The Nature Conservancy

Saint Lawrence Eastern Lake Ontario

environmental DNA

2021 Tributary Project

INVASIVE SPECIES MANAGEMENT
SAINT LAWRENCE
EASTERN LAKE ONTARIO

Report Prepared by:

Philippa Kohn, Sustainable Fisheries Ecologist
The Nature Conservancy

Brittney Rogers, Aquatic Restoration and Resiliency Coordinator
Saint Lawrence Eastern Lake Ontario Partnership for Regional Invasive Species Management
The Nature Conservancy

Jacob Wojcik, 2021 eDNA Project Technician
Saint Lawrence Eastern Lake Ontario Partnership for Regional Invasive Species Management
The Nature Conservancy

Acknowledgements

We wish to thank the partners, community scientists and volunteers, and all stakeholders who provided assistance over the course of this project; their project review, expert opinion and guidance was key to the success of this project. We also wish to thank The Nature Conservancy staff who worked on all aspects of this project; including grant administration, finance and field operations, and human resources. We also wish to thank the following individuals and organizations for assistance and guidance with this project:

SLELO PRISM Partners

The Nature Conservancy

Dr. Nicholas Sard, SUNY Oswego

The New York State Office of Parks, Recreation and Historic Preservation

The Nicandri Nature Center

Funding for this project provided through

The Arconic Foundation

New York State Environmental Protection Fund, NYS Contract No. C011256
New York State Department of Environmental Conservation

Contact Brittney Rogers Brittney.Rogers@TNC.ORG or Philippa Kohn, Philippa.Kohn@TNC.ORG with questions regarding the 2021 Tributary eDNA Project

Executive Summary

The tributary streams of Eastern Lake Ontario and the Upper St. Lawrence River flow through the most intact landscape in the bi-national Lake Ontario watershed. The Tug Hill, northern Adirondacks, and the Indian River chain of lakes along the St. Lawrence are still heavily forested regions that shelter over 30 creeks and rivers entering the embayment's of the lake and river. Fourteen tributaries along the eastern shore of Lake Ontario and the St. Lawrence River were sampled for environmental DNA between September – December 2021, to assess the presence of native and aquatic invasive aquatic species (AIS). In all, presence of four aquatic native species and nine aquatic invasive species were tested for.

eDNA detected the presence of at least one native or invasive species in all tributaries, resulting in there being a total of 93 positive detections during sampling. The tributaries that had positive hits for both a native and AIS included the Oswego River, Salmon River, Oswegatchie River, Sucker Brook, Grass River, Robinson Bay, and the Raquette River. Detection of the target species at the sampled tributaries was greatest in December, which in part reflects the greater sampling frequency during this month.

While the eDNA detections are promising, it is recommended that further work is done on developing the assays for Asian swamp eel, tubenose goby, rusty crayfish, and Atlantic salmon to avoid the potential for false positives. As such, the detections for these species from this study should be interpreted with caution. Local collection of tissue samples for these species would assist in this effort and would improve any additional eDNA studies with these species in this geographic area.

Community engagement was another goal of this project, and during the project, fifteen engagement events were offered bringing in 55 total participants and totaling 182.55 hours which exceeded our target objective of 150 volunteer hours.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	**Explanation**
AIS	Aquatic invasive species
eDNA	Environmental DNA
CTAB	Cetyl trimethylammonium bromide
ROV	Remotely operated vehicle
SLELO-PRISM	St. Lawrence Eastern Lake Ontario Partnership for Regional Invasive Species Management
TNC	The Nature Conservancy
UV	Ultraviolet

Introduction

Founded in 1951, the Nature Conservancy (TNC) is an environmental non-profit whose mission is to conserve the lands and waters on which all life depends. With staff located around the world and through collaborative partnerships, TNC has advanced conservation in 72 countries and territories. Within the United States, conservation work touches down in the Great Lakes in part through the Great Lakes Sustainable Fisheries Team. This team focuses efforts on helping to maintain and restore native fish populations in the Great Lakes, with a focus on coregonines, which are a sub-family of the Salmonidae family and include whitefishes and ciscoes. Further work in the Great Lakes region is done by the St. Lawrence Eastern Lake Ontario Partnership for Regional Invasive Species Management (SLELO PRISM). The SLELO PRISM was established in 2011 and is hosted by TNC in partnership with the New York State Department of Environmental Conservation, serving Jefferson, Lewis, Oneida, Oswego, and St. Lawrence counties. The program strives to protect native biodiversity and freshwater resources through a collaborative approach to invasive species management with an emphasis on core programming and multiple special initiatives. SLELO PRISM is a collaborative effort between numerous principal, at-large, and cooperating affiliate partners throughout the region. Contributions and expertise provided by our partners is the key to its success.

SLELO PRISM and The Nature Conservancy have been conducting eDNA monitoring for detection of native and non-native species in the SLELO PRISM alongside nineteen partners, including tribal communities, not-for-profits, government agencies and others located both regionally and internationally, since 2014.

Native Species

Within the Laurentian Great Lakes, there is a diversity of coregonine species that can be found in nearshore and offshore waters. Cisco (*Coregonus artedii)* and lake whitefish (*Coregonus clupeaformis*) are two coregonine species that have served as food staples for indigenous tribes and a focus of targeted commercial fisheries. Given the aggregations of these species in shallow nearshore areas during their spawning season, this enabled more efficient capture (Ebener et al., 2008). These species also play an important role in the lake food webs as forage fish, supporting top predators such as lake trout (*Salvelinus namaycush*) and Atlantic salmon (*Salmo salar*). However, populations of cisco and lake whitefish declined rapidly in the early 20th century due to overfishing, changes in environmental conditions and the introduction and spread of aquatic invasive species (AIS). While recovery of these species varies among the Great Lakes, in Lake Ontario, both species are the focus of restoration efforts.

Even though the importance of lake whitefish and cisco to Lake Ontario has been well established by researchers, there are still knowledge gaps pertaining to the spawning behavior of these species. The extent and condition of habitats used for spawning and whether there are bottlenecks (i.e. factors that could limit or impair rehabilitation of these species), have yet to be fully understood. Cisco and lake whitefish are known to spawn in shallow, near-shore lake habitats over rocky shoals or reefs. Within the Upper Great Lakes (i.e., Lakes Huron, Michigan, and Superior) there were lake whitefish populations that utilized rivers for spawning, but these populations were extirpated in the 1870s due to declining river conditions. Recently however, there has been a return of river running lake whitefish, with spawning being found to occur in all

the major tributaries of Green Bay (Ransom et al., 2021). Within Lake Ontario, whether whitefish and cisco utilize tributaries for spawning is not known. Given the ability of these species to modify their spawning behaviors and the occurrence of tributary use in the Upper Lakes, it is plausible and has yet to be investigated. If tributary use is occurring within Lake Ontario, this will not only change our understanding of how these species spawn but also provide information to better inform restoration efforts.

Aquatic Invasive Species

An invasive species is a non-native plant, animal, or other organism (e.g., microbe) whose introduction causes or is likely to cause economic or environmental harm, or harm to human health. Invasive species will often dominate an ecosystem to the detriment, and sometimes the exclusion, of native species. Invasive species can do this because the natural conditions, predators, parasites, and other organisms that keep them under control in their native range do not exist in the new environment where they have been introduced.

Invasive plants, animals, insects, and microorganisms are among the most serious threats to native species, habitats, ecosystems, and public health within the five-county area that defines the SLELO region. Invasive species are opportunistic and almost always out-compete, damage, or displace native species resulting in serious disruptions of ecosystem processes. Interdependency on food and habitat, hydrology, nutrient cycling, natural succession, soil erosion and water quality are among the processes impacted. At a global scale, invasive species are among the greatest threat to native plants, animals, and natural communities, such as forests, wetlands, streams, and ponds (Singh, 2005).

As it pertains to New York waters, as well as waters of neighboring states and Canada, invasive species have continued to proliferate. In Lake Ontario, as well as for other great lakes, pertains to ships entering the St. Lawrence River from all around the world (Ricciardi, A. 2006). As these ships move from port to port, many aquatic species will attach to hulls, and will be present in the ballast water taken on by the vessel as it enters or leaves any given port. Depending on the route taken, they can eventually take on many different species of non-native, and potentially invasive, species that are eventually discharged when they make it back to their home ports.

For more inland waters, dispersion of invasive species can be amplified by recreational activities. Recreational boaters frequently travel long distances, across several state lines, every year to fish and boat on many different bodies of water. In the process, if boats are not properly cleaned, drained and dried, aquatic species may be transported into non-native territories. The aquatic pet trade also poses threats as individuals carelessly dispose of unwanted organisms, which are generally exotic, into their local water ways. While many laws and regulations are in place to try and prevent the spread of AIS through ballast water dumping, watercraft inspection and aquatic pet trades, species are still being transported and introduced into new ecosystems. Many non-native species introductions often occur at such low quantities that newly established populations can go a long time without being detected.

Once AIS are established, dealing with infestations can become difficult and costly. Therefore, early identification of potential threats is crucial for managers. Given how quickly the spread and

establishment of invasive species can occur, utilizing innovative and effective early detection tools is essential.

eDNA

Like humans, aquatic species shed their DNA into their surrounding environment whenever they are present. This can be through the deposition of scales, feces, mucus etc. and is influenced by the type, size and characteristics of the organism. Environmental DNA (eDNA) is the genetic material that can be subsequently extracted from the environment. All that is needed is a single cell. For animals, there are two primary sources of DNA within a cell, mitochondrial and nuclear. Many studies use mitochondrial DNA, as the sequences are shorter, there are numerous mitochondria within a cell and there is significant divergence of this DNA among species (Billington and Hebert, 1991).

For aquatic species, the first step in conducting eDNA sampling is to collect a water sample as eDNA can persist in water for days (Dejean et al., 2011; Barnes et al., 2014). However, abiotic (e.g., temperature) and biotic (e.g., microbes) conditions, can affect the quantity and quality of DNA that can be present. Species behavior (e.g., spawning, migrations etc.) and activity can also affect the presence and persistence of eDNA. Once a sample is collected, it is then filtered or centrifuged to concentrate the DNA. Once in the laboratory, the DNA is extracted and amplified to detect species presence. Studying eDNA is non-invasive and offers a cost and time effective way to assess species presence when compared to traditional fisheries assessment methods, especially for rare and/or invasive species (Bessey et al., 2020).

Within the Great Lakes region, one of the first applications of eDNA in fisheries studies was to help with the detection of invasive carp. The need for early detection enabled eDNA to be an effective tool and helped foster further developments such as the exploration of a basin-wide surveillance program (Jerde et al., 2013). Currently, eDNA is in a burgeoning research space, with work taking place to further understand and improve its application in conservation (Barnes et al., 2016). For example, understanding detections if live fish are not present (e.g., introduction of genetic material via bird excrement, storm water discharge etc). Continued application of these tools in practical contexts is important as it can inform and improve future surveys and laboratory methodologies.

Objectives

The tributary streams of eastern Lake Ontario and the Upper St. Lawrence River flow through the most intact landscape in the bi-national Lake Ontario watershed. The Tug Hill, northern Adirondacks, and the Indian River chain of lakes along the St. Lawrence are still heavily forested in tact regions that shelter over 30 creeks and rivers entering the embayments of the lake and river. Considering the uncertainties around coregonine spawning behavior in Lake Ontario and the potential for these tributaries to harbor native and non-native species, the objectives sought in this study included:

* Determining whether coregonines are present in select tributaries during their spawning season (October – December).

* Understanding what other native fish species may be present in select tributaries during the coregonine spawning season.
* Understanding what AIS species may be present and the range of their presence in select tributaries during the coregonine spawning season.

Methods

Study Locations

All of the Great Lakes eventually flow into Lake Ontario before continuing through the Saint Lawrence River and into the Atlantic Ocean. Eastern Lake Ontario and the Saint Lawrence River up to Massena, New York were the focal area for this project. In total, 14 tributaries were sampled from September to December 2021 spanning the Eastern Lake Ontario shoreline up into the St. Lawrence River, from Rice Creek in Oswego, New York, to the Raquette River near Massena, New York (Appendix 1). This study timeframe was selected as it covers the period during which coregonines move into nearshore areas to spawn.

Prior to the commencement of sampling in August 2021, preliminary sites were selected through a combination of literature review and using a desk-based approach where GIS and aerial imagery was used to identify potential habitats and sampling locations. Field visits were conducted in August 2021 to verify suitability for sampling, potential species presence, site accessibility, and to gather some preliminary site data. All site verification data collected in the field was done using ArcGIS Survey123. After site verifications were completed, the specific locations to be sampled on each tributary were selected.

To assist with determining sampling frequency, tributaries were grouped into "priority" categories based on likelihood of detecting coregonines, as determined from a review of current and historical literature and consideration of habitat suitability. Two sampling sites were identified for each tributary, with three sampling sites being identified for priority tributaries (Table 1). Specific sampling site maps within the fourteen tributaries can be found in Appendix 1.

