Using environmental DNA (eDNA) and underwater video as early detection tools for invasive fish species in four Eastern Lake Ontario tributaries

Abstract

By convention, early detection of 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 species has likely populated, making management of aquatic invasives difficult at best. Genuine early detection confirms the presence of a species before it has the opportunity to populate and cause irreparable harm to the ecosystem of concern. Environmental DNA and underwater video surveillance advances our ability to detect species at their onset.

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1.0 Introduction and Purpose

The purpose of this project was to utilize technology that uses DNA from aquatic invasive species along with underwater video surveillance (UVS) to assess the feasibility of both as practical tools in the early detection of aquatic invasive species, while also engaging in citizen science-based partnerships. Environmental DNA (eDNA) has become a popular research subject among academic institutions. This project was guided by the need to determine if eDNA and UVS can be practical tools (rather than research topics) for the early detection toolbox. To be practical both must be relatively easy to use and costs must not be exorbitant.

Citizen Science

The growing interest of public participation in scientific fieldwork includes citizen science and volunteer monitoring in which members of the public engage in the process of making field observations, scientific investigations, collecting data and/or interpreting results. Collaboration between organizations and citizens yields new knowledge by providing access to more and different observations and data than traditional scientific research[3]. Under this proposal and by utilizing volunteer citizen science teams, we expanded our geographic range for early detection of aquatic invasive species, engaged new partnerships and demonstrated that new technologies can be effectively used in early detection efforts by layman.

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Focus Areas - Eastern Lake Ontario

As part of the Great Lakes system, the eastern portion of Lake Ontario's shoreline is host to unique habitats, such as streams, ponds, estuaries and embayments, as well as emergent marshes and forested and shrub swamps. Rare coastal fens, including globally rare Alvar communities, also exist in the region. The Eastern Lake Ontario barrier beach and coastal wetland complex includes a core of nearly 16,000 acres along 17 miles of Lake Ontario shoreline in Oswego and Jefferson Counties, New York.[4] Early detection of aquatic invasive species that have the potential to migrate into and out of these systems is an important element in preventing the spread of invasives throughout these regional ecosystems, the northeastern United States and the Great Lakes. Within the Eastern Lake Ontario region there are several significant tributaries that connect these ecosystems with Lake Ontario and inland waterways, including linkages to the St. Lawrence River and the Erie Canal.

The sites initially chosen, represent ecologically, recreationally and culturally important waterbodies in the St. Lawrence and Eastern Lake Ontario (SLELO) Region. We chose a downstream and upstream site for each of the four rivers selected. Downstream and upstream sites within each river are separated by an impassable barrier (i.e. dam, management structure) apart from French Creek. Chaumont River upstream site was not sampled in the 2017 field season.

Salmon River

The Salmon River, located along the eastern shore of Lake Ontario, is a valuable cultural and natural resource worthy of protection from the habitat-altering impacts of invasive species. As a cultural resource, the Salmon River is a multi-million-dollar fishery hosting in excess of 100,000 angler visitors annually. Angling enthusiasts travel from numerous regions across the United States, Canada and other parts of the world to fish the river. Many local businesses benefit monetarily because of this resource.

The 17-mile river system is rich in habitat and biodiversity and provides, both in the upstream reaches and within the estuary, spawning and nursery grounds for Pacific salmon (Chinook, Coho and Steelhead) and the native Atlantic salmon. The estuary provides shorebird nesting sites for species such as the Black Tern and the Least Bittern.[5]

Oswego River

The Oswego River, located in Oswego County, is formed by the joining of the Oneida River and the Seneca River. The Oswego River is approximately 23 miles long from its beginning at Three Rivers to the City of Oswego where it empties into Lake Ontario. The Oswego River provides a route from the Erie Canal to Lake Ontario and vice versa[6].

Chaumont Bay and Chaumont River

Chaumont Bay is a 9,000-acre embayment located on the east end of Lake Ontario. The bay receives waters from Guffen Creek, Three Mile Creek, and the Chaumont River, creating three smaller embayment's within Chaumont Bay on the northeastern side. Chaumont River, being the largest tributary connects Lake Ontario with hundreds of miles of inland waterways.

