Photo Credit: K. Williams, © Wells College

"Environmental DNA and Underwater Video Surveillance

USFW Agreement No. F14AP00482

St. Lawrence Eastern Lake Ontario
Partnership for Regional Invasive Species Management
in Cooperation with The Nature Conservancy, CWNY

Year 1 - Progress Report

By convention, early detection of invasive species including aquatic invasive species typically relies on visual observation of the species causing harm or the symptoms thereof. Unfortunately, by the time a species is visually observed, the invasive has likely populated and the management thereof becomes difficult at best. Genuine early detection means detecting the presence of a species before it has the opportunity to populate and cause irreplaceable harm to the ecosystem of concern.

Over the next two summers The Nature Conservancy, the Department of Microbiology and Immunology at Cornell University along with partners of the SLELO PRISM, will undertake a project to assess the feasibility of using environmental DNA or eDNA as an early detection tool for aquatic invasive species. 160 water samples will be collected from four strategic locations along Eastern Lake Ontario and analyzed using highly specialized processes for the presence of genetic material released by both invasive and native aquatic animals. High definition underwater video technology (HD-UVT) will also be used as a citizen science component to assess the validity of video technology as an early detection tool as well.

Sampling Locations

The eDNA project will assess downstream and upstream sites at four "key" tributaries of Lake Ontario: The Oswego River, Salmon River, Chaumont River, and French Creek. Samples will be collected regularly throughout the summers of 2016 and 2017, and subsequent genetic analyses will be performed at Cornell University in the lab of Dr. James Casey. The effort will also invite public participation through citizen science volunteer opportunities. This project includes an underwater video surveillance component, which aims to enhance public outreach through visual media. If successful, this project may serve as a model for the early detection of invasive species that can be replicated throughout the region. Ten samples will be collected at each location for a total of 80 samples per year.

Navigation

At each sampling site, transects were established in equal distances using Navionics® software. Samples were collected at even distances along each transect with the sample location being recorded in latitude and longitude using a Garmin GPS. The Navionics also shows the real-time travel route of the boat used during sample collection which allows for accurate straight-line travel translating into more evenly distributed sampling points. As the 2016 season progressed so did our confidence in using the Navionics software.

Above: Sample points near French Creek using Navionics.

Species of Interest

Under this project six invasive species of fish were investigated to include:

Northern snakehead (*Channa argus*)
Round goby (*Neogobius melanostomus*)
Bighead carp (*Hypophthalmichthys nobilis*)
Black carp (*Mylopharyngodon piceus*)
Grass carp (*Ctenopharyngodon idella*)
Silver carp (*Hypopthalmichthys molitrix*)

Additionally, two native species were investigated to include:

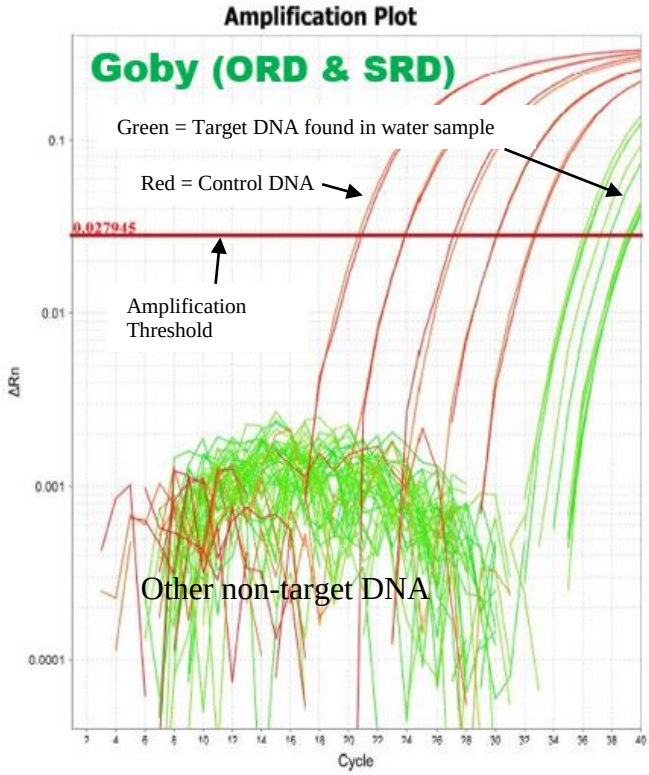
Rock bass (*Ambioplites rupestris*)
Cisco-Bloater species complex
(*Coregonus artedi and Coregonus hoyi*)

