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

Introduction

Aquatic Invasive Species (AIS) pose a serious threat to 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. 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. FishTracker uses a citizen-scientist-based approach to monitoring for the presence of invasive fish species all around New York State, combining the collection of water samples by teachers and students with state of the art eDNA analysis at Cornell.

As part of the FishTracker eDNA monitoring program, teachers and students play a critical role in tracking the spread of invasive fish species throughout New York State. Using materials and protocols supplied by the program, students collect multiple water samples from nearby lakes, streams, or ponds, noting the GPS coordinates of each site surveyed. The water is 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 are 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 (https://www.vet.cornell.edu/departments/microbiology-and-immunology/research/aquatic-animal-health-program).

In addition to the immediate analysis, duplicate sampling filters will be archived for long-term storage in freezers 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. 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.

FishTracker provides all 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 of the collection procedure (Water Collection Protocol)and a demonstration video are available on our website. A brief explanation of the qPCR protocol (Quantitative PCR Overview) describing what happens to water samples submitted for eDNA analysis is also available on the website. The results for each species analyzed and information on how to interpret the qPCR analysis output is also returned to teachers for further classroom analysis and discussion. Finally, the results of all eDNA testing are posted on the FishTracker website (https://fishtracker.vet.cornell.edu/edna-testing-results/).

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 viable 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 the FishTracker project we are focusing on invasive fish species, a growing problem nationwide, especially in coastal regions and in the Great Lakes watershed. Invasive fish species can cause serious environmental problems and 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 many important agricultural activities including aquaculture, fisheries, and the game fish industry, as well as tourism and recreational activities. Estimates of annual losses due to ship-borne invasive species in the Great Lakes region alone come to at least $200 million dollars, and estimates of overall economic losses in the United States due to non-native fish run as high as $5.4 billion annually. Prevention and early detection are cornerstones for the management of invasive species. 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 **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 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 led 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 Invasive Species

The FishTracker project uses the latest in scientific technology to check for the presence of the following invasive fish species, all of which pose a potential threat to New York waters.

Sea Lamprey (Petromyzon marinus)


NOAA Great Lakes Environmental Research Laboratory licensed under CC BY 2.0


NOAA Great Lakes Environmental Research Laboratory licensed under CC BY 2.0


"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 carp 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 added 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 goby (Neogobyus melanostomus)

NOAA Great Lakes Environmental Research Laboratory
licensed under CC BY 2.0

Round goby 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 goby have caused significant ecological and economic problems. Round goby have spread throughout the Mississippi River drainage area and into tributaries of the Great Lakes. Round goby are rapidly spreading waterways in northwestern New York, including the Finger Lakes.

Round goby, 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 goby also prey on small fish and eat the eggs and fry of larger native fish like lake trout. The increased presence of round goby has been shown to potentially impact the food chain that supports recreationally important fish like walleye and smallmouth bass. It has been noted that round goby 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 goby will have a significant impact on zebra mussel populations. Equally important, the zebra mussels eaten by round goby 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 goby, 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.

Northern snakehead (*Channa argus*)

Brian Gratwicke, licensed under CC BY 2.0

Northern snakehead fish are native to parts of China, Russia, and the Korean peninsula, where they are considered a valuable food fish. Snakeheads are also popular exotic additions to home aquariums and have been introduced into United States waters primarily by aquarium owners discarding their unwanted pets into local waterways. The fish may also have been intentionally released to provide a local source for fishermen interested in catching snakehead for consumption. As a result, the northern snakehead has successfully established wild breeding populations in some parts of the United States and is gradually expanding its range.

Snakeheads can live in freshwater lakes, ponds, rivers, streams, and wetlands, including slow moving or even stagnant water. They can also survive cold winters. Northern snakehead fish can spread by swimming in water, but they can also breathe air. Therefore, they are capable of surviving on land for up to four days, as long as they are wet, and they can move up to ¼ mile on land by wriggling their bodies and using their fins.

Northern snakeheads are aggressive predators that grow up to 4 feet in length, with large mouths and sharp canine-like teeth. Invasive snakeheads thrive in the absence of their natural enemies, with a single female capable of laying up to 150,000 eggs in just 2 years. Adult snakeheads eat other native fish, small amphibians, reptiles, crustaceans, and even some birds and mammals, while juvenile snakeheads pose a significant threat to zooplankton, larvae, small fish, and crustaceans. Snakeheads also compete with native species for food and habitat. Because snakeheads are considered injurious to wildlife, it is illegal to import the fish or viable eggs from other countries, or to transport them across state borders. Establishment of the northern snakehead in the United States could result in serious ecological and environmental damage, costing millions of dollars in management efforts and impacting aquaculture and the sport fishing industry.

