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

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Abstract

Hempstead Harbor (Cucumbers) were most prevalent within the waters, causing this drastic change in turbidity at Hempstead Harbor. This research project was completed to comprehend which organism(s) caused the pollen slicks to occur at the harbor. 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. It was hypothesized that plankton species was 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 plankton species were analyzed and identified. Results revealed that Bathycoccus prasioides (Peridinium) and Cucumis sativus (Cucumbers) were most prevalent within the waters, causing this drastic change in turbidity at Hempstead Harbor.

Discussion and Conclusion

The process of identifying organisms has become significant for scientists. DNA barcoding is a unique photo documentary system. The lab part of the project was partially funded by U.S. Department of Education QCC - MSEIP Grant to Dr. Gadura.

References


Materials

- Filter Paper
- Sample tubes
- Glacial Acetic Acid
- Pollen
- SiliCubes
- Microplate Kit
- BioRad and Gel Buffer
- Protease K
- Genome Binding Buffer
- Pre-Wash Buffer
- GDA Wash Buffer
- PCR Machine
- Premade Gd or agarose
- PBR322/Stbl3 Marker
- Thermal/Cycler
- UV Transilluminator

Methodology

DNA Extraction

1. Collect 100 mg of phytoplankton and zooplankton tissue from the water samples through filtration and separate into two tubes.
2. Separate DNA pellet from supernatant using silica resin columns.
3. Incubate tubes at 57°C for 5 minutes and centrifuge tubes for 30 seconds at maximum speed.
4. Centrifuge tubes for 30 seconds at maximum speed.
5. Separate DNA pellet from supernatant were then transferred from the tubes and into two fresh tubes. Store at -20°C.

PCR Amplification of rbcL

1. Obtain 5 PCR tubes and add 10 μL of an in vitro primer into each tube.
2. Obtain 5 PCR tubes and add 10 μL of a COI primer into the tubes.
3. Using a micropipette with a fresh tip, add 12.5 μL of the QiaAmp Master mix into each tube and mix by pipetting up and down.
4. Directly add 2 μL of DNA from the genomic DNA into the appropriate primer/Tag mix with a fresh pipette tip.
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 -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, setting the first well for the PBR322/Stbl3 Marker.
3. Store the remaining 20 μL 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 100 volts. Allow the dye front to move at 250 mm from the wells.
5. View the gel using a UV transilluminator. Take photographs using a photo-documentary system.

Hypothesis

It was hypothesized that the identification of either zooplankton or phytoplankton can lead to an explanation for the inconsistency in the turbidity. When phytoplankton, or algae grow in significant numbers and produce biotoxins, these blooms can have deleterious affect on both aquatic life and on those who depend on that water for sustenance. Stem from these algal blooms range from the colors in 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.