Sequencing New York Harbor: Effects of Salinity Gradients on Biodiversity (Hudson Raritan Estuary, 2019)

Photo Credit: United Nations Decade of Biodiversity

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Abstract

The New York Harbor (NYH) was once booming in biodiversity, but due to industrialization and urbanization, the immense variety of populations began to decrease over time. The NYH is a unique ecosystem, but beyond its uses for the economy and societal work, a distinguishing characteristic is its composition of brackish water- the mix of fresh and salt water, fluctuating based on tides, climate change and human interference. The levels of salinity in any waterbody directly affect the ecosystem since the marine life absorbs its surrounding waters. In the NYH, salinity is a crucial factor of physical-chemistry to study because of the constant changes in tide from ocean saltwater (during flood) and the freshwater (ebb), as well as to understand how to best promote the biodiversity.

This project assesses the effects of salinity on biodiversity from samples along the harbor's salinity gradient. The samples taken for the visual assessment revealed that species richness was similar across sites; The Upper West Side and Long Island City sites yielded a value of 5, Red Hook had a value of 6, and Great Kills with 4. Shannon entropy and inverse Simpson dominance (labeled respectively) were also similar: UWS: 0.826 and 1.7; LIC: 0.425 and 1.23; RH: 1.1 and 2.13; GK: 0.904 and 2.04. This occurred despite that the sites had very different salinity ranges and average salinity was fairly similar. Long Island City displayed having the greatest number of individuals over 1 millimeter per centimeters squared. Long Island City did have the greatest range of salinity which can act as an explanation, however, Red Hook was found to have the highest species richness with the second lowest range of salinity.

The genetic data sequenced yielded 4 main clusters of organisms. Sea squirt, jellyfish, bryozoans, colonial tunicates, it appeared that the species *Botryllus schlosseri* (colonial tunicate)

had black and black with white zooids morphologies, with few exceptions. *Botrylloides violaceus* (colonial tunicate) had red, pink, orange, and yellow morphologies.

Overall, this project suggests that environments that have a wide range of salinities have a greater number of species, but not necessarily a higher Shannon entropy. This supports the hypothesis that there is a positive relationship between salinity and the number of individuals in a community. It does not, however, support the hypothesis that there is a positive relationship between salinity and overall biodiversity (including; species richness, species evenness, and the number of individuals in a community). The data from the DNA analysis yielded that there are two separate species of colonial tunicates, each with their own color morphologies.

Introduction

New York Harbor's Estuary is a unique habitat and community that is home to thousands of marine species (Nyman, 2012). New York's Estuary is a small portion of the world's water bodies: there are 332,500,000 cubic miles (mi^3) of water in and on the planet Earth (Shiklomanov, 1993). New York used to be full of thriving fish and plentiful estuary life (Juet, 1609). When Henry Hudson and Robert Juet came to New York in 1609, Juet described the fish they caught and the biodiversity of the estuary: "[we] caught ten great Mullets, of a foot and a halfe [half] long a peece [piece], and a Ray as great as foure [four] men could hale into the ship." (Juet, 1609). As Europeans began arriving in the New World, they used New York's Estuary as a resource for food, water, transportation, and enjoyment (Alchin, 2014). When the Erie Canal was built in 1825, the waters became less about the marine life that lived there and more about shipping and transportation of goods (Finch, 1998). These waters were and are not only used by the marine species that live there but the human residents as well. As of 2010, 86% of people in the United States rely on public supply water, which comes from city or county water departments (Perlman, 2017). This water is used for domestic, commercial, and industrial purposes (Perlman, 2017). Not only do people rely on water, they heavily impact it as well, through nitrate infiltration (Re *et al.*, 2017) and runoff. New York's Estuary is and has been immensely important to the economy and social workings of New York. Between 1887 and 1996, the total production from the New York Harbor Estuary's most important commercial fish and fisheries decreased approximately 90%, attributed in large part to depleted dissolved oxygen (DO) levels caused by pollution (Tetra Tech and Stoddard, *et al.*, 2000). New York and its people traded natural resources benefits for trade profit.

