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

Introduction

Aquatic Invasive Species (AIS) pose a serious threat to the ecosystems around the world. The distribution of species can change rapidly, and early detection of an invasive species is a critical first step in monitoring spread and managing responses. Aquatic species are often difficult to detect, but new technologies monitoring environmental DNA (**eDNA**) show great promise for detecting the presence of a wide range of species. eDNA is nuclear or mitochondrial DNA that is released into the environment as a result of the constant shedding of cells by all organisms into the environment. Fish cells can be shed into the water in multiple ways, including in mucous, feces, urine, or blood, or as flaked off skin cells. The cells shed into the environment all contain genetic material (DNA) that is unique to their species. Therefore, the DNA in shed cells can be used to provide information about what organisms are or were recently present in a particular area. eDNA monitoring has been used in both fresh water and marine environments, and provides a highly efficient, sensitive, and cost-effective way to monitor for the presence of invasive species. We will use a citizen scientist based approach to monitoring for the presence of invasive fish species all around New York, combining the collection of water samples by teachers and students with state of the art eDNA analysis at Cornell.

Teachers and students will play a critical role in monitoring the spread of invasive fish species throughout New York State. Using materials and protocols supplied by this program, students will collect multiple water samples from nearby lakes, streams, or ponds, noting the GPS coordinates of each site surveyed. The water collected will be filtered through porous filters that will retain any cells shed by fish in the collection area. Since the half life of DNA (the amount of time required for the amount of measurable DNA to fall to half its value as measured at the beginning of the time period) from fish cells shed into the water is at least 4-6 hours following shedding, the presence of a fish can potentially be detected even if it swam by several hours earlier. The filters containing cells from the water sample will be placed into a vial containing a solution that will protect the DNA from further breakdown, and sent back to Cornell. The cells collected on the filter will provide the eDNA that will be analyzed by quantitative PCR (**qPCR**) analysis, which will be carried out in the laboratories of the Aquatic Health Program ((http://www.vet.cornell.edu/microbiology/FishDisease/AquaticProg/).

qPCR is a very sensitive test that can be used to identify specific fish species even when they are present at very low numbers. For example, qPCR analysis of grass carp eDNA in a single water sample can pick up one grass carp in a 50 acre pond 10 feet deep under good conditions, although sensitivity as low as one grass carp in a 2 acre pond has been seen if environmental conditions favor the rapid breakdown of shed cells. In addition to the immediate analysis, duplicate filters will be archived in long-term storage at -80˚C at Cornell. These samples will provide a "time capsule" view of the environmental DNA in the bodies of water sampled, and will be extremely valuable for future studies to document change in local ecosystems.

We will provide all of the basic materials needed to collect the water samples except water for control filtrations and a device for determining GPS locations. In addition to the information provided below, a more detailed description and a demonstration video of the collection procedure will be available on our website. A brief description of the qPCR protocol will also be available on the website so you will know what happens to your samples after you send the filters to us. We'll also provide a straightforward description of qPCR and information on how to interpret the qPCR analysis when your results are returned to you. Results of all sites tested will be posted on the website.

Background

For centuries human activities have helped spread plants and animals around the world, intentionally or unintentionally expanding the range of many organisms beyond their original locations.

Species (a group of similar animals, plants, or other living things that share common characteristics and can interbreed and produce young) living in an area where they are naturally and historically found are often called **"native" species**. Native species have adapted to their local habitats through a continuing interaction between their inherited characteristics and their environment. For thousands of years, natural barriers determined where organisms lived and helped defined their habitat, creating dynamic, natural ecosystems [an **ecosystem**, or ecological system, is a interactive community of living organisms (plants, animals and microbes) and the environment (including weather, earth, sun, soil, climate, atmosphere) in which they live]. Within natural ecosystems, native organisms develop unique balanced relationships with both their physical environments and with the other organisms around them. As human activities have expanded, things have changed.

The ability to move people and products around the globe has been the basis of some of mankind's greatest achievements, but increased mobility has also greatly increased the transfer of all types of organisms to new environments where they were not previously found. Species introduced into an area where they did not previously live are called **"non-native" species**, sometimes also known as exotic, nuisance, or non-indigenous species. Often, species introduced into a new wild environment will be unable to establish a viable population and will disappear with no ill effects. However, sometimes newly introduced species will thrive, outcompeting native species and destroying fragile ecosystems. Non-native species that damage the environment or disrupt existing ecosystems, or that result in economic loss or endanger human health, are generally referred to as "**invasive" species**. Many types of organisms, from plants to animals to microbes can be invasive. Invasive species can be introduced from other countries or from other parts of the same country.

