

Invertebrates in The Hudson-Raritan Estuary



Photo credit: Ernst Haeckel

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The Urban Assembly New York Harbor School

Marine Biology Research Plan

New York

2015

Summary

Invertebrates are one of the most diverse species. In this research there will be samples of invertebrates collected from the Hudson-Raritan Estuary. The purpose of this research is to learn how to extract DNA from invertebrates to barcode them and determine the biodiversity of the Hudson-Raritan Estuary.

Introduction

The Biodiversity in the Hudson-Raritan is very important to this research. Biodiversity is the variety of different types of life found on Earth and the variations within species. The more diverse the species are in the Hudson, the healthier the ecosystem. The biodiversity of an ecosystem is also a bioindicator of that specific ecosystem. A bioindicator is a living organism that gives us an idea of the health of an ecosystem. There are organisms that are sensitive to the pollution in their environment, so if pollutants are present, the organism may change its morphology, physiology and behaviour, or even worse it could even die. In this research there will be samples of invertebrates collected from the Hudson-Raritan Estuary. Invertebrates include insects, spiders, crustaceans, worms and corals. Invertebrates are one of the most diverse species. According to the International Union for Conservation of Nature (IUCN) over 1.3 million different invertebrates have been identified as of 2009. The reason Invertebrates are being used is because they have a relatively brief life cycle and their anatomy is not particularly complex, thereby allowing researchers to more easily study them and draw appropriate conclusions. When the sample is first collected a hypothesis will be made about what species it is.

Background + Data

The animals or organisms that classify as invertebrates are animals that neither possess nor develop a vertebral column. There are many different types of invertebrates like oysters, Polychaeta's, crabs and many more. There are also different types of Polychaeta. The differences can be seen in the anatomy of the Polychaeta. Depending on what kind it is, the head, the body and even the way it interacts with the world around it can be different. The purpose of this research is to learn how to extract DNA from invertebrates to determine the biodiversity of the Hudson-Raritan Estuary. To collect data, samples of invertebrates will be collected in the New York City area. There will be a maximum of thirty (30) samples collected and its corresponding sample bottle. The samples will be labeled (XRB) and numbered (031-060). The reasoning for those labels is to insure that the samples will not be mixed up with other groups' findings. The areas of collecting have already been chosen. With each sample being extracted from the water and placed in their corresponding sample bottle, then a hypothesis is made about the invertebrates that were collected. When the invertebrates are found at the research site, a picture will be taken with a Go-Pro camera, while the sample is alive. Its corresponding sample number will be in the picture along with a ruler measuring the sample. Then the specimen will be identified to a family; if not able to identify, it will be described in the observations part of the data table. With identification will be using two types of identification books, *Marine animal of southern new England and New York* by Howard M. Weiss, Ph.D. Along with Peterson field guide *Atlantic Seashore* by Kenneth L. Grosner. Then a sample size of a grain of rice (minimum) will be collected for DNA extraction and barcoding. The samples will then be stored in a freezer to maintain its DNA. While the site collecting samples, a table will be filled in with the sample name (or what it is thought to be) and if the name is not known there is a box for observations, the sample number, date it was collected and the location (site) it was collected in. Our field guides will be *Atlantic Seashore* by Kenneth L. Goner and *Marine Animals of Southern New England and New York* by Howard M. Weiss, Ph.D.

The table will look like the one below:

Sample Number	Name Of Organism	Old Code	UBP Code	Amp. Code	Observation	Location	Updated
XRB-031	colonial sea squirt	XRB 031			<ul style="list-style-type: none"> • redish • small • brown 	Pier 101	10-14-15
XRB-032	sea squirt	XRB 032			<ul style="list-style-type: none"> • algae covered • strong suction 	Pier 101	10-14-15
XRB-033	(to be determined)	XRB 033			<ul style="list-style-type: none"> • string like 	pier 101	10-14-15
XRB-034	mud snail	XRB 034			<ul style="list-style-type: none"> • moving • centipede 	pier 101	10-14-15
XRB-035	mud snail	XRB 035			<ul style="list-style-type: none"> • baby • small • stayed in shell 	pier 101	10-14-15
XRB-036	mud snail	XRB 036			<ul style="list-style-type: none"> • small shell • dirty 	pier 101	10-14-15
XRB-037	oyster	XRB 0037			<ul style="list-style-type: none"> • broken shell • dying 	East river	10-24-15
XRB-038	pollycate	XRB 038				East river	10-24-15
XRB-039	Snail	XRB 039				N 40.79394 W 073.93745	11-07-15
XRB-040	Pollycate	XRB 040				N 40. 79394 W 073.9314S	11-07-15
XRB-041	sponge	XRB 041				N 40. 79394 W 073..93745	11-07-15
XRB-042							
XRB-043							
XRB-044							
XRB-045							

XRB-046							
XRB-047							
XRB-048							
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Methods

As shown by DNA barcoding: To isolate the DNA of the invertebrate samples that have been collected, lysis solution will be added to it. The sample will then be grinded in the lysis solution. After it's grinded the sample will be incubated at 65 degree Celsius for 10 minutes and will then be 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 will then be transferred to a fresh tube and silica resin will be added to it. The supernatant and the silica resin will be mixed together and incubated for 5 minutes at 57 degrees Celsius. After it will be centrifuged for 30 seconds. The supernatant will be removed and wash buffer will be added to it. The sample tube will then be put into the vortex and be centrifuged for 30 seconds. After its finished being centrifuged the supernatant will be removed and wash buffer will once again be added. After the wash buffer is added, it will then be put into the vortex and once again be centrifuged for 30 seconds. Then the supernatant will be removed and distilled water (dH2O) will be added. It will be mixed by pipetting in and out until the pellet is dissolved. It will then be incubated for 5 minutes at 57 degrees Celsius. After that it will be centrifuged for 30 seconds and the supernatant will be transferred to a fresh tube and store at -20 degrees Celsius.

