Effects of Water Quality on Algae

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Abstract

Algae can be used as indicators for the presence of pollutants and detrimental environmental alterations. The presence of algal blooms indicate multiple factors such as pollution from runoff or environmental alterations that reduce water flow such as dams, weirs, and irrigation systems. During this experiment we aimed to determine the effects of pollution on algae populations and how they behave as parts of their ecosystem by barcoding algae samples and determining the quality of the water. The samples were obtained through collection from several different bodies of water with sterilized falcon tubes or water bottles and then transported back to our school where the DNA was extracted, purified, amplified (PCR), and electrophoresed. The samples were then compared with identified DNA sequences and the water quality was assessed to see if there was any correlation. The results of that we received after the sequencing were unexpected. While a multitude of our collected samples did not yield any identifiable results, the one result that we were able to obtain contained Drosophila melanogaster DNA. This could be the result of potential contamination or poor preservation of our collected samples. As a result, our procedure was unable to yield conclusive results; however, if the algae in our sample truly did contain melanogaster DNA, that could signify that there was either a coincidental mutation or that the sequence that was amplified was a conserved sequence.

Introduction

Algae are photosynthetic organisms that mostly exist in aquatic environments. While many of these organisms are beneficial for the environment, they can also be extremely harmful; some providing up to 70 percent of the oxygen that we breathe, while others appearing only when body of water is no longer healthy and scavenging what resources are left. During recent periods, scientist has observed an increase in the amount of nitrogen and phosphorous that are released from the usage of fertilizers into the native habitats of the algae. This excess of nutrients leads to rapid growths in the algae population, which leads to algae blooms: rapid algae growth which, when uncontrolled, can lead to anoxic conditions that kills both fish and invertebrates indiscriminately. However, it's also important to note that Algal blooms are also furthered by an environment with minimal turbulence and long retention times; all provided by the numerous ways of water retentions that we have crafted over the ages. When blooms are present, it can be said to indicate one or more of theses factors and thus algae can be used a indicators for pollution and detrimental environmental alterations. Our hope is that since they can be used as indicators for some types of pollution, one day, they might be able to show the effects of other types of pollution as well. These occurrences brings with them a multitude of questions such as: What does algae indicate about the pollution in the various waters of New York City? How does comparing algae from different bodies of water help us learn more about the water quality of the various bodies of water in New York City? The objective of our project was to not only determine the biodiversity of algae in New York City, but also to find out whether or not water pollution can impact the way algae acts as part of its environment.

Materials & Methods

Sampling:
The algae samples that were used for our experiment were obtained from several different bodies of water in New York City at surface level: for smaller bodies of water, one sample was collected and for larger bodies of water, multiple samples were collected from multiple locations. We collected from the Upper Bay, the Hudson River, the Little Neck Bay, the Meadow Lake, the Flushing Bay, the Central Park Lake, and the Alley Pond Park in Queens. Our goal was to collect from many sources throughout the boroughs in order to see if the algae species varies from one location to another in New York City. We also tested the water quality to see if it had any impact on the algae's genetics. We tested for pH, nitrogen and phosphorus percentage. In total, we collected eighteen samples which were usually obtained through getting close to the water and filling up sterilized Falcon tubes or water bottles with water and, if possible, large pieces of algae. In order to ensure that we've gotten enough DNA to work with, we tried to collect samples with visible pieces of algae or made sure that the water was at least green.

Laboratory and bioinformatics protocol:
In order to obtain the necessary DNA sequence, we first extracted the DNA by transferring 1.5 mL of the water samples into pre-labeled Eppendorf tubes, then we spun down the tubes in the microcentrifuge to separate algae protists from the water. The water was removed and saved for later testing while the resulting pellets containing the algae were exposed to lysis buffer and mashed to aid the lysis process. The mashed pellets in lysis buffer were incubated in a 67 degree Celsius hot water bath for 30 minutes. After the buffer had turned green, we retrieved the samples and removed the supernatant.
The DNA-containing supernatant was then subjected to silica resin beads, and incubated in a 57 Celsius degree hot water bath for 5 minutes. After centrifugation, the silica resin pellet, which should have the DNA bound to it, was washed in alcohol repeatedly to get rid of the excess protein and other cellular debris. This involved several centrifugation steps and waiting at the end to ensure alcohol evaporation. Then deionized distilled water was added to the dry pellets, and the pellets were incubated again at 57 degrees Celsius. In terms of storage, we stored the DNA in deionized distilled water in Eppendorf tubes. For long-term storage, the tubes were placed in a freezer. After extracting the DNA, we used PCR to amplify the desired sequence and performed gel electrophoresis to separate the DNA that we've amplified according to size. We used a Mastercycler for the PCR, as well as NEB Master Mix and TufA algae primer. We cast the 2% agarose gel and using micropipettes, loaded samples which have been mixed with tracking dye and SYBR Green to allow visual monitoring. We also loaded a 100 kb ladder standard. Viewing the gel under UV light, we were able to see faint bands of DNA. We photographed the gel and uploaded to UBP's database, and sent the PCR products to GeneWiz for sequencing.