In this project we are focusing on invasive fish species, a growing problem nationwide, especially in coastal regions and in the Great Lakes and surrounding areas. Invasive fish species can cause serious environmental problems and cause significant economic losses, often rapidly disrupting the fragile balance of natural ecosystems, and threatening the diversity and abundance of native aquatic species. The resulting loss of native fish can lead to important economic consequences, affecting a wide range of commercial, agricultural, aquacultural, and recreational activities. Globally, the introduction and spread of non-native species around the world has been described as a major threat to a stable natural environment and to global species **biodiversity** (biodiversity, or biological diversity, is the variety of life found on Earth).

The introduction of invasive fish species is of particular concern because of the ease and frequency of waterway contamination by non-native fish. Aquatic species are often introduced into new areas as a result of the dumping of large amounts of **ballast water** (the water that is pumped into huge tanks to stabilize unloaded ships, and discharged at the next port of call, along with any surviving organisms) by large ships. Around the world, millions of tons of ballast water are exchanged daily, transporting within it aquatic species from microscopic **plankton** (marine and freshwater organisms that cannot swim against the current and live in a drifting, floating state) to fish. On a smaller scale, commercial activities such as aquaculture and the aquarium trade in exotic fish species can sometimes lead to the accidental or purposeful release of fish species into areas where they have never been found before. Even well intentioned plans to introduce non-native species to control biological problems have backfired and resulted in serious damage by invasive species. For example, the introduction of grass carp to control the spread of unwanted aquatic plants has lead to the destruction of native plant species in inland lakes, resulting in tremendous damage to lake ecology and ecosystems at all levels. Recreational boaters and fishermen can also contribute to the problem by transporting fish (even baitfish) between rivers and lakes, resulting in cross-contamination of previously unaffected waters. Even the owners of household aquaria can potentially contaminate a waterway simply by dumping the contents of their home aquarium into a lake or stream. For example, a few pet goldfish released in Teller Lake in Colorado quickly reproduced, creating thousands of goldfish that destroyed many of the naturally occurring fish and plants in the lake.

Goldfish in Teller Lake. Courtesy Colorado Fish and Wildlife

Target species

This research project will use the latest in scientific technology to check for the presence of the following three invasive fish species, all of which pose a potential threat to New York waters.

Sea Lamprey (Petromyzon marinus)

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"Boca de lamprea. Licensed under CC BY-SA 3.0 via Wikimedia Commons -

Sea lamprey are native to the Atlantic Ocean but were introduced into the Great Lakes in the 1800s through a series of manmade locks and shipping canals. By the late 1940s all of the Great Lakes contained large populations of sea lamprey that caused serious damage to lake trout and other important fish species. For part of its life cycle, the sea lamprey feeds on the blood of host fish. Sea lamprey have a large sucking disc for a mouth, filled with sharp teeth and a file like tongue. They use the sucking disc and teeth to attach to prey fish, and rasp through the scales and skin to feed on blood and other body fluids, often resulting in the death of the prey. The lamprey attack is so destructive that only about 1 out of 7 fish will survive an attack. During its life, which can last from an average of 6 to as long as 20 years, a single lamprey can kill large numbers of native lake and rainbow trout, whitefish, chubs, walleye, and catfish. The economic effect of this invasive species has been enormous. For example, before the spread of the sea lamprey invasion, the United States and Canada harvested about 15 million pounds of lake trout from the upper Great Lakes each year. By the 1960s the total lake trout catch had dropped to only about 300,000 pounds. In Lake Michigan alone the catch dropped from 5.5 million pounds in 1946 to 402 pounds in 1953 (data from the Great Lakes Fishery Commission). Today there is an ongoing sea lamprey control program that is helping to reduce sea lamprey populations in many areas, but vigilant monitoring is still a key factor in controlling this highly destructive invasive species.