All sampling was conducted up to the location of the first barrier (i.e., dam), if there was one in the system. Of note, sampling was not able to commence in August as originally planned, thus some sites were added later in the season. Table 2 outlines the revised sample collection numbers by month and in total for the project. With regards to sampling frequency, priority tributaries were sampled each month, with sample collections increasing in October, November, and December, to reflect when native fish may move into tributaries and be present in the systems. During a sampling month, sample collections were aimed to be bi-weekly to ensure that there was temporal spread during the sample collections. For other tributaries, it was originally intended to sample every other month e.g., August, September, and December. As noted, this was revised due to the one-month delay in commencing sampling. As such, these sites were sampled once in September, October, and December.

*Table 1. Approximate site distances from the tributary mouth (miles). *Shaded cells in dark gray denote those designated as "priority" tributaries.*

Tributary	Sampling Site	Distance from tributary mouth (miles)
Rice Creek	A	1.66
	B	1.64
Oswego River*	**A**	**0.95**
	B	**0.90**
	C	**0.86**
Salmon River	A	0.36
	B	0.94
North Sandy Pond	A	0.25
	B	1.37
South Sandy Creek	A	1.35
	B	2.32
Stony Creek	A	0.35
	B	0.32
Kents Creek	A	1.31
	B	0.31
Chippewa Creek	A	0.00
	B	0.27
Oswegatchie River*	**A**	**0.15**
	B	**0.18**
	C	**0.25**
Sucker Brook*	**A**	**0.14**
	B	**0.20**
	C	**0.13**
Brandy Brook	A	0.63
	B	0.65
Robinson Bay	A	3.75
	B	2.03
	C	0.14
Grasse River*	**A**	**9.91**
	B	**7.76**
	C	**0.61**
Raquette River*	**A**	**11.99**
	B	**4.15**
	C	**15.78**

Table 2. Sample collection by month based on priority status. Sampling was originally intended to start in August 2021, but due to supply chain issues, was started in September, with sample collections being revised to meet the desired goal of collecting 140 samples.

Month Sampled	Number Samples Collected at Priority Tributaries	Number Samples Collected at all other Tributaries	*Total Samples Collected Monthly*
August	----	---	---
September	10	20	*30*
October	20	20	*40*
November	30	---	*30*
December	20	20	*40*
Total Number of Samples Collected	*80*	*60*	*140*

Target Species

Target species for this project included both native and invasive fish and crustacean species (Table 3). Atlantic Salmon and American Eel were included in the sampling panel due to the similarities in their habitat use and preferences with that of coregonines and potential for being present in these systems during the sampling period. Further, these species have also undergone population declines and are impacted by the presence of AIS in Lake Ontario. The selected AIS were chosen based on interest for monitoring and response efforts. These species have the potential to establish populations and proliferate in Lake Ontario, the St. Lawrence River, and their respective tributaries.

Table 3. List of target native and invasive species tested for in collected samples.

Native Species	Aquatic Invasive Species
• Cisco (*Coregonus artedi*) • Lake whitefish (*Coregonus clupeaformis*) • Atlantic salmon (*Salmo salar*) • American eel (*Anguilla rostrata*)	• Silver carp (*Hypophthalmichthys molitrix*) • Bighead carp (*Hypophthalmichthys nobilis*) • Northern snakehead *(Channa argus)* • Tench *(Tinca tinca)* • Tubenose goby *(Proterorhinus semilunaris)* • Asian swamp eel (*Monopterus albus*) • Rusty crayfish *(Orconectes rusticus)*

Field Sampling Methods

Field samples were collected from land, except for North Sandy Pond and Chippewa Creek, where a boat and kayaks were used respectively. At a given sample location, a previously decontaminated 1 L Nalgene bottle was used to collect a water sample. Before collecting the sample, the Nalgene bottle was rinsed with ambient water from downstream at the site to remove any residual bleach and to not contaminate sample location. After the sample was collected, it was subsequently filtered using a vacuum or hand pump with a 47 mm Sterlitech membrane filter. The membrane filter was contained within a Nalgene analytical filter funnel attached to a Buechner funnel. Once the entire water sample was processed, the filter was folded 3 times and placed into a vial with ethanol for preservation. To limit degradation of the DNA, all samples were filtered and preserved within 30 minutes of collection. Control samples were filtered through the same process using DI water to test for contamination in filter housing units and thus limit the chance of false positive detections. The negative controls were sampled at the end of a sampling day, to keep per sample cost as low as possible. Preserved and control samples were delivered to the laboratory on a weekly basis for extraction and analysis. All reusable field and lab equipment was sterilized with a 20% bleach solution and all single-use plasticware used in the lab was ultraviolet (UV) sterilized prior to use. Further information on sampling procedures and equipment can be found in Appendix 2, which contains the Sampling QAQC document for this project.

Laboratory Methods

Extraction of the DNA on the filters was conducted using a conventional cetyl trimethylammonium bromide (CTAB) method following Turner et al. (2014). To amplify the DNA of the target species, a quantitative polymerase chain reaction (qPCR) was done using the assays outlined in Table 4. Each qPCR assay was run in duplicate for each 1L water sample filtered. To estimate the starting amount of target DNA template in each qPCR, standard curves were produced using synthetic double-stranded DNA (gblocks).

Table 4. Assay sources and forwards used for the target species.

Species	**Assay Source**
Coregonine	Hernandez et al. 2020
Atlantic salmon	Atkinson et al. 2018
American eel	Hernandez et al. 2020
Rusty crayfish	Unpublished
Tench	Hernandez et al. 2020
Tubenose goby	Unpublished
Northern snakehead	Unpublished
Carp complex	Turner et al. 2014
Asian swamp eel	Moyer and Diaz-Ferguson, 2013

Volunteer Engagement and Community Outreach

Participation of volunteers and the community was an important pillar of this project. At the outset, there was a defined goal of seeing an engagement of 150 hours by volunteers during the

project. One of the first steps to build a volunteer base was conducting webinars over Zoom (teleconferencing software) during the beginning of the project to introduce the project and provide information on volunteering to interested stakeholders. Additionally, as volunteers were interested in assisting with field efforts, a hands-on volunteer training day was held on 2 September 2021, at the Nicandri Nature Center in Massena, New York. During this training participants were taught how to collect, filter, and preserve eDNA samples. A question-and-answer session was also held to address any questions volunteers had and ensure clarity on project specifics and field season plans.

During the field season, to help maintain engagement, volunteers were provided with regular emails containing information for upcoming field sampling and project updates. The application "ConstantContact" was used to create and send these emails automatically to the developed listservs. Additionally, social media posting on sites such as Facebook was used to further engage the community, and radio appearances were made by SLELO's outreach coordinator.

To calculate the amount of time volunteers and stakeholders were engaged in this project, time tracking was done using multiple different methods. For the conducted webinars, data was downloaded from the Zoom application, which provided information regarding the amount of time each participant spent on any call. For volunteer times at the outreach event and during field sampling efforts (including commute times), staff hosting those activities recorded those respective times. Information pertaining to engagement with the volunteer emails was downloaded from the ConstantContact application. Engagement is defined here as the estimated amount of time spent reading, reviewing, or interacting with the resource shared. A total of two minutes of engagement was estimated for each opened email (based on average of 500 words read in two minutes) with an additional three minutes for every click-through that was reported, meaning the volunteer clicked links or filled out further information via the volunteer sign-up form. Finally, Facebook data was collected using their feature that provides analytics. Regarding social media, engagement rate was estimated as the proportion of engagements or interactions e.g. likes, shares, click-through, etc. to reaches e.g. total number of people exposed to specific post.

Remotely Operated Vehicle (ROV)

A FIFISH V6 manufactured by QYSEA was used during the project to collect underwater footage at the sampling locations and, if possible, assist with observing species presence. During preliminary deployments, information gained from usage was utilized in the process of constructing an SOP for future use that can be found in Appendix 3.

There was no consistent operating pattern utilized when deploying the ROV (e.g., transects). The aim was to learn how to pilot the vehicle well enough to capture sufficient footage. During each ROV sampling event, staff piloted the vehicle across as wide of a spatial extent to view as much of the area as possible. All video footage was archived and is intended to be used in further citizen science projects. These projects may include such things as characterizing substrate and identifying species presence.

Results

eDNA

All fourteen sampled tributaries had at least one positive hit for presence of a native or AIS (Figure 1) resulting in there being a total of 93 positive detections during sampling. The Grass River (n=14), Sucker Brook (n=14) and Oswego River (n=13) had the greatest number of detections of all the tributaries. The tributaries that had positive hits for both a native and AIS included the Oswego River, Salmon River, Oswegatchie River, Sucker Brook, Grass River, Robinson Bay, and the Raquette River. Detection of the target species at the sampled tributaries was greatest in December (n=32). However, there was a peak initially in detections across the tributaries in September (n=31) (Figure 2), which was a month when fewer overall samples were collected. Of the positive detections, 35% had a positive detection in more than one replicate. See Appendix 4 for more details on sampling results.

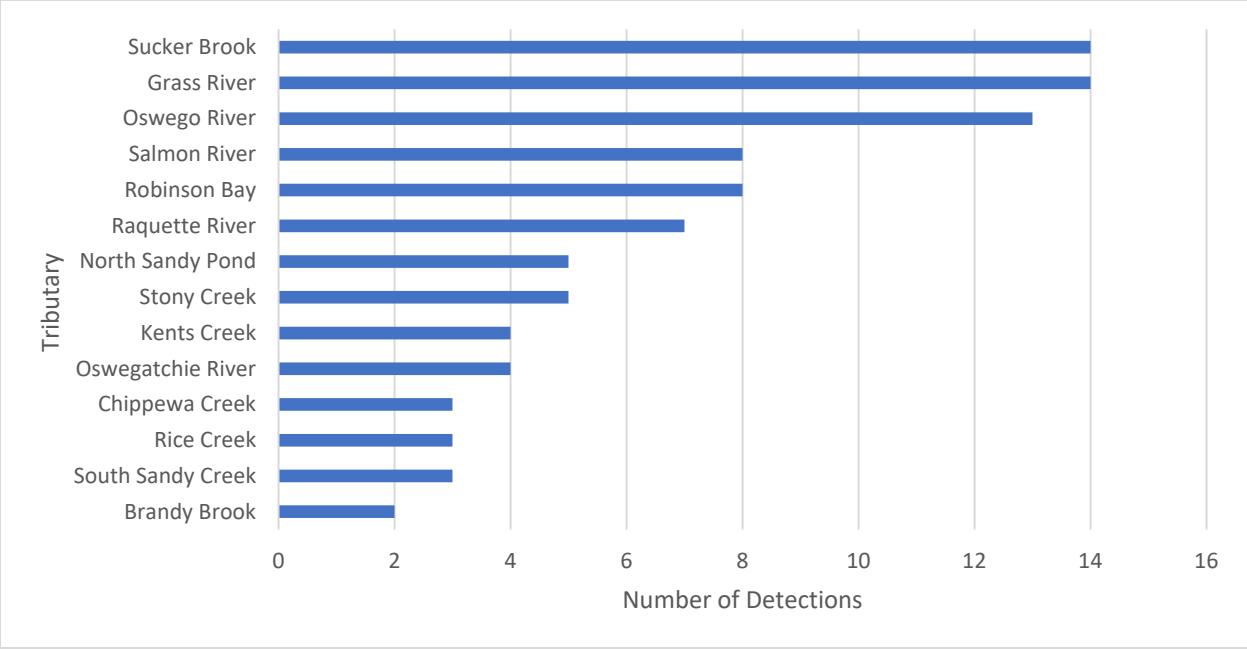


Figure 1. Total number of positive detections at the sampled tributaries during the sampling period.

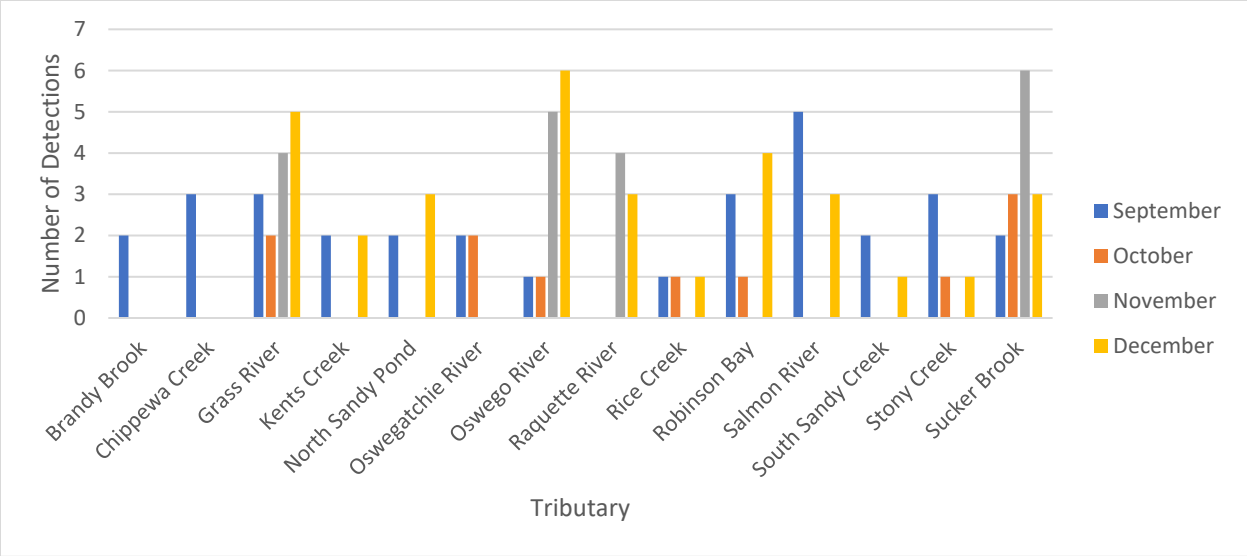


Figure 2. Total positive detections of all the target species by tributary and sampling month.

