Biodiversity of Macroinvertebrate Species within the New York Bay

Authors: Grace Carter[1], Jared Rosin[1], Erik Wiemer[1]
Mentor: Mauricio González[1]
[1] Urban Assembly New York Harbor School
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Abstract

The group collected, documented, and identified several different species of invertebrates in the Upper New York Bay. This will help to compare the level of biodiversity between the New York Bay now as well as in the future. This experiment could also help with restoring the Upper New York Bay. It was predicted that our team would find that the species found would be closely related, because they would have to have similar adaptations to survive the polluted habitat. It is also predicted that our team will find that the biodiversity of macroinvertebrates within the Upper New York Bay is significantly lacking, and that this has a major effect on the water quality.

Introduction

The New York Harbor Estuary is an amazing habitat where hundreds of different species reside. Along with the myriad of species that call the Estuary home, humans benefit from the cleanliness of this waterway as well. Even given this, the largest impact on the Estuary is human interaction. People dredge the bottom of the river, litter, and dump hazardous and biological waste into the Estuary, among many other detrimental activities, every day (EPA, n.d.). This project brings up questions on how the estuary is doing, in terms of ecosystem and biodiversity. This project is specifically about whether or not the invertebrate population within the Upper New York Bay covers a myriad of species and creates a healthy, self-sustaining ecosystem. The results of this experiment will also provide information on the quality of the water within the Upper New York Bay - information that could prove useful to anyone wishing to improve the quality of life in the regions surrounding this water body. There is an immense amount of biodiversity among invertebrates within the Upper New York Bay. However, the specific level of diversity within said Bay has not been documented anytime recently. The goal of our project is to document, as well as sequence the genetics of (identify), various invertebrate species within the Upper New York Bay, including, but not limited to: Flatworms (Platyhelminthes) (Berkeley, n.d.), leeches (Hirudinea) (SWCSMH, 2013), scuds (Amphipoda) (Fasulo, n.d.), true bugs (Hemiptera) (Discover Life, n.d.), and caddisflies (Trichoptera) (Meyer, 2009). This project is important because the level of biodiversity within a water body is an indicator of how well people are doing in terms of ecosystem preservation and restoration, as well as how well the ecosystem is operating in a given water body. Our group will be sequencing the DNA of invertebrate organisms from the Upper New York Bay acquired from Pier 101 which is on Governors Island, including a larger project, “How Native Organisms of the New York Harbor Estuary are Affected by Building Materials Used in a Recreational Area” (Civitas n.d.) . This project could very well benefit the health of the New York Harbor in the years to come, in that it will collect data on not only biodiversity within the Upper New York Bay, but also provide indicators of water quality, which could help to understand what exactly needs to be done to improve the quality of the ecosystem. It is predicted that our team will find that the biodiversity of macroinvertebrates within the Upper New York Bay is significantly lacking, and that this has a major effect on the water quality. If we were to know just a few of the macroinvertebrates that reside in the Upper New York Bay, we or any other organization or group could help further the health of the Upper New York Bay and, therefore, the whole of the New York Harbor. If we were to study and learn what invertebrate organisms live in the Upper New York Bay, we would be better educated on how we can further benefit the water coming into the Bay, and know of any oyster, mussel, or other shellfish reefs that have formed and prospered in the area. We could also find out where oyster reefs would thrive in this area in the future with the information that we have gathered. Oysters are a keystone species of the Upper New York Bay and their reefs

provide habitats for many different organisms including fire plankton and zooplankton in general.