[4] Sargis, et.al. Invasive Species Management and Wetland Restoration in the Eastern Lake Ontario Barrier Beach and Coastal Wetland Complex. The Nature Conservancy. 274 N. Goodman St. Rochester, NY 14607

[5] Chapman, G and R.K.Williams. Managing Japanese Knotweed (*Polygonum cuspidatum)*In the Salmon River and Salmon River Estuary. 2012. The Nature Conservancy/SLELO PRISM.

[6] http://www.dec.ny.gov/outdoor/41044.html

French Creek

French Creek is the main tributary of the French Creek Wildlife Management Area near Clayton, New York. The main channel of French Creek is approximately 6 miles in length and connects the St. Lawrence River to inland waters. The Wildlife Management Area (WMA) is comprised of 2,300 acres and supports cattail marsh bordering open water areas.

Collaboration

Responding to the growing invasive species problem, New York State passed legislation in 2003 that created the New York Invasive Species Task Force (ISTF). The ISTF final report led to a 2008 statute, known as Title 17 of ECL Article 9, which established the New York Invasive Species Council and Invasive Species Advisory committee. The Council is co-led by the NYS Departments of Environmental Conservation (DEC) and the New York State Department of Agriculture and Markets. Among the Council's numerous statutory responsibilities is the requirement to encourage and support, within available funds, Partnerships for Regional Invasive Species Management (PRISMs) in their efforts to address invasive species through coordination, recruitment, training of volunteers and citizen scientists, education, early detection and rapid response.

The entire eastern portion of Lake Ontario is represented by one such collaborative partnership known as The St. Lawrence Eastern Lake Ontario Partnership for Regional Invasive Species Management (SLELO PRISM). The SLELO region encompasses a 7,387-square mile region and includes the counties of St. Lawrence, Jefferson, Lewis, Oneida and Oswego outside of the Adirondack Park. The SLELO region includes portions of the Lake Ontario watershed and shoreline as well as Oneida Lake. The northern and western ends of the region correspond to the county boundaries of Jefferson, St. Lawrence and Oswego Counties along the Lake Ontario coastline.

2.0 Project Objectives and Accomplishments

Under this project, members of the SLELO partnership engaged citizen scientists, incorporated underwater video technology, exposed volunteers to the concept of environmental DNA and its place in invasive species detection and developed a reference guide for other volunteer groups to participate. Citizen scientists were also given the opportunity to provide feedback as to the ease of implementation of both project components.

The value of utilizing citizen scientists exceeds scientific posterity and allows for a cost-effective means by which to conduct aquatic invasive species early detection on a broader level because citizen scientists 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. For this project a modified field kit was utilized to ensure quality control and to reduce cross contamination of samples. This capability allows for aquatic invasive species 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. The production of our Citizen Science Reference Guide provides a framework for which citizen scientists can start their own sampling initiatives without direct guidance from an organization or traditionally trained scientist.

In 2016-17 a sufficient number of volunteer citizen scientists provided support to this project by assisting with sample collection and filtration and underwater video recording. Additional citizen science metrics (including cost metrics) are as follows:

Number of volunteers = 8
Hours contributed = 47.5
Hourly rate equivalent = $641.25

Environmental DNA

Environmental DNA analysis relies on the use of species-specific genetic markers to identify genetic material shed from an aquatic organism (i.e. skin cells, feces, and mucus). Genetic material exists in suspension until it is degraded by UV, temperature, pH and other water quality conditions. By collecting water samples and filtering out suspended genetic material, laboratory technicians are able to use species-specific probes to identify target organisms within a sample.

During the summers of 2016-2017, partners of the SLELO PRISM along with The Nature Conservancy and the Department of Microbiology and Immunology at Cornell University, implemented a project to assess the feasibility of using eDNA as an early detection tool for aquatic invasive species. 160 water samples were collected from four strategic focal areas along Eastern Lake Ontario and were analyzed using highly specialized processes for the presence of genetic material released by both invasive and native aquatic animals. For this project, testing targeted six invasive species and two native species of fish (Table 1). The native species were included to validate qPCR[7] methodology.

