risk regions of NY state**

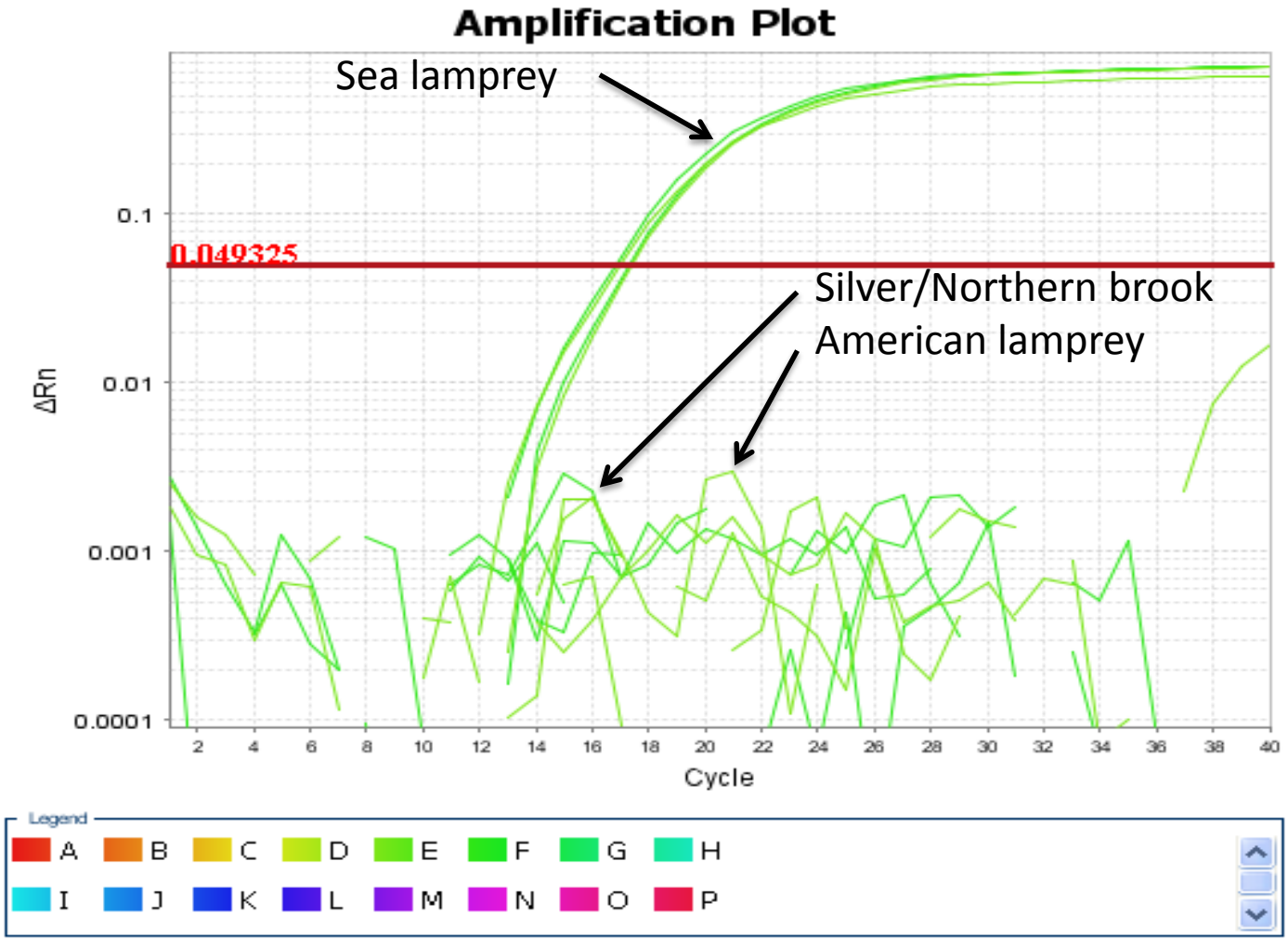
# Sea Lamprey (eDNA)

| | |
|---|---|
| Sea lamprey | ACCCCTAATACTTGGTGCTCC |
| Chestnut lamprey | ACCACTAATACTAAGCGCCCC |
| N. brook lamprey | GCCACTAATACTAAGCGCCCC |
| Silver lamprey | GCCACTAATACTAAGCGCCCC |
| Am. brook lamprey | GCCTATAATACTTAGCGCCCC |

