

Restoring the Harbor, Invertebrate by Invertebrate



Ernst Haeckel

Nailea Rodriguez
Advisor: Mauricio Gonzalez
New York Harbor School
Marine Biology Research Program
New York City
2016

Abstract

This project was done in collaboration with CIVITAS- a nonprofit organization that aims to rebuild the Hudson Estuary.

For this project 19 invertebrate samples from the Hudson Raritan Estuary were collected. These samples were then taken to the Harlem DNA Lab where the DNA was extracted, amplified and was then sent to be sequenced. Out of the 19 samples only 2 came out with a positive DNA result; samples ZNR002A and ZNR014A, because of complications during the extraction of the DNA sample ZNR002A had very low quality sequences which caused it to be unidentified. Sample ZNR014A was identified at *Tubular Hydroid* also known as *Ectopleura Crocea* (iplant collaborative 2016). This organism is sensible to water changes; due to the size of it when it was found it's safe to conclude the Harbor Raritan Estuary is not stable .

Introduction

The Biodiversity in the Hudson-Raritan Estuary 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 (Tilman, 1999). A bio-indicator 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 behavior, or even worse it could even die (Miller JH). 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. The animals or organisms that classify as invertebrates are animals that neither possess nor develop a vertebral column (Webster, 2011). There are many different types of invertebrates like oysters, Polychaeta's, crabs and many more. This means that if invertebrates that detect water pollution are found, then the Harbor Raritan Estuary ecosystem isn't stable nor is it healthy.

Specific Aims

The purpose of this research is to identify which invertebrates can serve as a bio-indicator for the Hudson-Raritan Estuary. This will then help to determine the biodiversity of the Hudson-Raritan Estuary depending on the results acquired; this will be done by collecting samples from Governors Island -Pier 101- and East Hudson River.

Procedures

To isolate the DNA of the invertebrate samples that have been collected, lysis solution will be added to it and grinded. The sample will then be incubated at 65 degree Celsius for 10 minutes. 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. The supernatant will be removed and wash buffer will be added to it. The sample tube will then be centrifuged for 30 seconds. The supernatant will be removed and wash buffer will once again be added. Then the supernatant will then be transferred to a fresh tube and stored at -20 degrees Celsius.

To amplify the DNA the isolated DNA sample will be used; primer mix LepR1_t1 will be added to a clean tube and the DNA will then be added to it. Then it will be placed in the thermal cycler to heat the sample so the DNA separates into two pieces of single-stranded DNA. Then the PCR products will be analyzed using gel electrophoresis. The samples found with positive DNA will be sent to sequence and the results will be analyzed using bio-informants. The program that will be used is DNA Subway by bringing together bio-informants to assemble gene models, which makes it easier to identify the organism.

Analysis

Out of the two positive DNA sample-ZNR002 and ZNR014A- identified during Gel Electrophoresis only one DNA sample had a strong base sequence to be identified; the organisms was *Tubular Hydroid or Ectopleura crocea* (DNA subway,2016). However sample ZNR002A had less than 20 base pairs, making it low quality sequencing. In the end there was only 1 identified organism out of 19; this was sample ZNR014A. It was then found that Tubular Hydroid negatively affects the suspended culture of the mussel *Mytilus galloprovincialis*. It mostly polluted the body, edge and dorsal of the mussle shell. This caused the mussel t have a reduction in length by 4% and a reduction in weight by 23% in young mussels (fitridge).

Conclusion

There were 19 invertebrate samples that were collected in the East Hudson River and along pier 101 off of Governors Island. Out of the 19 samples only 2 came out with positive DNA. But there were complications through the extraction of DNA that may have caused sample ZNR002A to have a low quality sequence. Although some data was collected it was not enough to support my hypothesis. In the future certain collecting sites should be set up along the East River and pier 101 and the organisms identified should be compared to try and identify the health of the Hudson Estuary based on the bio-indicator of said organisms.

Annex



Figure 1: Gel Electrophoresis results where sample 2 (ZNR002A) and sample 14 (ZNR014A) are shown to be positive DNA samples.

