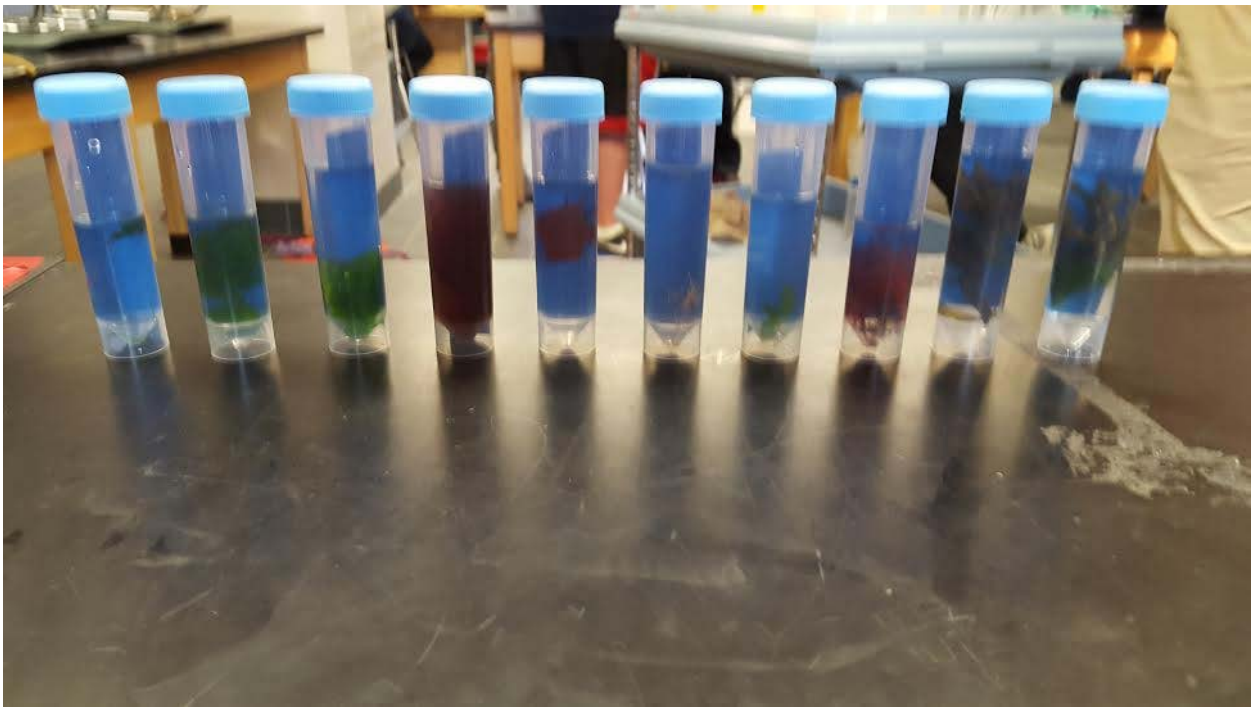
Cleaning the Harbor: A Method to Check the Mess



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Abstract

The purpose of this project is a way to check the damage that has been done using different species of algae as bioindicators to make a baseline of the water quality inside of the New York Harbor. Most of the species found were of the *Ulva* family, which usually survive and live in low nutrient waters and waters that have low human contamination, and the overall result was that the water is quite healthy and not as bad as it has been thought by the large population

Introduction

The New York City harbor has been flourished with events and creatures. For instance, geoscientists Jonathan Woodruff and Christine Brandon stated in 2016 that "The Harbor we had was amazing because of its protection. The thousands of oysters and oyster reefs protected the Harbor, even in the event of major storms and extreme waves" (J. Lathrop, March 7, 2016). Now it is faced with a much bigger event, It's death. For many years the river that has surrounded modern New York City has been in a constant state of deterioration and no one has done much to change that. In recent years, however, people have sought to heal the harbor. Organizations like the River Project and the Billion Oyster Project have attempted to soothe the wounded harbor.

While their efforts have been seen, not much is known about how poor the Harbor truly is. The project being presented is an attempt to make a baseline of the water in New York City based off of the specimen of algae found in Pier 101. These species can be considered bioindicators because they need a large array of physical chemical parameters that will affect their tolerance for the water quality they live in, while other fish and marine animals can usually tolerate much more and survive in a larger range of waters with different levels of dissolved oxygen, pH and more. The objective would be to compare the water that the algae are usually found in and the difference between the water quality there and in Pier 101. Our hope is to see a difference between those places and to set a criterion, and an ultimate goal for our neighboring water body.

Materials and Methods

Nine samples of algae were collected from the water in Pier 101 using dissection kits to cut a piece of the sample off. Photos of the samples were taken next to a ruler on a clipboard and were uploaded to the sample database. Then, pictures of the samples were uploaded as well as the locations where they were found, and an educated guess was made of what kind of species of algae were extracted.