Native Species

American eel had the greatest number of detections and was detected each month sampled (Figures 5 and 6). In all, American eel was detected in eight of the fourteen tributaries. For the other species, Atlantic salmon was detected at two of the fourteen tributaries and coregonines were detected in one tributary. For the coregonine detection, during both sampling events in December, there was a positive hit in the Raquette River. Of all the sampled tributaries, the Oswego River had the greatest number of hits for native species, with both American eel and Atlantic salmon being detected there. As far as total number of hits, the Oswego River and Salmon River were the sites with the greatest number of detections.

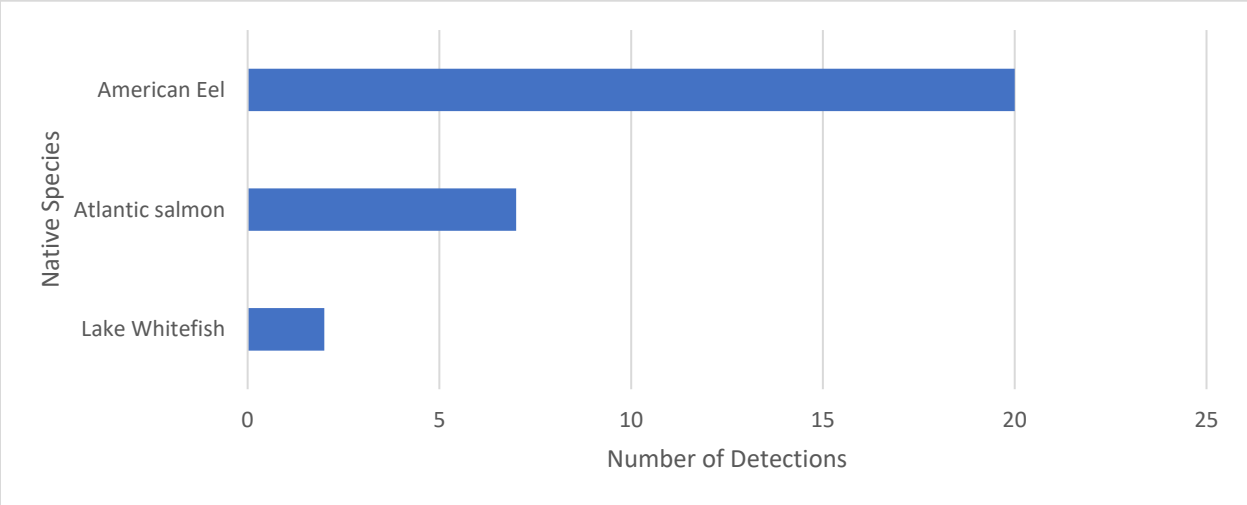


Figure 3. Total number of positive detections of native species at the sampled tributaries during the project.

	American eel				Atlantic salmon				Coregonine			
	S	*O*	*N*	*D*	*S*	*O*	*N*	*D*	*S*	*O*	*N*	*D*
Rice Creek												
Oswego River												
Salmon River												
N Sandy Pond												
S Sandy Creek												
Stony Creek												
Kents Creek												
Chippewa Creek												
Oswegatchie River												
Sucker Brook												
Brandy Brook												
Grasse River												
Robinson Bay												
Raquette River												

Figure 4. Positive detections for the target native species by location and month (gray cell = positive detection; S= September, O=October, N=November, and D=December).

AIS

Of the AIS sampled for this project, there was the greatest detection of tubenose goby (Figure 3), which were detected at ten of the fourteen tributaries (Figure 4). There were no detections of carp species or northern snakehead. Of the sampled tributaries, Sucker Brook was the location with the most detections during the sampling period and tubenose goby were consistently detected there. From a diversity standpoint, Robinson Bay was the location that had the greatest number of AIS species being detected (n=3). This was also the only location where there was a detection of tench. Currently, the tubenose goby, rusty crayfish and northern snakehead assays developed and used for this project are unpublished. Of note, there were several false detections of Asian swamp eel. After thorough review of Moyer and Diaz-Ferguson 2013, it seems the samples amplified after 40 cycles, which is the total number of cycles recommended from that study. This amplification is considered to be a false positive and further analysis needs to occur in this qPCR methodology.

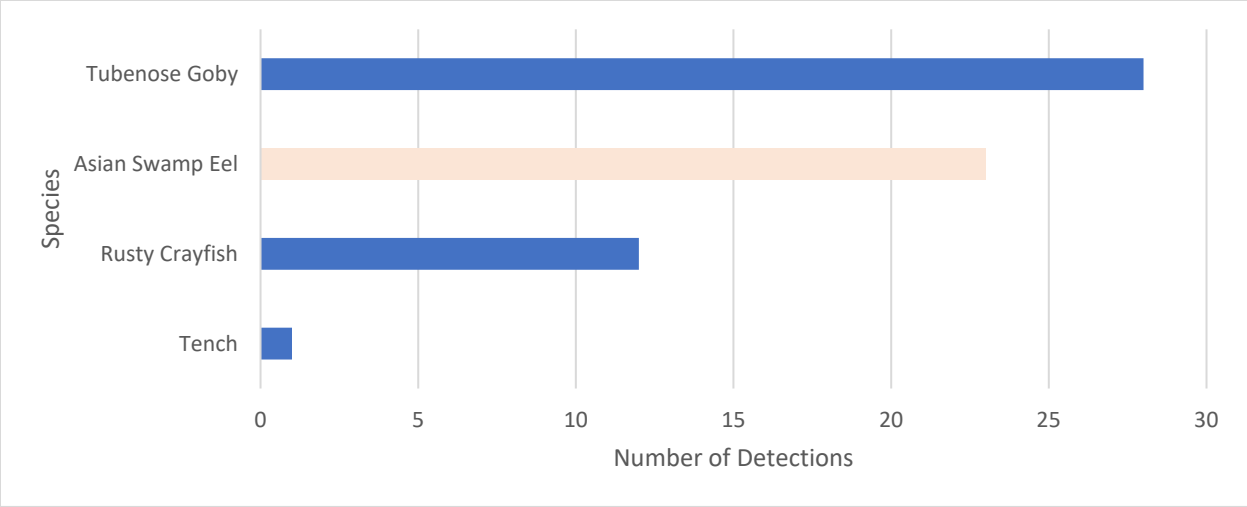


Figure 5. Total number of positive detections of invasive species at the sampled tributaries during the project.

	Carp Complex				Snakehead				Tench				Tubenose goby				Asian swamp eel				Rusty crayfish			
	S	*O*	*N*	*D*	*S*	*O*	*N*	*D*	*S*	*O*	*N*	*D*	*S*	*O*	*N*	*D*	*S*	*O*	*N*	*D*	*S*	*O*	*N*	*D*
Rice Creek																								
Oswego River																								
Salmon River																								
N Sandy Pond																								
S Sandy Creek																								
Stony Creek																								
Kents Creek																								
Chippewa Creek																								
Oswegatchie River																								
Sucker Brook																								
Brandy Brook																								
Grasse River																								
Robinson Bay																								
Raquette River																								

Figure 6. Positive detections for the target invasive species by location and month (gray cell = positive detection; S= September, O=October, N=November, and D=December). Cells shaded beige indicate potential false positives and the data should thus be interpreted with caution.

Volunteer Engagement/Community Outreach

A total of 25 emails were sent out between April and December 2021 to engage volunteers in the project. In all, 3,482 recipients were contacted with a pooled open rate of the emails being 37.22%. Volunteer engagement with the emails was estimated to total 57.55 hours (Table 5). Seven social media posts were made on Facebook, which resulted in reaching 8,430 people. However, there were only 120 engagements with these posts e.g. like, comment etc., resulting in an engagement rate of 1.42% for these social media posts. Fifteen engagement events were offered bringing in 55 total participants and totaling 125 hours invested (Table 6). Of the eleven field sampling events where volunteers assisted, all six volunteers repeatedly helped, with three of those individuals representing a partner organization.

Table 5. Summary of project email engagement.

Total Emails Sent	Total # of Recipients	Total # of Opened Emails	Pooled Open Rate	Total # of Clicks	Estimated Engagement Time per Email (Hours)
25	3,482	1,296	0.3722	287	57.55

Table 6. Summary of results for volunteer and partner engagement. Of note, total engagement time reflects the sum of engagement hours and any time spent commuting.

Type of Engagement	Number of Engagement Events	Number of Participants Involved	Total Engagement (Hours)	Total Commute (Hours)	Total Engagement (Hours)
Webinar	3	39	33.50	NA	33.50
Volunteer Training	1	10	20	18.5	38.50
Field Sampling	11	6	24.75	28.25	53.00
Totals	**15**	**55**	**78.25**	**46.75**	**125.00**

Remote Operating Vehicle (ROV)

The ROV was deployed a total of eight times across six tributaries. Across all deployments, a total of 2 hours, 26 minutes, and 24 seconds of video content was captured (Table 7). Unfavorable water conditions resulted in several surveys where the ROV was not able to be deployed.

Table 7. Tributary, number of ROV deployments and total video coverage gathered during the project.

Tributary	Number of Deployments	Footage Duration (min and sec)
North Sandy Pond	2	46m 50s
Oswego Harbor	1	53m 21s
Oswego River	1	6m 35s
Rice Creek	1	12m 12s

Salmon River	2	25m 36s
Oswegatchie River*	2	7m 40s
Totals	8	2hr 26m 24s

* *The conditions during both deployments were not suitable for long term usage; one deployment resulted in no footage.*

Conclusions and Recommendations

eDNA

All 14 tributaries were found to have a positive detection for at least one of the species tested. However, caution is needed in interpreting these results, especially for the Asian swamp eel. As this was the initial year of sampling it is advisable that these results are not taken or perceived as direct evidence of presence of any species. While the noted detections are promising, the detections for Asian swamp eel appear questionable and it is recommended that further work is done on developing the assay to avoid the potential for false positives. Specifically, the assay used in this study was designed in a different system in the southeastern United States and could be detecting other species found locally, triggering inaccurate amplifications, and resulting in false-positive hits. It would be beneficial in future projects to further test this assay against genomic DNA of local species and determine the cause of amplifications. Similarly, there were positive detections for some of the other species, such as tubenose goby, rusty crayfish and Atlantic salmon that would benefit from additional assay development given the potential for genetic similarities and false positives in the detections. Locally collecting tissue samples for these species would assist in this effort and is recommended for any additional eDNA studies with these species in this geographic area.

In all, 140 samples were collected from the fourteen tributaries covered in this study. While this is a good start, additional sampling would benefit the objectives of this study. More specifically, increasing the number of samples collected within each individual tributary would be advantageous. Now that initial detection probabilities can be inferred from this year's data, using this within an occupancy model is suggested to help determine ideal sample numbers in each tributary for future studies.

Native Species

There was a detection for coregonines in this study, which is promising. The detection occurred during both sampling events in December in the Raquette River. This detection could be indicative of lake whitefish being present in the system or result from the introduction of whitefish DNA into the system. Historically, it was noted that for lake whitefish, "young were dip-netted from the river" in reference to the Raquette River (Greeley, 1934), lending support to fish being present in the system. Further, in the 1970s there were reported commercial catches of lake whitefish from the Ontario portion of the St. Lawrence River (Carlson and LaPan, 1997). Given that this was the only detection for a coregonine in this study, further work is required to elucidate whether tributary use by coregonines is occurring in Lake Ontario and the St. Lawrence River. It is possible that given the low sample sizes and volume of water collected overall, coregonines may have been present in some of the other sampled tributaries but were not detected.

American eel were detected in eight of the sampled tributaries, including the Oswego River. Historically, eels were known to be present in major tributaries of Lake Ontario including the Oswego River with the Saint Lawrence River being a major migration route (Busch et al., 1997; Beak International Inc., 2001). Eels have been known to use the Oswego River to move into the Finger Lakes, making it an important tributary in terms of migrations and connectivity to inland freshwater bodies as there is doubt that Lake Ontario is the final destination for eels migrating through the Saint Lawrence River (Busch et al., 1998). However, more recent surveys support that while eels are in the Oswego Harbor, there are limited numbers moving up the Oswego River via the locks (Busch et al., 1997). Currently, there are reduced number of eels in Lake Ontario and its tributaries due to artificial barriers, such as the Moses-Saunders Dam, which can inhibit migrations and affect habitat connectivity (Beak International Inc., 2001). These detections support that eels are likely present in the system but additional research would be needed to elucidate further aspects of the population.

