

Cornell University College of Veterinary Medicine



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

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United States Department of Agriculture National Institute of Food and Agriculture



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 <u>cells that are</u> <u>collected</u>.
- 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





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

DONY

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.

	330	340	350	360	370	380	390	400
375085109.seq CAGGAT	GAACAGTTT		TCGCGGGCAAT	CTTGCCCACG	CAGGAGCA	TCCGTAAACCTA	ACAATTTTC	TCTCTT 378
63081821.seq CAGGGT	GAACAGT <mark>C</mark> T/		TCGCAGGCAAT	CTTGC <mark>A</mark> CACG	CAGGAGCA	ГС <mark>Т</mark> GTA <mark>G</mark> ATCTA	ACAAT <mark>C</mark> TT <mark>T</mark>	тс <mark>сста</mark> 5947
359326305.seq CAGGAT	GAACAGTTT	TCCACCA	TCGCGGGCAAT	CTTGCCCACG	CAGGAGCA	ΓCCGTA <mark>G</mark> ACCTA	ACAATTTTC	ICTCTT 400
314907005.seq CAGG <mark>G</mark> T	GAAC <u>A</u> GT <u>T</u> T/		TCGC <mark>A</mark> GGCAAT	<u>CTTGCCCACG</u>	CAGGAGCA	TC <u>C</u> GTA <mark>G</mark> ACCTA	ACAATTTTC	TCACTC 385
321118705.seq CAGGAT	GAAC <mark>C</mark> GT <mark>A</mark> T/		.T <mark>T</mark> GC <mark>AGG</mark> AAC	T <mark>T</mark> AGCCCACG	CAGGAGCA	ГС <mark>А</mark> GTA <mark>G</mark> ACCTA	ACAATTTTC	TC <mark>A</mark> CTT 400
186883156.seq C <u>A</u> GGAT	GAACAGTCT/	αςαταστ	T <mark>G</mark> GC <mark>A</mark> GGCAAC	CT <mark>C</mark> GCCCACG	CAGG <mark>G</mark> GC	TCCGT <mark>CG</mark> ATTTA	Αστατττς	тс <mark>с</mark> ст <mark>с</mark> 400
186883068.seq C <mark>C</mark> GGAT	GAACAGT <mark>C</mark> T/	CCCGCCT	TGGCAGGTAAT	CT <u>T</u> GCCCACG	CCGGAGCG	TCCGT <mark>CG</mark> ATCTA	AC <mark>T</mark> AT <mark>C</mark> TTC	TC <u>T</u> CT <u>T</u> 400
186883196.seq CAGGAT	GAACAGT <mark>C</mark> T/	АТССТССТ	T <mark>GGCA</mark> GGCAAC	CT <mark>C</mark> GCCCACG	CAGG <mark>G</mark> GC	TCCGT <mark>CG</mark> ATTTA	Αστατττς	TCCCTC 400
186883228.seq CAGGAT	GAACAGT <mark>C</mark> T/	ΑΤΟΟΤΟΤΑ	T <mark>GGC</mark> AGGCAAC	CT <mark>C</mark> GCCCACG	CAGG <mark>G</mark> GC	TCCGT <mark>CG</mark> ATTTA	Αστατττς	TCCCTC 400
186883248.seq CAGGAT	GAACAGTCT/	ΑΤΟΟΤΟΤΙ	T <mark>GGCA</mark> GGCAAC	CT <mark>C</mark> GCCCACG	CAGG <mark>G</mark> GC	TCCGT <mark>CG</mark> ATTTA	ACTATTTTC	TCCCTC 400
186883328.seq C <mark>G</mark> GG <mark>G</mark> T	GAAC <mark>C</mark> GTTT/	ACCC <mark>C</mark> CCA(T <mark>G</mark> GCTGGAAAC	TT <mark>AGCACAT</mark> G	CCGC <mark>G</mark> GCA	TC <mark>T</mark> GT <mark>GG</mark> ATCTA	AC <mark>C</mark> ATTTT <mark>T</mark>	TC <mark>C</mark> CT <mark>A</mark> 400
186883482.seq C <mark>G</mark> GG <mark>G</mark> T	GAACAGT <mark>A</mark> T/	ACCCCCCA(TTGCGGGTAAT	CT <mark>C</mark> GCCCACG	CCGGAGCT	TC <mark>A</mark> GTA <mark>G</mark> ACTTG	ACTATTTTC	TCACTT 400
186883552.seq C <mark>T</mark> GG <mark>C</mark> T	GAACCGTCT/	ATCC <mark>C</mark> CCC	CT <mark>TGCAA</mark> GCAA <mark>C</mark>	CTTGC <mark>A</mark> CACG	CAGG <mark>T</mark> GCA	TC <mark>T</mark> GTA <mark>G</mark> ACTTA	ΑCΑΑΤΤΤΤΤ	TCTCTC 400