Sea Lamprey primers and MGB probe

# Sea Lamprey (eDNA)

## Lamprey Test Amplification



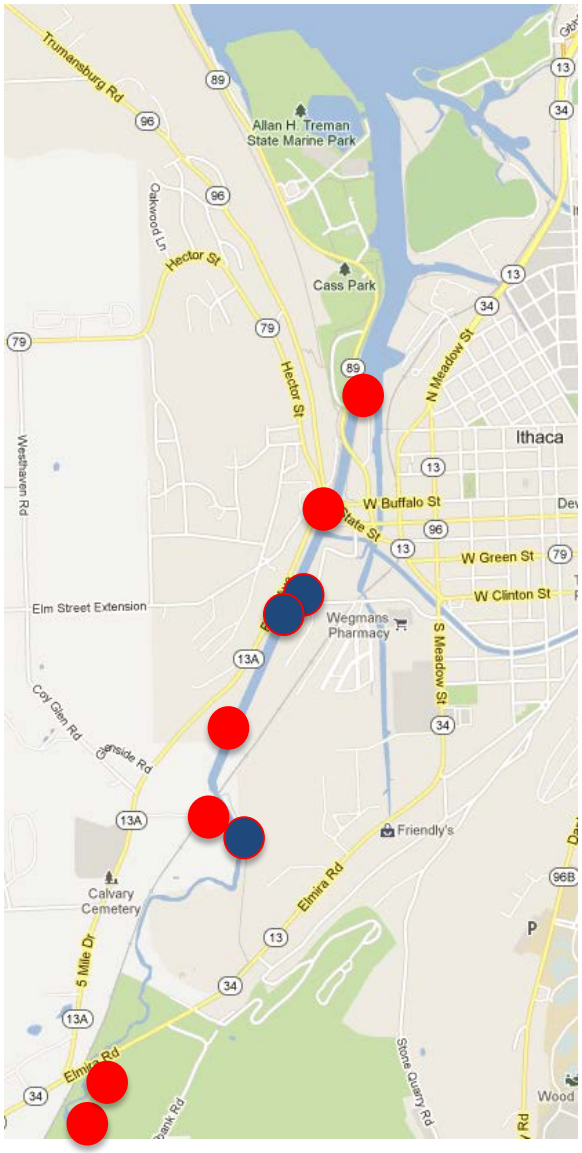
# Sea Lamprey eDNA Collection

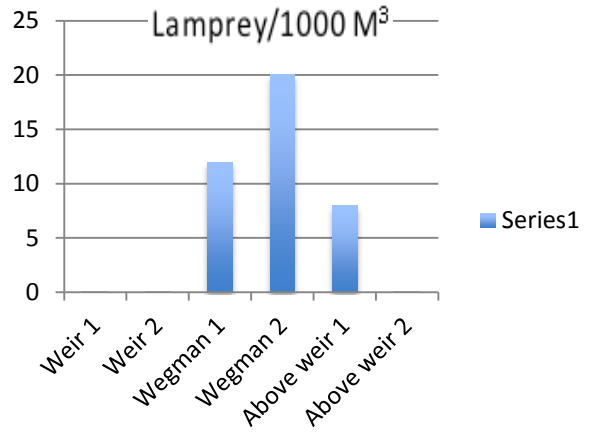
Winter 2012 collections

Greg Wooster risking it all

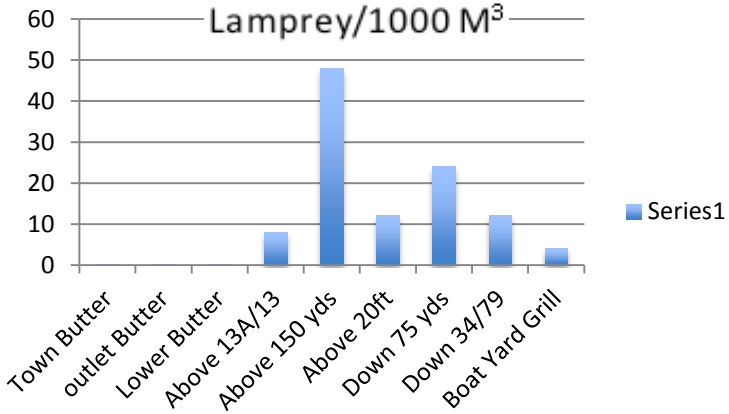
# Sea Lamprey (eDNA)