Further, the detections of American eels at locations such as Chippewa Creek, Brandy Brook and Robinson Bay, make sense when considering habitat preferences. These sites are slower moving and shallow. American eels are warmwater species and prefer to reside in shallow and warm environments, which support their prey of benthic organisms. As far as Atlantic salmon, they were detected in the Salmon River, which is plausible given that they are the namesake for this tributary. It is known that Atlantic's can occasionally be found there. While stocking has occurred, continued restoration of this species will depend on natural spawning occurring which in part entails suitable spawning habitats, emphasizing the importance of this type of study and further work to better understand the habitats being used for spawning and their quality.

Volunteer Engagement/Community Outreach

Pooling together email and engagement times, we were able to total 182.55 hours which exceeded our target of 150 hours. This supports using a multifaceted approach such as in person events, webinars and use of social media to engage the community. The attendance on the conducted webinars was relatively strong but could possibly be improved by sharing through more avenues as well as sharing the information earlier. The volunteer event conducted in Massena was well attended however only a handful of the attendees subsequently assisted in the field. In future, it would be helpful to conduct this type of training again but to do it in a different location, such as further south, as this may help draw more individuals. Massena is quite north and many of the project volunteers were located more central to Oswego and Syracuse. Developing a stronger relationship with SUNY-Oswego could also help to build the volunteer base through student engagement. In all, there were six volunteers who routinely assisted with the project. If the volunteer base continues to grow, it would be advantageous to develop a framework for gathering more information on the volunteers such as demographics, understanding why they choose to volunteer and what aspects they like best and to solicit feedback on ways to increase volunteer participation. In summary, we were fortunate to have a strong showing of support from the local community and exploring ways to effectively express this to the volunteers and thank them for their assistance is advised.

ROV

The use of the ROV in this project was exploratory but proved to be a beneficial tool that can be further incorporated in future efforts. Ideally, all sites would have had the ROV deployed, and footage gathered. Although this was not able to be done, it did serve the purpose of learning the bounds of the ROV use and application. For the collected footage, it is envisioned that a community science project could be undertaken to help review and assess the footage both for presence of species but also to ascertain habitat attributes. In future, once confident pilots are trained with the use of the ROV, it will be important to develop and implement a standard methodology for conducting assessments.

References

Barnes, M.A. et al. 2014. Environmental conditions influence eDNA persistence in aquatic systems. Environmental Science and Technology 48(3):

Barnes, M. A., & Turner, C. R. 2016. The ecology of environmental DNA and implications for conservation genetics. *Conservation genetics*, *17*(1), 1-17.

Beak International Incorporated. 2001. The decline of American eel (*Anguilla rostrata*) in the Lake Ontario/St. Lawrence River ecosystem: a modeling approach to identification of data gaps and research priorities. White Paper. 70 pgs. http://www.glfc.org/pubs/lake_committees/ontario/eel.pdf

Bessey, C. et al. 2020. Maximizing fish detection with eDNA metabarcoding. Environmental DNA 2: 493-504.

Billington, N., and Hebert, P.D.N. 1991. Mitochondrial DNA diversity in fishes and its implications for introductions. Canadian Journal of Fisheries and Aquatic Sciences 48(1): 80 -94.

Busch, W.N. et al. 1997. American eel in Lake Ontario and its tributaries: distribution, abundance, essential habitat and restoration requirements. Technical Report. 24 pgs.

Busch, W.N. et al. 1998. Distribution and availability of Atlantic coast freshwater habitats for American eel. Administrative Report #98-2, U.S. Fish and Wildlife Service. 28 pgs.

Carlson, D.M. and LaPan, S.R. 1997. Fish Species Inhabiting the International Portion of the St. Lawrence River. NYSDEC Report.

Dejean, T. et al. 2011. Persistence of Environmental DNA in Freshwater Ecosystems. PLoS ONE 6(8): e23398. https://doi.org/10.1371/journal.pone.0023398

Ebener et al. 2008. Management of Commercial Fisheries for Lake Whitefish in the Laurentian Great Lakes of North America. International Governance of Fisheries Ecosystems: 99-143.

George, E.M. et al. 2018. Identifying research priorities for Cisco in Lake Ontario: a workshop summary report. Workshop held at the Cornell Biological Field Station at Shackelton Point, Bridgeport NY, 31 May 2018.

Greeley, J.R. 1934. Fishes of the Raquette watershed with annotated list. Pages 53-108 in A biological survey of the Raquette watershed. Suppl. to 23rd Annu. Rep. (1933), N.Y. Conserv. Dep.

Jerde, C.L. et al. 2013. Detection of Asian carp DNA as part of a Great Lakes basin-wide surveillance program. Canadian Journal of Fisheries and Aquatic Sciences 70(4): 522-526.

Pagnucco, K.S. et al. 2015. The future of species invasions in the Great Lakes-St. Lawrence River basin. Journal of Great Lakes Research. Volume 41, Supplement 1. 2015. Pages 96-107. ISSN 0380-1330. https://doi.org/10.1016/j.jglr.2014.11.004.

Ransom, A.L. et al. 2021. Recolonization of lake whitefish river spawning ecotypes and estimates of riverine larval production in Green Bay, Lake Michigan. Journal of Great Lakes Research 47(1): 213-225. https://doi.org/10.1016/j.jglr.2020.11.011

Ricciardi, A. 2006. Patterns of invasion in the Laurentian Great Lakes in relation to changes in vector activity. Diversity and Distributions, 12: 425-433. https://doi.org/10.1111/j.1366-9516.2006.00262.x

Singh, J. et al. 2005. Plant invasions: Emerging trends and future implications. Current Science 88:726-734.

Thalinger B. et al. 2021. A validation scale to determine the readiness of environmental DNA assays for routine species monitoring. *Environmental DNA*. 2021; 3:823–836. https://doi.org/10.1002/edn3.189

Turner, C.R., Miller, D.J., Coyne, K.J., and Corush, J., 2014. Improved methods for capture, extraction, and quantitative assay of environmental DNA from Asian bigheaded carp (Hypophthalmichthys spp.). *PloS one*, *9*(12), p.e114329.

United States Forest Service. “Invasive Plants”. *https://www.fs.fed.us/wildflowers/invasives* Accessed 1/9/2021.

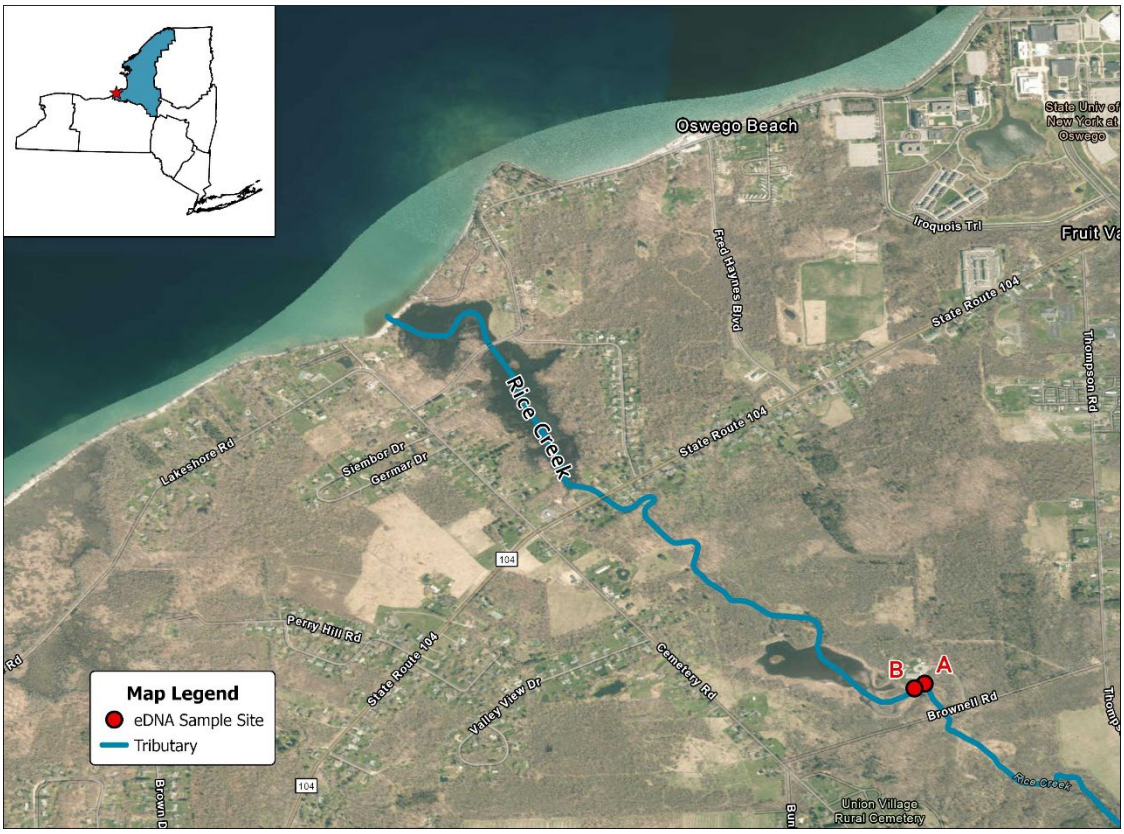
Appendices

Appendix 1. Sample Locations

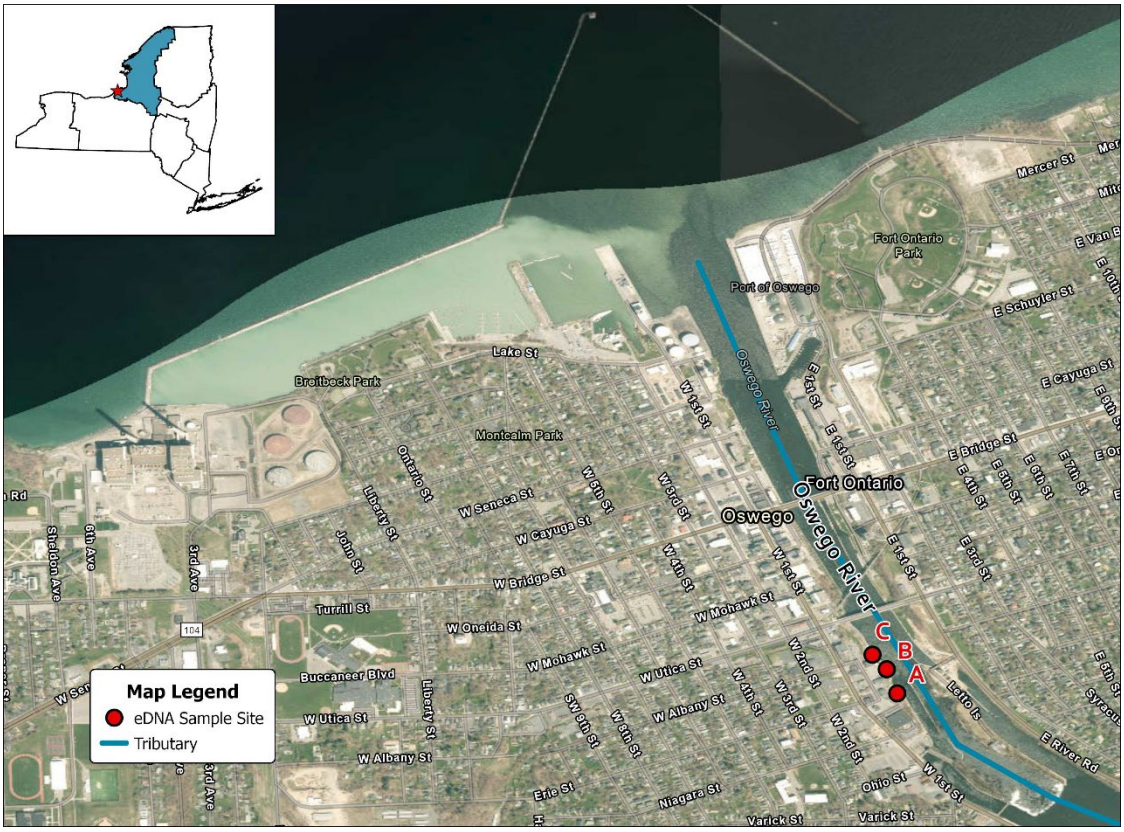
The following figures outline the specific sampling locations e.g. A, B and where relevant C, within a tributary, for each of the fourteen tributaries.

