The Effect of Invasive Fish on United States Waters

Victoria Arlia, MaryKate Clark, Christina DeAngelis, Jaclyn Deteskey and Grace Perez

Fontbonne Hall Academy - Biology Honors Class

Teacher: Mrs. Ashkenazy

Abstract

On September 26, 2017, our Biology class went to Plumb Beach, Brooklyn, NY as well as Marine Park Beach, Brooklyn, NY to test the eDNA of invasive fish species in the Atlantic Ocean. Invasive fish pose serious threats to our ecosystems and the health of humans and other animals within the environment. This means that these fish are eating a majority of the food source, causing the native fish to die out. As these invasive fish have no found predators, our ecosystems are being disrupted. Invasive fish mainly cause damage because they have a tendency to grow tremendously and reproduce, causing these species to compete for necessary resources of native species. We took samples from Plumb Beach and Marine Park Beach water and filtered it with a Buchner funnel, filter paper discs, flask, and hand held vacuum. The eDNA was sent into Cornell University where they tested our results to check for the presence of invasive fish in the eDNA.

Introduction:

Foreign species are introduced into a new specific location, but does not certainly mean they have negative impacts; when foreign species begin to have negative impacts they are known as invasive species. Invasive fish are most commonly found in the United States that pose a serious threat towards our native ecosystems. Our existing ecosystems are constantly being disrupted because the invasive fish have no predators. Like some invasive species, fish can alter the environment to their liking and make it less favorable to the native species, which is called ecological facilitation. As a result, invasive fish are reproducing and using up necessary resources for native fish .[1] Not only does the spread of invasive species affect ecosystems, but also the health of humans and other animals within the environment. The most dangerous effect of these species is as the carrier of diseases. Invasive species pose a serious threat to both humans and animals within the environment.[2]

These invasive fish migrate into U.S. waters through human activities, such as boating and fishing. The introduction of invasive fish into new areas is developed through ballast water, water carried in ships ballast tanks to improve stability and balance. When the ships take on this ballast water, plants and animals in the ocean are taken in and is carried to the next port[5]. These ships dump this ballast water into the ocean at the next port of stop causing the introduction of foreign fish. Instead of making these invasive fish beneficial to our ecosystems, it causes serious damage to the environment. A major contribution to this environmental destruction is through

the fisherman's process of transporting fish between lakes and rivers leading to cross-contamination of immune waters, which means these waters were not affected or influenced. The discovery of Sea Lampreys, Asian Carps and Round Gobies are the main invasive fish threats to U.S. waters.[8]

In this research project, one of the invasive fish we will be testing for in the eDNA is the Sea Lamprey. The Sea lamprey is native to the Atlantic Ocean and migrated into the Great Lakes through manmade locks and canals in the 1800s. These invasive fish began to grow and develop in these new waters and started to cause serious damage to the environment and other types of fish, such as the lake trout. The Sea Lamprey may be known as America's first destructive invasive species. Sea Lamprey has an eel shaped body and feeds on the blood and bodily fluids of host fish. The Sea Lamprey is so destructive that only 1 out of 7 of their prey survive these attacks. The population of the Sea Lamprey got so out of control that a program was started to help reduce the Sea Lamprey population in many areas. [8]

The Asian Carp species was brought to the United States in the 1970s. They migrated into these waters in order to control algae growth on catfish farms and in wastewater treatment ponds. Two types of Asian carp were released into the wild in the 1990s due to flooding. Since the carp had been released, their invasion has been spreading North along the Mississippi River. Asian Carp's have now been identified in the canals that connect the Mississippi and the Great Lakes. Typically, these types of fish are about four feet long. However, ever since they have been introduced into American waters these fish have been able to weigh up to 100 pounds. An

individual carp is able to eat from 5% - 10% of their body weight in plankton daily. Due to this, the carp are consuming a majority of the plankton, which is causing this native fish to rapidly die out. In order to prevent Asian carp from wiping out more native fish species and from spreading into more waters, the U.S Fish and Wildlife Service have put several types of Asian Carp on the injurious wildlife. As a result, it's illegal to transport Asian Carp and move viable eggs into American waters.[8]