186884096.seq C <mark>I</mark> GGAT	G <mark>G</mark> ACCGTTT/	ACCOTCOT	T <mark>AGC</mark> CGGCAAC	CT <mark>AN</mark> CCCACG	CAGGTGCA	TC <mark>T</mark> GT <mark>CG</mark> ACCTA	ACCATCTTC	TCCCTT 400
186884104.seq CAGGAT	GAAC <mark>T</mark> GT <mark>A</mark> T/	ATCC <mark>C</mark> CCA(CTTGCTGGCAAC	CTTGC <mark>A</mark> CA <mark>T</mark> G	CAGGAGC	TC <mark>T</mark> GTA <mark>G</mark> ATCTA	ΑΟΤΑΤΟΤΤΤ	TC <mark>A</mark> CTT 400
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186884132.seq CGGGAT	GAAC <mark>T</mark> GT <mark>A</mark> T/	ATCC <mark>C</mark> CCA(CTTGCTGGCAA <mark>C</mark>	CTTGC <mark>A</mark> CA <mark>T</mark> G	CAGGAGC	TC <mark>T</mark> GTA <mark>G</mark> ATCTA	Αστατόττη	TCACTT 400
186884136.seq C <mark>C</mark> GG <mark>C</mark> T	GAAC <mark>T</mark> GT <mark>A</mark> T/	ACCCCCCT(T <mark>GGCAA</mark> GCAAC	CTTGC <mark>A</mark> CACG	CAGG <mark>C</mark> GCA	TC <mark>AGTAG</mark> ATTTA	AC <mark>C</mark> AT <mark>C</mark> TTC	TCCCTA 400
186884244.seq CAGGAT	GAAC <mark>G</mark> GT <mark>G</mark> T/	ACCC <mark>C</mark> CCA(TCGCAGGTAAC	CTTGCCCATG	CAGGAGCA	TC <mark>AGT</mark> TGACCTT	ACAAT <mark>C</mark> TTC	TC <mark>A</mark> CT <mark>C</mark> 400
186884328.seq CAGG <mark>C</mark> T	GAACAGT <mark>A</mark> T/	ATCC <mark>T</mark> CCA(CTCTCAGGT AAT(CT <mark>C</mark> GC <mark>T</mark> CACG	CCGGCGCA	TC <mark>A</mark> GTA <mark>G</mark> ACCTA	ACAATTTTC	TCCCTT 400
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186884868.seq CAGGAT	GGACTGTTT/	ACCC <mark>C</mark> CCA(. TTGCAGGTAAC	CTTGCCCACG	C <mark>TGGG</mark> GCA	TC <mark>A</mark> GTA <mark>G</mark> ACCT <mark>C</mark>	ACAAT <mark>CTT</mark> T	ICTCTT 400
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186884924.seq C <mark>T</mark> GG <mark>G</mark> T	GAACTGTCT/	ACCCCCTC	. TTGCCGGCAAC	CT <mark>G</mark> GCCCA <mark>T</mark> G	CAGGAGCA	TCCGT <mark>TG</mark> ACCTA	ACCATCTTC	ICTCTT 400
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186885412.seq CAGGGT	GAACAGT <mark>A</mark> T/	ACCCCCCA(TTGCAGGTAAC	CT <mark>C</mark> GCCCACG	CIGGAGCA	TC <mark>A</mark> GTA <mark>G</mark> ATCTA	ACAATTTTC	FC <mark>G</mark> CTT 400
186885460.seq C <mark>T</mark> GGAT	G <mark>G</mark> AC <mark>C</mark> GTTT/	ACCCCCTC	TAGCCGGCAAT	CT <mark>G</mark> GCCCACG	CAGGAGC	TCCGT <mark>TG</mark> ACCTA	ACCATTTTC	TCCCTT 400
186885472.seq C <mark>T</mark> GGAT	GAAC <mark>C</mark> GTTT/	ATCCCCCT	IT <mark>G</mark> GC <mark>C</mark> GGCAA <mark>C</mark>	CTTGCCCATG	CAGGAGCA	TCCGT <mark>TG</mark> ACTTG	AC <mark>C</mark> AT <mark>C</mark> TTC	ra <mark>c</mark> ct <mark>c</mark> 400
186885528.seq CAGG <mark>G</mark> T	GAACTGTAT/	ACCC <mark>C</mark> CC <mark>G</mark> (TCGCAGGCAAC	CT <mark>C</mark> GCCCACG	CAGGAGCA	TC <mark>A</mark> GTA <mark>G</mark> ACCTC	ACAAT <mark>C</mark> TTC	TCTCTA 400
186885536.seq C <u>A</u> GG <u>A</u> T	GAAC <mark>T</mark> GT <mark>A</mark> T/		TCGCAGGTAAC	CT <mark>C</mark> GCCCACG	C <u>A</u> GGAGCA	TC <mark>A</mark> GTA <mark>G</mark> ACCT <mark>C</mark>	ACAATTTTC	ГС <mark>СС</mark> Т <mark>А</mark> 400

