Utilizing DNA Barcoding to Identify Plankton Species in the Long Island Sound and Hempstead Harbor Region

Denise Obaji[1], Desiree Obaji[2], Oscar J. Zagalo[3], Nidhi Gadura PhD[3]

[1]Townsend Harris High School Flushing, NY, [2]Queens Gateway to Health Sciences Jamaica, NY, [3]Queensborough Community College, CUNY

Abstract

The Coalition to Save Hempstead Harbor (CSHH) has recently worked as a partner to develop a report card for Hempstead Harbor, located in New York State. However, in the beginning of May 2015, records of a sludge had been reported at the harbor. This sludge was discovered to be pollen slicks, mixed with algal bloom. These pollen slicks appeared to rise to the water's surface. When the pollen begins to decay, the sludge turned brown, mixing with the vegetation and algae in the area, affecting the turbidity of the water. Water turbidity signals the amount of sunlight which reaches underwater plants, depending on the clarity of the water. The cloudier the water, the more turbid it is classified. In result, aquatic plants will die if they do not receive enough sunlight. Fewer plants also means less food for aquatic species. It was concluded that this situation is occurring at Hempstead Harbor due to the fact that when water samples collected at the harbor were measured for temperature, salinity, dissolved oxygen (DO), pH, turbidity, all parameters were consistent throughout the season except for turbidity.

This research project was completed to comprehend which organism(s) caused the pollen slicks to occur at the Harbor. The water samples collected at Hempstead Harbor were measured for temperature, salinity, dissolved oxygen (DO), pH, turbidity, etc. In result, all parameters were consistent throughout the season except for turbidity. It was hypothesized that plankton species were responsible for the increase in water turbidity. Through DNA extraction, PCR for the *rbcL* and *COI* gene and the sequencing of the PCR product, various plant species were analyzed and identified. Results revealed that *Bathycoccus prasinos* (Picoplankton) and *Cucumis sativus* (Cucumbers) were most prevalent within the waters, causing this drastic change in turbidity at Hempstead Harbor.

Introduction

The process of identifying organisms has become significant for scientists. DNA barcoding is a unique method utilized to identify living species. DNA barcoding depends on short regions, between 400-800 base pairs long, of the genome. Through the polymerase chain reaction **(PCR)**, thousands of copies per cell, mitochondrial and chloroplast sequences are amplified. An area of the chloroplast gene ribulose 1, 5 bisphosphate carboxylase/ oxygenase (RUBISCO), is coded into the *rbcL* gene, which is used to recognize plant species. An area of the mitochondrial gene, called *COI,* is used to recognize animal species.

In this research project, an experiment was completed to identify the factors causing the change in turbidity at Hempstead Harbor. Through the usage of DNA extraction, PCR for the *rbcL* and *COI* gene and the sequencing of the extracted DNA, various plant species in Hempstead Harbor region were analyzed and identified.

