Species Richness and Interaction of Benthic Marine Macroinvertebrates within the Lower Hudson River Estuary

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Abstract. Looking out over the New York Harbor, one would never expect to find it teeming with life. However, there is an entire ecosystem of benthic invertebrates that are essential to the estuary. Invertebrates filter out the New York estuary water every day of harmful chemicals and algae (Nigro, 2011). This project is a baseline study to observe the species richness in the ecosystem, compare 3 different stations to determine how they are affecting the organisms, and to monitor the list of species that was generated over the course of a year. There are many studies on biodiversity of freshwater macroinvertebrates in New York State (DEC, 2016.). Species included within this study are flatworms, scuds, and leeches, of which similar organisms have been discovered already within the baseline study proposed within this research project. An Ekman Grab was used to collect samples and they were identified using the jgLCO1490 and the jgHCO2198 primers (revisions of the Folmer Primers). All three stations were then compared to get an idea of the health of the Estuary. Out of 42 organisms that were found at Pier 101 on Governors Island and between 106th street and 120th street in Manhattan, 30 organisms representing 10 different species were successfully identified using genetic sequencing. The remaining 12 did not have conclusive data. Of the identified species 5 are indicator species for a "healthy" Estuary including *Hediste diversicolor, Crepidula cf. plana, Amphibalanus improvisus, Mytilus edulis and Streblospio benedicti.*

Introduction

Looking out over the New York Harbor, one would never expect to find it teeming with life. However, there is an entire ecosystem of benthic invertebrates essential to the estuary. Invertebrates that continuously filter the New York estuary's water every day of harmful chemicals and algae (Nigro, 2011). This project was a baseline study to observe macroinvertebrate species richness at 4 sites: 116th, 111th, 103rd Street on the East River and a control at the Governors Island Oyster

Reef. This baseline study of the Lower Harlem River took place from October 2015 to October 2016.

Results from the three stations were compared to determine which station has the most richness. The DEC, (2016) has a list of freshwater macroinvertebrates that can be found in New York State. Examples include flatworms, scuds and leeches. Unfortunately, there are no studies regarding the Macroinvertebrates in the Lower Hudson River Estuary (LHRE). This project used the jgLCO1490 and the jgHCO2198 primers (revisions of the Folmer Primers) at the NYU lab to identify the organisms that were found. The species were then compiled in a list of species and a food web.

With improved and current biodiversity data, the river ecosystem's health and viability can be improved, and ultimately a new urban development plan can be constructed to provide New Yorkers with the opportunity to interact with a new space along the waterfront for recreation, education, and to increase the public's stake in the harbor. CIVITAS, a New York City based non-profit organization dedicated to improving the quality of life on the Upper East Side and in East Harlem, has studied the East River Esplanade, the land adjacent to the stations sampled in this project, with the goal of identifying the work that needs to be done to begin the process of restoring both the Esplanade and waterfront as a whole.

Methods

Goals

The goal of this project is to determine the species richness of macroinvertebrates within the Lower New York Estuary. This will be done by collecting samples from the 4 sites, and analyzing the genetic makeup of the aforementioned samples using genetic amplification followed by the employment of bioinformatic technology to organize and identify genus and species based entirely on codified DNA.

Sample collection and processing

The specimens were collected from 4 locations on the East River. Sample Site 1 is on 103rd Street in Manhattan, Sample Site 2 is on 109th street, and Sample Site 3 is on 116th Street (Map 1), along with the Governors Island Reef control site. The invertebrate species were removed from the benthic layer with an Eckman grab and placed in collection tubes. Each tube has a code ex: [B1S1S1], which was processed as Biodiversity 1 Station 1 Sample 1.

After the benthos was removed from the water, it was placed into the sift bucket. The sift bucket is rotated 180 ° 14 times to move the benthic sediment through the sift, while letting the remaining invertebrates stay in the sift bucket.

In the New York University (NYU) lab, forceps and scissors were used to cut away a sample from the specimen. The specimen was frozen at -20℃, until preparations for isolation were completed. Then the sample was then placed into a 1.5 ml eppendorf tube. 1 ml solution A and 10µl RNase A were added. After adding 200µl saturated NaCl, samples were rocked for approximately 10 minutes. Test tubes were then spun at top speed in a centrifuge for 5 minutes. After this, 500µl of the supernatant was transferred to a new tube and precipitated with 50µl isopropanol. The precipitated solution was spun at top speed for 5 minutes. Once that was completed, the solution is washed with 100µl of 70% ethanol. Once again, the solution was spun for 5 minutes and then left out to air dry. Then, the solutions were re-suspended in 50µl ddH_2O. Next, a primer was added to the sample solution for gel electrophoresis and sequencing of the DNA.