Asian Carp (Several species of carp are collectively known in the United States as Asian carp)

Asian Carp Regional Coordinating Committee

Asian carp were originally brought to the United States in the 1970s to help control algae growth on catfish farms and in wastewater treatment ponds. Two species of Asian carp were released from southern aquaculture facilities following flooding in the 1990s, and the invasion has been spreading north along the Mississippi ever since. In some areas of the Mississippi River, Asian carp have become the most abundant fish species, having already out-competed native fish. Asian car have been identified in the canals connecting the Mississippi River to the Great Lakes. Unfortunately, Asian carp, which can grow up to four feet long and weigh more than 100 pounds, have no natural predators in their new environment. A single carp can eat up to 5 -10% of their body weight in plankton each day. By consuming nearly all of the available plankton, the primary food source for most of the native fish, the Asian carp can rapidly wipe out entire populations of native fish. In an effort to decrease the spread of Asian carp into new rivers and lakes, the U.S. Fish and Wildlife Service has placed several species of Asian carp to the federal list of injurious wildlife, making it illegal to transport live Asian carp, including viable eggs or hybrids of the species, across state lines except by special permit for zoological, education, medical, or scientific purposes.

Round Gobi (Neogobius melanostomus)

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Round Gobi were introduced into the Great Lakes through the ballast water from large cargo ships and were first identified here in 1990. Since their introduction, round gobi have caused significant ecological and economic problems. Since then round Gobi have spread throughout the Mississippi River drainage area and into tributaries of the Great Lakes, including a sighting in one of the New York Finger Lakes (Cayuga Lake). Round Gobi, which are bottom dwellers, compete very successfully with native bottom dwelling species like sculpins and darters for food, habitat, and spawning areas, and can cause substantial decreases in local populations of native fish. Round Gobi also prey on small fish and eat the eggs and fry of larger native fish like lake trout, and the increased presence of round Gobi has been shown to potentially impact the food chain supplying recreationally important fish like walleye and smallmouth bass. It has been noted that round Gobi eat large amounts of zebra mussels, which in the short term may seem like an unexpected benefit. But, as with most environmental and ecological issues, it is important to look at the broad picture. Despite their large appetites, it is unlikely that round Gobi will have a significant impact on zebra mussel populations. Equally important, the zebra mussels eaten by round Gobi contain large amounts of various toxins that are found throughout the Great Lakes. Following intake of the zebra mussels, the toxins become concentrated in the Gobi, which are in turn eaten by a variety of sport fish, including smallmouth and yellow bass, walleyes, yellow perch, and brown trout. This

food chain can lead to high concentrations of dangerous toxins in sport fish that are consumed by humans, increasing health concerns related to consuming sport fish.

Environmental Monitoring

A critical part of environmental monitoring is the widespread, accurate collection of water samples. The distribution of the invasive species can change very rapidly, and the presence and abundance of invasive species is often unknown in a particular area. Monitoring waterways for invasive fish species plays a key role in understanding the scope and extent of invasion, a critical first step in determining appropriate responses. Other than direct prevention, early detection and rapid response is the most cost effective method for dealing with invasive species. For many species, early detection can result in earlier and often less costly control. If new invasions can be detected before they become established, the chances of eliminating the problem are greatly increased, minimizing ecological and economic impacts and potentially resulting in significant savings in long-term control and management costs.

The collection of water samples is at the heart of the project. The engagement of citizen scientists has been important in monitoring both aquatic and terrestrial invasive species. Your participation in this project will help us track invasive sea lamprey, Asian carp, and round gobi throughout New York and will greatly contribute to efforts to control the rapid spread of these destructive, costly pests.

There are a number of ways of monitoring a body of water for invasive fish, including visual sightings, catching or trapping, and, more recently, monitoring of species specific DNA from cells shed into the environment. We will use a very sensitive technology that monitors DNA found in environmental samples (**environmental DNA or eDNA**). eDNA is genetic material that is found in environmental samples like water, soil, or air. When eDNA is collected, it is made up of DNA from all the different organisms present in the environment, including plants, animals, singled celled organism like protozoa, and bacteria. When you isolate eDNA you don't know what DNA is in the sample until you conduct a genetic analysis. For example, if you collect a bucket of water from a lake and collect DNA from that water, you have eDNA. If that lake has fish in it, that eDNA will contain some fish DNA, along with many other DNAs from unknown sources. eDNA enters the environment in many ways, including in waste products, blood, or bits of tissue or cells that are shed into the environment. Although there is free eDNA in the environment, for this test we will actually be looking at DNA contained in cells released into the environment. That's because the filters we are using will generally not trap free DNA molecules, but will retain intact cells. eDNA testing has been successfully used to detect the presence of a wide range of plant and animal species in both fresh water and marine environments.