Winter 2012



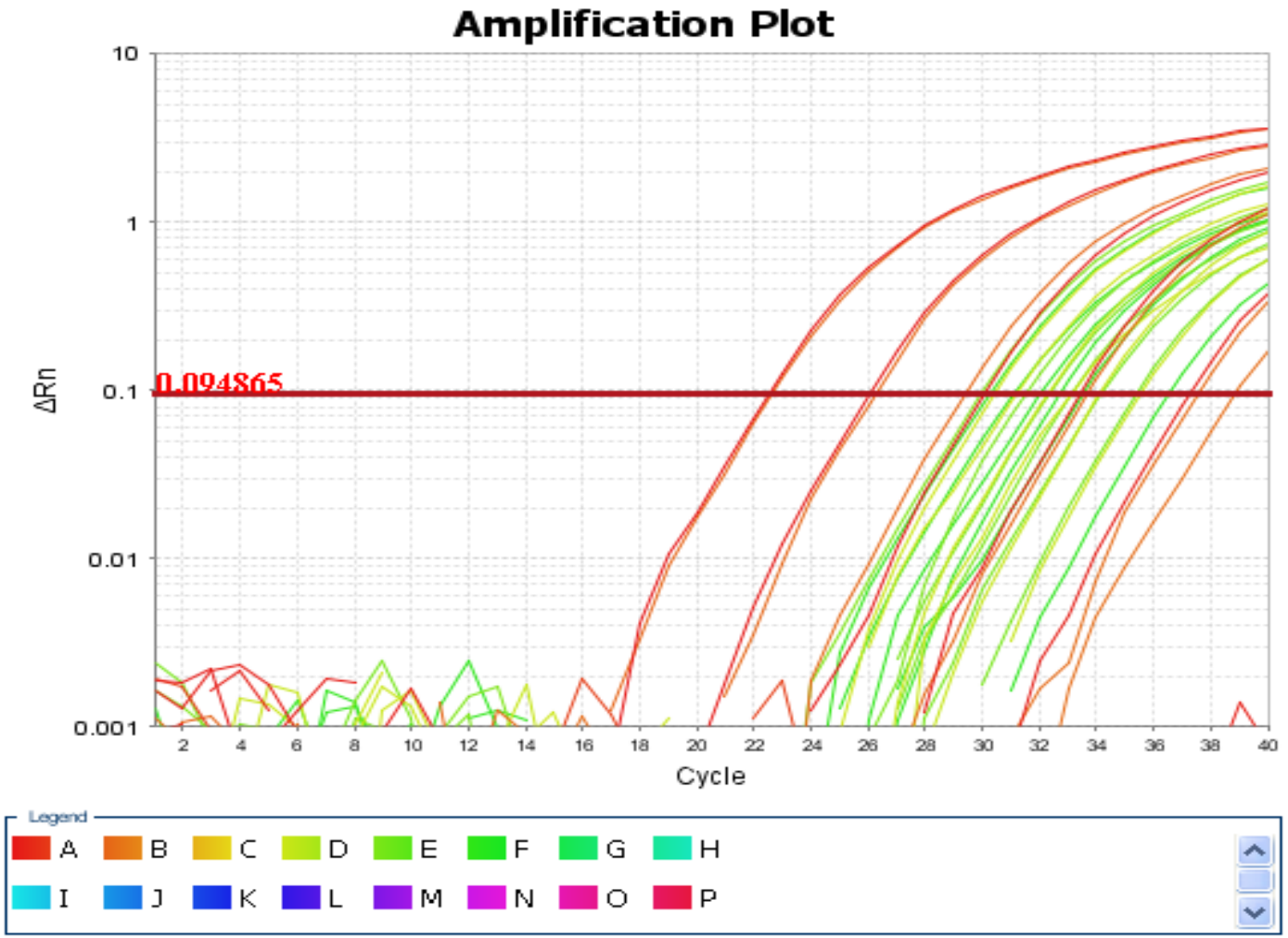




Spring 2013

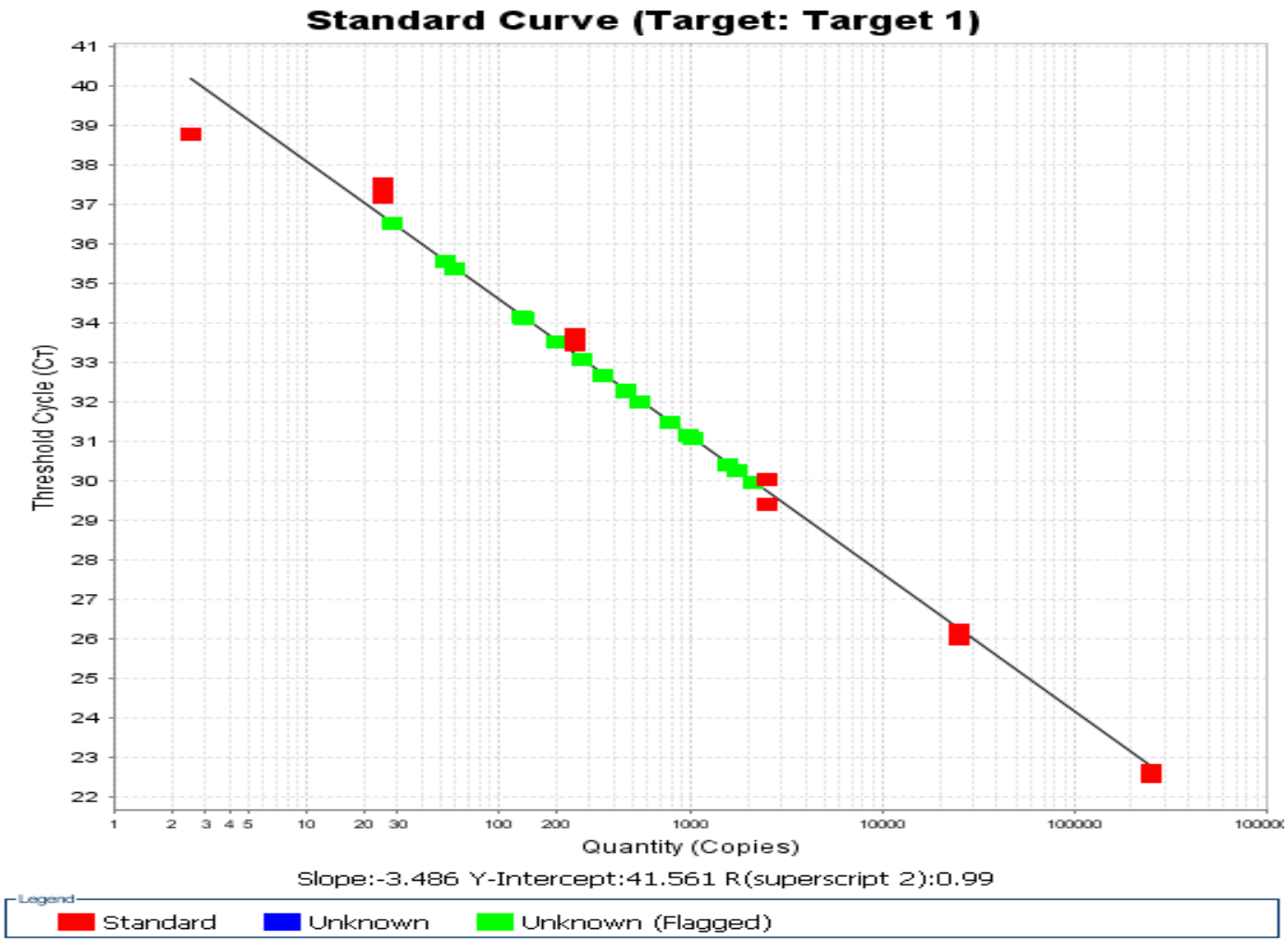


# Round Goby eDNA Collection

# Round Goby qPCR Amplification



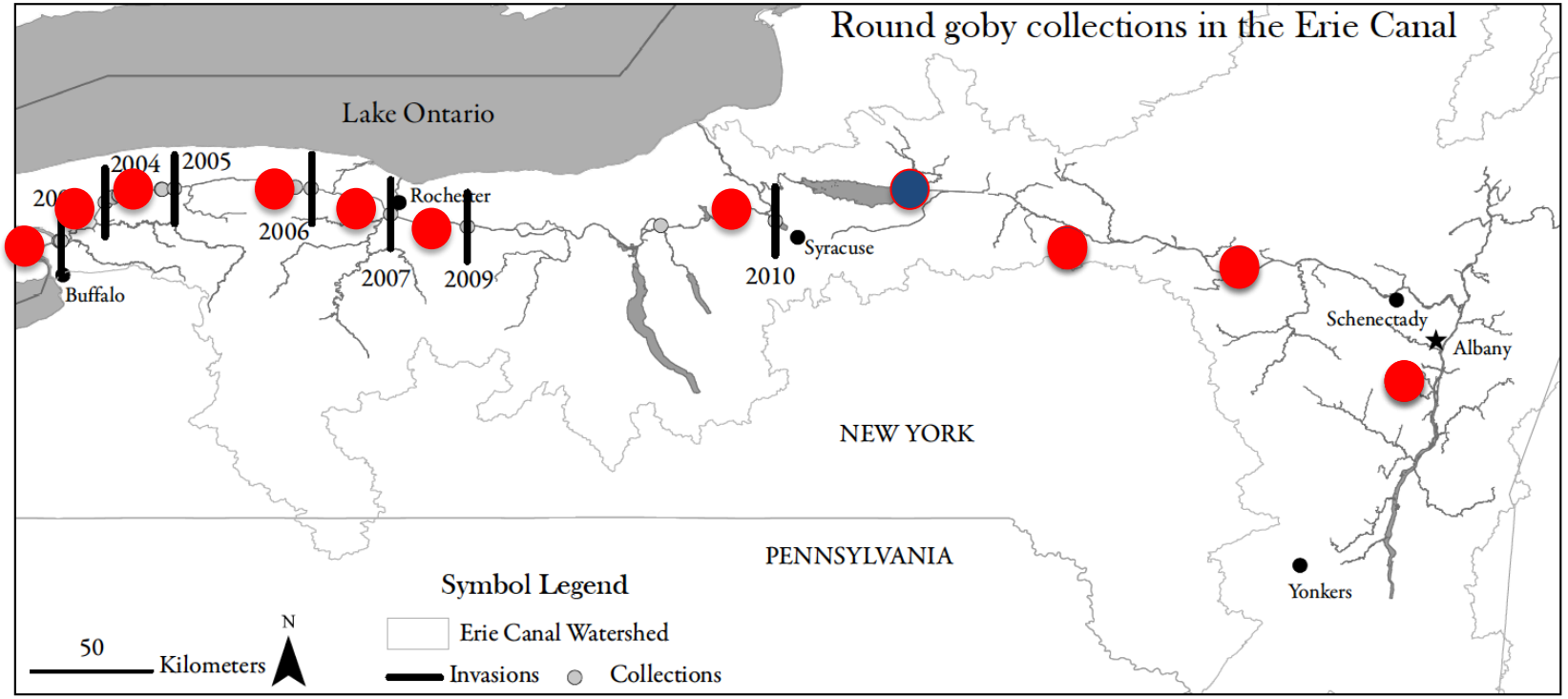
# Round Goby qPCR Sensitivity




Analytical sensitivity is one copy of target

# Round Goby Invasion of the Erie Canal

## Goby eDNA collection sites as of 2014




Positive

Negative