Finally, an unpurified primer mix (gLCO1490 and the jgHCO2198 primers revisions of the Folmer Primers) were added to the final sample of DNA and sent to another lab for the retrieval of the samples of amino acid streams (DNA).

Image capture

Images of the organism were taken on an iPhone 6s at a 90° angle above the specimen. Image quality would've been improved if pictures were taken with a waterproof camera on a flat white surface with a ruler, with the specimen fully stretched out and including images of various angles and close-ups of the mouth and any other identifying features.

Identification process

Barcoding uses a very short genetic sequence from a standard part of the genome the way a supermarket scanner distinguishes products using the black stripes of the Universal Product Code (UPC). Two items may look very similar to the untrained eye, but in both cases the barcodes are distinct. Barcoding relies on short, highly variable regions of the genome. With thousands of copies per cell, mitochondrial sequences are readily amplified by polymerase chain reaction (PCR), even from very small or degraded specimens. A region of the mitochondrial gene *COI* (cytochrome C oxidase subunit I) is used for barcoding animals. Cytochrome C oxidase is involved in the electron transport phase of respiration. Thus, the genes used for barcoding are involved in the key reactions of life: storing energy as carbohydrates and releasing it to form ATP.

The amino acid streams were put into and processed by a worldwide bioinformatics database (BLAST), the sequenced results were then used to search a DNA database. A close match quickly identifies a species that is already represented in the database. However, some barcodes will be entirely new, and identification may rely on placing the unknown species in a phylogenetic tree with near relatives.

Locality

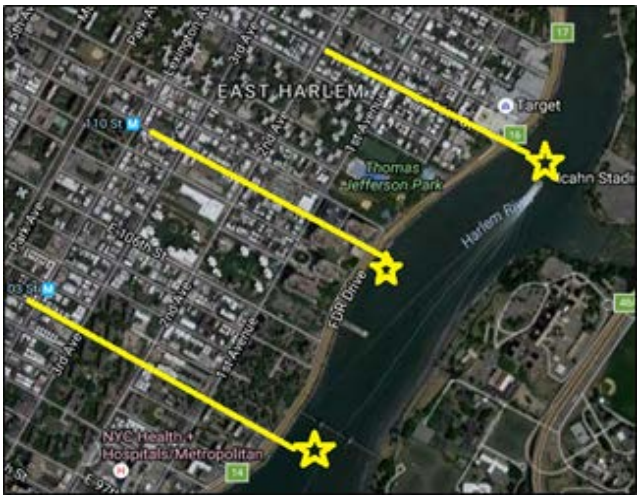
The project was conducted between 103rd and 116th Streets along the Harlem/ East River Esplanade bulkhead structure bordering the Harlem River (Figure 01.) Site 03. (40°47.210192'N, 73°56.301825'W) is located at approximately 103rd Street, along the bulkhead just north of where

the Harlem River opens up into Rheinlander Bay, leading to a change in the current's speed as it picks up with changing water flow dynamics. Moving further north, Site 02 (40°47.490298'N, 73°56.109390'W) is located at the periphery of the 111th Street Pier; a small, closed off, dilapidated pier built off of low-level relieving platform bulkhead construction. This site offers a habitat bulkhead construction that differs from Site 03 further south, in addition to encompassing the added variable of spatial complexity contributed by a pier that has been left vulnerable to the forces of nature for many years. Site 01 (40°47.641665'N, 73°55.863572'W) is situated slightly further north between 115th to 116th Streets and is also along low-level relieving platform bulkhead construction, but without the added spatial complexity. The fourth site is located at the Governors Island Reef in Buttermilk Channel. Site 04 (40°41.20781666'N, 74°0.7383'W) serves as the control for the project, as it is subjectively far away enough from the other sites to serve as a possible reference point for comparison (Smith, 2016).

Table 01. Exact locations of experimental and control sites. They are expressed by street location, exact GPS coordinates, and the water body they are a part of.

Site	Street Location	GPS Coordinates	Water Body
1	**115th-116th Streets**	**40°47.641665'N, 73°55.863572'W**	**Harlem River**
2	**111th Street**	**40°47.490298'N, 73°56.109390'W**	**Harlem River**
3	**103rd Street**	**40°47.210192'N, 73°56.301825'W**	**Harlem River**
4 (Control)	**Governors Island**	**40°41.20781666'N, 74°0.7383'W**	**Buttermilk Channel**

Map 01. Sample stations in relation to Manhattan. The section labelled as “A” refers to the Harlem/ East River experimentation sites, while “B” refers to the Governors Island control site.



Map 02. Sample Sites along East River Esplanade. 103rd Street, 110th Street and 116th Street.

Results

Table 02. The list species found at each site. 2 species were found at Site 1, 5 species at Site 2, 0 species at Site 3 (this was the site under the bridge, no benthic material found) and 6 species at Site 4.