Salinity is the measurement of dissolved salt in the body of water (Narragansett Bay Commission, 2009). They can fluctuate based on natural occurrences or human interference

(Ojaveer, *et al*., 2005). Salinity changes are being heavily influenced by climate change (Curry, 2003). New York City's main waterways are considered brackish because of the saline water coming from the ocean and the freshwater coming from the Hudson River. Due to natural ocean currents and the ebb and flow of the water, salt levels throughout the system change daily. Marine species can live in a wide variety of salinities (Murray, 1991), however they do have salinity tolerances (Bayly, 1972). If the salinity levels go below 10 ppt biodiversity can decrease in some macroinvertebrates (Ahmadi, *et al.,* 2011).

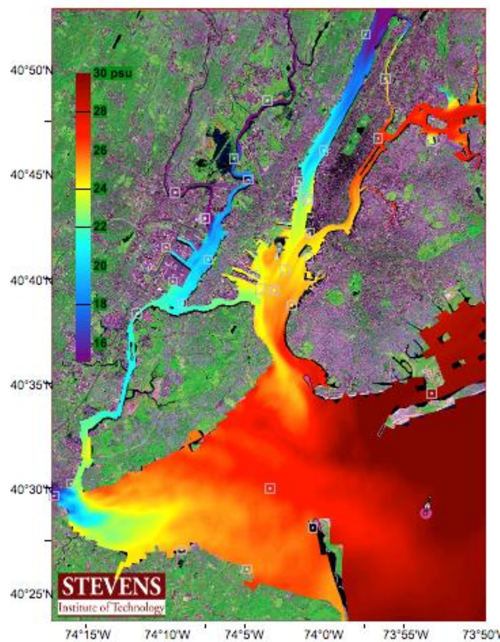

Figure 01: NY/NJ Harbor Estuary Surface Salinity (psu), July 20, 2017, 00:00-01:00 (Stevens Institute of Technology)

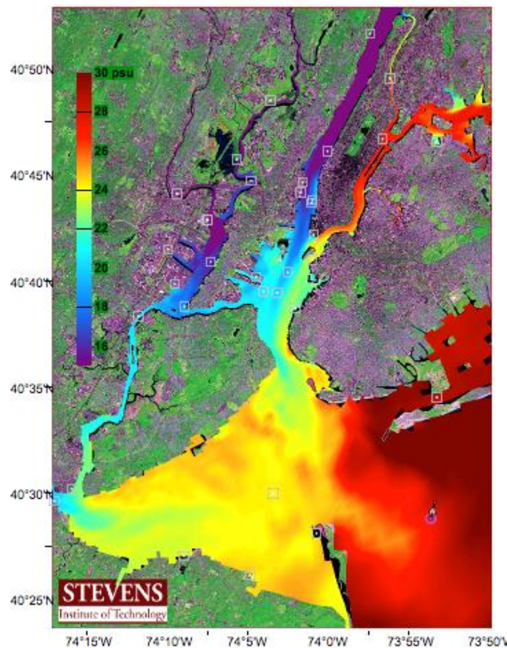

Figure 02: NY/NJ Harbor Estuary Surface Salinity (psu), August 20, 2017, 00:00-01:00 (Stevens Institute of Technology)

Invertebrates are one of the most diverse groups of organisms, with over 1.3 million different identified marine invertebrates as of 2009 (IUCN, 2009). Common marine macroinvertebrates include crustaceans, Polychaetas, sponges, and corals (Thorson, 1950). Macroinvertebrates were used as an indicator of environmental health because they have a relatively brief life cycle, and their anatomy is not particularly complex (Thorson, 1950). Invertebrates are very useful to the study of genetics because of their gene traceability (Wilson-

Sanders, 2011). Thereby allowing researchers to more easily study them and draw appropriate and more accurate conclusions. Invertebrates have been used in an abundance of studies in the past; examples being: Sea Urchin and Clam Eggs: Key Regulators of the Cell Cycle (Hartwell, Hunt, and Nurse; 2001); *Caenorhabditis elegans*: Genetic Regulation of Organ Development and Programmed Cell Death, (Brenner, Horvitz, and Sulston; 2002); Aplysia: Signal Transduction in the Nervous System (Carlsson, Greengard, and Kandel; 2000); Squid: Ionic Mechanism Involved in Excitation and Inhibition in the Peripheral and central portions of the nerve cell membrane (Eccles, Hodgkin, and Huxley; 1963).