Another type of invasive fish that over populates U.S waters is known as the Round Goby. The Round Goby were introduced into the Great Lakes in 1990, through ballast water from large cargo ships. Ever since they have been introduced into these new waters, they have done severe damage to the ecosystem. From the Mississippi river, the Round Goby have migrated into streams and rivers that flow into the Great Lakes. The Round Goby has caused a decrease to the native species of the areas they have moved into. They compete successfully with native fish like Sculpins and Darters who live on the bottom dwellings of the water, for food, habitat, and places to lay eggs. Round Goby typically prey on small fish and eggs of the larger fish in the area, such as lake trout. These Round Goby fish are apart of a food chain with other fish that contain toxins that are very dangerous to other fish and can even increase health concerns in humans.[8]

DNA is a molecule that carries specific information about an organism's genetic uniqueness. The DNA from cells released into the environment is called Environmental DNA. When collecting eDNA it is important to realize that indirect contacts with that area can affect

your sample. eDNA can be found in experimental samples such as water, soil, or air. In this experiment, using several different materials and methods, we indirectly tested for the eDNA of invasive fish harming our U.S. waters. We indirectly tested for these invasive fish because we didn't come in direct contact with these species.

As ‘citizen scientists’ we have a moral obligation to try to help our environment. It is our duty to understand what poses negative and positive effects on our environment, so we can learn how to make our environment more efficient. The importance of contributing to a project bigger than yourself is to help someone or something more than you imagine. For example, in this experiment we were ‘citizen scientists’ using samples from Plumb Beach and Marine Park Beach; our few samples helped a much larger experiment that pertained to all of New York. The number of trials affect the outcome because the more trials you have the more relevant the data/evidence becomes. Working on Marine Park Beach and Plumb Beach has inspired us to continue being a ‘citizen scientist’ in many ways, whether it is continuing to collect water samples to test for invasive fish or collecting samples of soil to test for microorganisms. We learned that researching to better our environment was fun because we were able to tangibly contribute to a much larger experiment. This opportunity allowed us to work on an experiment out of the ordinary for us and it opened our eyes to the many ways we can help our environment. As ‘citizen scientists’ we collected samples from two beaches; although this may seem like very little data we contributed to a much larger project and our experiment will affect Cornell University's research.

In addition to our invasive fish experiment, the Horseshoe Crab Spawning Survey is another example of a 'citizen scientists' project. The Horseshoe Crab Spawning Survey, occurs during the months of May and June, where people gather at twelve different beaches in New Jersey and thirteen different beaches in Delaware to count the number of spawning crabs, which means the number of reproducing crabs. Many people contribute to this experiment because Delaware Bay is home to the largest population of horseshoe crabs.[4]

Atlantic horseshoe crabs, also known as Limulus polyphemus, are a common sight at Plumb Beach. Horseshoe crabs are invertebrate species who spend most of the year in deep water, but sometimes emerge from the seas in the warmer months to mate along the beach on moonlit nights.[6] Horseshoe crabs are ironically not crabs, they belong to a group known as arachnids, which include spiders, ticks, mites and scorpions. The most obvious and remarkable aspect is its blue blood with antibacterial properties. Therefore, their transportation of oxygen comes from copper-based hemocyanin, unlike vertebrates who use iron in the hemoglobin. Unlike most animals who use white blood cells to fight infections, horseshoe crabs have amebocytes that extract the negative bacteria. Horseshoe crabs have evolved to become enormous medical value. Coagulan, the chemical inside amebocytes, is used for medical equipment and vaccine testings. Today, without the horseshoe crabs' blood many more people would die from infections. The harvesters extract 30% of each crabs' blood before returning back to the ocean; many crabs don't recover from this extraction. As a result, there has been a decline of crab populations in North America.[7]

Our group did not measure the temperature at Plumb Beach, but we did observe that the ocean water was slightly colder than comfortable to the touch. However, we researched that average temperature of the Atlantic Ocean on September 26 was 18.3 degrees Celsius. Besides the temperature of the water at Plumb Beach, the surrounding area had some pollution from humans and the amount of animals nearby, such as the dog we spotted when experimenting. The ocean and the surrounding area was covered in debris and garbage. For example; there were wrappers and garbage found in the surrounding area, and even a plastic bag floating in the ocean which is very harmful to ocean life. The water was a greenish-brown color due to seaweed and pollution, which is common in New York waters.