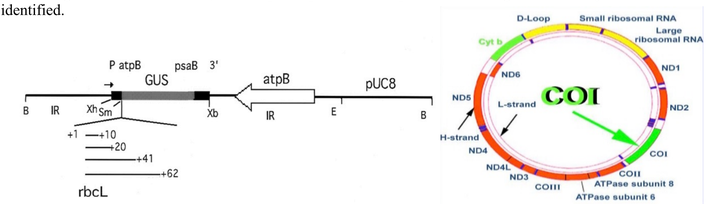


Figure 1; CSHH Stations at Hempstead Harbor *(#1-#16)*

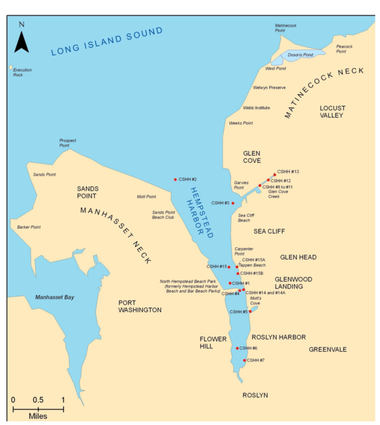


Figure 2; Pollen Slicks Discovered at the Surface of the Water

Hypothesis

It was hypothesized that the identification of either zoo or phytoplankton can lead to a explanation for the inconsistency in the turbidity. When phytoplankton, or algae grow in significant numbers and produce biotoxins, these events are termed harmful algal blooms or HABs. These blooms can have deleterious affects on both other aquatic life and on those who depend on that water for subsistence. Scum from these algal blooms range from the colors is green to blue-green, have an oily shine, resembling that of a motor slick, and form a thick, soupy mass on the surface of the water.

Materials

- Filter Paper
- Samples
- Guanidine Hydrochloride
- Pestle
- Silica Resin
- Centrifuge
- Micropipette Kit
- BioFluid and Cell Buffer
- Proteinase K
- Genomic Binding Buffer
- Zymo-Spin IIC-CL Columns
- Pre-wash Buffers
- G-DNA Wash Buffers
- PCR Machine
- Premade Gel or agarose
- PBR322/BstNI Marker
- Thermal Cycler
- UV transillumination

Methodology

DNA Extraction:

1. Collect 10-20 mg of phytoplankton and zooplankton tissue from the water samples through filtration and into separate tubes.
2. Separate DNA pellet from supernant using silica resin incubated at 57°C. Discard of the supernant.
3. Add 500 uL of ice cold wash buffer into the pellets of each tube and mix.
4. Centrifuge tubes for 30 seconds at maximum speed.
5. Repeat steps 6-8 again, then add 80 uL of distilled water into the silica resins within each tube and mix well.
6. Incubate tubes at 57°C for 5 minutes and centrifuge tubes for 30 seconds at maximum speed.
7. Add 70 uL of the supernatant were then transferred from the tubes and into two fresh tubes. Store at -20°C.

PCR Amplification of *rbcL;*

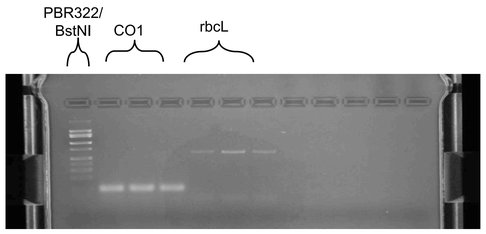
1. Obtain 6 PCR tubes and add 10 uL of an rbcL primer into each tube.
2. Obtain 3 PCR tubes and add 10.5 uL of a *C01* primer into the tubes.
3. Using a micropipette with a fresh tip, add 12.5 uL of the Taq 2x Master mix into each PCR tube and mix by pipetting up and down.
4. Directly add 2 uL of DNA from the Genomic DNA into the appropriate primer/Taq mixture with a fresh tubed micropipette.
5. Store the samples within ice until ready to begin the PCR.
6. Place the tubes within the PCR, programmed at the appropriate PCR protocol.
7. After thermal cycling, store the amplified DNA in ice or at -20°C until ready to go on to gel electrophoresis.

Gel Electrophoresis;

1. Create a gel by pouring agarose into a flat container and allowing it to set for 20 minutes.
2. Using a micropipette with a fresh tip, load each sample into its assigned well, saving the first well for the PBR322/BstNI marker.
3. Store the remaining 20 uL of PCR products on ice of -20°C until the samples are to be submitted for sequencing.
4. Run the gel for 30 minutes at 130 volts. Allow the dye front to move at least 50 mm from the wells.
5. After, view the gel using a UV transillumination. Take photographs using a photo documentary system.