Materials and Methods

The materials used to conduct this experiment include a collection tube, jar or bag to hold the specimen and to transport it from its habitat. Forceps are used to help pick the specimen up from its habitat. A scalpel or scissors are used to create the sample- a small piece of the specimen. A camera was used to take images of the whole specimen. A coin and a ruler are also used to compare the organism's size. A field guide or a Taxonomic Key is used to aid in the identification of species. A freezer that can get down to -20 °C to preserve and specimens. Distilled water is used as a reagent in the isolating process. The Lysis Solution is used to break up membranes and to release the DNA of the samples. Silica Resin was used to bind the DNA from the solution. A cold water wash buffer was used to decontaminate the resuspension of the DNA pellet.A container of crushed ice was used to cool the wash buffer. Micropipettes (P1000,P100,P10) were used to measure the different solutions and disposable micropipette tips were used to prevent contamination. A Microcentrifuge was used to separate the DNA from the other tissue and debris.A plastic pestle was used to grind the specimens. A hot water bath was used to incubate the samples. A vortexer was used to dilute the samples into the solutions. Test tubes were used to hold samples and waste. The personal protection equipment (PPE) included gloves, goggles and a smock. Research journals were also used to take notes/ observations in.

The specimens were collected from their environment from two locations. One of these locations was Pier 101 which is located on Governors Island. The invertebrate species were removed from bricks that were submerged in the Upper New York Bay's water. and placed in collection tubes. The other location was Push Terminal Piers and the samples were collected from the water and placed in collection bags.

Collection, Identification, and sample preparations

Collect specimens, from varying sources, using a collection tube. Using a camera, take pictures of each specimen in its natural habitat. Take close-range, mid-range, and long-range pictures. In the close-range shots, use a coin or a ruler for size comparison. In mid- and long-range shots, use a person. Include the sample code in the picture. Use a field guide or a taxonomic key to identify the specimen as accurately as is possible. Use forceps and scissors or a scalpel to cut away a sample from the specimen. Freeze the specimen at -20 °C, until prepa are complete for isolation of the DNA.When ready, take the samples out of the freezer, and place each into a separate test tube. Label each test tube, using a permanent marker, with sample number, type of sample, etc (any other identifying features worth noting).

Isolation of DNA

Using the P1000 micropipette and a fresh tip, add 300 microliters (µl) of lysis solution to each test tube, changing the tip after each tube. Discard used tips. Twist a clean plastic pestle with force into each test tube, until each sample is completely ground into the solution. Use a different pestle for each test tube, and discard of used pestles immediately. Incubate each test tube in a water bath or heating block for approximately 10 minutes at 65 °C. After incub tubes into a microcentrifuge in a balanced formation, with cap hinges pointed upward. Turn the centrifuge on for 1 minute at maximum speed to pellet debris. Label new test tubes for each sample, with all of the same information. Using a P1000 micropipette and a fresh tip for each

tube, transfer 150 µl of the supernatant (clear solution above the pellet at the bottom of the tube) to each new tube. Do not touch or disturb in any way the pellet at the bottom of the tube. Discard of all old tubes. Using a P10 micropipette and a fresh tip for each tube, add 3 µl of silica resin to the tubes. Mix well with a vortexer (if available), finger vortexing (flicking the bottom of the test tube until sufficiently mixed), or pipetting up and down. Close and incubate each tube in a water bath or heat block for 5 minutes at 57 °C. Place the microcentrifuge, again with cap hinges pointed outward. Centrifuge for 30 seconds at maximum speed. Using a P1000 micropipette and a fresh tip for each tube, remove the supernatant from each tube without disrupting the pellet at the bottom of the tube. Using a P1000 micropipette and a fresh tip for each tube, add 500 µl of cold wash buffer to the pellets. Close the tube and mix well by vortexing, either with the machine or by finger, or pipetting up and down so as to resuspend the silica resin. Place each tube into the microcentrifuge in a balanced formation, with cap hinges pointed outward. Centrifuge at maximum speed for 30 seconds. Using the P1000 micropipette and a fresh tip for each tube, remove the supernatant from each tube without disrupting the pellets. Using the P1000 micropipette and a fresh tip, add 500 µl of cold wash buffer to each pellet. Close the tube and mix well with finger/machine vortexing or pipetting up and down.Place the tubes in a balanced formation in the microcentrifuge, with cap hinges pointed outward. Centrifuge at maximum speed for 30 seconds. Using a P1000 micropipette and a fresh tip for each tube, remove the supernatant from each tube without disturbing the pellets. Briefly spin the tubes to collect any drops of supernatant, removing these drops with a micropipette and a new tip. Using a P100 micropipette and a fresh tip for each tube, add 100 µl of distilled water to each silica resin pellet. Mix well by vortexing or pipetting up and down. Incubate each test tube at 57 °C for 5 minu microcentrifuge with cap hinges pointed outward. Centrifuge at maximum speed for 30 seconds. Label new tubes with the same information as the previous tubes, and transfer 90 µl of the supernatant from each tube to its respective new tube without disturbing the silica resin pellet. Discard of any old tubes. Freeze the new samples or put them in the fridge to preserve tissue.