qPCR

PCR is a method where an enzyme (polymerase), amplifies a short specific part of the DNA in cycles. In every cycle the number of short specific sections of DNA is doubled, leading to an exponential amplification of target DNA. The methods of choice for nucleic acid (DNA, RNA) quantification in all areas of molecular biology is real-time PCR or quantitative PCR (qPCR). The method is so-called because the amplification of DNA with a polymerase chain reaction (PCR) is monitored in real time (PCR cyclers constantly scan PCR plates). In qPCR, the amplified DNA is fluorescently labelled and the amount of the fluorescence released during amplification is directly proportional to the amount of amplified DNA. Fluorescence is monitored during the whole PCR process. The higher the initial number of DNA molecules in the sample, the faster the fluorescence will increase during the PCR cycles[1]. These are known as amplification plots.

Amplification Plots

In the following example the red horizontal line on the graph labeled .027945 represents a fluorescence threshold. The red lines that rise above the threshold represent the control DNA (actual DNA of the target species). Green lines that rise above the threshold represent matching DNA that is in the water sample. All other lines below the threshold are non-target DNA.



2016 eDNA Results

Round Goby – found in all downstream sites.
Rock Bass – found in all downstream sites.
Northern Snakehead – no detection.
Silver Carp – no detection.
Black Carp – no detection.
Bighead Carp – no detection.
Grass Carp – one low level detection at Oswego River downstream.
Cisco complex – samples not yet run due to assay.

[1] *Credit: http://biosistemika.com/workshops/qpcr-basics*

Laboratory Response Time

In order for eDNA to be effectively used as an early detection tool and to allow for an appropriate response to a newly discovered invasive species, the PCR analysis needs to be completed and the results reported in a timely manner. Early detection of aquatic invasive species at a molecular level assumes that the species may not be well established and therefor receiving the laboratory results prior to the reproductive period and/or the sudden increase of the target population would be desirable. A laboratory response time of days or at most a few weeks would generally be desirable verses a response time of months. Delays in PCR results may cause delays in developing a rapid response thus allowing for the population of the species in question to increase along with the ecological damage associated with that species.

During the first season of this project PCR was initially completed with an approximate ten-day period. This turnaround time improved to a period of four to seven days by mid-summer.

Underwater Video Surveillance

As part of our Eastern Lake Ontario eDNA initiative, underwater video technology is being used not only as a hands-on citizen science tool but also to determine its practicality as an early detection tool. Over this past summer nine species of fish have been videotaped from four locations documenting at least one invasive species (Round Goby). Noteworthy is the capture of two native species; the

Above: Video snippet of Bowfin (Amia calva) taken from Chaumont River downstream.

Bowfin (*Amia calva*) -which is considered as primitive and the River Redhorse (*Moxostoma carinatum*) native but not common. eDNA work has also captured a bulk of information on a total of eight native and non-native species including the common crayfish (*Decapoda spp*).