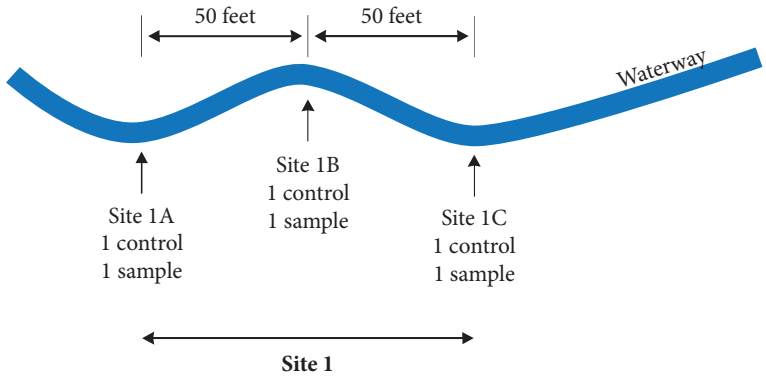
Overview of the Collection Process

All of the materials needed for the successful collection of eDNA samples will be provided. A detailed description of the collection process can be found in the accompanying eDNA **Water Collection Protocol**.

Contamination. The most important thing you can do to ensure that your eDNA results are accurate is to avoid contamination of the control and field sample. Anything that has come in contact with fish, or water that may have at some point been exposed to fish DNA, is a potential source of contamination. This includes (but is not limited to) your hands, clothes, boots, waders, the car you are traveling in if water containing eDNA is sloshed onto a surface, used sampling equipment (such as forceps, tubing, funnels), and the environment around you (i.e., the field site itself). Since it is critical that the water samples collected at any site contain only DNA that is actually found in the water at that site, a control sample of distilled or tap water should be processed before collecting and processing water from each location. A negative result from the control filter will help to ensure that to no invasive fish DNA has accidently contaminated any of the collection materials. If you are processing at the collection site,

process your control before you process your collected sample. If it is not possible to process your sample immediately at the collection site, you can put the collected water on ice, store it in the refrigerator, and process it in your classroom up to 24 hours after collection. Collected water cannot be stored at room temperature for more than 4 hours since there is a high probability that any eDNA present will be degraded. When processing your collected samples in the classroom, it is still necessary to process a control water sample for each location. If you collect from multiple sites, all of the samples from each site should be processed on a separate desk or table to prevent cross site contamination.

Water sample collection. To increase our chances of obtaining a positive reading, when terrain permits, whenever possible 3 water samples will be collected, one from each of three locations ~ 25 to 50 feet apart, as soon in the diagram below. It is important to remember that the 3 filters containing eDNA from 3 adjacent locations all represent one collection site.



An example of the type of materials that will be used for sample filtration and a cartoon of the basic filtration process are shown below (Figures 1 – 3). Detailed instructions are presented in the eDNA Water Collection Protocol that is provided with the collection kit.

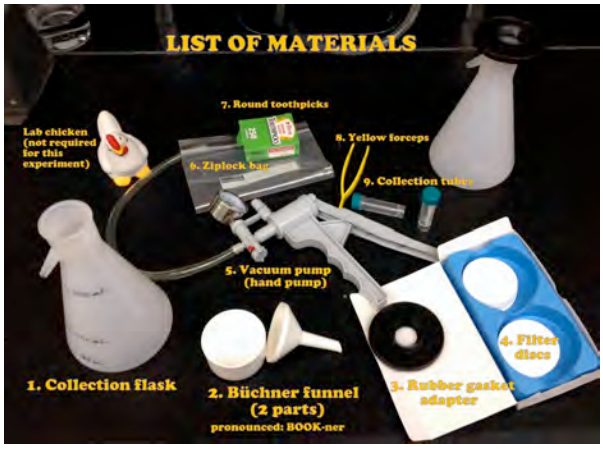


Figure 1. The materials.

Figure 2. The apparatus.

