Species Richness of Macroinvertebrates in New York Harbor: Buttermilk Channel

ImageCredit: Ernst Haeckel

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Abstract

The purpose of this research was to learn how to extract DNA from invertebrates, and to discover the species richness of the Buttermilk Channel. Another goal of this research project was to compare the effectiveness of the Folmer and Leroy primers. We had hypothesized that there would be little

biodiversity because of New York's history of dredging and heavy boat traffic. This turned out to be true as we found many of our tentative sponges and tunicates to be of the Botrylloides and Botryllus genus. We also found many amphipods that were closely related if not the same exact species. This indicates that the Buttermilk Channel is not very diverse but will with more and more awareness it will become so.

Introduction

Invertebrates are one of the oldest and most diverse group of organisms (Alroy, Aberhan, and Bottjer, 2008). Per the International Union for Conservation of Nature (IUCN) over 1.3 million different invertebrates have been identified as of 2009. The biodiversity in the Hudson-Raritan estuary is a key factor in creating a healthy and sustainable ecosystem. The biodiversity index is an overarching topic that includes; biodiversity, species richness and species evenness. In this project, we focus on the species richness. The more species that are present in a single area, the healthier the ecosystem. The biodiversity of an area is also a bioindicator of that ecosystem. A bioindicator is a living organism(s) that shows how healthy an ecosystem is, because it may not be noticeable to the naked eye. Many organisms are sensitive to the slightest change in their environment, so if pollutants are present, the organism may change its morphology, physiology and behaviour, or simply die. In this research, we collected samples of invertebrates from the Hudson-Raritan Estuary, specifically from Buttermilk Channel. Invertebrates include polychaetes, sponges, tunicates, colonial sea squirts, and mollusks. Invertebrates are one of the most diverse organisms. The reason invertebrates are being utilized is because they have a relatively brief life cycle, and their anatomy is not particularly complex, thereby allowing the researchers to more easily study them and draw appropriate and accurate conclusions.

Background Information

The gene that was studied was Cytochrome Oxidase subunit 1 (CO1). This is the primary gene used when genetically barcoding animals because of its fast duplication rate. This allows for little confusion when differentiating closely related species (Rach, Bergmann, Paknia, DeSalle, Schierwater, and Hadrys, 2017). The animals or organisms that classify as invertebrates are animals that neither possesses nor develop a vertebral column. There are many different types of invertebrates like oysters, polychaetes, crabs and many more. There are also different types of polychaetes. The differences can be seen in the anatomy of the Polychaeta. Depending on what species it is, the head, the body and even the way it interacts with the world around it can be different.

Methods

As shown by DNA barcoding: To isolate the DNA of the invertebrate samples that were collected, lysis solution was be added to it. The sample was then ground in the lysis solution. After this the ground the sample was be incubated at 65 degree Celsius for 10 minutes and was then centrifuged for 1 minute to separate the supernatant on top of the DNA sample which is at the bottom of the sample tube. The supernatant was then transferred to a fresh tube and silica resin was added to it. The supernatant and the silica resin were mixed together and incubated for 5 minutes at 57 degrees Celsius. After it was centrifuged for 30 seconds, the supernatant was removed and a wash buffer was added to it. The sample tube was then put into the vortex and was centrifuged for an additional 30 seconds. After this process was completed, the supernatant was removed and wash buffer was once again added. After the wash buffer was added for a second time, the sample was put into the vortex and once again centrifuged for 30 seconds. Then the supernatant was removed and distilled water (dH20) was added. It was mixed by

pipetting in and out until the pellet was dissolved. It was then incubated for 5 minutes at 57 degrees Celsius. After that it was centrifuged for 30 seconds and the supernatant was transferred to a fresh tube and stored at -20 degrees Celsius.

To amplify the DNA and sequence and analyze the PCR product there were nine (9) steps that needed to be completed in order to get accurate results. The isolated DNA sample was first defrosted. Then primer mix LepR1_t1 (used for Mammals, reptiles, fish, amphibians, and some insects) was added to a clean tube and the DNA added. Then it was placed in the thermal cycler to heat the sample so the DNA could separate into two pieces of single-stranded DNA. The gene being separated will be Cytochrome oxide subunit 1. Then the PCR products were analyzed using gel electrophoresis. Gel electrophoresis is a method used to separate and analyze micromolecules (DNA, RNA and proteins) and their fragments, based on their size and charge. The gel was poured into the gel electrophoresis chamber and let set for 20 minutes. After the gel set, the electrophoresis machine was set 130 volts for 30 minutes. Then the sample was sent to be analyzed using bioinformants.

Results

The software program that was used was DNA Subway. This program brings together the key bioinformants to assemble gene models which makes it easier to analyze data and study the organism's genetic makeup. As you can see in the consensus chart, samples with similar base pair patterns were grouped together. We found that FCT-003, 004, 006, and 007 were most likely *Botryllus sohlosseri* (golden star tunicate) and closely related species. In many of our samples there are large chunks where there is no data, this is likely due to human error. If the sample had too many missing gaps, the program could not generate a possible species and was left blank, this was the case with FCT-010 and 021. As for the Folmer and Leroy primers; the Leroy primer produced species names when the Folmer primer could not, however the same thing happened when the Leroy primer had no answer. We did not have the opportunity to accurately test whether these results were correct.