# Correlation of eDNA & Electroshocking

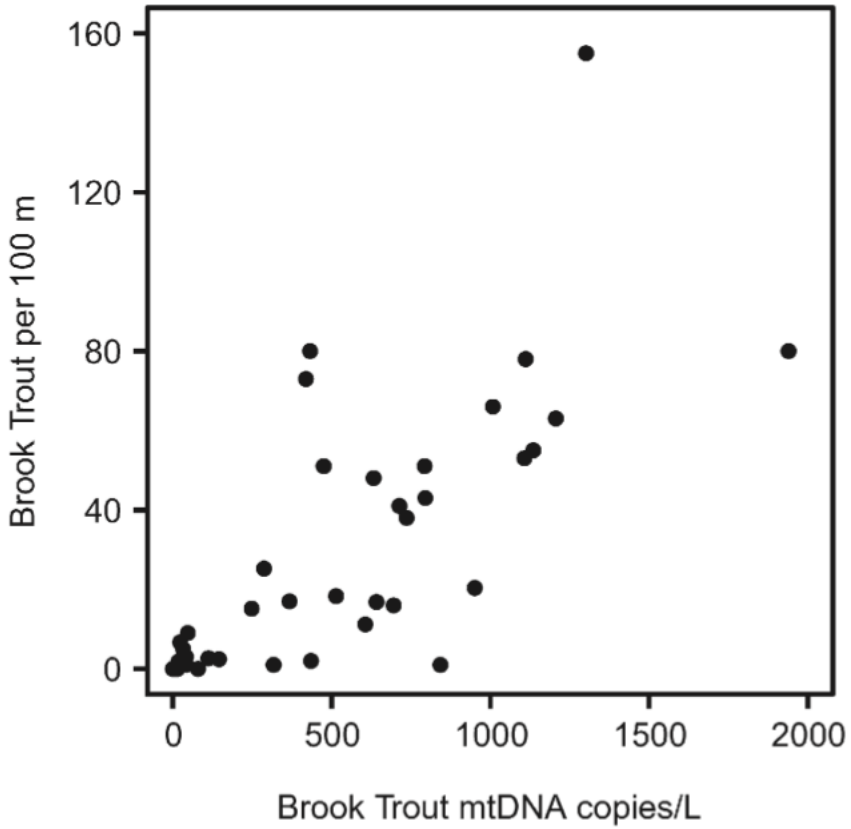



**Fig 1.** Brook trout eDNA concentration (mitochondrial DNA copies/L) versus estimated number of brook trout ≥75 mm TL per 100 m of stream as estimated by backpack electrofishing in the observational study. Environmental DNA concentration and brook trout number were positively correlated ($P < 0.001$, $r^2 = 0.592$, $n = 46$ stream reaches).