These procedures were derived from “Using DNA Barcodes to Identify and Classify Living Things”, an article on DNA 101 of the Urban Barcode Project Website.

Results

Sample Number	**Tentative Identification**	**Bioinformatics Identification**
XRB-001	Snail	N/A
XRB-002	Green Algae	N/A
XRB-003	Arthropod	N/A
Sample Number	**Tentative Identification**	**Bioinformatics Identification**
XRB-004	Red Algae	N/A

XRB-005	Arthropod	Red Algae - Genus *Ceramium*, species *secundatum, diaphanum,* or *ciliatum*
XRB-006	Sea Squirt	N/A
XRB-007	Snail Gonads	N/A
XRB-008	Worm	N/A
XRB-009	Sea Squirt (tent. *Ciona intestinalis)*	Sponge - *Halichondria panicea, Terpios hoshinota, Suberites pagurorum,* or *Hymeniacidon perlevus*
XRB-010	Arthropod	Snail - *Ilyanassa obsoleta*
XRB-011	Arthropod	N/A

Despite the challenges of collecting solid data from tedious procedures, progress has been made. We have successfully gathered strands of DNA, Shown in the Annex, Figure 1 via Gel Electrophoresis. This data was then inputted to the DNA subway and three organisms have been successfully sequenced. The bioinformatics of the DNA subway have been shown in the Annex, Figures 2,3, and 4. Figure 2 is Sample organism 5. It was presumed to be an arthropod, and was confirmed to be of genus *Ceramium*, and most likely species *secundatum* - an algae. Figure 3 is Sample organism 9, thought to be a sea squirt- it had similar characteristics such as body shape and color as a common sea squirt *Ciona intestinalis*. It is most likely of the genus *Halichondria* and species *panicea*, a sponge. Figure 4 is Sample organism 10, thought to be an arthropod. It was confirmed to be of genus *Ilyanassa* and species *obsoleta*, a sea snail.

Discussion

Among the organisms sequenced properly, we have concurred from the bioinformatics in the Annex that Sample 5 is most likely the algae, *Ceramium secundatum* (Lyngbye, 1819) a form of red algae; Sample 9 is most likely a sponge, *Halichondria panicea* (Pallas, 1766) which is most commonly known as the breadcrumb sponge; Sample 10 was identified as a sea snail, *Ilyanassa obsoleta*(Say, 1822). The data shown in Annex Figure 1 are possible DNA strands of organisms that we properly extracted and amplified the DNA of. The Gel Electrophoresis image shows a “P” for Positive DNA for invertebrates/plants Control . The M means Marker, which shows DNA. Some possible DNA bars are hazy within samples 002 and 004, but still present – other numbers have relatively clear bars such as Samples 5, 9, and 10. These organisms were found on bricks that have been resting in the harbor for weeks, so the possibility of finding sessile and/or slow moving organisms was to be expected. The data gathered shows us of possible organisms that we could of found living in our Estuary. Also there was another organism (Sample 7) that we presumed sequenced properly but a believed error DNA subway or inputting data into the DNA subway could have been the cause of not receiving this organism’s

genetic information Further testing is needed to learn more on the biodiversity of our Estuary, but the data gathered is still useful for the future of this project.

The organisms we found would show the health of the Upper New York Bay. *Ceramium secundatum*, the species that Sample 005 was most likely, generally reside in sandy wave-protected beds, and act as food for fish and invertebrates. *Halichondria panicea*, the species that Sample 009 was most likely, offer habitats for many different organisms, and also act as filter feeders - they absorb nutrients from the water around them. *Ilyanassa obsoleta*, the species that Sample 010 was, eat many microscopic marine plants and are primary consumers - they are an integral part of an ecosystem in that fish use them as a source of food.