White perch (*Morone americana*)

Ohio Sea Grant, licensed under CC BY-NC 2.0

White perch are native to the east coast of the United States and Canada and are often found in fresh, brackish and coastal waters. They have invaded other areas in the United States, primarily through bait fish release and movement through watersheds. Access to the Great Lakes probably occurred via the Welland Canal and the Erie Barge Canal starting in the 1930s.

White perch reproduce rapidly since they can spawn multiple times per year under optimal conditions, and can successfully breed in a variety of water conditions. There are several important negative impacts associated with the introduction of white perch, including predation, competition for food, resources, and habitat, and hybridization. Fish eggs are a substantial part of the white perch diet, and predation on the eggs of walleyes and white bass have caused a decline in some fisheries. White perch also eat small fish like minnows, and successfully compete for food with native species such as yellow perch, white bass, and black bullhead. As a result, a decrease in the abundance of native fishes often follows the introduction of white perch. White perch also consume large amounts of zooplankton, which may contribute to algal blooms in waters where they become heavily established.

White perch are closely related to white bass, but are normally not found in the same waters. When non-native white perch are introduced into an area where white bass are found, fertile hybrid offspring can be produced that are capable of reproducing with the parent species, potentially diluting the gene pool of both parent species. Because of the problems resulting from the introduction of non-native white perch, possession of live white perch is illegal in several states, and release of captured white perch back into the water is not recommended.

Asian swamp eel (*Monopterus albus*)

U.S. Geological Survey, licensed under CC public domain

Asian swamp eels are native to Asia and were first introduced into the continental United States from multiple geographic areas in the mid 1990s as a food source and also for use in home aquaria. Escape from fish farms and intentional release of pet fish have resulted in the establishment of wild populations in Florida and Georgia. Although not yet found in New York state, the presence of swamp eel was confirmed in New Jersey in 2008.

The swamp eel lives in fresh water, including stagnant waters, marshes, shallow wetlands, streams, rivers, lakes and ponds, but can also tolerate brackish and saline conditions. Swamp eels can survive cold temperatures and a wide range of oxygen levels since they can absorb up to 25 percent of the oxygen they need from the air. As a result, swamp eels can migrate short distances over land, increasing their ability to disperse to new habitats. They have a voracious appetite and eat a wide range of prey, competing with native species for food, and potentially disrupting fragile ecosystems. Swamp eels are hermaphroditic, with all young initially being female. As they mature, some fish become male, but are capable of reverting to the female form when females become rare. Females can lay up to 1000 eggs each time they spawn, and spawning can occur throughout the year. This reproductive flexibility can provide a survival advantage over native fish. To help limit the spread of this invasive species, the United States Geological Survey discourages catching and transporting the eel for use as bait, food, or aquarium pets.

Tench (Tinca tinca)

Christophe Quintin licensed under CC BY-NC 2.0

Tench, a member of the minnow/carp family, is a freshwater game fish native to Europe that has been introduced to many different countries around the world, including the United States. Tench are potamodromous, meaning they migrate and complete their life cycle in fresh water only. Tench average about 10 inches in length, but can live up to 20 years and grow to nearly 3 feet in length and weigh up to 16 pounds. Tench mature between 2 and 7 years of age and breed in the late spring or summer.

Females lay a new clutch of eggs every 15 days for about 2 months. Tench prefer to live in shallow lakes and rivers in areas of heavy vegetation with a weedy, muddy bottom. Tench can survive in low-oxygen environments and in some areas spend the winter buried in mud. Tench are voracious eaters, consuming huge quantities of insect larvae, snails, mollusks, plants, algae, and organic debris that native species depend on. Tench also eat the eggs of other fish species, placing further stress on native fish like yellow perch and the copper redhorse that are already struggling with pollution and habitat loss. Tench are also a source of fish parasites that can spread to other species. In 2018 the Great Lakes St. Lawrence Governors & Premiers coalition added tench to the "least wanted" fish list and the St. Lawrence–Eastern Lake Ontario Partnership for Regional Invasive Species Management (SLELO-PRISM) in New York is trying to raise awareness of the impending problems that could result from invasive Tench.