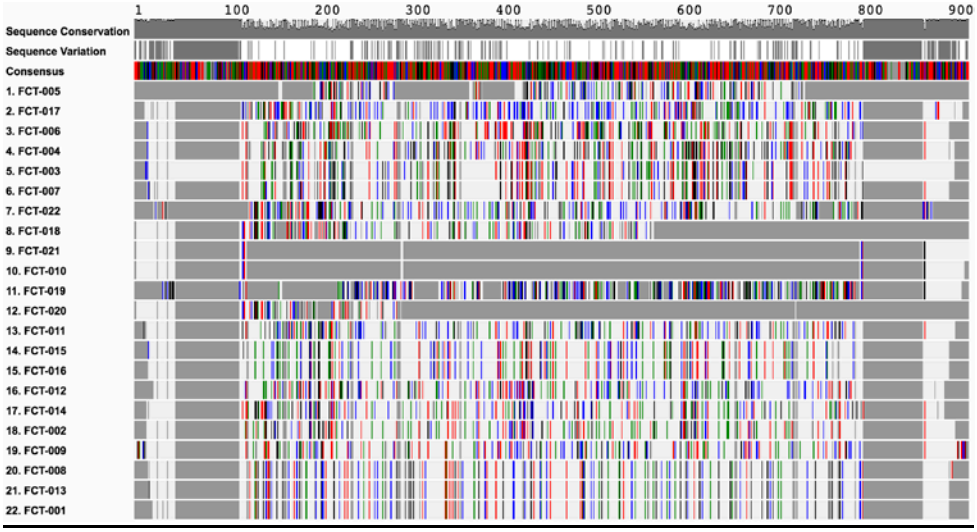

Figure 1: Consensus chart for all samples sequenced from the Folmer primer

Anemabuddies Gel 1		Folmer et al Primer 700bp	Comments		Leroy Primer 400pb	Comments
FCT-001	Halichondria panicea	sponge		FCT-001	Halichondria panicea	
FCT-002	Urosalpinx cinerea	snail		FCT-002	Urosalpinx cinerea	
FCT-003	Botryllus schlosseri	golden start tunicate			no PCR product	
FCT-004	Botrylloides violaceus	colonial tunicate		FCT-004	Botrylloides violaceum	
FCT-005	Pione sp./Stephanauge nexilis (?)		many mismatches	FCT-005	Bonneviella regia	
FCT-006	Molgula manhattensis	Sea Grape Tunicate/sea squirt		FCT-006	Hydra vulgaris	
FCT-007	Botryllus schlosseri	golden start tunicate		FCT-007	Botryllus schlosseri	
FCT-008	Halichondria panicea	breadcrumb sponge		FCT-008	Diadumene lineata	green sea anemone
FCT-009	Ectopleura crocea	In Hydrozoans genus		FCT-009	Botrylloides violaceum -	
FCT-010	No Match	match on GenBank: Cyanea sp. (jellyfish)/ questionable		FCT-010	Ampithoe valida	
FCT-011	Ampithoe valida			FCT-011	Lepidonotus squamatus	
FCT-012	Lepidonotus squamatus			FCT-012	Halichondria panicea	
FCT-013	Halichondria panicea	breadcrumb sponge		FCT-013	Crepidula fornicata	
FCT-014	Crepidula fornicata			FCT-014	Amphibalanus sp.	
FCT-015	Amphibalanus sp.	barnacle				
FCT-016	Amphibalanus sp.	barnacle		FCT-016	Amphibalanus sp.	
FCT-017	Parasabella microphthalma			FCT-017	Parasabella microphthalma	
FCT-018	Jassa marorata	amphipod		FCT-018	Jassa marorata	
FCT-019	Stenotrophomonas sp. / vibrio	bacteria- contamination/ often found in brackish saltw ate		FCT-019	Halichondria panicea	
FCT-020	Lepidonotus squamatus			FCT-020	no seq available	
FCT-021	No Match	match on GenBank: match on GenBank: Cyanea sp. (jell		FCT-021	Zeuxis siquijorensis	sea snail
FCT-022	Monocorophium insidiosum	Amphipodalamphipod		FCT-022	Sternomoera rhyaca	amphipod

Figure 2: Table comparing sequenced results from both the Folmer and Leroy primers

Discussion

The phylogenetic tree (fig. 1), shows the evolutionary relationships between all our samples and many of them are very closely related. In the consensus chart (fig. 2) the closer a species' sequence is to another, the more likely they are to be related. We had a fairly low rate of biodiversity, which supported our hypothesis, this is likely due to the extreme dredging in the 1900s. Buttermilk Channel also happens to have violent currents, being right next to the Atlantic Ocean, which makes it difficult for invertebrates like mollusks to attach to something solid In regards to the different primers, the Leroy primer did come up with possible species names where the Folmer primer did not. However, on some samples we knew were amphipods the Leroy gave a result for a sponge (FCT-019). However, overall, I would recommend using the Leroy primer because it gives more results that appear to be accurate more of the time. One of the things we would have liked to do, but couldn't, was to take more samples on order to get a clearer and more accurate final result.

Conclusion

Our project was set to answer a few things; what is the species richness of the Buttermilk Channel? Why? Finally, for genetically barcoding macroinvertebrates, what primer is the best, the Folmer primer or Leroy primer? It was found that the Buttermilk Channel is not very biodiverse but through looking at previous data we can determine if it has been improving.

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