The samples were stored in ice and taken to a DNA lab in Harlem. Here DNA was extracted from algae samples using pipettes to separate the DNA from the pellet. This is done by adding solutions to the vile such as lysis solution. The pellet was then grinded up. The sample was incubated for 10 minutes and centrifuged for 1 minute. Next the supernatant is transferred to a fresh tube and silica resin was added. The solution was mixed in, incubated (10min) and centrifuged (30sec) again. The supernatant is then removed and wash buffer is added and mixed in. The supernatant is removed again for a second round of wash buffer to be mixed in. After that the supernatant and dH_2O is added. It is incubated once more for 5min and centrifuged for 30 seconds. Finally the supernatant goes to a fresh tube and stored at -20^o Celsius. This is done to isolate the DNA from the samples and to move on to the next step. To do this PCR reagents and the DNA were added to the samples. The sample is then amplified in the thermal cycler and then stored at -20^o Celsius. DNA by PCR were amplified and prepared for Gel electrophoresis using the primers ITS and Tuf-A. The gel was created and sent to be sequenced through the DNA subway. Once the results arrived students were able to identify if the algae reflected the harbor's current condition.

Table .01: The table below shows all of the samples that received results for both primers out of the 9 samples collected. They showed an array of results and proved the high chance of most species being of the *Ulva* family.

Sample Code	KNP-002	KNP-003	KNP-004	KNP-007	KNP-008	KNP-009
Hypothesis	*Chlorophyceae*	*Chlorophyceae*	*Chlorophyceae*	*Unknown*	*Chlorophyceae*	*Rhodophyta*
Tuf-A Primers	-*Ulva laetevirens* -Uncultured Basidiomycete -*Trametes versicolor*	-*Ulva laetevirens* -*Ulva fasciata*	-Uncultured Fungus -*Muggiaea atlantica* -Uncultured eukaryote	*No sequences or results were found for the* Tuf-A *primers in sample KNP-007*	-Uncultured fungus -*Ulva procera* -*Ulva linza*	-*Trametes versicolor* -Uncultured fungus -*Ulva laetevirens* -*Ulva fasciata*
ITS Primers	-*Ulva laetevirens* -*Ulva californica* -*Ulva beytensis*	*No sequences were found for the* ITS *primers in sample* KNP-003	-*Ulva laetevirens* -*Ulva procera* -*Ulva linza*	-*Ulva laetevirens* -*Ulva fasciata*	*No DNA results came back for the* ITS *primers of sample* KNP-008	-*Terana caerulea* -Uncultured Fungus -*Terana caerulea* -Uncultured fungus -*Terana caerulea*

Results

From the DNA that was extracted, many results were obtained. Most of the species that were found were using the primers ITS and Tuf-A. The specimen found were mostly from the *Ulva* family, including the *Ulva laetevirens, U. californica, U. beytensis, U. lactuca, U. fasciata, U. procera* and *U. Linza.* These varied from the vials of KNP-002 to 004, 007-009 and most of them were of the chlorophyceae family, or green algae, giving indication that the water is healthier than bodies of water that could include giant algal blooms or high quantities of cyanobacteria, and because these species live in low nutrient waters (M.D. Guiry, 2016) or waters that have low human contamination, they show greater indication that the New York Harbor is not in as much peril as many people thought. However, while there were mostly results for chlorophyceae, there were other results, mostly from fungi, such as uncultured fungus, *Trametes versicolor,* and *Terana caerulea* that looked like terrestrial fungi and a few unexpected bacteria, such as the *Muggiaea atlantica*.

Discussion

From the results that were conceived, the ultimate answer was that there were mostly species of the *Ulva* family, which signified that the Harbor was doing better than expected. No cyanobacteria was seen that could have shown a decrease of dissolved oxygen. (A. Brown & R.Carpenter, 2013) There was no excess of algae or signs of an algal bloom in Pier 101 the days that the samples were collected, and many chlorophyceae samples were found. However, while

most of the results were of the *Ulva* family, there were several results that were unexpected. For one, some species extracted were fungi, such as *T. versicolor* or *T. caerulea* were found, species of fungi that looked a lot like terrestrial fungi. Also, some species found were unknown and even unexpected, like the *M. atlantica* a species of zooplankton found or the uncultured eukaryotes in the Tuf-A primer of sample KNP-004. While being out of the norm, these species could have reached the lab for several reasons. For instance, the tide was unknown when the samples were collected. If there was a flood tide during the time we collected samples, small zooplankton could have floated up to Governors Island. Another reason why these species could have been found may have been with the equipment. It is not known if the dissection kits used to take samples were disinfected, so there could have fungi or samples of completely different species of animals on them. One of the final explanations thought of for why there were unexpected results could have been found inside of the primers given. The ITS primers used are specified for fungi and the Tuf-A was made for microalgae, being one of the possible causes for why terrestrial fungi like *T. caerulea* was found in sample KNP-009 for both ITS-F and ITS-R. Lastly, for many species

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