Sampling Location Maps

Rice Creek

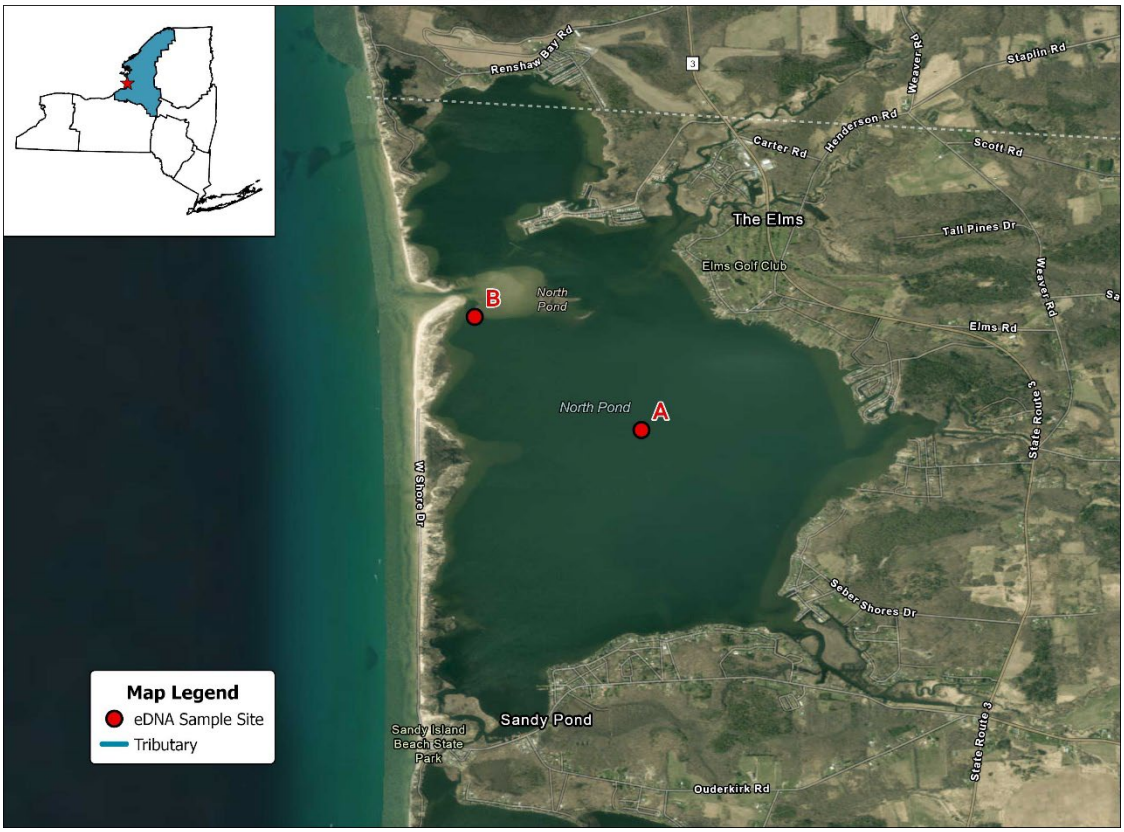


Oswego River

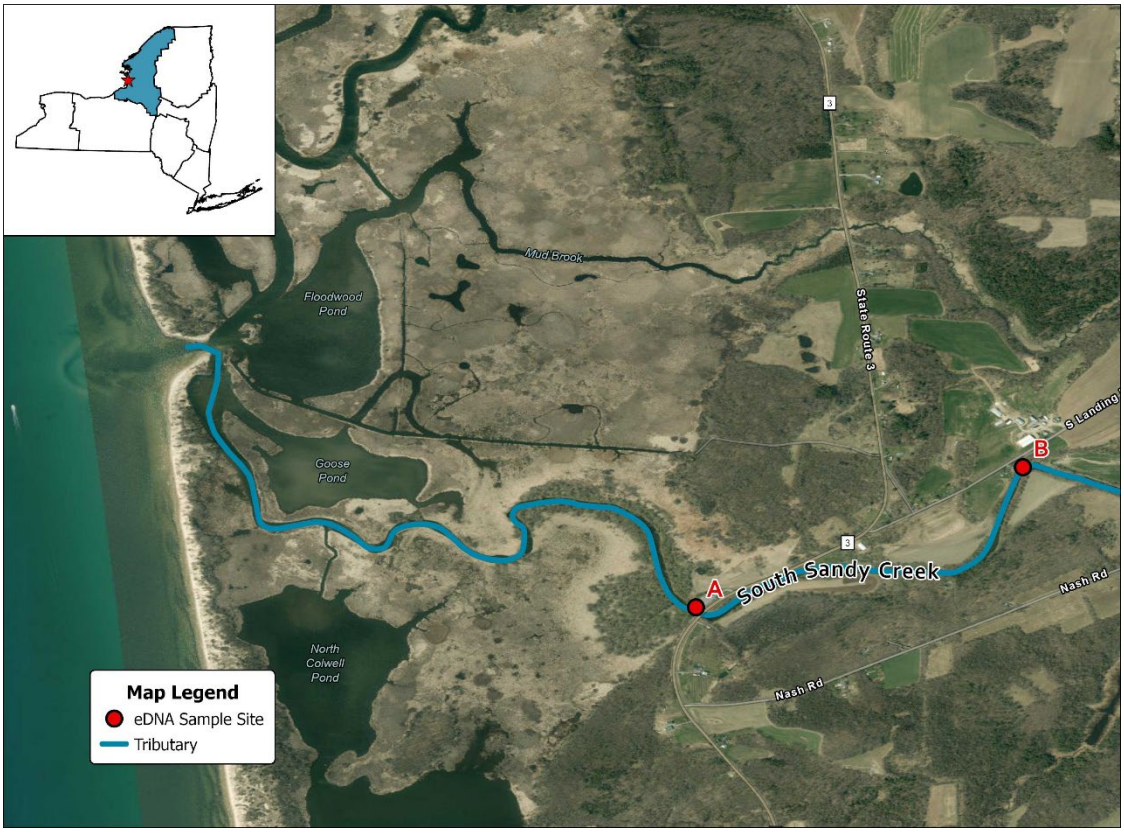


Salmon River

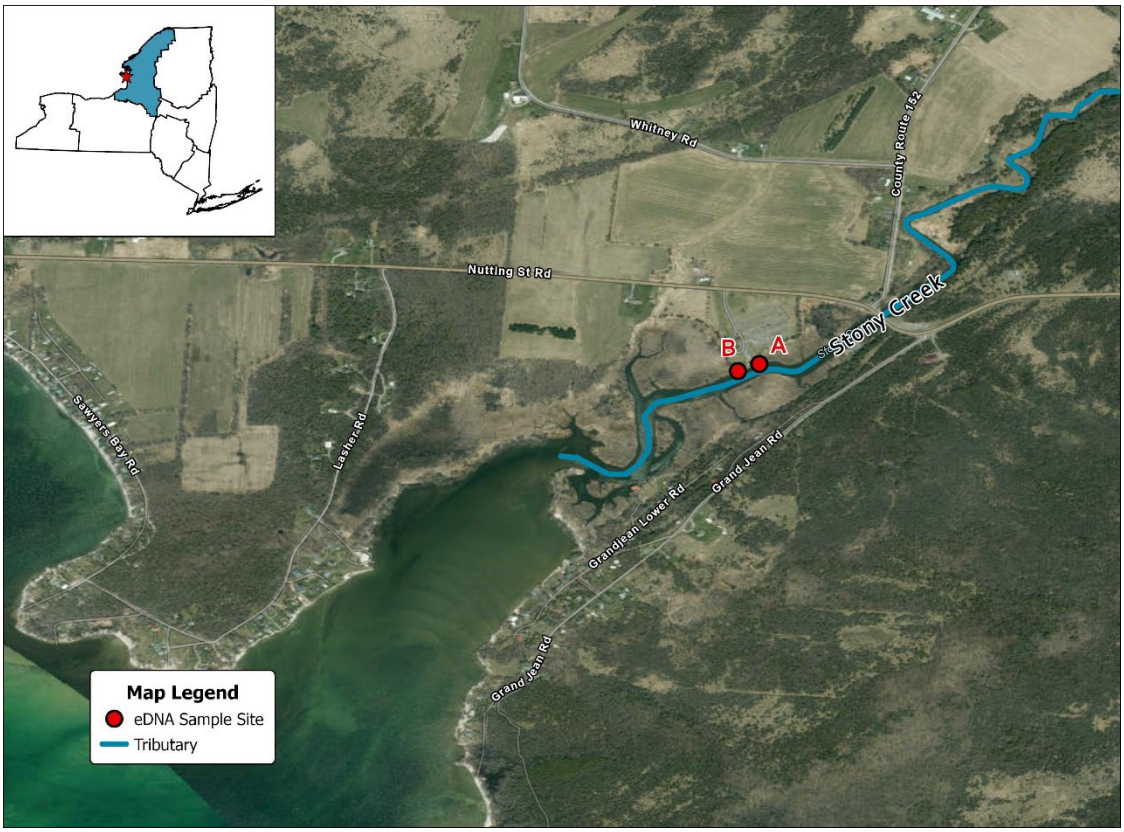
North Sandy Pond



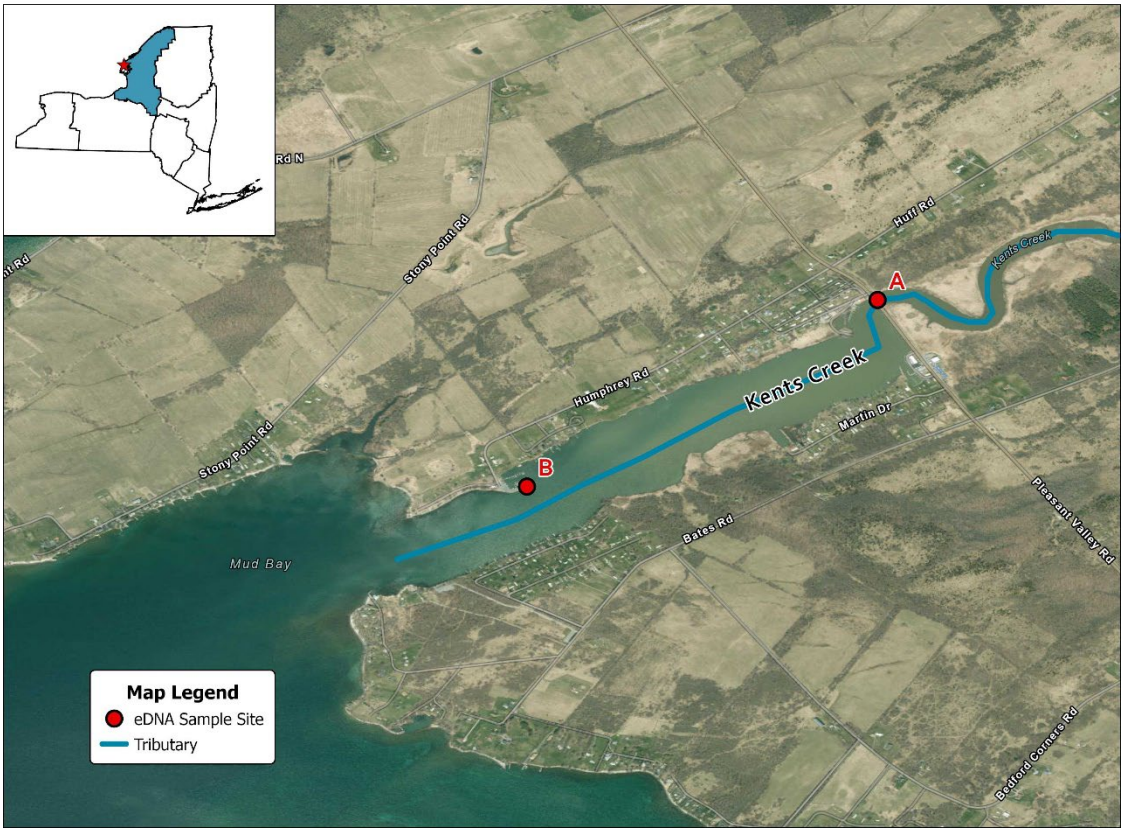
South Sandy Creek



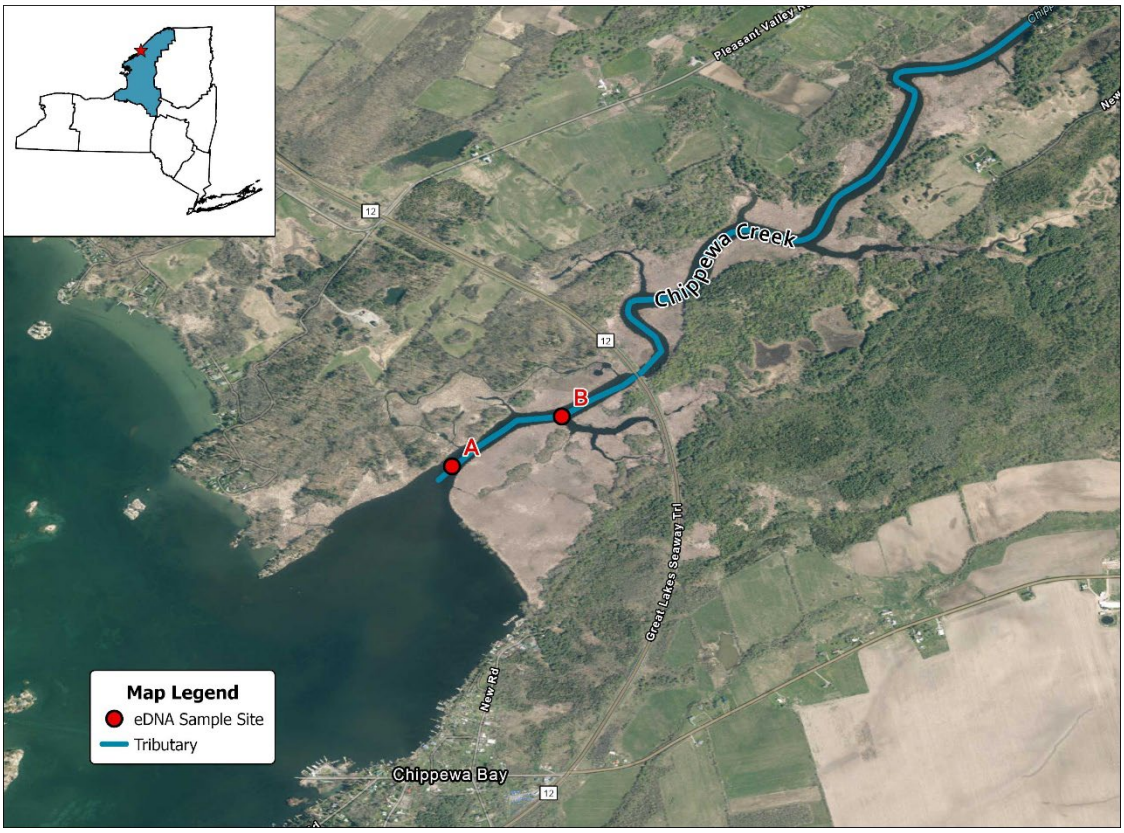
Stony Creek



Kents Creek

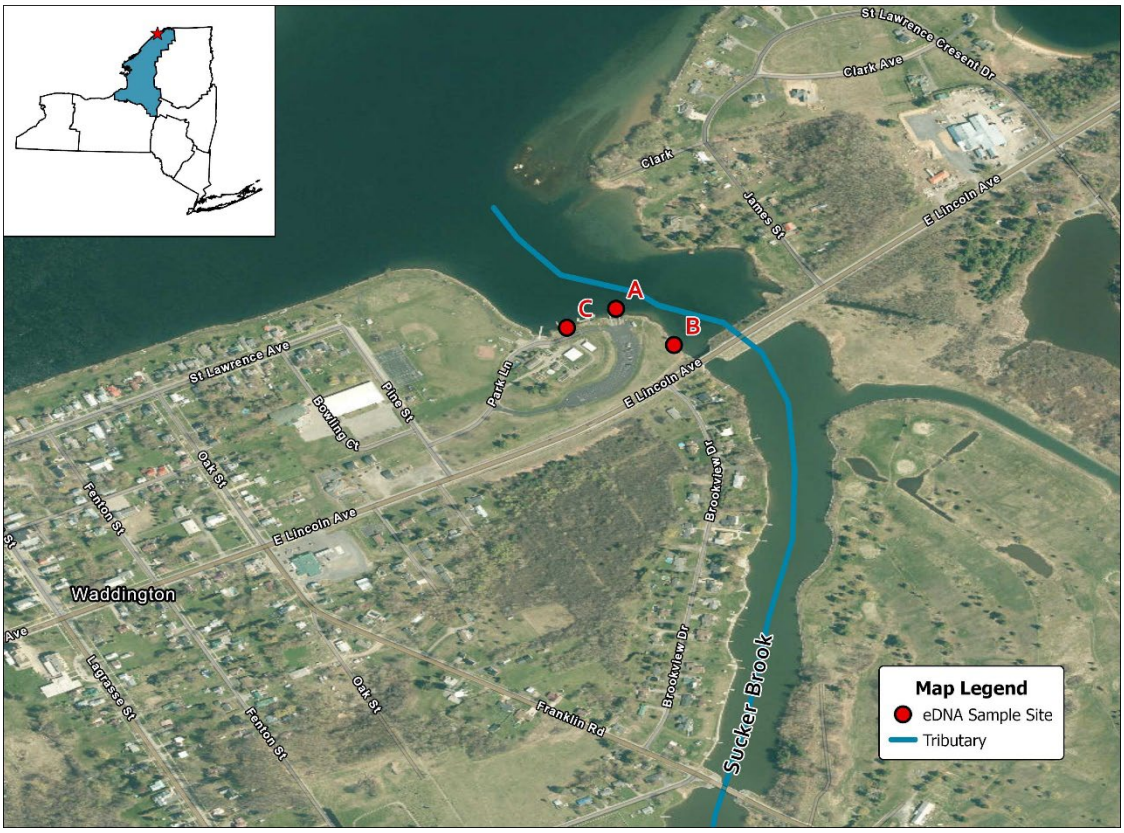


Chippewa Creek

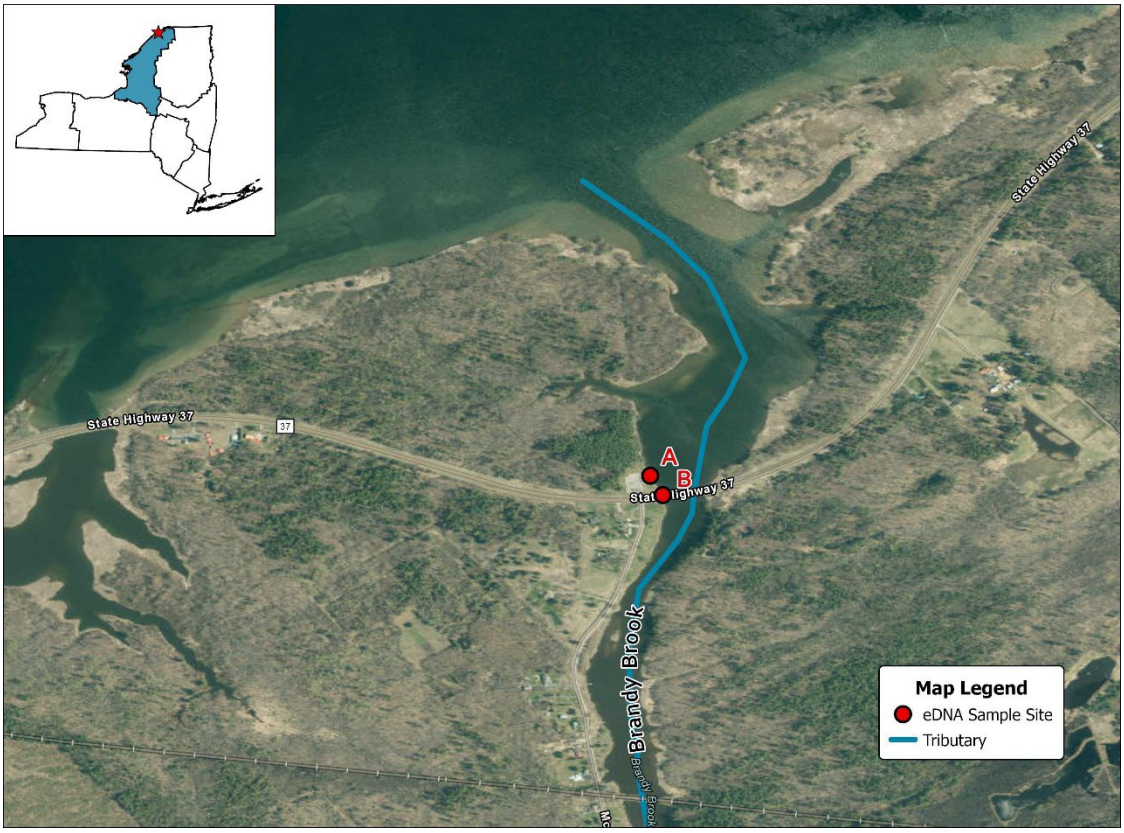


Oswegatchie River

Sucker Brook

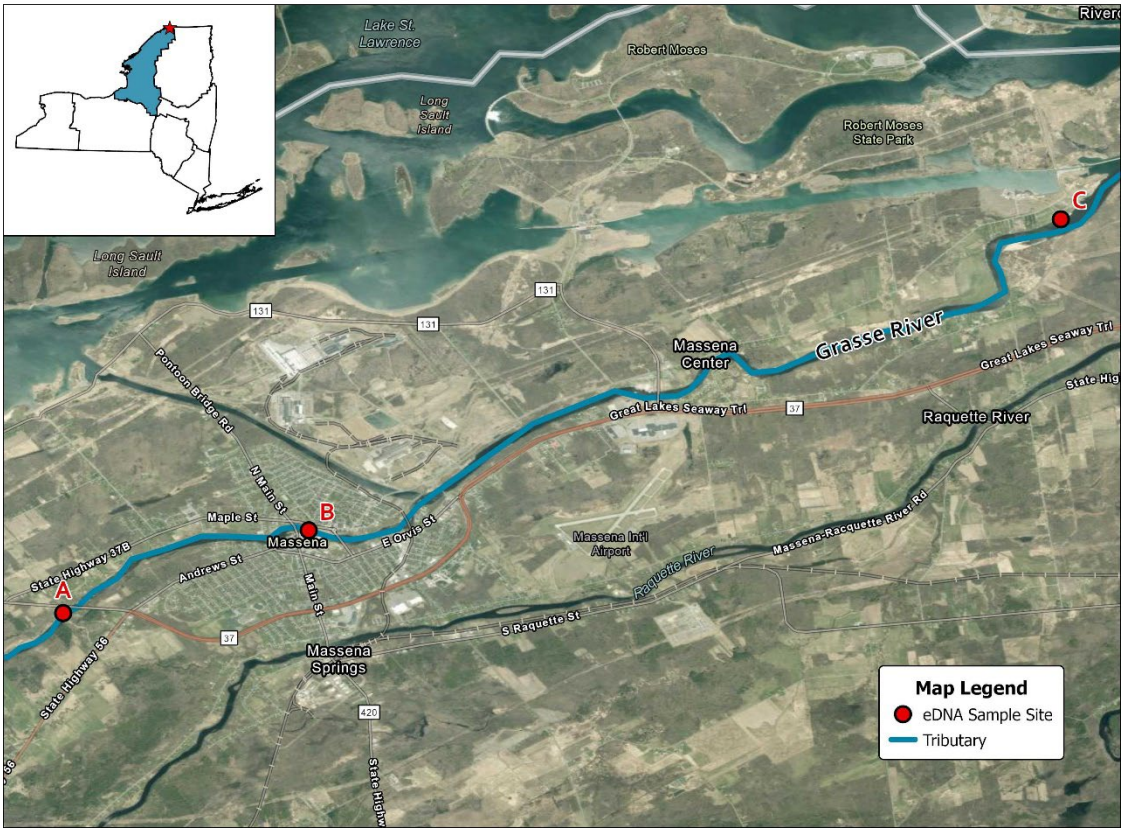


Brandy Brook

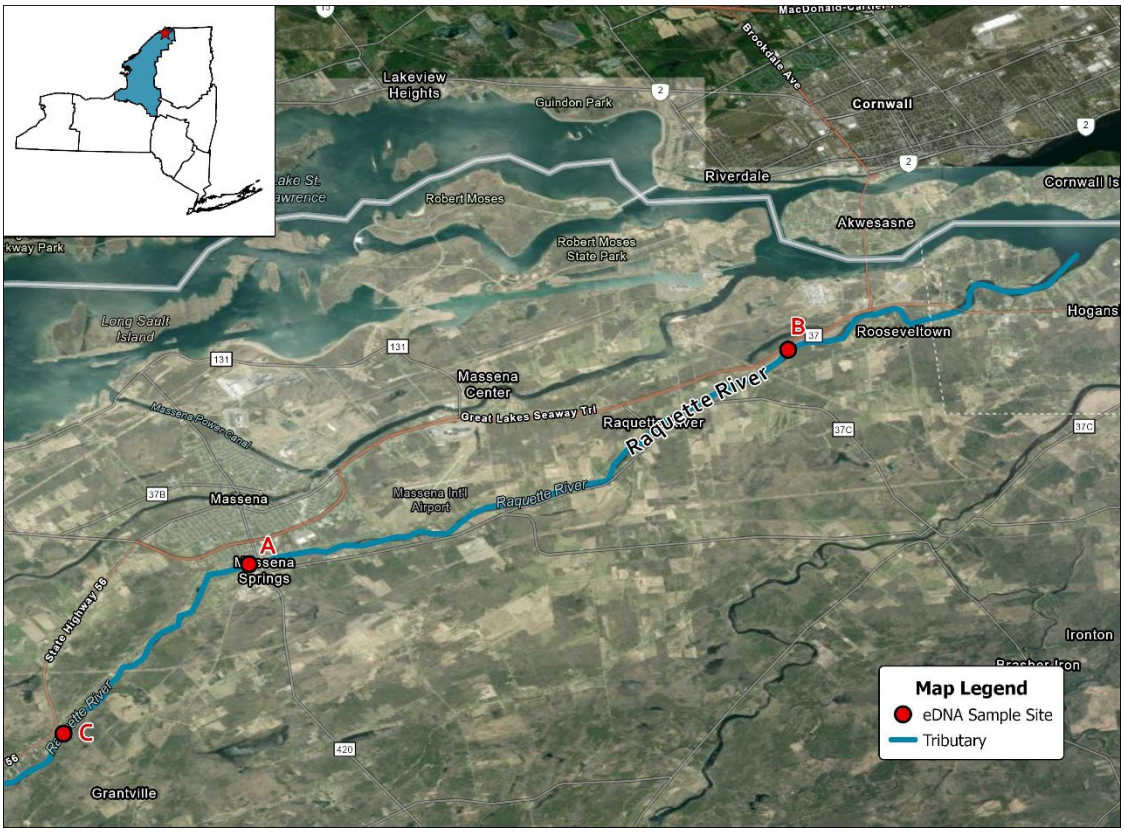


Robinson Bay

Grasse River



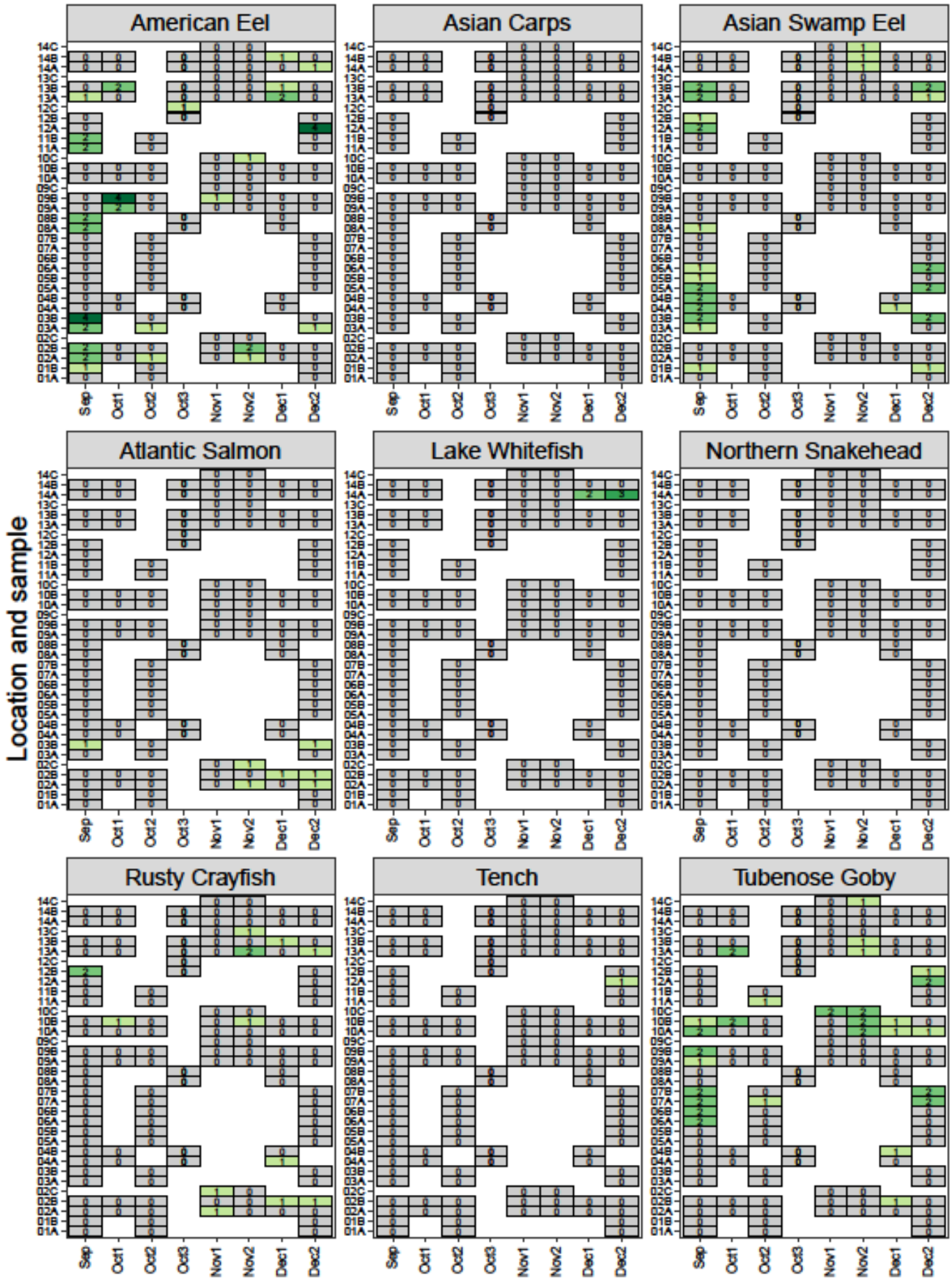
Raquette River



Appendix 2 Species Detections

The following figure outlines detections by species, tributary, sampling site e.g. A, B or C, within a tributary and sampling event. Of note, the counts in the cells are the number of PCR replicates that were positive e.g., 0= none, 1= one replicate, and 2= two replicates etc. Please refer to Appendix 1 for the exact information on sample sites A, B and C (where relevant) within a tributary. The site codes used in the figure are as follows:

Code	Tributary
01	Rice Creek
02	Oswego River
03	Salmon River
04	North Sandy Pond
05	South Sandy Creek
06	Stony Creek
07	Kents Creek
08	Chippewa Creek
09	Oswegatchie River
10	Sucker Brook
11	Brandy Brook
12	Robinson Bay
13	Grasse River
14	Raquette River


American Eel
Asian Carps
Asian Swamp Eel
Atlantic Salmon
Lake Whitefish
Northern Snakehead
Rusty Crayfish
Tench
Tubenose Goby
Location and sample
14C
14B
14A
13C
13B
13A
12C
12B
12A
11B
11A
10C
10B
10A
09C
09B
09A
08B
08A
07B
07A
06B
06A
05B
05A
04B
04A
03B
03A
02C
02B
02A
01B
01A
Sep
Oct1
Oct2
Oct3
Nov1
Nov2
Dec1
Dec2

eDNA Sampling Protocol

The Nature Conservancy
SLELO PRISM

Equipment Overview:

A. Disposable face masks
B. Hand sanitizer
C. Paper towels
D. Nitrile gloves
E. Spray bottle
F. 5-Gal bucket w/ lid
G. Bleach (20% solution)
H. Forceps
I. 1L Nalgene bottles
J. Sampling pole
K. Polypropylene vacuum flask
L. Rubber stopper
M. Vacuum tubing
N. Vacuum hand pump
O. Motorized vacuum pump
P. Buchner (filter) funnel
Q. Nalgene filter funnel housing
R. 0.45 µm filter paper
S. Ethanol
T. Pipette
U. Sample vial
V. Cooler
W. Totes

Preparation:

- Determine that conditions are suitable for sampling.
- Using the tablet, complete the eDNA sampling form for each site-sample pairing.
- Consolidate all equipment to be used at respective sites (Site-sample specific kits consisting of all gear to be used should be available to you).
- Make sure that sample-specific vials are labeled appropriately (This includes writing the date in the spaces that are present on each label – there should be 6 total spaces following each sample locations alpha numeric code).
- Face masks **MUST** be worn throughout the entire process.
- Paper towels may be used during clean up.

If the filtration unit is not pre-assembled, follow the steps below (* Make sure a fresh pair of gloves are worn during this process):

1. Make sure all items are accounted for: flask, filter funnel with filter paper (Nalgene filter funnel housing with filter paper as applicable), rubber stopper, tubing, and vacuum pumps (hand-held or motorized as applicable).