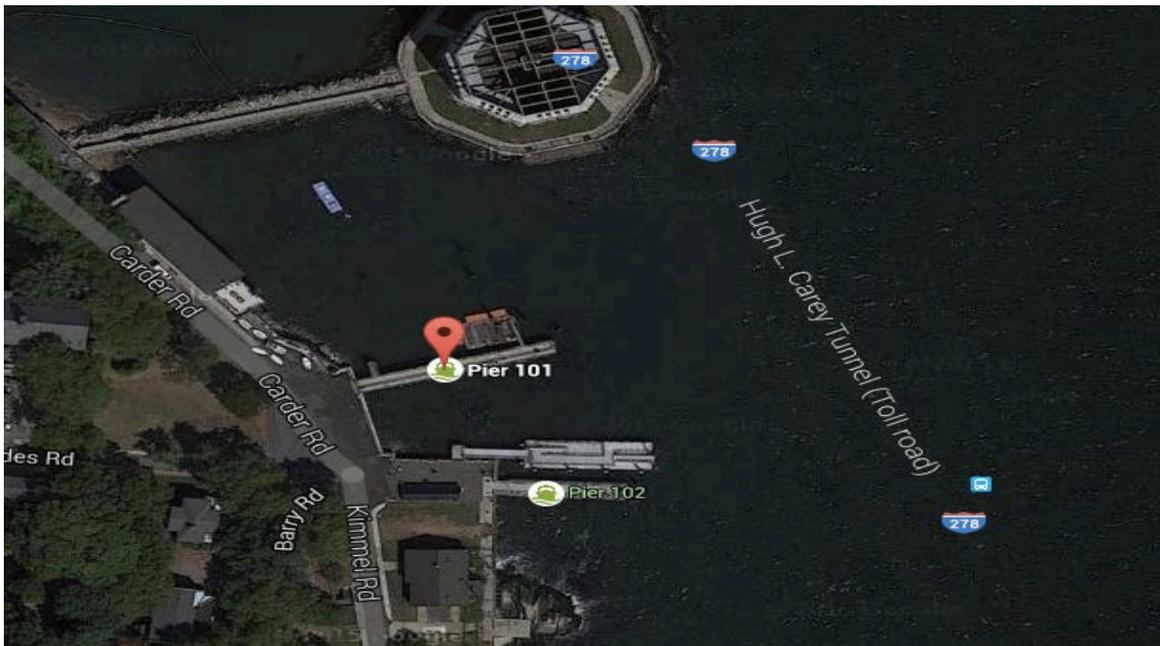


Figure 2: Pier 10 off of Governors Island where sample ZNR-014A was found.

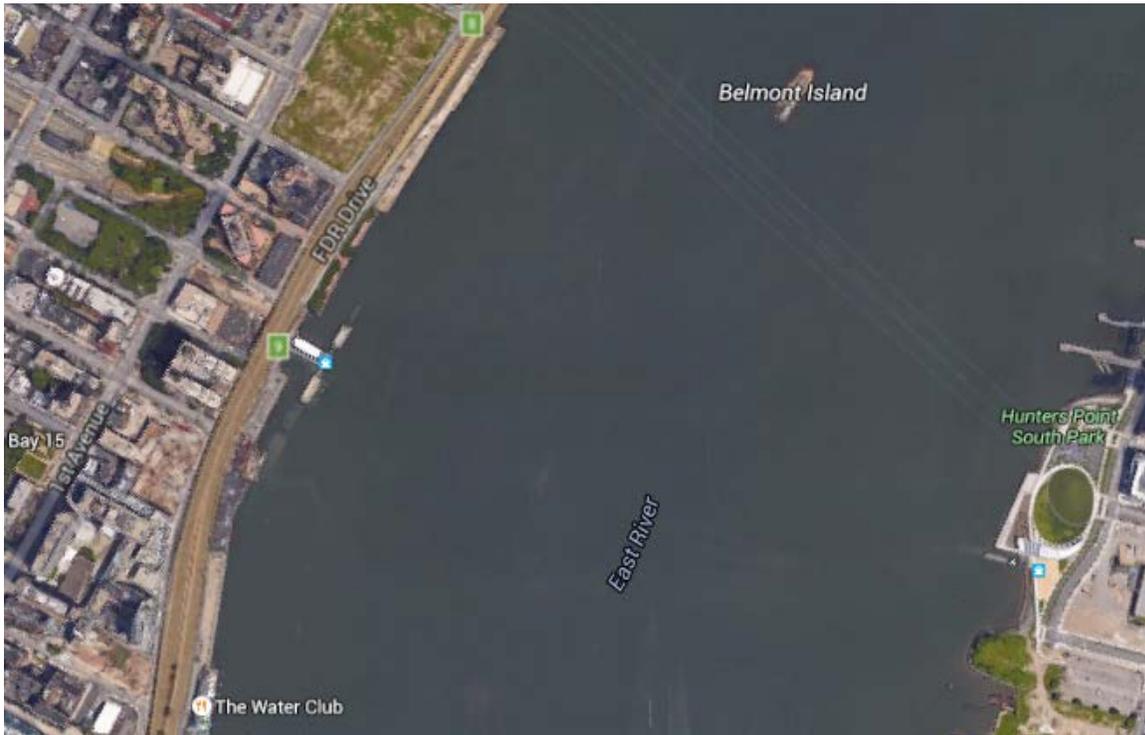


Figure 4: Shows where the other 18 unidentified invertebrate samples were found.

Table 1: Identifies the difference between the visual identification and the genetic identification of the organisms.

Sample #	Visual Identification	Genetic Identification
ZNR014A	Tubular Spongue Hydroid/ <i>Zyzyzus warreni</i>	Tubular Hydroid/ <i>Ectopleura Crocea</i>

Bibliography

- Allen, C. M., 1900. A contribution to the development of *Parypha crocea*. Biol. Bull., 1: 291-315.
- An Introduction to Genetic Analysis-2000; 7th edition; Griffiths AJF, Miller JH
- Berrill, N. J., 1952. Growth and form in gymnoblastic hydroids. V. Growth cycle in Tubularia. J. Morph., 90: 583-601.
- Biodiversity and Ecosystem Functioning: Maintaining Natural Life Support Processes; David Tilman, 1999
- Cold Spring Harbor Laboratory Bioinformatics; steps 1-8
<http://www.dnabarcoding101.org/bioinformatics.html>
- Cold Spring Harbor Laboratory “protocols” PDF, 2014
- DNA Subway Manual; blue line. Pages 17-21
http://dnasubway.iplantcollaborative.org/files/pdf/DNA_Subway_Guide.pdf
- DNA Subway- Blue Line
<http://dnasubway.iplantcollaborative.org>
- "Invertebrate." *Merriam-Webster.com*. Merriam-Webster, 2011. Web. 8 May 2011.
- Invertebrate Zoology (third edition) by Paula A. Meglitsch
- Ruinous resident: the hydroid *Ectopleura crocea* negatively affects suspended culture of the mussel *Mytilus galloprovincialis*. Department of Zoology, University of Melbourne, Victoria, Australia; Frittridge
- Seavolt H, H. 2001 “*Ectopleura crocea*” (on-line)
http://animaldiversity.org/accounts/Ectopleura_crocea/
- Urban Barcode Project- Barcoding Protocol
<http://www.urbanbarcodeproject.org/experiment.html>