Tench were first introduced into the Great Lakes basin in the 1980s, likely after escaping from aquaculture ponds in Quebec, and became established in Lake Champlain in 2000.Tench have expanded their range into Lake St. Francis in the St. Lawrence Seaway and are steadily pushing upstream into Lake Ontario. Unfortunately, habitat conditions that were expected to limit feeding and spawning have not halted their expansion. The spread of tench has also been facilitated by illegal fish transport, illegal pond stocking, and use of tench as baitfish. Currently tench are a potential problem, but not yet a direct threat to New York waterways. However, tench are already found in the Great Lakes, and given their ability to spread and thrive in a wide variety of conditions, tench are poised to become a major invasive problem in New York waters. Increasing community awareness, developing strategies to slow the invasion, and early monitoring are important factors in limiting the tench invasion while there is still time.

Threatened, Endangered, and Declining Native Fish Species

Invasive species often contribute to the decline of important native fish species. To help monitor levels of currently declining, threatened, or endangered species, we are adding the following native fish species to our monitoring program. According to the Federal Endangered Species Act of 1973, two categories of declining species of plants and animals need protection – endangered species and threatened species. Endangered species are defined as "any species that is in danger of extinction throughout all or a significant portion of its range", while a threatened species is defined as "any species that is likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range". Simply put, endangered species are at the edge of extinction now, and threatened species are likely to be at the edge of extinction in the near future. Declining species not yet characterized as threatened or endangered can show significant decreases in population, and will benefit from monitoring information to help understand population levels.

Fish Species Declining in New York

Deep water cisco, also called lake herring (Coregonus artedii)

Ciscoes are freshwater schooling fish that are predominantly found in waters below 18°C (~65°F). These cold-water fish breed once a year, spawning in shallow coastal waters in the winter and returning to deeper waters during the spring. Cisco eggs develop slowly, hatching in the spring as the ice starts to thaw. Juveniles usually live in shallow bays for their first month, and mature within 1 to 4 years.

Cisco. USFWS Fish and Aquatic Conservation, licensed under CC BY-NC-ND 2.0

Ciscoes play an important role in the ecosystem. They feed predominantly on zooplankton and insect larvae and are a primary food for other economically important fish species like lake trout, yellow perch, walleye, and northern pike. In the 1940s, ciscoes were one of the most commercially important fish in the Great Lakes, where fisheries produced an average of 19 million pounds annually. However, the combined pressures of over-fishing, competition by invasive species like rainbow trout and alewives, and pollution have caused a significantly **declining** population.

Once abundant in all five Great Lakes, ciscoes are now common only in Lake Superior, in part due to the effects of invasive species like round goby, which eat their eggs, sea lamprey that attack adults, and alewives that eat their food. In addition, one of the greatest risks to ciscoes in the Great Lakes is the increasing level of nutrients being added to the lakes each year, which results in a decrease in the amount of oxygen in the water. Ciscoes are sensitive to changes in temperature and levels of dissolved oxygen. Rising temperatures resulting in lowered levels of dissolved oxygen can cause large die-offs in cisco populations, especially in the summer.

In New York, ciscoes are native to ten watersheds in Great Lakes drainage, including the Finger Lakes, lower elevations of the Adirondacks, and Chautauqua Lake. However, populations have declined in the westernmost watersheds and in lower elevation lakes, including Lake Erie. Re-establishing ciscoes in the lower Great Lakes would help improve connections in the food web. Additional information on location and abundance of native populations will aid efforts to protect and restore this important native species.
A map of cisco range in New York pre/post 1976-1977 is shown here http://www.dec.ny.gov/animals/94547.html.

American eel (Anguilla rostrate)
American eels are the only freshwater eel native to North America. American eels hatch in the Sargasso Sea in the North Atlantic. Larvae drift with the Gulf Stream, reaching the US Atlantic coast about a year later. American eels undergo several distinct morphological stages as they mature. Depending on the environment, they can take up to 40 years to reach sexual maturity, at which time they return to the Sargasso Sea where each female lays 20 -30 million eggs.


American eel. Chesapeake Bay Program, licensed under CC BY-NC 2.0

Although the American eel remains widely distributed throughout much of its historical range, the population has decreased significantly over the last several decades, a result of the combined effects of habitat destruction, dam construction, water pollution, parasites, and overfishing, along with a huge increase in demand by Asian food markets following large decreases in the native Asian eel populations. Although the U.S. Fish and Wildlife Service has not yet provided endangered species protection to the American eel, populations are **declining** and according to the International Union for Conservation of Nature (IUCN), the American eel is at very high risk of extinction.