Summary of species observed on video:

Blacknose dace – *Rhinichthys atratulus*
Bluegill – *Lepomis macrochirus*
Pumpkinseed – *Lepomus gibbosus*
Round Goby – *Neogobius melanostomus*
Yellow Perch – *Perca flavascens*
Brook Silverside – *Labidesthes sicculus*
Crayfish (native) -*Decapoda spp.*
Sculpin – *Cottus sp*.
River Redhorse - *Moxostoma carinatum*
Round Goby – *Neogobius melanostomus*
Bowfin – *Amia calva*
Largemouth Bass – *Micropterus salmoides*

Citizen Science

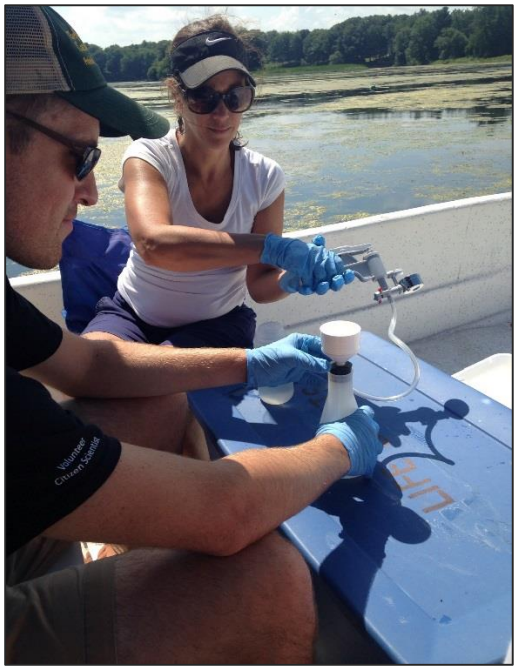
The growing interest of public participation in scientific fieldwork includes a type of volunteerism known as citizen science, in which members of the public engage in the process of scientific investigations, collecting data, and/or interpreting results. This type of collaboration yields new knowledge by providing access to more and different observations and data than traditional scientific studies[2]. Under this project volunteer citizen science teams were utilized as a way to engage, learn and demonstrate that new technologies can be effectively used in early detection efforts.

The value of utilizing citizen scientists exceeds scientific posterity and allows for a cost effective means by which to conduct AIS early detection on a broader level because citizen scientist typically volunteer without compensation. Faculty at Cornell University have developed a stand-alone field kit including step-by-step instructions that allow citizen scientists to conduct early detection using eDNA with nominal guidance. This capability allows for AIS early detection on a much broader scale whereby citizen scientists can adopt a local waterbody and implement eDNA sampling thus expanding early detection work to waterbodies that may otherwise not be searched due to insufficient funding.

In 2016 volunteer citizen scientists provided support to this project by assisting with sample collection and

[2] *http://www.birds.cornell.edu/citscitoolkit/about/defining-citizen-science/*

underwater video recording. Additional citizen science metrics (including cost metrics) are being evaluated.



Above (and on front page) citizen volunteers assisting with eDNA and video-taping.

People and Nature

The Lake Ontario fisheries provides food and income to individuals, families and water-based business's. This lake-based way of life occurs throughout the Great Lakes and locally along Eastern Lake Ontario. When combined with the economic benefits from water-based recreation and tourism, providing healthy, sustainable freshwater resources becomes a local, regional and global priority.

Aquatic invasive species have and will continue to have a negative effect on our freshwater resources and the benefits these resources provide. Although prevention is the best means by which to reduce invasive species impacts, early detection and rapid response provides the next level of defense towards minimizing their impacts and keeping our freshwater resources healthy. Using environmental DNA provides us with a strategic advantage towards achieving this goal.

Summary

Work completed in 2016 was instrumental and will allow us to meet the objectives of this project. Accomplishments include:

- Engaging Citizen Scientists towards a better understanding of eDNA and video technology as early detection tools.
- Test underwater video and its usefulness as an early detection tool.
- Determine the practicality of eDNA as an early detection tool.
- The fourth objective is to develop a "reference guide" for others to follow. This guide will be a final deliverable of this project.

Several DNA samples were not analyzed do to laboratory constraints. These samples have been frozen for future analysis. Additional results in the 2017 field season shall supplement current efforts.

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