2. Attach the rubber stopper to the top of the flask until a sufficient seal is achieved.
3. Attach the tubing to the connection port on the side of the flask, and then attach the vacuum pump (hand-held or motorized as applicable) to the other end of the tubing.
4. Attach the filter funnel adapter to the top of the rubber stopper and then attach the filter funnel (Nalgene filter funnel) to the adapter.

Collection:

Equipment: A, B, C, D, E, G, I, J

* Each site will have two sampling locations. You will be collecting your primary sample and a replicate at each location. If this is the last site you are sampling for the day, you will also be running a blank sample which will be provided in your initial set of sampling kits (A total of four bottles will be used at each site – five if it is the last site of the day).

If wading into the water for sampling (Make sure boots or waders to be worn are sterile and clean of any form of debris):

1. Sanitize hands and put on a fresh pair of Nitrile gloves.
2. Grab site-specific/sample-specific Nalgene bottles.
3. Get into the water below your sampling location.
4. Wade up to the sampling location.
5. With the flow of the water coming towards you, use the following rinsing steps:
    a. Extend your Nalgene bottle out in front of you with the current of the water flowing into your bottle (This will add an extra layer of caution to reduce the chances of contaminating your sample with any form of particulate that may be present on your boots, waders, or other forms of clothing).
    b. Fill the bottle, cap the top, and shake the bottle.
    c. Empty the bottle and repeat step “b” two more times.
        i. These steps will ensure that any residue from prior decontamination (i.e. Bleach) will be rinsed from your bottle.
6. After rinsing your bottle, collect and cap your sample.
7. Repeat the rinsing and collection steps for this location’s replicate as well as the other location’s sample and replicate.

If not wading into the water and using the sampling pole for sampling:

1. Sanitize hands and put on a fresh pair of Nitrile gloves.
2. Grab site-specific/sample-specific Nalgene bottles.
3. Attach Nalgene bottle to the sampling pole.
4. Extend sampling pole out to the sampling location.
5. With the flow of the water coming towards the sampling pole, use the following rinsing steps:
    a. With the current of the water flowing into your bottle, fill the bottle.
    b. Retrieve the filled bottle, cap the top, and shake the bottle.
    c. Empty the bottle and repeat steps “a” and “b” two more times.
        i. These steps will ensure that any residue from prior decontamination (i.e. Bleach) will be rinsed from your bottle.
6. After rinsing your bottle, collect and cap your sample.
7. Repeat the rinsing and collection steps for this location’s replicate as well as the other location’s sample and replicate.

If not wading into the water and using a kayak or boat:

1. Grab site-specific/sample-specific Nalgene bottles.
2. Kayak or boat to your sampling location.
3. Sanitize hands and put on a fresh pair of Nitrile gloves.
4. With the flow of the water coming towards the kayak or boat, use the following rinsing steps:
    a. With the current of the water flowing into your bottle, fill the bottle.