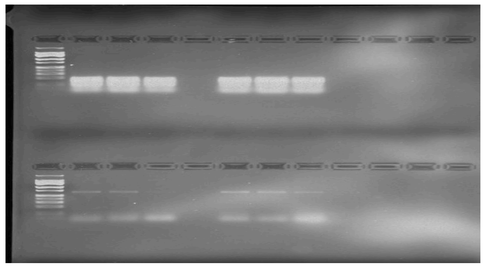
Results

Figure 3; Gel Electrophoresis for Stations #1 and #16



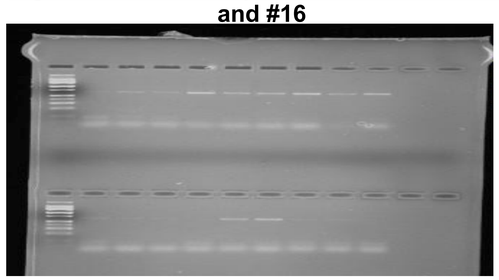
11-03-2015 (DNA 1B) CO1:1, 2, 3 RbcL: 1, 2, 3

Figure 4; Gel Electrophoresis for Stations #1 and #16



11-17-15 *Top: CO1 (DNA 1D): 1, 2, 3/(DNA16D): 1, 2, 3*
Bottom: rbcL (DNA 1D): 1, 2, 3/(DNA 16D): 1, 2, 3

Figure 5; Gel Electrophoresis for Stations #1 and #16



2-3-15 Top: CO1 (DNA 1A,B,C)
Bottom: rbcL (DNA-16A,B,&C)

Figure 6; DNA Subway BLAST Sequence Data

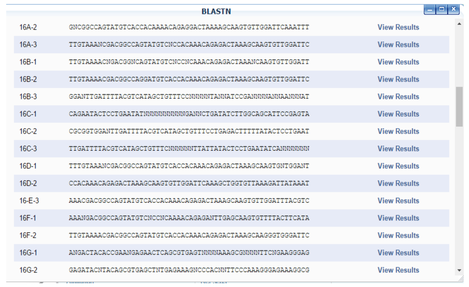


Figure 7; SPECIES IDENTIFIED BY THE STUDY

1B	1B-1	Bathycoccus pra	Picoplankton
	1B-2	Bathycoccus pra	Picoplankton
	1B-3	Bathycoccus pra	Picoplankton
	1C-3	Cucumis sativus	Cucumber
1D	1D-1	Cucumis sativus	Cucumber
	1D-2	Cucumis sativus	Cucumber
	1F-3	Cucumis sativus	Cucumber
1G	1F-4	Bathycoccus pra	Picoplankton
	1G-2	Cucumis sativus	Cucumber
	1G-3	N/A	N/A
1F	1F-1	N/A	N/A
	1F-2	N/A	N/A
	1F-3	Cucumis sativus	Cucumber
1G	1G-1	Bathycoccus pra	Picoplankton
	1G-2	Cucumis sativus	Cucumber

Discussion and Conclusion

- The gel electrophoresis analysis of the PCR products displays that the DNA extraction procedure worked, yet only the *rbcL* primers were able to yield a PCR product.
- The *rbcL* primers, which is utilized by researchers to detect plants species, detected species such as the picoplankton, which is a phytoplankton, and the cucumbers within the water. We don't fully understand why plants like cucumbers were detected.
- Though the *CO1* primer did not work well, it was hypothesized that the *CO1* primers were not at the right concentration.
- Also, the PCR products using the *rbcL* primers were sent out for sequencing, but the sequencing results were unclear and some PCR products were not able to be sequenced.
- Perhaps DNA Barcoding with rbcL primers is not the appropriate way to identify plankton species. Further testing is needed to clarify these results.

Acknowledgments

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- We would like to thank Carol DiPaolo, CSHH Programs Director and Water-Monitoring Coordinator for collecting all water samples for our project from 7/15/15 to 10/29/15. http://coalitiontosavehempsteadharbor.org
- Reference: Harbor News June 18, 2015 http://coalitiontosavehempsteadharbor.org/wp-content/uploads/HarborNews-2015-June-18.pdf
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