Site 1 (116th St)		**Site 2 (111th St)**		**Site 3 (103rd St)**		**Site 4 (GI Oyster Reef)**	
Genus	**species**	**Genus**	**species**	**G**	**s**	**Genus**	**species**
Hediste	*diversicolor*	*Hediste*	*diversicolor*	*none*	*none*	*Hediste*	*diversicolor*
Trinta	*obsoleta*	*Streblospio*	*benedicti*	*none*	*none*	*Mytilus*	*galloprovincialis*
none	*none*	*Crepidula*	*plana*	*none*	*none*	*Mytilus*	*edulis*
none	*none*	*Trinta*	*obsoleta*	*none*	*none*	*Amphibalanus*	*variegatus*
none	*none*	*Urosalpinx*	*cinerea*	*none*	*none*	*Botrylloides*	*violaceus*
none	*none*	*none*	*none*	*none*	*none*	*Amphibalanus*	*improvisus*

Table 03. Total number of samples - 18, while the total number of different species is 10. The most commonly found species is *Hediste diversicolor*.

Date	Location	Sample #	Common Name	Phylum	Genus	species
April 15	GI Reef	P1	Tunicate	Chordata	Botrylloides	violaceus
April 15	GI Reef	P2	Mediterranean Mussel	Mollusca	Mytilus	galloprovincialis
April 15	GI Reef	P3	Blue mussel	Mollusca	Mytilus	edulis
April 15	GI Reef	P4	Barnacle	Arthropoda	Amphibalanus	variegatus
April 15	GI Reef	P5	Ragworm	Annelida	Hediste	diversicolor
April 15	GI Reef	P6	Tunicate	Chordata	Botrylloides	violaceus
April 15	GI Reef	P7	Bay Barnacle	Arthropoda	Amphibalanus	improvisus
April 15	GI Reef	P8	Ragworm	Annelida	Hediste	diversicolor
April 15	GI Reef	P9	Tunicate	Chordata	Botrylloides	violaceus
April 15	GI Reef	P10	Tunicate	Chordata	Botrylloides	violaceus
Sept. 17	Site 1	P11	Eastern Mud Snail	Mollusca	Trinta	obsoleta
Sept. 17	Site 1	P12	Ragworm	Annelida	Hediste	diversicolor
April 16	Site 2	P13	Mud Tube Worm	Annelida	Streblospio	benedicti
April 16	Site 2	P14	Slipper Snail	Mollusca	Crepidula	plana
April 16	Site 2	P15	Eastern Mud Snail	Mollusca	Trinta	obsoleta
Oct. 15	Site 2	P16	Oyster Drill	Mollusca	Urosalpinx	cinerea
Oct. 15	Site 2	P17	Ragworm	Annelida	Hediste	diversicolor
Oct. 15	Site 2	P18	Eastern Mud Snail	Mollusca	Trinta	obsoleta

Analysis

Hediste diversicolor (Ragworm), was the species most present at each site. *Hediste diversicolor* is a predatory polychaete and scavenger, as well as being able to adapt its diet to whatever is currently available. It spins a mucus net at the entrance of its burrow in which it traps phytoplankton, zooplankton, diatoms, bacteria and other small particles. If this fails then it will venture outside of its burrow (Vismann, B. 1990). Many different type of fish and birds eat this organism and it seems to be an important player in the New York Estuary.

By far, the next most commonly found organism found at half of the sites was *Trinta obsoleta*, also known as the Eastern Mudsnail. The invading eastern mudsnail has been found in several Pacific locations, but its ecological effects have yet to be evaluated. It is believed to be transported along with Atlantic oysters, and the eastern mudsnail poses a threat to new habitats (Race, 1982). The mud snails have no natural predators because they are invasive, making it impossible to control the population.

Streblospio benedicti (found at Site 2) was relatively tolerant to elevated levels of sediment organics (Reish 1979), a trait that contributes to its success as an opportunistic species. *Streblospio benedicti* is consumed by a variety of epibenthic predators, such as grass shrimp (*Palaemonetes pugio*), blue crabs (*Callinectes* spp.), and juvenile fish like spot flounder (*Leiostomus xanthurus*), (Virnstein 1977, Kneib and Stiven 1982, Posey and Hines 1991), making it an important part of food chain in the Estuary.

Crepidula cf. plana prefers well oxygenated conditions. (Zimmerman, Pechenik. 1991). Filter-feeding bivalves that feed mostly on phytoplankton. It is known that *M. galloprovincialis* is able to outcompete and displace native mussels and become the dominant mussel species in certain localities. This is because *M. galloprovincialis* may grow faster than native mussels, be more

tolerant to air exposure and have a reproductive output of between 20% and 200% greater than that of indigenous species (Van Erkom Schurink and Griffiths 1993, in Branch and Stephanni 2004).