Testing water quality:
We placed pH paper in the water sample and wait for the color to change. Then we matched the color to the given pH color chart and determined the pH of each sample. We used a Phosphate Test Kit to measure the amount of phosphate in each sample. We put six drops of each phosphate test solution into 5ml of each sample and matched the color with the chart after shaking the solution and waiting 3 minutes. To do a Nitrate Test we used a similar kit which instructed us to put 5 drops of the testing solution into 5ml of the water sample and wait 5 minutes after shaking and then match the color of the solution with the color chart.

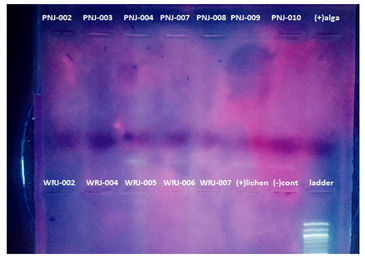
Picture of Hudson Samples

Results

The sequences were too short to determine the identity of the organisms that were in our water samples. The only identifiable one was *Drosophilia melanogaster,* an insect, not algae. It was found in specimen number PNJ-008 which had a pH of 6.5 and 4 ppm of Phosphate. None of the water samples tested positive for Nitrate, they all contained 0 ppm. The pH varied among the samples, ranging from 5 to 8. The amount of Phosphate varies the most, ranging from 0 to 10.

Tables & Figures

Figure 1: Gel Electrophoresis of Algae Samples after PCR



Very faint bands were visible for PNJ-004, 007, 008, 009, and 010. Primer dimers are visible, and brighter than the product bands. Positive algal control did not show a band.

Table 2: Quality of Water Specimens

Specimen Number	pH	Phosphate	Nitrate
PNJ-004	7.5	0.25 ppm	0 ppm
PNJ-005	6	0.25 ppm	0 ppm
PNJ-009	8	0 ppm	0 ppm
PNJ-011	7	7 ppm	0 ppm
PNJ-013	5	4 ppm	0 ppm
PNJ-014	6	8 ppm	0 ppm
PNJ-015	8	10 ppm	0 ppm

Figure 2: Sequence for PNJ-008-F TTGGTGGATTTGGAATTTGATTANTGCC TTTAATNTTAGGTGCTCCTGATATAGCATTCCNACGAATAAATAANATAAGATTTTGACTACTACCTCCTGCTCTTTCTTTACTANTANTAAGTAGAATAGTTGAAAATGGAGCTGAAACATGATGAACTGNTTATCCANCTTTATC

BLAST Results for PNJ-008-F

#	Accession #	Details	Aln. Length	Bit Score	e	Mis- matches
1(1).	U37541.1	Drosophila melanogaster - genome	175	273	2e-70	11
2(2).	NC_028518.1	Drosophila formosana - genome	175	273	2e-70	11
3(3).	M57910.1	Drosophila melanogaster - c oxidase subunit I (COI) gene, 5' end, Trp-, Cys-, and Tyr-tRNA genes,NADH dehydrogenase subunit 2 (ND2) gene, 3' end	175	273	2e-70	11
4(4).	KY559392.1	Drosophila melanogaster - mitochondrion, partial genome	175	273	2e-70	11
5(5).	KY559391.1	Drosophila melanogaster - mitochondrion, partial genome	175	273	2e-70	11
6(6).	KY559390.1	Drosophila melanogaster - mitochondrion, partial genome	175	273	2e-70	11
7(7).	KY559389.1	Drosophila melanogaster - mitochondrion, partial genome	175	273	2e-70	11
8(8).	KT174472.1	Drosophila melanogaster - genome	175	273	2e-70	11
9(9).	KR265324.1	Drosophila formosana - genome	175	273	2e-70	11
10(10).	KP843852.1	Drosophila melanogaster - mitochondrion, complete genome	175	273	2e-70	11

Discussion

There were many unforeseen obstacles and astonishing results that came with our experiment. After running the gels we discovered that the PCR bands were smaller than they should've been and our sequences were too short to be identified. However, what was truly unexpected was that one of the samples that had an identifiable result seemed to have *Drosophila melanogaster* DNA. While there is a possibility that invertebrates might have been present in our sample, it was surprising that there was enough of the DNA in our PCR product to identify it clearly as Drosophila DNA. Furthermore, our primers were algae specific and the bands produced during electrophoresis didn't match up to the size of normal Drosophila bands either. This had led to a question: is it possible for algae and Drosophila melanogaster to have the same DNA? And if not, could the environment have indirectly caused a mutation to occur? It has been shown to be possible for organisms to have similar, if not the same, sequences in areas that are important for general life function. During our experiment we only utilized BLAST to help find and identify matching sequences and we might be able to find a match between our samples and other algae if we use other programs.

Our samples did not give us the expected results due to multiple possible sources of error. There was a risk of contamination since some of the samples were stored in plastic bottles and others were not refrigerated. They were not stored in the ideal environments for preservation so algae that may have originally been in the sample may have been contaminated. In addition, when collecting samples, water was collected in large amounts from the surface so it is possible that there were invertebrates mixed in with the water. This method of collection also allowed debris to be in the sample, making it more difficult to isolate the algae. We should have washed the algae or cleaned it properly before extraction. In addition, the DNA we extracted was not very clean. We also can't discount the possibility of errors during the sequencing setup.
It is also possible, though unlikely, that the algae primers were mixed up with the mosquito primers which were stored in the same freezer.
Because of the fact that we only used one type of primer and were not able to attempt multiple extractions and PCRs due to a lack of time and resources, we were not able to get any conclusive results.

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Acknowledgements

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