Wilcox et al 2016 Biological Conservation 194 (2016) 209–216

# Correlation of eDNA & Electroshocking

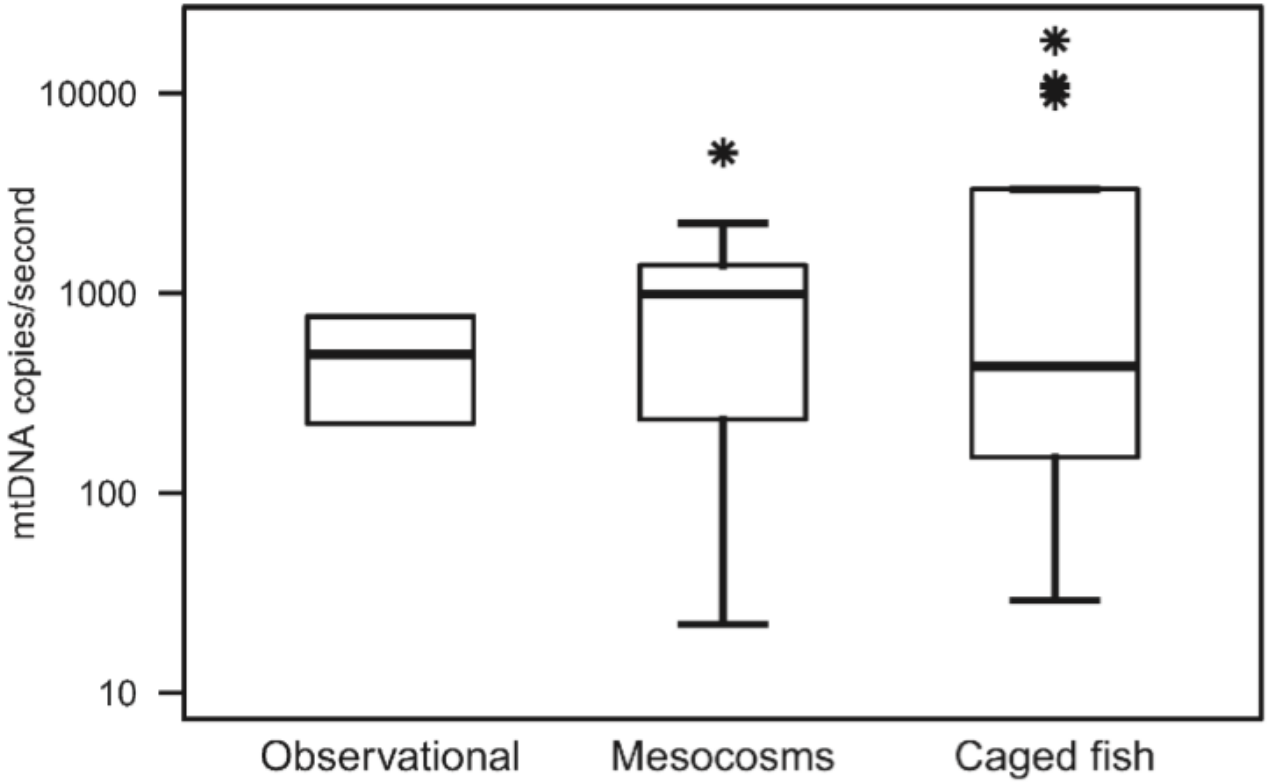



**Fig 2.** Estimated eDNA production rates per fish from the observational study (mean = 495 copies/s), mesocosm (median = 990 copies/s), and caged fish (Jane et al., 2015; median = 430 copies/s) experiments. For the observational study, the middle line and box represent the model coefficient estimate and 95% CI. For the caged fish and mesocosm experiments the boxplots represent the minimum, median, maximum, and interquartile ranges of the observed data.

Wilcox et al 2016 Biological Conservation 194 (2016) 209–216

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# Students Engaging the Environment: A Student/Scientist Collaboration to Assess Aquatic Invasive Species

Donna Cassidy-Hanley, Rod Getchell, Fina Casey, Jim Casey, and Dave MacNeill,

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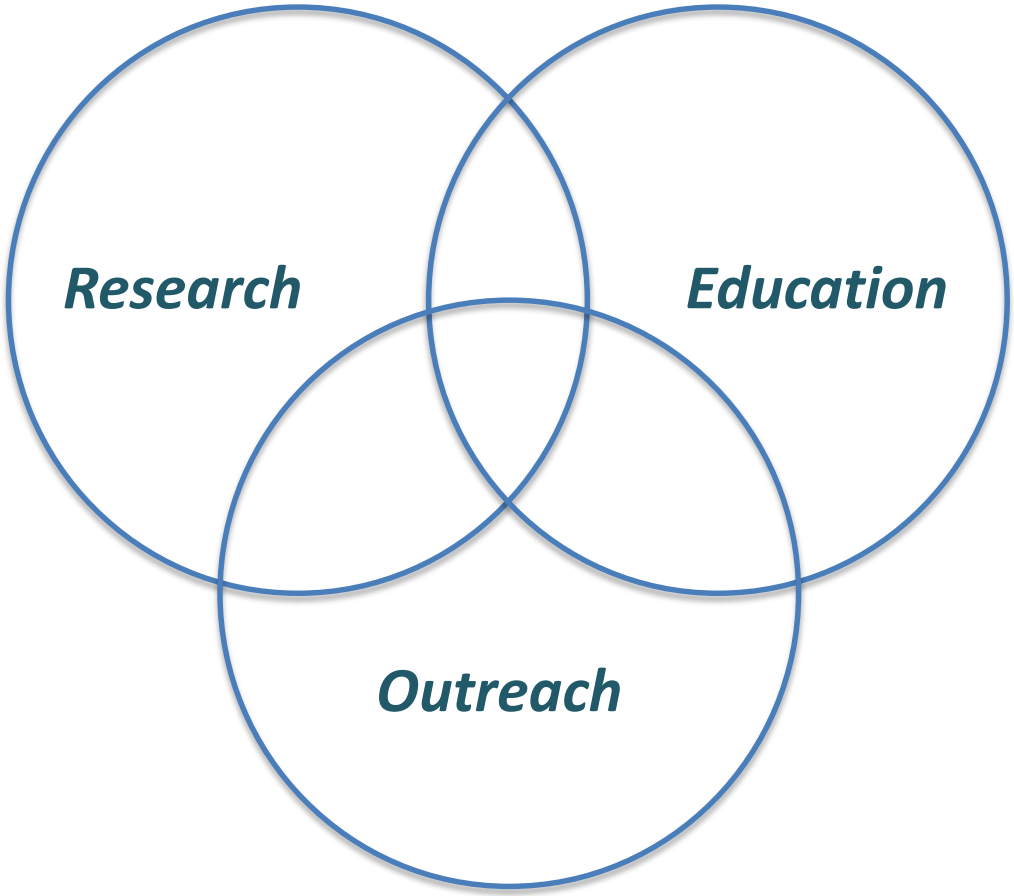
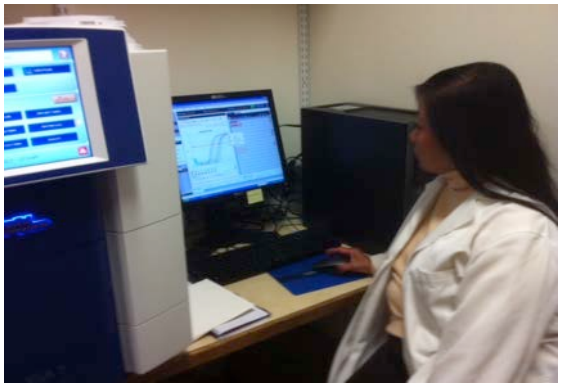
# Citizen Science




**Creating real life connections**

# Students Engaging the Environment: A Student/Scientist Collaboration to Assess Aquatic Invasive Species

The project brings together Cornell researchers using cutting edge eDNA analysis for detecting invasive fish species…..and student and teacher citizen scientists collecting samples from local waterways.



# Sharing ASSETs: Expanding Science Opportunities in K-12 Classrooms

# Outreach: ASSET

## Sharing ASSETs: Expanding Science Opportunities in K-12 Classrooms

Outreach Infrastructure

***Initial program impacted >300 teachers and 11,000 students***

Teacher Contacts

Outreach Experience

**Funded by NIH SEPA**

# Education: Schools, Teachers, and Students

Teachers provide educational experience, expertise, and critical feedback.