September 26th, 2017 was the day we went to our two locations to collect our samples. It was 30.6 degrees Celsius, slightly humid, with some wind at our locations. We came across different animals at our locations. At Plumb Beach, we found two washed up horseshoe crabs, and we saw a dog. The dog could affect our samples because we don't know if the dog had been in the water we were sampling before we got there. If it had been, there could be eDNA from the dog along with the fish we were testing for in our samples. However, there is a not a good chance of this happening, because the primers that are provided in this experiment specifically target the 3 types of fish being tested. After Plumb Beach, we went to Marine Park Beach, where we saw different animals at this location. We saw nine seagulls, also known as Laridae, along with a dead animal that we were unable to identify due to it's face being in the ground.

Figure 1

During our school walkathon on Friday, October 6, we noticed a sign on Shore Road, (see Figure 1) which explained different invasive species in our local waters. Some of these species included Zebra Mussels, European Green Crab, Shipworms and Gribbles. Zebra Mussels were introduced into New York through ballast waters. These mussels have no known predators, which causes them to rise in population, reduce the number of plankton in the water, as well as oxygen and room for native species. European Green Crabs were introduced into the Atlantic coral 150 years ago. This species eats clam beds and oysters. Shipworms and Gribbles can be found in wooden pilings. This causes severe damage to these pilings. Shipworms are worm like clams while Gribbles are shrimp-like crustaceans. Through ballast waters, new species were entered into a non-native ecosystem. These invasive species entered our waters like Round Goby, Sea Lamprey and Asian Carp fish we discussed earlier. They were able to enter from water carried in ships in ballast tanks to improve stability and balance. These species are harming the ecosystems in New York just as they are everywhere else.

Our group hypothesized that there would be different types of invasive species in Plumb Beach and Marine Park Beach ocean water, including Sea Lamprey, Asian Carp and Round Goby. We believed that there would be plenty of Round Goby because they were discovered in

New York lakes. There may be some Sea Lamprey because they were discovered in Lake Ontario, which borders New York. Lastly, we thought there would be very little of Asian Carp fish because they were discovered in the Mississippi River which hasn't reached near New York yet.

Materials:

1. 3 Polypropylene suction flasks
2. 3 Buchner funnel(2 pieces)
3. 3 Rubber stopper adapters for funnel
4. 6 Glass fiber discs
5. 3 Hand held Vacuum pump
6. 3 Ziploc bags for water collection
7. Round toothpicks
8. 2 Forceps(1 for control, 1 for sample)
9. 6 collection tubes with buffer for storing filters after use
10. 3 Gloves for water collection
11. 1 Black bag for storing used equipment in the field
12. 1 small trash bag for disposable waste
13. A second black plastic bag for return shipment of wasted used equipment
14. 2 Red ties for closing the black bags
15. 300 ml of water for each control sample

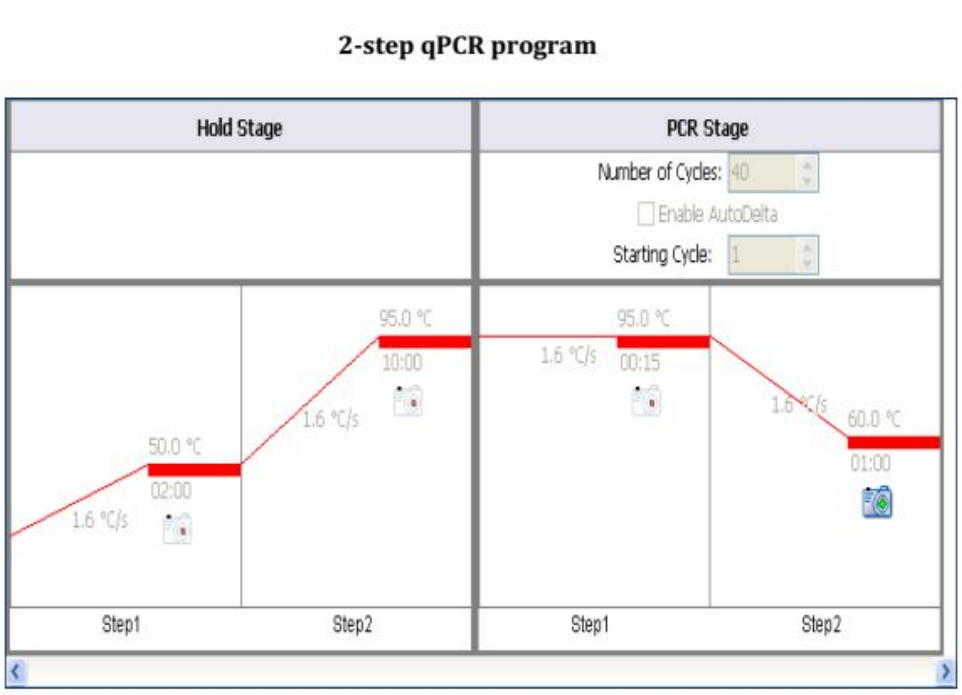
16. Clipboard and pen/pencil
17. Tape measure
18. GPS unit