Conservation of the American eel is important for many reasons. American eels are an ecologically important species, playing various roles as prey, predator, and host species that help maintain ecosystem balance. In New York, the American eel is native to 17 of 18 watersheds. It was introduced to Lake Erie but the population was not sustained. American eel numbers have been greatly reduced throughout the watersheds of the Great Lakes. It has been completely eliminated from the Allegheny, and has declined to levels below detection in the Chemung and Susquehanna.

Although conservation efforts are now underway, much more will need to be done to restore the American eel to healthy, sustainable levels. Efforts to protect and restore this important native species will benefit from additional information on current locations and abundance of native populations.

Brook trout (*Salvelinus fontinalis*)

National Fish Habitat Partnership

Brook trout are popular small game fish generally weighing two pounds or less and seldom living more than five years. They require clean high-quality cold water (below 72 degrees Fahrenheit) to survive and depend on clean gravel areas for spawning. Once widely distributed, brook trout are now most commonly found in small streams and the headwaters of larger streams in mountainous areas. Brook trout are so sensitive to environmental conditions that they are considered an environmental indicator species. A large number of brook trout generally indicates a relatively healthy aquatic environment while a decline in the brook trout population often indicates a deteriorating habitat and decreased water quality. Brook trout are native to all 18 New York watersheds and have been introduced to additional areas throughout the state where they are not native. However, intact stream populations of brook trout now exist in only 5% of sub-watersheds, and the vast majority of large rivers that were historically home to thriving brook trout populations no longer support self-reproducing populations.

Although not listed under the Endangered Species Act, the brook trout have suffered **decline** due to a combination of factors. Brook trout evolved in waters where there were few other fish species competing for resources. Brook trout do not compete well when non-native fish like yellow perch, bass, golden shiner, and various baitfish (small fish used as bait to attract larger fish, often game fish) are introduced into their waters. In the presence of non-native fish, brook trout populations generally experience a serious decline. As a result, the use of baitfish is prohibited in many brook trout ponds and it is illegal in New York to introduce any fish into waters they did not come from. Brook trout are also particularly sensitive to the effects of acid rain, especially in areas low in limestone that cannot naturally buffer increases in acidity. As a result of acid rain coupled with illegal fishing, in some areas of the Adirondacks, only ~3% of the waters that previously supported brook trout still maintain a viable population. To help counter this problem, New York State has stringent acid rain control requirements and is working to support reductions in power plant emissions outside the state.

An active brook trout restoration program has been established in the Adirondack Park. To secure the survival of the species in this area, ponds are selectively treated with lime each winter to decrease acidity levels, and competitive non-native fish populations are eliminated. When acidity levels suitable for fish survival are reached and competing fish species have been eradicated, ponds are restocked with brook trout.

Threatened and Endangered Fish Species in New York

Sturgeon

Three types of sturgeon are found in New York waters, the Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*), the Lake Sturgeon (*Acipenser fulvescens),* and the Shortnose sturgeon (*Acipenser brevirostrum*). The FishTracker sturgeon primers detect all three species.

Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*).

Atlantic sturgeon, Arthur Chapman, licensed under CC BY-NC 2.0

The Atlantic sturgeon is the biggest and longest-living of the sturgeons found in New York, with adults often reaching 6 to 8 feet or more in length and weighing over 100 pounds. Atlantic sturgeon are anadromous fish, which means that they are born in fresh water, but spend most of their lives in the ocean. Adults migrate from the salty ocean into rivers to lay their eggs in fresh water, sometimes traveling hundreds of miles to spawn, and then returning to the ocean when spawning is complete.

After hatching, young Atlantic sturgeon remain in the fresh water environment for several years before migrating to the ocean, where they live for several more years before reaching sexual maturity. When ready to spawn, each generation of Atlantic sturgeon briefly returns to fresh water to lay their eggs. Female sturgeon can lay up to 2 million eggs during a single spawning season, but females do not spawn every year.