Students participate in a research project with clear aims.

# Educational Aims

- Introduction to Ecology and Environmental Stewardship.
- Introduction to molecular biology and bioinformatics.
- Bring real life impact of science to students who might otherwise not be engaged in science.
- Address Common Core standards.

# Research: Cornell Aquatic Animal Health Program

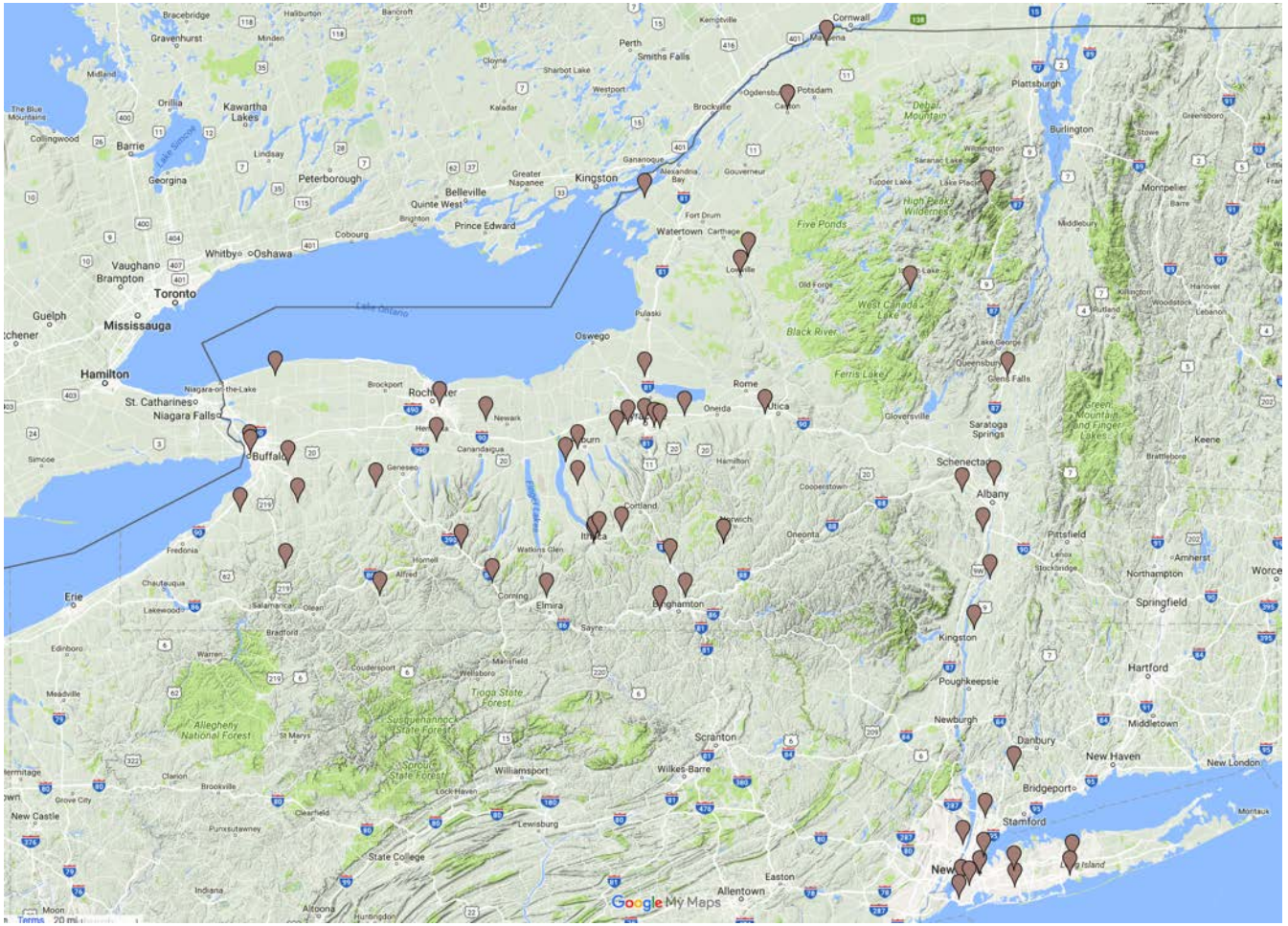
Research Infrastructure

Research Facilities

Scientific Expertise

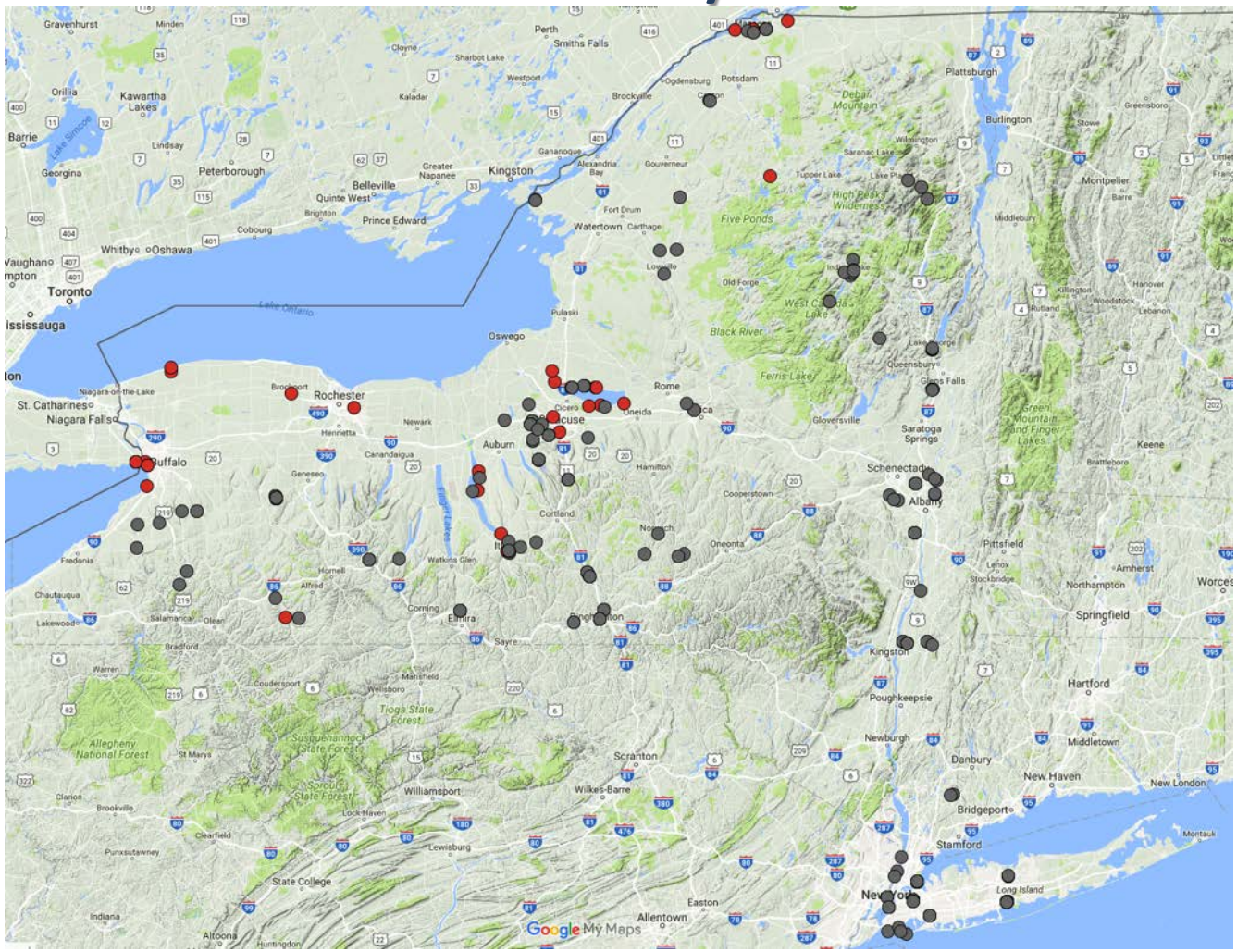
