Sea Creatures In New York: Comparing The Phylogeny Of Saltwater And Freshwater Aquatic Invertebrates

Arham Alrowmeim, Lance Cruz, Gregory Francis, Anahi Joya, Ciel Tejera

Sunset Park High School; Genspace

Cold Spring Harbor Laboratory DNA Learning Center

Funded by the Thompson Family Foundation

Abstract

New York City homes a variety of aquatic ecosystems — perfect for scientists looking to conduct their research right here, in the heart of New York.

Of the inhabitants in these ecosystems, aquatic invertebrates are one of them, each invertebrate population different in its own ways.

Some invertebrates are accustomed to salty environments, while others are suited for freshwater. Our team took aquatic invertebrates samples from two different ecosystems, Prospect Park and Bush Terminal Park, hoping to extract DNA from our samples and use their genetic data to create a phylogenetic tree to compare saltwater and freshwater invertebrates.

However, our team ran into a problem — sequencing failure. Our team looked back at our experiment, troubleshooting the DNA extraction process. Why did we receive uninformative sequences, could it have been cross contamination or human error? Or perhaps our experiment failed because of something beyond our control — DNA degradation.

Introduction

Bush Terminal Park and Prospect Park are field sites near our school, Sunset Park High School. Science enthusiasts and citizen scientists travel to these sites to conduct research, such as the many students that engaged in the Billion Oysters Project, a project dedicated to introducing oysters back to the Hudson Estuary.

By investigating the biodiversity of these two sites, our group hoped to not only compare and contrast the populations of the two ecosystems, but to also determine the health of the ecosystems by measuring its biodiversity. Concluding that Bush Terminal Park and Prospect Park are two different and healthy ecosystems could be enough to inspire more New Yorkers to utilize their neighborhoods for the good of science.

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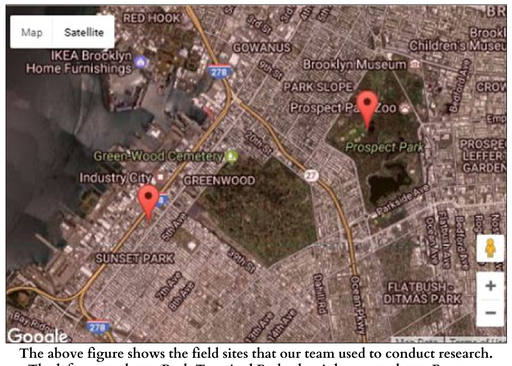
Materials & Methods

Our research was conducted at Prospect Park and Bush Terminal Park, two field sites near Sunset Park High School. Our team was granted access to use the field sites for research, as our project mentor received a New York City park permit. To collect the aquatic invertebrates at both sites, our team used a plankton net, which was dragged through each body of water multiple times for one minute. To accurately determine the identity of our specimen, its genetic makeup had to be obtained through DNA extraction. Our group is networked with Genspace, a BSL-1 laboratory located in Brooklyn; the lab allowed our group to use their equipment for DNA extraction:

1. To begin the DNA extraction process, our specimen's DNA must be isolated.
2. The following cycles involved using a wash buffer, which acts on the pH of the solution and further cleans the DNA.
3. Polymerase Chain Reaction, or PCR for short, is the next step in the DNA extraction process. PCR amplifies the DNA of specimen, which will allow a solution to be sequenced.
4. Before sending our amplified DNA to be sequenced, we had to ensure that our PCR was successful. We ran our PCR products through gel electrophoresis, a process that allows us to determine the molecular weight of our product.

Unfortunately, our PCR results for some of our samples were faint; DNA was clearly lost somewhere in the DNA extracting process. Despite the faint DNA, our group sent some of the strongest solutions out for sequencing. The following results were shown on DNA subway, a bioinformatic web tool.



The above figure shows the field sites that our team used to conduct research. The left arrow shows Bush Terminal Park; the right arrow shows Prospect Park.

Results

SAMPLE	CONSENSUS SEQUENCE
WNJ-002	TTAGTGAGGCCCTCCGATCNGCCCCNC CNNNNNNNNGNCNCTTGNNAAANGAC GGNCCAGTGGTCAACAAATCATAAAG ATATTGGCCTTCGTTTTCTTGGTGATTT TTTGGTCACCCTGAAGTTTAGTCATAG CTGTTTCCTGANCNNTNNAACGTGACT ATCCAAAGGAAGTAAAACTCGAAACA AGGCTTCCGTAAGTGAACCTGGACAA GGATCAAA
WNJ-004	GGCCTTCGTTTTCTTGGTGATTTTTTGG TCACCCTGAAGTTTAGTCATAGCTGTT TCCTGAGCCNTNNNGGCCGGCGGGGC CGATCCGAGGGCCNCACTAAACCACC CTNCCGGAGNTTTGCTCTTTTCCCAGG TTCACTTNCGNAAACGAGGTTACGNCT TGCACTTCGCTCAGATCGTCAANNTCN NNNNNNNNCNTTGTAAAACGNNNNNN CAGTGGTCAAC
WNJ-006	TTGTAAAACGACGGCCAGTGGTCAAC AAATCATAAAGATAATGGCCTTCGTTT TCTTGGTGATTTTTTGGTCACCCTGAA GTTTAGCCATAGCTGTTTCCTG

Of our sequenced data, all samples were too short or uninformative to identify a species. The above figure shows the consensus sequence of each sample; however, the forward and reverse sequences each had their own areas of no DNA, making the consensuses insignificant. With uninformative sequences, our team was unable to match our specimen to species, create an evolutionary tree, and compare freshwater and saltwater invertebrates.

Discussion

DNA extraction is not always easy. Often students, citizen scientists, and even professional scientists take the simplicity of the DNA extraction process for granted and attempt to rush through the procedure. **Any slight variation in an amount of a reagent or to an incubation temperature can ruin a project** — a probable reason for the unsubstantial sequences from our solutions. For example, too little of primer mix will cause amplification to not occur during Polymerase Chain Reaction; too much primer mix can lead to primer dimerization of samples (bioSynthesis, n.d.)

Human error could have also come to play; using wrong pipettes or entering the wrong volume on the device could significantly skew reagent concentrations.

Will Schindel, laboratory manager at Genspace, suggested **cross contamination** as the reason behind our sequencing failure. Our team shared the space with other students and laboratory members, it was very possible for cross contamination to occur between samples.

PCR inhibition could have also disturbed the success of our DNA amplification. A PCR inhibitor is a substance that inactivates the enzymes necessary for DNA amplification to occur. According to the Journal of Applied Microbiology: "PCR inhibitors can be found in a variety of biological materials (organs, blood, body fluids etc.), environmental samples (water, soil, air etc.) and food (meat, milk, fruits, vegetables, seafood etc.)" (Schrader, n.d.). The probability of a PCR inhibitor being in our solution is high, as we collected our samples at parks — areas with soil and water.

Sample degradation is another possible reason for the lack of DNA in our amplified solutions. Due to scheduling issues, our team could only take weekly trips to Genspace. The extended time between steps of the procedure may have had an effect on the quality of our DNA; it could have possibly caused the DNA to degrade.

Acknowledgements

Dr. Ellen Jorgensen and William Shindel of Genspace NYC for their advice and guidance during the DNA Extracting process; New York's Park Department for granting us a permit; our project mentor, Ms. Megan Wallner, for her incredible support throughout our project.