[7] Quantitative polymerase chain reaction. A technique used in molecular biology to amplify a single copy of a segment of DNA

eDNA Sampling Protocol

500 mL of water were collected at each sampling point. A 1.5µm glass fiber filter was placed into the filter platform of a Buchner filter funnel and 300 mL of water were drawn through the filter using an attached vacuum hand pump. The filter was then placed in a tube of Longmire's solution and kept on ice. Longmire's solution is a cell lysis buffer that preserves DNA. This procedure traps cells from water samples in the filter and preserves genetic material for analysis in the laboratory. Field equipment required for this project included the following, also shown in (Table 2):

Table 2:

- 500 mL plastic sampling bottles
- 500 mL plastic vacuum flask with rubber stopper
- Buchner filter funnels
- 1.5µm glass fiber filters
- Vacuum hand pump
- Sample containers with Longmire's solution
- Forceps
- Nitrile gloves

Laboratory Analysis

Samples were transported to the Cornell University Department of Microbiology and Immunology laboratory of Dr. James Casey the day after collection. Samples can also be mailed to the laboratory at a flat rate postage. The laboratory then extracted DNA from our sample filters and tested the DNA using qPCR methodology. We received all test results within ten days.

Genetic Markers

Genetic markers used for invasive and native species presence testing are mitochondrial

cytochrome c oxidase 1 (COX1) gene sequences extracted from tissue samples collected from target fish samples. Cornell designed the PCR primers and probes and tested the resulting species-specific probes on target tissue DNA and eDNA that they collected from contaminated waters. Ten controls with varying amounts of target genetic material are run in each of our PCR plates along with duplicates using 1.2-micron, glass fiber filters[8].

The qPCR assay in our eDNA analysis targets mitochondrial sequences of the gene COX1. This gene is extremely species-specific, allowing us to identify DNA from our target species with a strong level of confidence. TaqMan (a common qPCR reagent) is used to flag these positive sequences within our samples. A fluorescent probe selectively binds only to DNA corresponding to the target COX1 sequence.

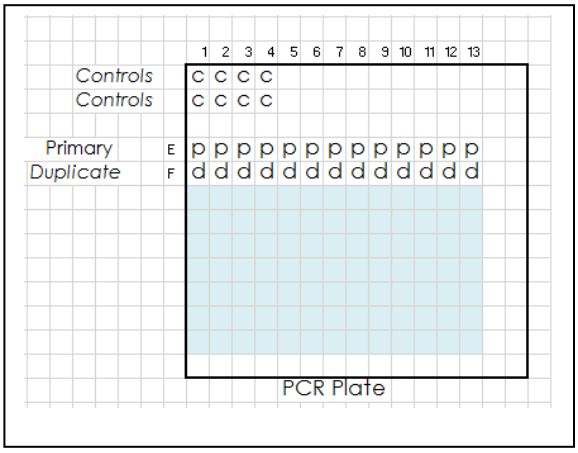
Different probes are used for each test species, as a probe is specific to a single species. Once the probe binds to a target sequence, the fluorescent unit of the probe detaches from the species-specific primer and fluoresces. The qPCR machine then detects this fluorescence and records the result. Fluorescence readings directly correspond to the quantity of target sequence present in the sample. Any recorded target sequence is considered a positive result. When we designate a result as a low-level positive, we are referring to a quantity reading of less than twenty copies of target sequence. Given the high sensitivity of qPCR, low-level positives are still considered detection events.

It is important to note that eDNA is not finite and is used as an indicator only. A positive test result indicates that a species might be present and further testing and possible fish/organism sampling may be warranted.

Reporting

Results were received in several formats. This includes the plate layout (Table 3), amplification plots (Figure 2), standard curves (not referenced), and quantities of genetic copies of target species in a tabular format (Table 4). The amplification plots provide visual representation of target sequence amplification. Exponential curves indicate amplification of target sequence. Each qPCR test includes known standards tested alongside samples to extrapolate sample target DNA quantity, precision, and efficiency. Clear exponential curves that pass the algorithm determined threshold represent the successful amplification of standards and any positive detection results.

Table 3. Basic PCR plate layout - example



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

[8] Casey J.W. Personal Communication. July 18, 2017.

below the threshold are non-target DNA (Figure 2).

Figure 2. Example amplification plot.