qPCR Specificity

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



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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"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 Chestnut lamprey N. brook lamprey Silver lamprey Am. brook lamprey ACCCCTAATACTTGGTGCTCC ACCACTAATACTAAGCGCCCC GCCACTAATACTAAGCGCCCC GCCACTAATACTAAGCGCCCC GCCTATAATACTTAGCGCCCC

Sea Lamprey primers and MGB probe



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Sea Lamprey eDNA Collection

Winter 2012 collections





Greg Wooster risking it all



Round Goby eDNA Collection



Round Goby qPCR Amplification



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Round Goby qPCR Sensitivity



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Analytical sensitivity is one copy of target

Round Goby Invasion of the Erie Canal Goby eDNA collection sites as of 2014





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Correlation of eDNA & Electroshocking



Brook Trout mtDNA copies/L

Fig 1. Brook trout eDNA concentration (mitochondrial DNA copies/L) versus estimated number of brook trout \geq 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,



United States Department of Agriculture National Institute of Food and Agriculture



Supported by the National Institutes of Health

Citizen Science



DONY

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



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ASSET: Advancing Secondary Science Education thru Tetrahymena



Resources & Videos

Home About the ASSET program Modules Workshops

Workshops Photos

Invasive Fish Program

- Older posts

ASSET is BACK!!

Posted on July 10, 2015 by admin

ASSET has received a new 5-year NIH SEPA grant! We hope you will join us as we expand the ASSET program in exciting new directions. Here are some of the opportunities ASSET will offer.

Science modules for elementary and middle school students. We expanding our materials for middle and elementary level students, using previously tested modules as a starting point. We are excited at the opportunity to work with middle and elementary school teachers and students to develop innovative activities that use a hands-on, inquiry-based approach to introduce younger students to the wonders of biology using living protozoa.

Classroom research opportunities for high school students using Tetrahymena.

Every so often something in class will catch a student's interest and imagination. We intend to help capture that moment by making it possible for motivated students to follow up on an idea that interests them. Using our existing high school modules as an introduction to the use of *Tetrahymena* to explore biological phenomena, we will provide the opportunity for expanded independent investigation among a limited number of interested, motivated students, either individually or as part of a research class. We are especially interested in providing opportunities for independent inquiry to students at under-resourced schools. The student-designed projects will be teacher supervised, with support from the ASSET program.

LOGIN/REGISTER

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Forms

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- Contact Us
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 - Science modules
 - Request a science module
 - Biology & Society Modules
 - Science module protocols
- Photos
- Resources & Videos
 - ASSET How-to videos and documentation
 - ASSET Multimedia
 - Online research resources
 - Tetrahymena people &
 - news
- Invasive Fish Program
- TWITTER

Y Follow @sepaass

Outreach: ASSET

Sharing ASSETs: Expanding Science Opportunities in K-12 Classrooms

Outreach Infrastructure

Teacher Contacts

Initial program impacted >300 teachers and 11,000 students





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 Infrastructure

Research Facilities

Scientific Expertise









Citizen Scientist eDNA Collections Schools Involved in Pilot Study of Invasive Fish Species



DOOT

Citizen Scientist eDNA Collections Round Goby results



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



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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

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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 Forcers
- 9. Collection tubes for storing filters after use
- 10. Waterproof marker (not shown)
- Figure 1. The materials 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



LIST OF MATERIALS 7. Round toothpicks Lab chicke 8. Yellow forceps (not requ for this experiment 5. Vacuum pump (hand pump **1.** Collection flask 2. Büchner funnel (2 parts) pronounced: BOOK-ner

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.



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.