Methods:

1. Take the Polypropylene suction flask and place it on a flat surface
2. Place the rubber stopper at the top of the flask
3. Place the Buchner funnel into the rubber stopper for more support of the funnel
4. Gently, place the glass fiber disc into funnel
5. Take the clear tube and connect it to the side of the flask where it projects out and connect the other side of the tube to the hand held Vacuum pump
6. After you have assembled your pump, assign two people to go into the water with the plastic bag while the rest of the group stays at the station.
7. Take the plastic bag into the water and fill up completely until you have no more room to close the bag
8. Now begin to filter the water by poking a small hole into the plastic bag and carefully pouring it into the funnel.
9. Continue this process until you have reached the black line on the flask.
10. When completely done you should notice the residue left behind on the fiber disc. To keep from affecting the results, use the forceps and gently pick up the fiber disc.

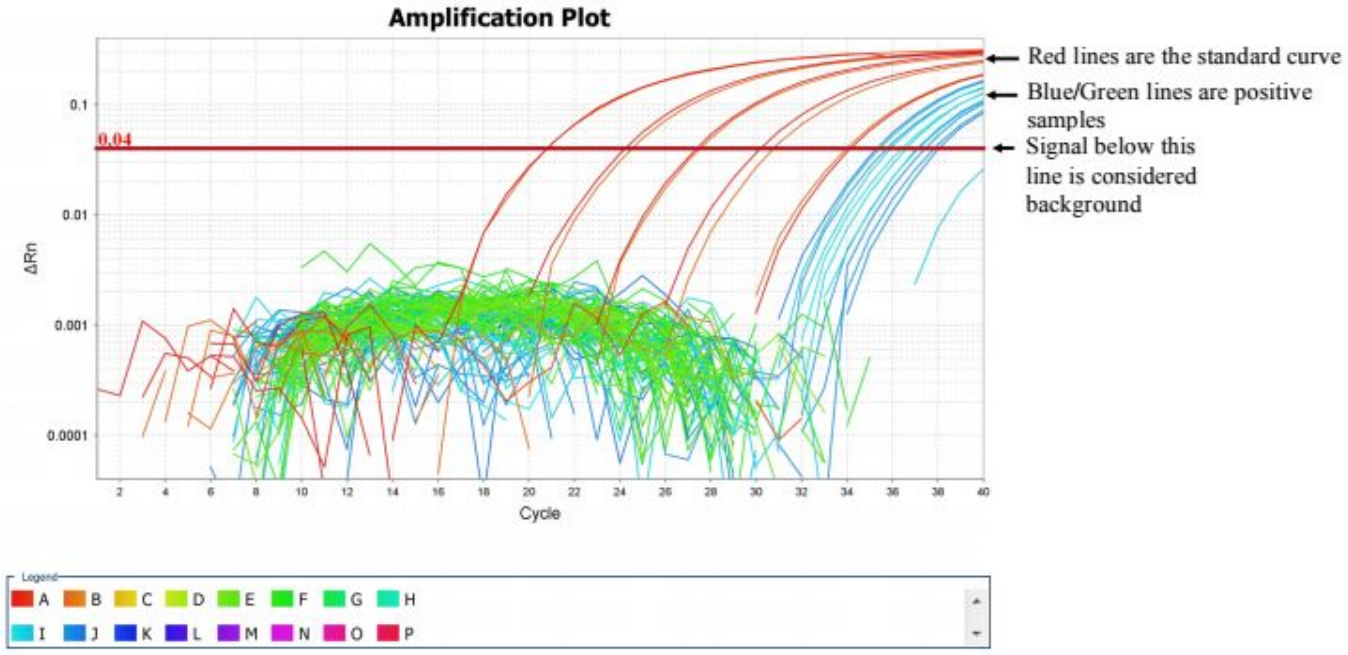
11. Place the disc into the collection tube, which should be labeled, and tightly secure the tube for no further contamination. Your label should also state the location.