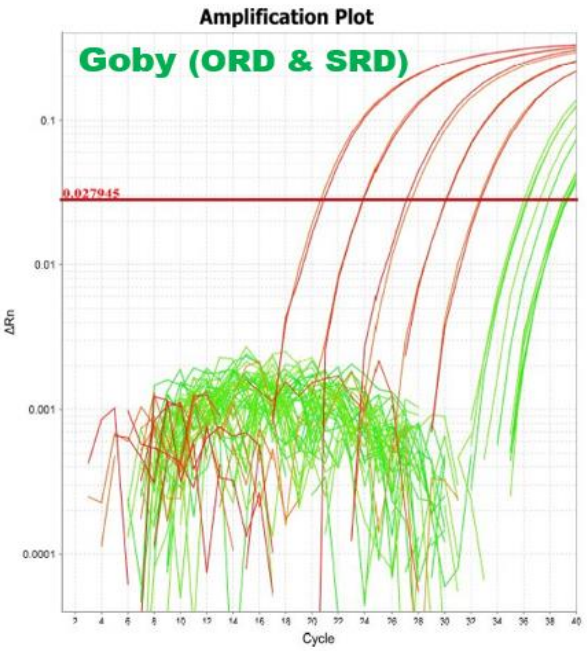


Table 4 (below). Quantity of target genetic copies in tabular format.

Plate Well	Target	Quantity
10	Control	1,000,000
11	Control	1,000,00
12	Control	10,000
13	Actual	0
4	Actual	72.411
15	Actual	88.354
16	Actual	0
17	Duplicate	0
18	Duplicate	72.411
19	Duplicate	12.332
20	Duplicate	0

A standard curve (not shown) was developed for each sample tested. This is a regression line generated using the known concentrations of the standards. Using this regression model, sample concentration can be determined from fluorescence readings and the number of target DNA copies is calculated. The tabular format shows the quantity of target species DNA in each sample (each sample is tested twice). Samples with blanks in the quantity columns are negatives, meaning no target species DNA was detected in the sample.

eDNA Results

Detection of round goby was very common, which is not surprising given their establishment throughout Lake Ontario. Native rock bass presence was less consistent than round goby presence but was still a common occurrence. In 2016, a low-level amount of grass carp DNA was detected in a single sample from our Oswego River downstream site. In 2017, one low-level northern snakehead positive was observed from our Oswego River upstream site, and three low-level northern snakehead positives were observed from our Oswego River downstream site. All positive test results and corresponding locations for the duration of this project are presented in (Table 5).

Species	Where detected
Bighead carp (*Hypophthalmichthys nobilis*)	*Not detected*
Black carp (*Mylopharyngodon piceus*)	*Not detected*
Grass carp (Ctenopharyngodon Idella)	Oswego River
Silver carp (*Hypophthalmichthys molitrix*)	*Not detected*
Northern snakehead (*Channa argus*)	Oswego River downstream
Round goby (*Neogobius melanostomus*)	Oswego River, Salmon River, Chaumont River, French Creek
Lake herring (*Coregonus artedi)*	Oswego River downstream, Chaumont River downstream, French Creek downstream.

Rock bass (*Ambloplites rupestris)*	Oswego River, Salmon River, Chaumont River, French Creek

Underwater Video Surveillance

As a component of this project initiative, underwater video technology was used not only as a hands-on citizen science tool but also to determine its practicality as an early detection tool. The video surveillance initiative used a high resolution SeaViewer® underwater color video camera and recording unit. The unit was either suspended into the water column via cable or attached to a holding bracket when suspended into moving water for camera stability. This was done in such a way as to not disturb spawning areas.

A small bait bag was suspended independently in front of the camera lens. This was done to increase observations and to decrease time needed waiting for fish to move within viewing range of the camera. The lens and camera recorder were originally to be deployed and monitored in two-hour intervals; however, the time of suspension was reduced to 30-minute intervals due to time constraints of citizen science volunteers.

An adjustable LED lighting system is included for dark and dirty water viewing. Frozen chicken wings were placed into the bait bag made from cheese cloth or plastic netting and weighted with small stones. The bait bag was suspended separately from the camera lens approximately 15 inches from the camera lens. The camera lens and bait bag were positioned at or near the benthic area of the waterway. In some areas the lens and bait were placed just above stone substrate as to capture occurrences (if any) of Rusty Crayfish (*Orconectes rusticus*), Round Goby (*Neogobius melanostomus)* and native organisms.

Site Selection & Video Analysis

Field sites were predominately selected to match eDNA sampling sites. However, a small number of sites where no eDNA sampling took place were selected for video in both the 2016 and 2017 field seasons. Videos were analyzed for species identification, and presence of species at each filming location was recorded. Notable excerpts from videos were edited into short videos.

Video Results

Common species, such as pumpkinseed sunfish, bluegills, and yellow perch were seen regularly during filming. Round goby were extremely common and were the most common invasive species sighted using underwater video. Video was captured of rust crayfish at Oneida Lake after confirmation of its presence by the 2017 early detection team. Other less common native species were recorded as well, including smallmouth bass, red horse and bowfin. Table 6 refers to the species identified with video throughout the project.