To amplify the DNA and sequence and analyze the PCR product there will be nine (9) steps that need to be completed in order to get accurate results. The isolated DNA sample should first be de-frozen. After it's defroze a primer mix LepR1_t1 (used for Mammals, reptiles, fish, amphibians, and some insects) will be added to a clean tube and the DNA will then be added to it. The mineral oil, a distillation product of petroleum, used as a lubricant or moisturizer will be added to it (only if we're unable to go to the Harlem Lab, beside that mineral oil will not be needed). Then it will be placed in the thermal cycler to heat the sample so the DNA separates into two pieces of single-stranded DNA. The gene being separated will be Cytochrome oxide subunit 1, it will be stored at -20 degrees Celsius. Then the PCR products will be 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 will be poured into the gel electrophoresis chamber and will set for 20 minutes. After its set the electrophorese machine will be set 130 volts for 30 minutes. Then the sample will be sent to sequence and the results will be analyzed using bioinformants. The program that will be used is DNA Subway. This program brings together the key bioinformants to assemble gene models which makes it easier to analyze data and analyze the organism.

Bibliography

Invertebrate Zoology (third edition) by Paula A. Meglitsch

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<http://www.aboutanimaltesting.co.uk/invertebrates-used-testing.html>

<http://www.dnabarcoding101.org>

http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html

Biography

My name is **Nailea Rodriguez**. I was born on March 8, 2000 and I'm fifteen years old I'm from Dominican Republic .I have one brother and one sister but, they live with my mom in Dominican Republic (D.R). I live with my dad (Robin Rodriguez), my stepmom (Indira Vallejo) and my step-sister and step-brother (Kylie Marte and Danniell Paulino) in the Bronx, New York.

When I first came to this country I was 9 years old. I didn't speak English and I was dealing with the change in not only location but weather and language. It was hard for me at first but by the summer of 2011 I was fluent in English, made friends and was doing very good in school. When I started 6th grade I became very serious about my school work because I had decided that I wanted to become a doctor More specifically, a surgeon. I then realized that my education to achieve my dreams would cost a lot of money; money that my family doesn't have.

I never gave up on my dreams of becoming a surgeon but, this summer I had a bicycle accident while biking along the Underground Railroad in Ohio. I fell off my bike and shattered my elbow. I had to have surgery, and 2 metal plates and 6 screws. I lost feeling in three of my fingers (middle, ring and thumb). I realized that if my fingers didn't get back to normal I would have to have a backup plan; I know that you can't do science if you don't have both your mind and heart in it. So I decided that I should transfer High School and go to one where it would benefit me. I decided marine biology was something

that I would love to do, mostly because of my love for animals and the curiosity I have about all the things we can discover that would advance the human race.

Apart from science I have many other things that make me happy. I love to read. I can read anything and everything. Apart from loving to read I love to watch sci-fi television. My favorite TV shows are Doctor Who and Star Wars. I know they're not scientifically correct but I can't help my love for it. I also like practicing yoga, most specifically Vinyasa. It relaxes me but, I can't do it at the moment because my arm isn't strong enough to hold my body weight. I still haven't lost my love for it. There is also one thing that makes me happy beyond words and that is becoming someone in life while I have people telling me I can't do it because "I'm not smart enough."

My name is **Zen Mena-Rodriguez**. I am seventeen, Dominican, Mexican and native American. I have one older sister. My mother is an anthropologist and my sister is a zoologist. My sister and I grew with a single mother working three jobs but still raising her children with the installment and importance of education. I've always wanted to become a marine biologist. When I was younger my mother use to take me to the museum of natural history and the aquarium in the little spare time she had between jobs. I would spend hours there, just staring up at the massive whale hanging from the roof in the middle of the exhibit or even the giant squid attacking the whale. The background was so dark I use to think it was real and at any second they would come to life. Every Sunday night my sister would turn on the BBC nature shows before going to bed.

The late nights would be filled of silly things my sister would do for me to keep us distracted from the hard adult reality of life. We've been through a lot but that's never kept my family down. My mother worked three jobs but not in her field Instead as a bartender in highly established restaurants. But even with those jobs she pushed my sister and I to go places. She sent my sister to James Cook University in Queensland Australia. Collage is important to me. I want to go places. I don't want others to look at me like another statistic. I am a Latina woman who has ambition and dreams. Going somewhere is not the question at hand the real question is just where. My ambitions are not arbitrary- They'll truly happen. I'm working towards these goals. There is a start and end to my thoughts. My ideology towards attending college are fairly straight forward I would like to attend university overseas. The university of Sorbonne in Paris, Trinity university of Dublin, university of Saint Andrews and Glasgow university both located in Scotland. A few others in Amsterdam, Iceland and London. I was raised by not just my mother but my sister too. My sister played a massive roll for my love and passion for science but not just science for art as well. Being born and raised in New York City, an area that polytechnic, has fueled the love of art and science. My sister is not just a scientist but an artist as well, as children she would draw on my faces making me into a different creature every day. Once when I was a kid I couldn't fall asleep so my sister made me a whole suit out of tinfoil. Over the last two years my sister and I have started a collaboration of sticker art that at first was only supposed to be for us but over time we enjoyed it so much that she brought up the idea to head over to Logan square in Chicago and hang up one of our stickers. Right now in several different locations of Chicago and New York both our sticker art is .hanging around on building walls, storefronts and construction sites. Anywhere you felt was right to sticker our little bits of art up.