Results:

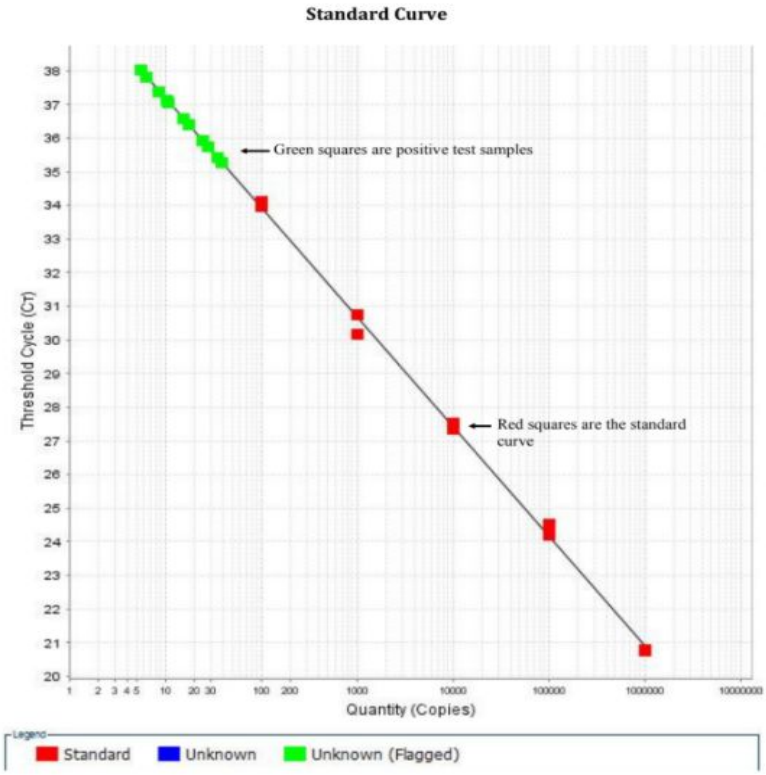


The image above depicts the qPCR program used in our experiment. The Hold Stage, on the left, includes 2 steps. The first step has uracil-N-glycosylase (UNG) activity. The UNG prevents increasing of PCR products and decreases the chance of cross contamination. In the second step of hold stage, it activates the Gold enzyme used in the reaction to split the dU-containing PCR products that are created in low temperatures, and to denature the the native DNA in the area. To

the right is the PCR stage. The 2 steps are two different temperatures. During this process, the PCR product is amplified.



The image above depicts the output of the qPCR machine. Multiple samples, positive and negative, are shown at the same time in this plot. The different colored lines represent the DNA tested from various bodies of water, which are labeled at the bottom of the image. The green lines are the results of our school, Fontbonne Hall Academy. The red and blue/green colored lines above the thick red line are positive and anything below it is considered background. For example, above the line are the blue/green and red lines. The red lines are the standard curve and the blue/green colored lines are a positive sample of DNA, meaning there was DNA of an invasive fish species in the area. According to the graph, our results did come back negative However, our lines are above the bottom of the graph which means that we did obtain some eDNA and correctly pumped the water in the experiment.



The image above shows the standard curve and sample readings. The curve provides us with an approximate number of copies of DNA in a tiny sample of water tested. Each sample that is tested is represented differently on the graph. The green blocks are the positive samples and the red samples are the control samples.