    b. Retrieve the filled bottle, cap the top, and shake the bottle.
    c. Empty the bottle and repeat steps “a” and “b” two more times.
        i. These steps will ensure that any residue from prior decontamination (i.e. Bleach) will be rinsed from your bottle.
5. After rinsing your bottle, collect and cap your sample.
6. Repeat the rinsing and collection steps for this location’s replicate as well as the other location’s sample and replicate.

Filtering:

Equipment: A, B, C, D, E, G, K, L, M, N, O, P, Q, R, H

1. If the gloves you used during collection encountered potentially contaminated surfaces, re-sanitize your hands, and put on a fresh pair of Nitrile gloves.
2. Grab the pre-assembled flask, funnel, rubber stopper, and tubing.
3. Connect the Nalgene filter funnel housing if applicable.
4. Filter paper will either be placed inside the filter funnel or filter housing unit as applicable.
5. Once the filtration unit is ready, begin to pour collected water samples into the funnel.
6. Using the vacuum pump (hand or motorized), draw water through the filter
7. Depending on the rate of filtration, sample contents may need to be shook to stir up settled materials.
8. Repeat steps 5-6 until the entire sample has been filtered.
    a. Use forceps to remove any large particulate that builds up on the filter paper.
9. Once the entire sample has been filtered, acquire the equipment to be used for preservation.
    * Spray bottle with 20% bleach solution may be used to sanitize surfaces which may have become contaminated throughout the process.
    * Any piece of equipment used during this process must be submerged in the 5-gallon bucket of bleach solution for at least 1 minute for decontamination.

Preserving:

Equipment: A, B, C, D, E, G, H, S, T, U

1. If the gloves you used during collection and or filtering encountered potentially contaminated surfaces, re-sanitize your hands and put on a fresh pair of Nitrile gloves.
2. Inside the filter funnel, using forceps, fold the filter paper in half a total of three times.
    * When folding the filter paper, make sure that you are folding inward (i.e., fold one side of the paper over to the other side).
3. Using forceps, place folded filter paper into respective ethanol filled vials and cap.

Storage:

- All samples must be stored in a cool container sheltered from light, and in an upright position.

Sanitation:

- All equipment used in the field for collection, filtering, and preservation **MUST** be sanitized.

1. Place equipment in 5-gallon bucket with 20% bleach solution.
2. All equipment must be submerged for at least 1 minute.
3. Once sanitized, place equipment in equipment-specific totes with drying racks.
    * Make sure all sanitized equipment remains SEPARATE from any other unused/used equipment.

Transport:

Key points:

- All samples must remain upright on their way to the lab.
- All samples must be kept at a sufficient temperature.
- Avoid samples encountering any form of light exposure.
- Samples will be transported to our contracted lab.

References

The following document was utilized during the preparation of this protocol, USGS: http://dx.doi.org/10.3133/tm2A13.

For additional references or to contact staff, visit our website www.sleloinvasives.org/eDNA.