Urosalpinx cinerea (Atlantic oyster drill) preys upon oysters, barnacles and other bivalves including mussels. Young snails feed on bryozoans, ectoprocts, small snails and barnacles.The optimal temperature for the development of the Atlantic oyster drill is reported to be 20°C (Ganaros 1958). *Urosalpinx cinerea* can live in salinities as low as 13 to 15 PSU.

Mytilus edulis is also a filter feeding bivalve that feeds mostly on phytoplankton and is a close relative to Mytilus galloprovincialis. Mytilus edulis is eurythermal and are able to withstand freezing conditions for several months. Blue mussels are well acclimated to a 5 to 20 °C temperature range, with an upper sustained thermal tolerance limit of about 29 °C for adults. Blue mussels do not thrive in salinities of less than 15%, but can withstand wide environmental fluctuations. Their depth ranges from 5 to 10 meters. ("Fisheries Global Information System (FIGIS)", 2006; Tyler-Walters and Seed, 2006).

Amphibalanus reticulatus is a frequent fouler of ships and marine structures worldwide in warm subtropical-tropical waters (Utinomi 1970; Henry and McLaughlin 1975). *Amphibalanus reticulatus* prefers saline (30-40 PSU), subtidal habitats in subtropical and tropical seas, although it has been found at salinities as low as 10 PSU (Utinomi 1970). So finding this wasn't a good sign for the harbor. They are both invasive and dangerous to the Maritime Industry.

The Bay Barnacle, *Amphibalanus improvisus,* is characteristic of estuaries and brackish waters, although it can also tolerate high salinities, up to 40 PSU (Foster 1970). In estuaries, it can survive weeks of exposure to freshwater, but requires salinities of at least 2 PSU for reproduction (Dineen and Hines 1992).

Suggestion for improvement

Improvements for this study would include increasing the number of sampling sites and the number of sample days. Also to increase the number of students working on the project because if more people work on this project then more data could be gathered in the allotted time.

Suggestion for future research

A suggestion for a future study is to do more species richness research and organize each of the organisms found by genus and species so that its an observational study that possesses a good amount of both qualitative and quantitative data. Species richness should continue to be measured in sampling sites and compared with this baseline study as well as others previously conducted. It could be beneficial to expand the scope of this project beyond the East River, measuring species richness in places such as the Upper New York Bay or the Long Island Sound. Also using the BugID method which is a system that identifies organisms using images and minimal user input might be a good way to identify the invertebrate samples visually.

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Biographies

Gracie Carter is a senior from the Urban Assembly New York Harbor School. She is apart of a program by the name of Harbor Seals that is restoring the New York City Estuary. She is also currently in her school's rowing team and has won several regional championships as a certified Coxswain. Along with being an advocate for the Billion Oyster Project she is a core member of her school's community and is seen volunteering often. She loves biology and plans on majoring in it at a liberal arts college.

Jared Rosin is a senior within the Urban Assembly New York Harbor School. I have participated in the Rowing team, Student Council, Boat Building, and Harbor Seals, as well as working with the Billion Oyster Project during my time at the Harbor School. My hobbies are powered by my curiosity of this world: Being in nature and preserving it, playing video games and sports, All of the sciences and new/interesting scientific as well as engineering discoveries, reading, creating stories of my own, and spending time with my family. Getting my hands dirty is a way of life for me because i'm always busy.

SAMPLE DAY 9/17/16

NYHS Harbor SEALs – NYHF – CIVITAS
Revision Number: 02
September 14th, 2016

Biodiversity Field Data

Name(s): Bella, Grace
Date: 9/17/16 Time: 11:11
Location:
Station: 3

Weather:
Wind speed/direction: 10 mph Waves/tide/current: Flooding
Air temp: 70° F % cloud: 10%
Moon phase: Full

Water @ start (from 11:17 to 11:30):
Salinity: 22.6 °C: 23.3 Secchi Depth:
pH: 7.77 DO: 78%
Comments:

Method of Sampling:
A) Eckman Grab
B) Line
C) Oyster Cage
D) Tile

Water @ end (from 11:30 to 11:40):
Salinity: 23.5 °C: 23.2 Secchi Depth:
pH: 7.77 DO: 69.2
Comments:

KEY

AE = Aquatic Earthworm; ADWS = Atlantic Dog Winkle Snail; BM = Blue Mussel; BS = Brania Spp; Melampus = Eastern Melampus Snail; Mud = Eastern Mud Snail; HC = Hermit Crab; LDS = Lunar Dove Snail; NHM = Northern Horse Mussel; OW = Opal Worm; SG = Sea Grape/Squirt; SBP = Streblospio Benedictia; TMS = Threeline Mud Snail; TOW = Typical Oligochaete Worm; TTO = Typical Tubified Oligochaete

	ABW	ABWS	BM	BI	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	DW	SD	SW	[illegible]	[illegible]	[illegible]	Other
1										X				X	X	
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