# Citizen Scientist eDNA Collections

## Schools Involved in Pilot Study of Invasive Fish Species

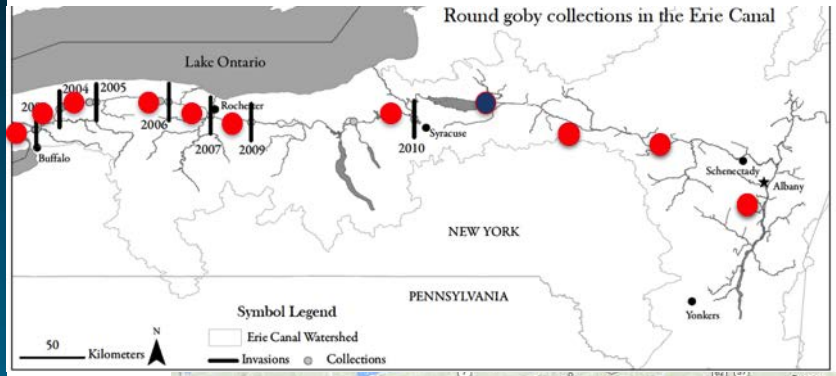


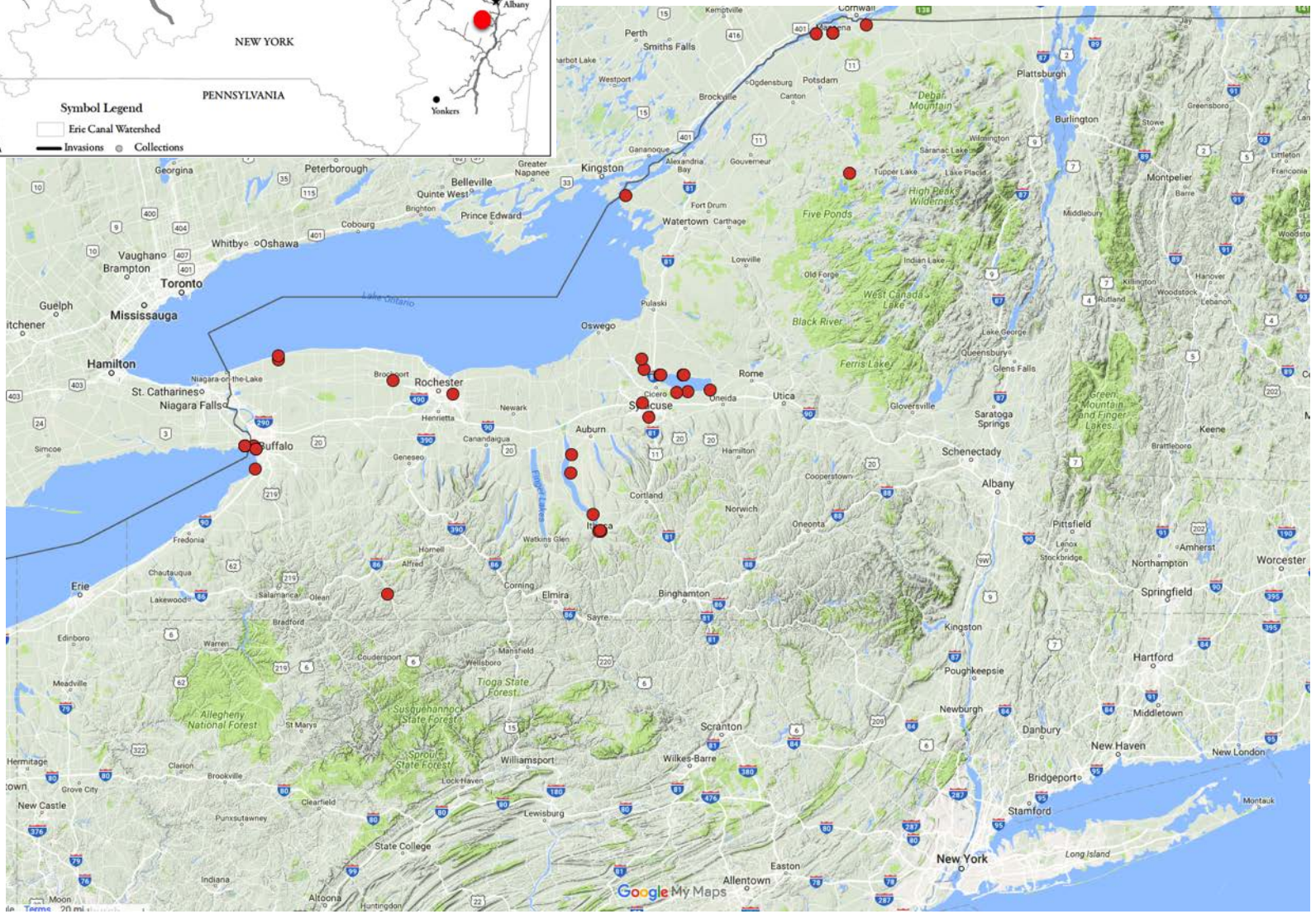
# Citizen Scientist eDNA Collections

## Round Goby results



# Round Goby Results 2014 (scientist) vs 2016 (student)



# Other fish species

**Asian Carp** all negative.

Asian carp are easily detected using eDNA due to their life history, distribution, and DNA shedding characteristics. Probably not here yet in significant numbers.