ROV ("Remotely Operated Vehicle") Usage Protocol

The Nature Conservancy
SLELO PRISM

Equipment Overview:

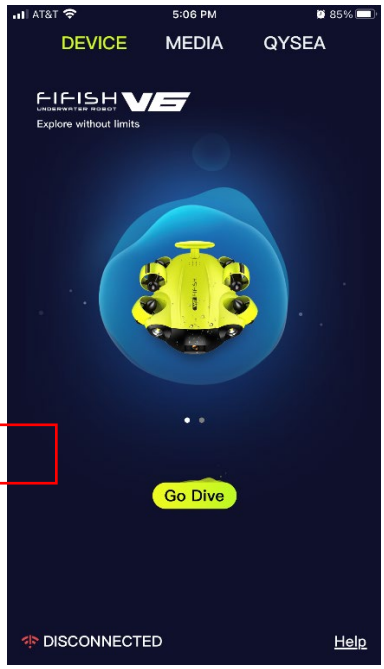
- Vehicle
- Controller
- Smartphone
- Spool and tether
- Towels

Smartphone set-up:

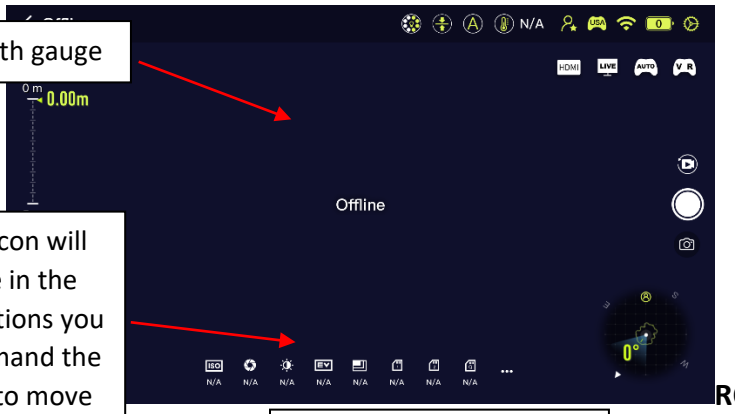
If you are using your smartphone, you will need to have downloaded the "FIFISH" application for IOS or Android. If you do not have the app, search "FIFISH" in your respective app store to download it. This application provides several options, but the most salient ones for your usage are the interface to view what the ROV is capturing with its camera and the options to take photos and videos (very similar layout to your phones camera).

The ROV has built-in Wi-Fi that you will need to connect to. Go into your phones settings for Wi-Fi and connect to "FIFISHRC_xxxx". The x's will appear as a string of numbers. The password is: 1234567890.

Once you have downloaded the app and are connected to Wi-Fi, you may open the app to view this screen:

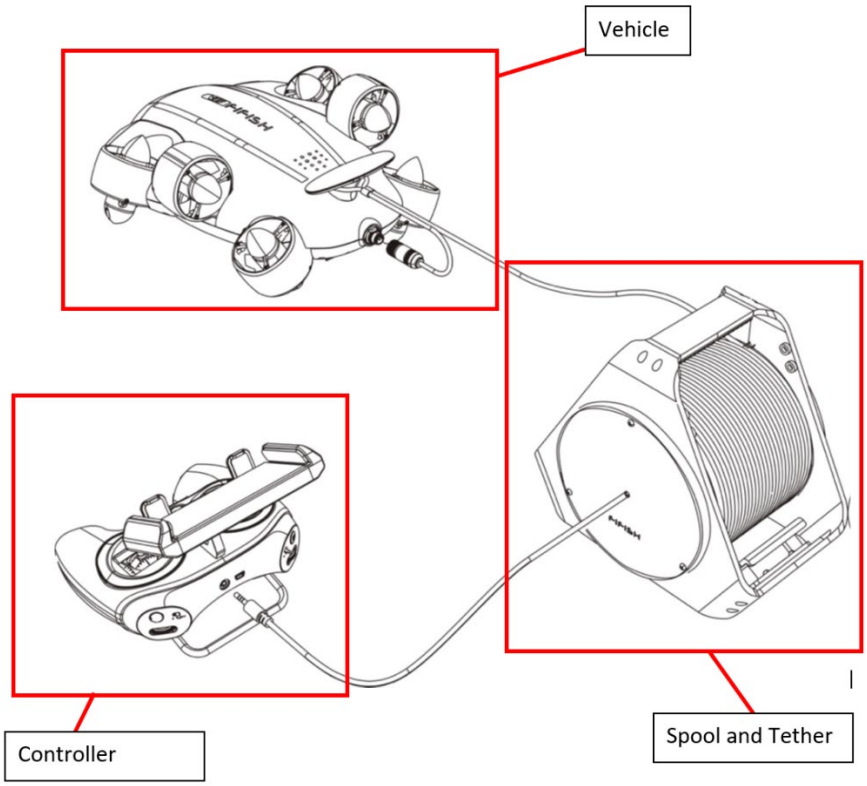


Click "Go Dive" to view the camera interface:



ROV Set-up:

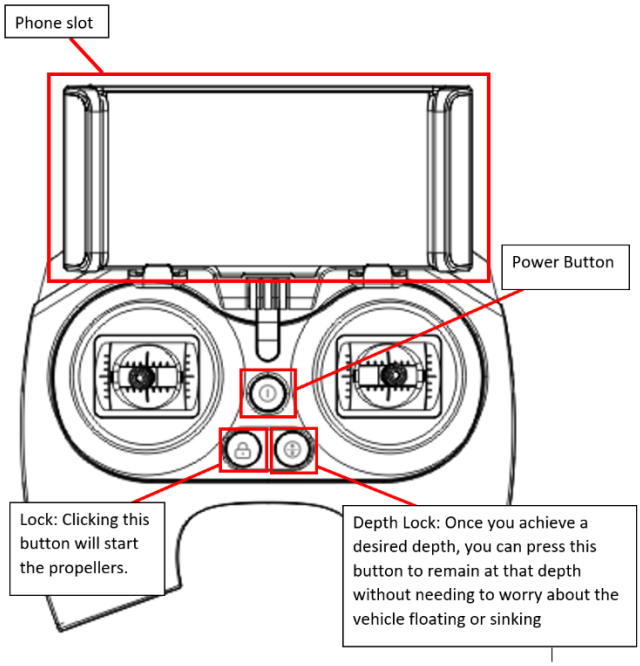
The vehicle, spool with tether, and controller must all be connected like so:



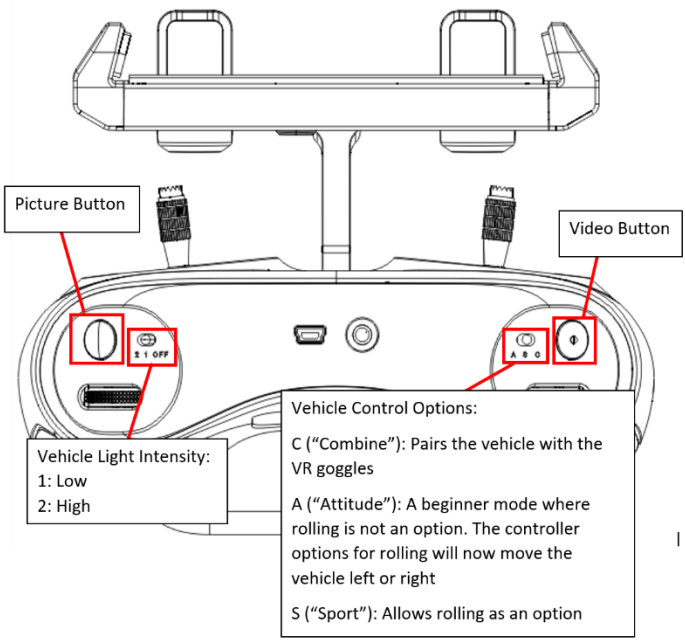
ROV deploying and control:

When deploying the ROV, it is important that you do not hold the vehicle itself but the tether that it is connected to. Even when it is “locked” this is an added level of safety from the propeller blades.

When you are ready to deploy, you need to turn on the controller using the power button. The buttons in the middle of the controller will illuminate in succession indicating that the controller can be connected. The power and lock buttons will then stay illuminated when connection is achieved. This indicates that the ROV is now ready to be deployed and piloted (See image)



Other important controller options to be aware of:



When the vehicle is in the water and the controller is on and connected, you may click the lock button (as seen in the above image) to start the propellers.

This ROV has 6 "degrees of freedom", meaning that it can move in 6 general directions:

1. Up and Down
2. Left and Right
3. Forward and Reverse
4. Roll Right and Roll Left
5. Pitch Up and Pitch Down
6. Yaw Right and Yaw Left

The last page of this document provides a table that shows which controller wheels and joysticks correspond to the directions listed above.

In general, it is useful to follow the ROV's camera on your phones interface to explore the environment you are in.

Maintenance:

After each deploy, be sure to dry all equipment with the provided towels and make sure as much debris is removed from the propeller as possible (IMPORTANT: Make sure the vehicle is locked and or off when removing debris from the propellers. It is safer to use a tool like a flat-head screwdriver than your hands to remove debris).

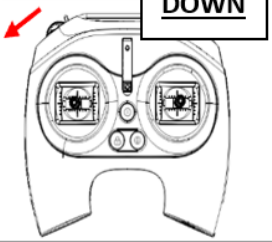
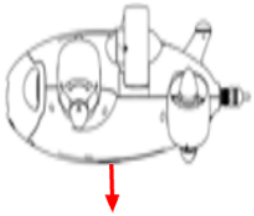
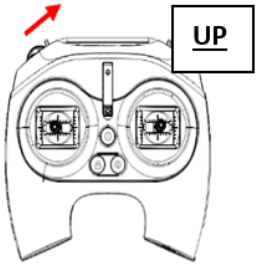
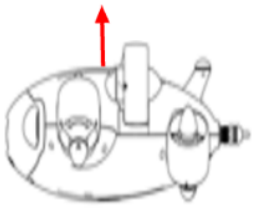
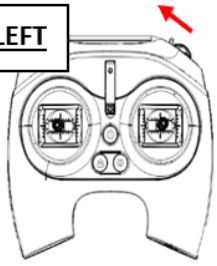
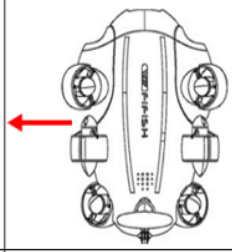
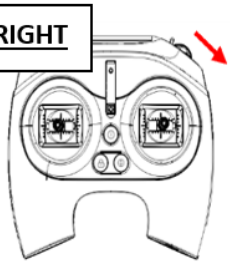
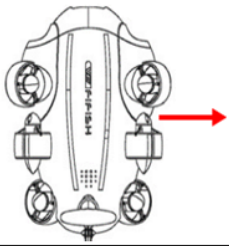
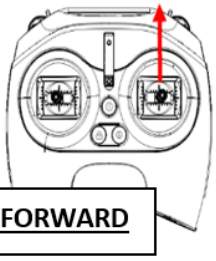
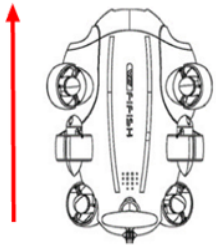
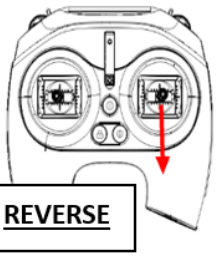
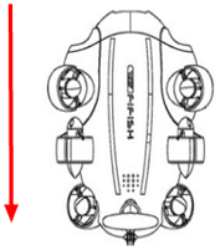
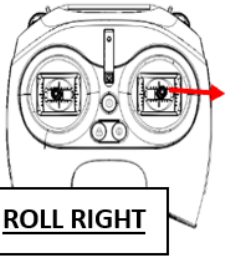
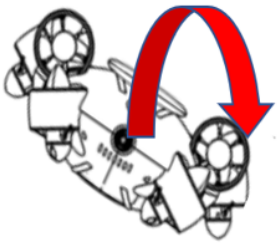
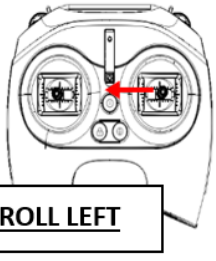
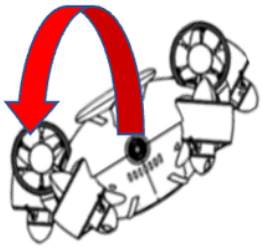
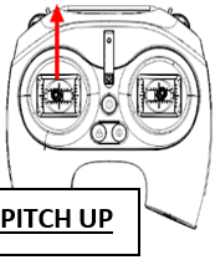
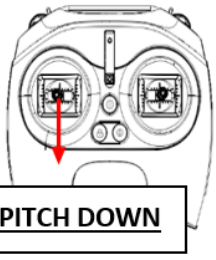
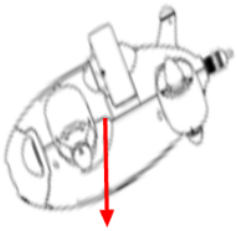
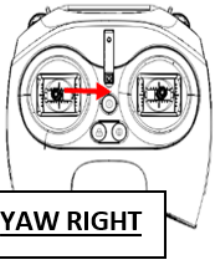
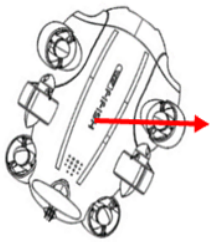
Field observation: There may be instances where the propellers shift a little bit when removing debris, so please be cautious.

As applicable, you may also place the vehicle in a large tub of tap water to further clean the propellers. With the vehicle in the tub, put it into forward and reverse several times to dislodge excess debris.

Sources:

The black and white images as well as bits of narrative reference the following source:

https://www.qysea.com/support/user-manual/ (Option: "FIFISH V6/V6s: Quick Start Guide V1.4(EN)")

DOWN 		UP 	
LEFT 		RIGHT 	
FORWARD 		REVERSE 	
ROLL RIGHT 		ROLL LEFT 	
PITCH UP 		PITCH DOWN 	
YAW RIGHT 		YAW LEFT