Atlantic sturgeon are toothless bottom feeders, using a tubular mouth to suck up food including plants, invertebrates such as mollusks, worms, and crustaceans, as well as small bottom-dwelling fish. Atlantic sturgeon were once an abundant, commercially important fish in the Hudson river, especially prized for their meat, tough skins that were made into leather, and eggs (caviar). However, over the span of 100 years the catch of Atlantic sturgeon dropped from nearly 7 million pounds in1887 to only 400 pounds in 1989. As a result of this dramatic decrease, Atlantic sturgeon are now considered an **endangered** species in New York and are protected from fishing. The New York Department of Environmental Conservation, NOAA (National Oceanic and Atmospheric Administration) Fisheries and other groups are attempting to conserve and rebuild Atlantic sturgeon populations along the East Coast. However, the species still faces a number of threats including entanglement in fishing gear, bycatch (the unintentional capture of non-target species), dams and other barriers that impede habitat access, and being hit by boats.

Lake sturgeon (*Acipenser fulvescens*)

Lake sturgeon, NYS DEC, licensed under CC BY-NC-ND 2.0

The lake sturgeon is New York's largest fresh water fish, averaging between 3 to 5 feet in length and weighing up to 80 pounds. Lake sturgeon are bottom feeders, eating primarily insect larvae, worms, leeches, other invertebrates including snails and clams, and algae. Lake sturgeon can live up to 80 years or more, and take 4 to 23 years to reach sexual maturity. Once mature, a female can lay 200,000 to 800,000 eggs during a single season, but lake sturgeon only spawn every four to six years. Lake sturgeon lay their eggs from April to June in rapidly moving water, like rocky island shores and quickly moving gravel streams where, after being scattered by currents, the eggs stick to rocks and logs.

The lake sturgeon population underwent a precipitous decline in the 1900s. From 1879 – 1900 the commercial catch of lake sturgeon in the Great Lakes averaged over 4 million pounds. However, by 1929 the commercial harvest of lake sturgeon in Lake Michigan was only 2000 pounds, a result primarily of high demand for their eggs (caviar) and meat. More recently the lake sturgeon population has been further impacted by the construction of dams that cut off spawning and nursery areas, pollution, degradation of habitat due to channelization, and invasive aquatic species. Remnant populations of lake sturgeon are believed to currently exist in several waterways in New York, including the St. Lawrence River, Niagara River, Oswegatchie River, Grasse River, Lake Ontario, Lake Erie, Lake Champlain, Cayuga Lake, and in the

Seneca and Cayuga canals. However, the lake sturgeon is currently listed as **threatened** by the American Fisheries Society since the population has not recovered from the losses that occurred in the 1900s. To help protect and manage lake sturgeon populations, the New York State Department of Environmental Conservation (DEC) is researching lake sturgeon populations, and attempting to reestablish lake sturgeon populations in some tributaries of Lake Ontario, the St. Lawrence River, the Oswegatchie River, Black Lake, the St. Regis River, Oneida Lake and Cayuga Lake.

Shortnose sturgeon (*Acipenser brevirostrum*)


Shortnose sturgeon, Chesapeake Bay Program, licensed under CC BY-NC 2.0

The shortnose sturgeon is the smallest sturgeon found in New York waterways, averaging under 4 feet long and less than 14 pounds. Shortnose sturgeon are bottom feeders, primarily eating mollusks, worms, insects and crustaceans. Like other sturgeon, the shortnose sturgeon is a long-lived, slow maturing fish. Females take 6 to 17 years to become sexually mature, and can live nearly 70 years. Adult females can lay up to 200,000 eggs in one season, but only breed every 3 to 5 years. In New York, the shortnose sturgeon is found only in the lower part of the Hudson River, from the southern tip of Manhattan to the Federal Dam at Troy. Shortnose sturgeon overwinter in the Hudson River and spawn in fresh water areas north of Coxsackie.

The shortnose sturgeon population in New York was decimated as a result of over-fishing in response to demand for its meat and caviar. In addition, during the 1800s and early 1900s tidal rivers like the Hudson were used as dumping grounds for pollutants, causing severe decreases in water oxygen levels that resulted in high die-offs of shortnose sturgeon and many other fish species. Damming of the Hudson River, which cut off upriver spawning grounds, also contributed to the shortnose sturgeon decline. Currently the shortnose sturgeon is listed as **endangered** by the National Marine Fisheries Service and is therefore protected by the Endangered Species Act. State and national agencies are working to re-establish the shortnose sturgeon population through research and strict regulations prohibiting fishing of this endangered species.