References

Berkeley. Introduction to the Platyhelminthes. (n.d.). Retrieved January 20, 2015, from http://www.ucmp.berkeley.edu/platyhelminthes/platyhelminthes.html

DEC. Biomonitoring. (n.d.). Retrieved November 6, 2014, from dec.ny.gov/chemical/23847.html

Discover Life. Hemiptera. (n.d.). Retrieved January 20, 2015, from http://www.discoverlife.org/mp/20q?search=Hemiptera

DNA Subway. Fast Track to Gene Annotation and Genome Analysis - DNA Subway. (n.d.). Retrieved January 20, 2015, from http://dnasubway.iplantcollaborative.org/

EPA. Dredged Material Management Program. (n.d.). Retrieved December 12, 2014, from http://www.epa.gov/region02/water/dredge/intro.htm#Management

Meyer, J. (n.d.). Classification & Distribution. Retrieved January 20, 2015, from http://www.cals.ncsu.edu/course/ent425/library/compendium/trichoptera.html

CIVITAS. Reimagining the Waterfront. (n.d.). Retrieved October 21, 2014, from http://reimaginethewaterfront-civitas.com/

Riverkeeper Organization. Combined Sewage Overflows (CSOs). (n.d.). Retrieved November 12, 2014, from http://www.riverkeeper.org/campaigns/stop-polluters/sewage-contamination/cso/

Soil and Water Conservation Society of Metro Halifax. Class Hirudinea. (n.d.). Retrieved January 20, 2015, from http://lakes.chebucto.org/ZOOBENTH/BENTHOS/xxvi.html

Urban Barcode Project. Using DNA Barcodes to Identify and Classify Living Things: I. Collect, Document, and Identify Specimens. (n.d.). Retrieved October 25, 2014, from http://www.dnabarcoding101.org/protocol_collecting_specimens.html

Urban Barcode Project. Using DNA Barcodes to Identify and Classify Living Things: II. Isolate DNA from Plant, Fungal, or Animal Samples. (n.d.). Retrieved October 25, 2014, from http://www.dnabarcoding101.org/protocol_isolating_dna.html

Urban Barcode Project. Using DNA Barcodes to Identify and Classify Living Things: III. Amplify DNA by PCR. (n.d.). Retrieved October 25, 2014, from http://www.dnabarcoding101.org/protocol_amplifying_dna.html

Urban Barcode Project. Using DNA Barcodes to Identify and Classify Living Things: IV. Analyze PCR Products by Gel Electrophoresis. (n.d.) Retrieved October 25, 2014, from http://www.dnabarcoding101.org/protocol_analyze_pcr_products.html