**Sea lamprey** all negative. More complicated story reflects life cycle.

Can detect eDNA from spawning and larval sea lamprey in streams.

**Key criteria**

**Location**

- larval lampreys are primarily found in stream sediments and are relatively sedentary
- successful eDNA monitoring for lamprey larvae is inversely correlated with the size and discharge of stream

**Density**

- medium to high density of larvae give most dependable results

**Seasonal impact**

- lampreys die after spawning, high detection with eDNA assays during this seasonal window

# Educational Concerns

- Student engagement and positive learning outcomes.
- Relevant grade-appropriate background information, collection protocols, and clear interpretation of results.
- Teacher support.
- Ease of implementation.
- Equipment availability.

# Materials and Protocols

**CORNELL INVASIVE AQUATIC SPECIES PROGRAM**
**eDNA Collection Protocol**

**Before Heading into the Field**

1. Be sure to read the introductory handout that accompanies the kits before engaging in any fieldwork. It will provide you with background information on the aquatic invasive species we will be looking for, and why they pose potentially serious ecological and economic problems.

2. This protocol is a step-by-step description of how to collect water samples and minimize cross contamination. Please go over the complete protocol carefully before beginning any field collections. A short video demonstrating the assembly of kit materials and the collection technique is also available on our website (https://tetrahymenaasset.vet.cornell.edu/ ).

3. Check the eDNA Kit Contents list to ensure that you have all of the materials required **before** going into the field.

**Kit Contents**

The kits contain all of the materials needed to collect eDNA except distilled water for the field control and a GPS unit to identify the sampling location. For each field control, you will need to bring ~ 300 ml of distilled water (which can be purchased at a gas station or grocery store). If you do not have a source of distilled water, tap water can be used but care must be taken to prevent the introduction of any contaminants in the water or by the bottles in which it is transported. Use clean, well-rinsed bottles that have not been exposed to lake or stream water or any potential source of fish DNA.

Kits should be stored at room temperature, and used and returned within a 2-week period. Each kit contains enough materials to process one site at 3 closely adjacent locations.

**Please keep all packaging and the return shipment label since you will need these for FedEx return shipment of samples.** Directions for return shipment are included in the kit.

Complete eDNA Sampling Kit contents:

1. Polypropylene suction flask
2. Buchner funnel (2 pieces)
3. Rubber gasket adapter for funnel
4. Glass fiber filter discs
5. Hand held vacuum pump (most will not have a gauge)
6. Ziploc bags for water collection
7. Round toothpicks
8. Forceps
9. Collection tubes for storing filters after use
10. Waterproof marker (not shown)
11. Gloves for water collection (not shown)
12. Black bag for storing used equipment in the field. (Not shown)
13. Small trash bag for disposable waste (not shown)
14. Blue plastic bag for return shipment of washed used equipment (not shown)
15. Water for control samples **Not provided**
16. GPS unit **Not provided** (Many cell phone apps will provide this information)
17. Clipboard and waterproof pen/pencil **Not provided**

Figure 1. The materials.

Figure 1. The materials.

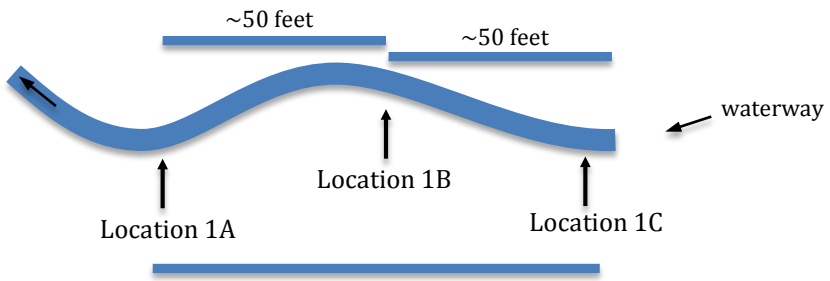
- **Detailed protocols and all materials are provided to facilitate use in a class or extracurricular setting.**

# Minimizing Cross-Contamination

- Complete materials for each site packaged separately and opened immediately before use.
- After collection, used equipment immediately sealed in return bag. No sharing of equipment between sites.
- Between uses, returned equipment decontaminated in 10% bleach.

# Other Research Concerns

- Sample Preservation: Buffer for short term storage, freezer for long term archiving.
- Accurate identification of sites and samples: GPS coordinates.
- Repeatability: Multiple samples at each site plus negative control.




**Site 1**

# Results

**Educational**

- Over 60 teachers (many repeat participants) and schools involved across the state
- Active engagement with teachers and students to optimize protocols
- Participating schools range from large NYC schools to very small rural
- schools
- Very high levels of student interest and enthusiasm

**Scientific**

- >280 sites sampled across the state
- Good data

**Overall**

- Increased interest in and awareness of invasive species issues
- Increased interest in science and the environment in students not
- typically engaged by traditional science education

# Student Reactions

- I loved the lab it was incredible.
- We all really had fun and enjoyed every moment.
- It was a very interesting and intriguing experiment.
- I highly recommend next year the 9th graders do it.
- I learned a lot and found it very enjoyable!
- In my opinion, the lab was really amazing.
- It was really cool to see how we test water for many different types of DNA.
- It was cool to explore the world outside of our classroom or school labs.
- We actually got to test something in the real world that would be helpful to scientists.

**… next year I think we should do it on a nicer, sunnier day.**