Common Native Species

Rock Bass *(Ambloplites rupestris)*

Rock Bass are found in streams and lakes throughout New York, generally in areas of clear water, abundant vegetation, and rocky or sandy bottoms. Rock bass average 6 to 8 inches in length but can grow to 10 to 12 inches long and weigh over 1 pound, feeding mostly on aquatic insects, crayfish, and other small fishes. Rock bass take 2–3 years to become sexually mature. Adult females lay 2,000 to 11,000 eggs between April and early June, after which the male guards the eggs and the newly hatched young for a brief time. Rock bass are found in all New York watersheds, but are not native to the Adirondacks and to Atlantic slope watersheds where they were introduced between 1889 and 1936 by the United States Fish Commission. Because of their population stability, rock bass are listed as a **species of "least concern"** by the International Union for Conservation of Nature and


Rock Bass, Chesapeake Bay Program, licensed under CC BY-NC 2.0

currently do not require monitoring or conservation management plans, although due to their status as a sport fish their population is managed in some areas. Because of their abundance and widespread distribution, rock bass can be used for comparative purposes when analyzing results for declining, threatened, and endangered fish species.

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, so 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 goby throughout New York and will greatly contribute to efforts to control the rapid spread of these destructive, costly pests.

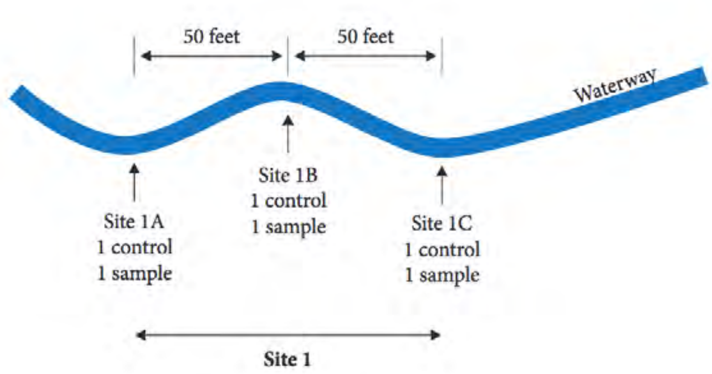
There are a number of ways of monitoring a body of water for invasive fish, including visual sightings, catching or trapping, and, more recently, monitoring of species-specific DNA from cells shed into the environment. We will use a very sensitive technology that monitors DNA found in environmental samples (**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 brought to the site must be processed before collecting and processing water from the site itself. 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.

Water sample collection. To increase our chances of obtaining a positive reading, when terrain permits, 2 additional water samples will be collected at locations ~ 25 to 50 feet on either side of the original collection site. Collection should start at the most downstream location and move progressively upstream to prevent the flow of any dislodged debris or sediment that might clog the filter to the adjacent site. It is important to remember that the 6 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.

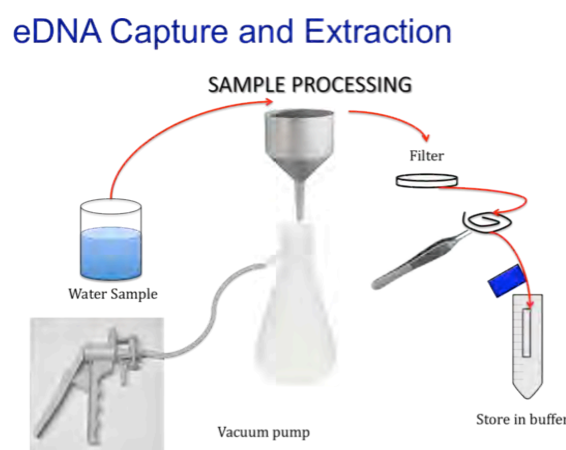
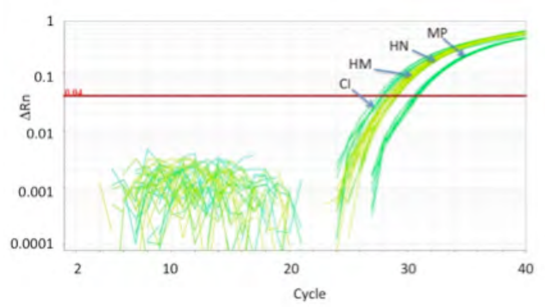


Figure 3. The collection process.

Filters carrying the eDNA from each site will be shipped to Cornell and tested for the presence of environmental DNA from all of the invasive and native species mentioned above. 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 a 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 compared to positive and negative controls, 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 detailed 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.

This work is supported by the USDA National Institute of Food and Agriculture HATCH project 2017-18-128.