Discussion

Results generated by eDNA analysis may come as a surprise to managers and citizen scientists, since they may indicate the presence of a species that has yet to be seen in the focal area. These results work well as a tool to highlight species to be on the lookout for. Early detection at the molecular level provides significant lead time for communities to gather resources for more extensive detection efforts and public advisories.

Underwater video presents a uniquely visual method of connecting citizen scientists to freshwater ecosystems. Seeing the species present within a waterbody in real-time is an appealing method of species monitoring. Video analysis promotes visual identification skills (of aquatic plants and animals) and allows citizen

scientists to engage with wildlife professionals if they are unsure what species they have captured.

Video is time dependent. For effective coverage, longer recording time is recommended. Depending on the target species and biology of focal organisms, filming during different times of day may be effective. Video quality is also affected by water quality. Turbidity can decrease clarity and make visual identification difficult. Regardless of its limitations, video represents a stimulating and visual tool for citizen science use. This tool can be used repeatedly over long periods of time to connect stewards and citizens with aquatic environments, while at the same time, monitoring for invasive species.

Table 6 – presents a summary of species observed using underwater video during the project term.

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.*
Rusty Crayfish – *Orconectes rusticus*
Sculpin – *Cottus sp*.
Redhorse Spp. - *Moxostoma Spp.*
Round Goby – *Neogobius melanostomus*
Bowfin – *Amia calva*
Largemouth Bass – *Micropterus salmoides*
Smallmouth Bass - (*Micropterus dolomieu*)
Mooneye – (*Hiodon tergisus)*
Rock Bass – *Ambloplites rupestris*
Freshwater Drum – *Aplodinotus grunniens*
Common Minnow – *Phoxinus phoxinus*
Common Musk Turtle – *Sternotherus odoratus*

Working with other members of the SLELO partnership, this project serves as a pilot project that could be adopted by other partnerships and expanded to a broader scale, for example, use by lake associations. A deliverable of this project component was to develop a Citizen Science Reference Guide that could be used to guide other participants or programs towards developing a similar effort using either environmental DNA and/or underwater video technology. A Citizen Science Reference Guide has been prepared and is a separate document from this technical report. Copies of the guide can be downloaded from www.sleloinvasives.org or by contacting the SLELO PRISM Coordinator.

Financials

Total project costs to deliver this initiative was 95% of the total grant which is less than originally projected. The lower costs were associated with reduced laboratory costs that were estimated greater than the actual delivery costs. We used the best information available at the time of proposal development to determine expenses.

Field Operations

Most of the field data collected throughout this project was collected by seasonal field staff and volunteers. At the start of each field season staff were trained on project protocols, sample collection, camera deployment and data management. In addition to field training, crews were trained on safe use of equipment, organizational policies and procedures and with first aid/CPR training.

Conclusions

Today's approaches to genetic analysis make working with eDNA relatively easy. Collecting and filtering water samples and sending the samples to a lab is straight forward and easy to implement by volunteer citizen scientists. The laboratory (which you will need) is tasked with processing the samples and reporting the results. Costs for laboratories varies, in many cases however, samples can be processed for under $150.00 per sample, which includes multiple species. A lake association, for example, could collect 10 samples each year for an approximate cost of $1,500. Some university programs also

supply a box containing all field sampling equipment along with instructions, at no or little cost.

Using underwater video technology can be an exciting way to engage citizen scientists in aquatic invasive species observations. Real time video allows for close observation of aquatic plants and animals. During the term of this project, citizen scientists were able to identify several aquatic macrophytes in addition to fish and crayfish. Recorded video can be a practical means by which to taxonomically determine fish species passing the viewing range of the video camera, and the videos can be preserved as historical documents. This technology offers citizen science teams a (hands-on) and (real-time) means by which to embrace technology and to test its usefulness as an aquatic invasive species early detection tool. High definition underwater, color video cameras range in cost from a few hundred dollars and up. Most cameras are very easy to use.

Environmental DNA and underwater video technology can act as practical early detection tools for aquatic invasive species. Both techniques are easy to implement with the caveat being that you will need a laboratory that conducts qPCR analysis. Costs vary but can be found at reasonable rates that are not exorbitant.

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