Annex

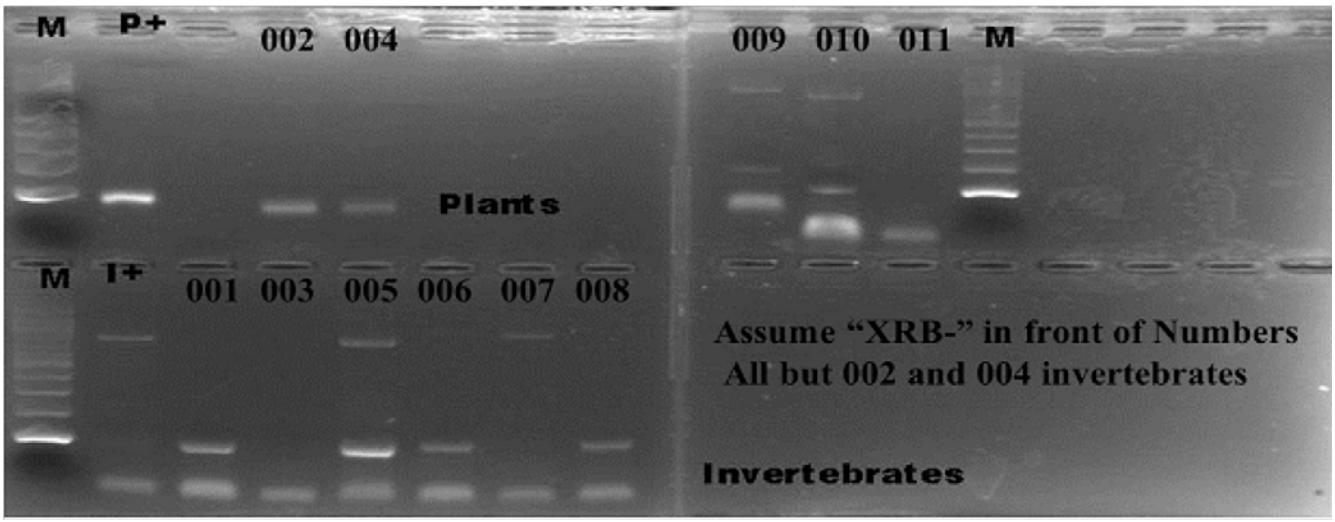

Figure 1 – Gel Electrophoresis - I+/P+ = Positive DNA for invertebrates/plants (Control); M = Marker (Shows DNA). DNA bars hazy within 002 and 004, but still present – other numbers have relatively clear bars.

Gonzalez_XRB-005

#	Accession #	Details	Aln. Length	Bit Score	e	Mis-matches
1(1)	gi|168418717|gb|EU194972	Ceramium secundatum - cytochrome c oxidase subunit I (cox1) gene, partial cds; mitochondrial	548	751	0.0	51
2(2)	gi|545746628|gb|KF367751	Ceramium diaphanum - cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	547	645	0.0	74
3(3)	gi|545746636|gb|KF367755	Ceramium diaphanum - cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	547	645	0.0	74
4(4)	gi|633289469|gb|KJ179929	Ceramium ciliatum - cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	547	618	1e-174	80
5(5)	gi|633289473|gb|KJ179931	Ceramium ciliatum - cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	547	618	1e-174	80

Figure 2 - Bioinformatics - Sample 5 - Tentative arthropod, confirmed of genus *Ceramium*, most likely species *secundatum*; algae. (iplantcollaborative.org)

Gonzalez_XRB-009

#	Accession #	Details	Aln. Length	Bit Score	e	Mis-matches
1(1).	gi|512764451|gb|KC869423	Halichondria panicea - subunit I (COI) gene, partial cds; mitochondrial	656	998	0.0	32
2(2).	gi|597437709|gb|KJ008098	Terpios hoshinota - oxidase subunit I (CO1) gene, partial cds; mitochondrial	686	875	0.0	67
3(3).	gi|50083203|gb|AY561979	'Protosuberites' sp. - oxidase subunit I (COI) gene, partial cds; mitochondrial	688	843	0.0	75
4(4).	gi|512764449|gb|KC869422	Suberites pagurorum - subunit I (COI) gene, partial cds; mitochondrial	652	816	0.0	76
5(5).	gi|430768101|gb|JX477016	Hymeniacidon perlevis - cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	676	812	0.0	77

Figure 3 - Bioinformatics - Sample 9 - Tentative sea squirt, most likely of genus *Halichondria* and species *panicea*; sponge. (iplantcollaborative.org)

Gonzalez_XRB-010

#	Accession #	Details	Aln. Length	Bit Score	e	Mis-matches
1(1).	gi|564734267|gb|KF643603	Ilyanassa obsoleta - cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	654	1146	0.0	8
2(2).	gi|564734447|gb|KF643693	Ilyanassa obsoleta - cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	654	1146	0.0	8
3(3).	gi|564734717|gb|KF643828	Ilyanassa obsoleta - cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	654	1146	0.0	8
4(4).	gi|564734849|gb|KF643894	Ilyanassa obsoleta - cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	654	1146	0.0	8
5(5).	gi|564735017|gb|KF643978	Ilyanassa obsoleta - cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	654	1146	0.0	8

Figure 4 - Bioinformatics - Sample 10 - Tentative arthropod, confirmed of genus *Ilyanassa* and species *obsoleta*; sea snail. (iplantcollaborative.org)