Conclusion:

In conclusion of this lab, our hypothesis was incorrect according to the results from Cornell University. We hypothesized that there would be several different types of invasive fish including Sea Lamprey, Round Goby, and Asian Carp at Plumb Beach and Marine Park Beach,

however, Cornell University collected the data that found that there are no invasive fish present in those waters. Even though our results came back negative, we were able to help Cornell University monitor the spread of invasive fish throughout New York.

Discussion:

'Citizen scientist' projects allow you to contribute to a project bigger than yourself; to help someone or something more than you can imagine. The role of 'citizen scientists' is to try to make the environment more efficient and comprehend what poses positive or negative effects to the environment. Our 'citizen scientists' experiment of testing samples for eDNA of invasive fish from Plumb Beach and Marine Park Beach helped a much larger experiment that pertained to all of New York.

Us 'citizen scientists' worked together to to test the types of fish in the Atlantic Ocean and if any of them were invasive fish causing threats to the area. Our samples from water collection filters tested as negative for the three fish that were tested, which included; Round Goby, Sea Lamprey and Asian Carp. Negative results are as equally important as a positive result. The ability to know where, what and how many of these invasive fish there are provides the knowledge to minimize damage to our ecosystems and the environment. Testing for eDNA of these invasive fish in other areas might have different results. Periodic testing each month for eDNA can assist in the research of invasive fish locations to limit damage to the environment.

Our ‘citizen scientists’ experiment of testing samples from the Atlantic Ocean went well overall, however, there were some complications in constructing the experiment. In this experiment, we had access to a limited amount of filter paper and moreover this paper cannot be touched for fear of contamination. The windy weather at Plumb Beach caused the filter paper to fly away and become contaminated from the sand or the fingerprints from the one’s hands that picked it up off the ground. In addition, the filtration of water process was very time consuming and difficult. A singular pump from the hand held vacuum pump only produced a minimum amount of water to be filtered. With an entire ziploc bag filled with water from Plumb Beach, the filtration process was very time consuming and not efficient.

Overall this was a fun experiment to perform, however, if we were to do it again it would need to be performed with better organization and a less windier environment. In addition, we should bring extra supplies in case of any complications or mishaps. We would definitely encourage others to do this experiment in their local waters to investigate the development and growth of invasive fish. Invasive fish have a tendency to grow tremendously and reproduce, causing these species to compete for necessary resources of native species. Although we would alter some aspects to improve this experiment, we enjoyed constructing and testing this experiment. Being able to travel to a destination to test samples for real-life problems was impressive and inspiring. It allowed us to realize that we can make a change and help our environment, even in a small way.

Bibliography:

[1]Invasive Species: How They Affect the Environment. (n.d.). Retrieved October 09, 2017, from http://www.environmentalscience.org/invasive-species

[2]Invasive alien species: a growing problem for environment and health. (2016, June 21). Retrieved October 09, 2017, from https://www.eea.europa.eu/highlights/invasive-alien-species-a-growing

[3]US Department of Commerce, NOAA National Centers for Environmental Information. (2017, October 09). Coastal Water Temperature Guide. Retrieved October 09, 2017, from https://www.nodc.noaa.gov/dsdt/cwtg/catl.html

[4]Feldkamp, L., Cornett, M., Sankar, C. L., & Byington, C. (2017, April 27). ReTurn the Favor: Horseshoe Crab Survey. Retrieved October 09, 2017, from https://blog.nature.org/science/2014/05/20/citizen-science-horseshoe-crab-shorebirds-nature-conservation/

[5]Ballast water. (n.d.). Retrieved October 09, 2017, from http://www.epa.vic.gov.au/your-environment/water/ballast-water

[6]Facts About Horseshoe Crabs and FAQ. (n.d.). Retrieved October 09, 2017, from http://myfwc.com/research/saltwater/crustaceans/horseshoe-crabs/facts/

[7]Luntz, S. (2016, August 15). How Horseshoe Crab Blood Saves Millions Of Lives. Retrieved October 09, 2017, from http://www.iflscience.com/plants-and-animals/how-horseshoe-crab-blood-saves-millions-lives

[8]W. (n.d.). Invasive Fish. Retrieved October 09, 2017, from http://invasivefish.com/