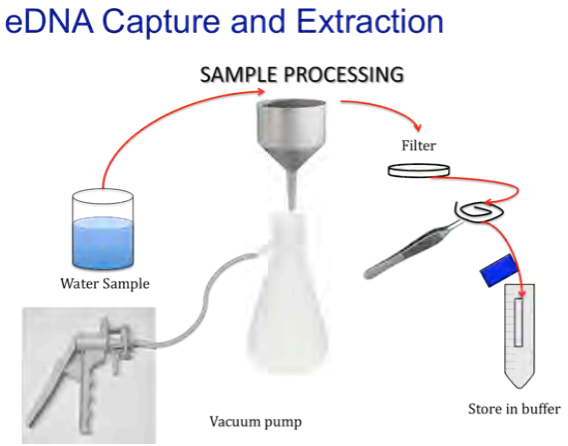
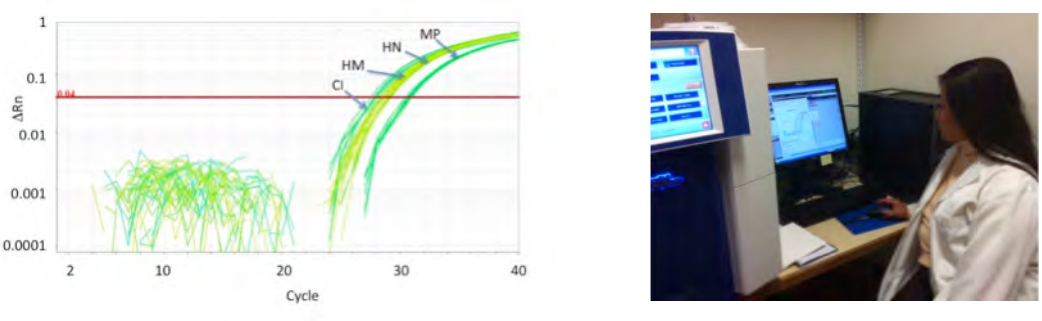


Figure 3. The collection process.

Filters carrying the eDNA from each site will be shipped to Cornell and tested for the presence of environmental DNA from sea lamprey, Asian carp, and round gobi, and in some cases, snakehead fish. Duplicate filters will be archived in long-term storage at -80 ^{0}C at Cornell. These archived samples will provide a "time capsule" view of the environmental DNA in the bodies of water sampled, and will be extremely valuable for future studies to document change in ecosystems.

DNA analysis

qPCR. At Cornell, samples will be tested by the Aquatic Animal Health Program using a variant of the polymerase chain reaction (PCR) known as quantitative PCR (qPCR). PCR is a way to make thousands or even millions of copies of a particular DNA sequence, starting with a very small amount of DNA. Usually PCR is done in a small tube and the copies of DNA made are analyzed at the end of the reaction, usually by running them on an agarose gel. Using standard PCR methods, only the final product made during the PCR reaction can be analyzed. qPCR is different in that it in allows an analysis of the copies being made as the reaction is actually going on. The reaction is monitored by incorporating a fluorescent dye into the newly made product. The DNA containing the dye can be measured on a special instrument that provides real time information about what DNA containing the dye is present in the reaction and how much. An example of qPCR machine and the kind of data it generates is shown in Figure 4.



The qPCR instrument and representative data collected on Asian carp eDNA.

Results of the qPCR analysis from student collected eDNA will be analyzed, and all positive samples will be retested to make sure they are repeatable. Data collected will be electronically put together, compared with existing information, and shared with teacher and student collaborators, along with a description of how to interpret the results.

One common question is what does a qPCR test of eDNA actually tell us? The qPCR results are basically a snapshot in time of the site sampled. The eDNA signal relates to the presence or absence of a species and the number of fish at a given location at the time the sample was taken. The detection of eDNA from an invasive species does not provide information about the age or sex of individuals present at the time of sampling, and does not indicate whether the DNA came from a live organism or a recently dead one (for example a bait fish). In the environment DNA breaks down over time, and detection sensitivity is limited by distance away from the original source of the DNA. The dispersal of eDNA in the environment is affected by factors like rapid water flow or wind, and the rate at which eDNA degrades is affected by things like temperature and the local bacterial community.

If the qPCR test signal does not give a positive signal, that may mean that the species being tested for is not present, or is there in such low abundance that the signal cannot be picked up. A weak eDNA signal could represent a few cells from a non-resident fish that has left the site or a fish that has just entered the site so there are not many shed cells in the water. A strong signal suggests a larger population. Testing the same site later in time will help establish patterns of fish populations. Experiments looking at the sensitivity of qPCR detection suggest that it can pick up one grass carp in a 50 acre pond 10 feet deep under good conditions, although sensitivity as low as one grass carp in a 2 acre pond has been seen if environmental conditions favor the rapid breakdown of shed cells.

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