As the United States of America became an industrialized country in the mid-1700s, pollution started to get worse (Dwyer, 2017). Most marine organisms are sensitive to the pollution in their environment, so if pollutants are present, the organism may change its morphology, physiology/behavior, or as well as cause mortality (Wilson, 1925). The rising effects of pollution have affected the biodiversity of all invertebrates in the Hudson Estuary (Dugan, *et al*., 2011). Identifying invertebrates through DNA barcoding and interpreting the data found, makes it possible to create accurate assumptions about the health of the New York Harbor Estuary through biodiversity and then take steps to improve based on that data.

Objectives/Hypothesis

Category	Entry
Scientific Problem:	Does salinity affect biodiversity?
Hypothesis 01:	There is a negative relationship between salinity and biodiversity.
Hypothesis 02:	There is a positive relationship between salinity and biodiversity.
Null Hypothesis:	There is an insignificant relationship between the salinity at sample sites and biodiversity.
Objective 01:	Determine the salinity gradient of New York Harbor.
Objective 02:	Determine the biodiversity of all organisms at sample sites.
Objective 03:	Determine the biodiversity of tunicates at sample sites.
Objective 04:	Determine the genetic diversity of tunicates at sample sites.

This project documents and analyzes through genetic sequencing the biodiversity of various macroinvertebrate species within the Upper New York Bay. Biodiversity in an aquatic community is an indicator of health and water quality. The information provided by this project could prove crucial to businesses, the government, and citizen scientists. It is anticipated that there will be different levels of biodiversity in different levels of salinities of New York Harbor. It is also expected that environments with changing salinities will have greater invertebrate biodiversity.

Locality

Samples were taken from four sites around New York Harbor. Site 1 (S1) was the Upper West Side's 79th Street Boat Basin (40.786201, -73.986065) (UWS). Site 2 (S2) was Long Island City's Anable Basin Sailing Bar and Grill (40.749514, -73.956246) (LIC). Site 3 (S3) was Red

Hook's IKEA (40.66931, -74.010893) (RH). Site 4 (Listed S5 in photographs) was the Richmond County Yacht Club (40.543373, -74.138919) (GK).

Figure 03: Map of all sites labeled with Site Code

Methods

Sample Collection

Invertebrate samples were collected from tiles hanging off of four bulkheads (see Figure 3) in the Hudson Raritan Estuary. Invertebrates were collected off of overturned tiles on 45.72cm by 20.32cm by 20.32cm cages with *Crassostrea virginica* growing inside. Each tile was photographed with the site code: dateSite#Tile#Cagetype (ex. 180821S1T1B). Three samples of

each of the visually dominant species from each tile were photographed with the sample code: dateSite#Organism#Tile#Cagetype (ex. 180821S1A063T1B), detached from the tile, and placed in a 10mL glass vial in 5mL of 100% ethanol. Any other visible organism was also labeled, photographed, and placed in 100% ethanol. Sample vials were stored at -20 °C. Percent cover was calculated through Coral Point Count.

Figure 04: Example tile and code

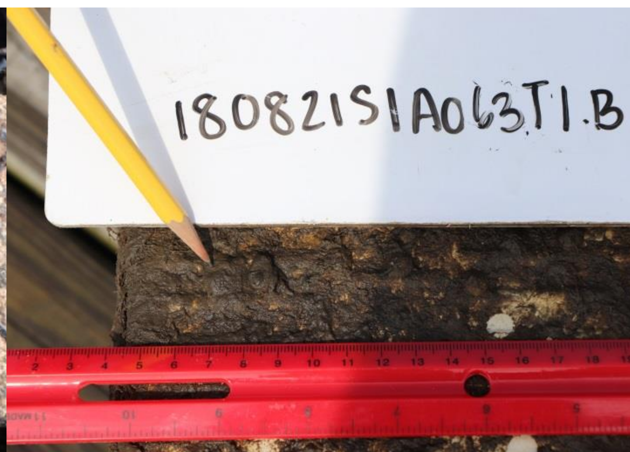

Figure 05: Example organism and code

DNA Isolation

DNA isolation was started by a sample being cut into small pieces and placed into a 1.5 mL microcentrifuge tube. 180 µl Buffer ATL was added. 20 µl proteinase K was added and mixed thoroughly via vortexing, and incubated at 56°C for 2 hours until the tissue was completely lysed. Sample was vortexed occasionally during the incubation process to disperse the sample. 200 µl Buffer AL was added to the sample, and mixed thoroughly via vortexing. 200 µl ethanol (100%) was then added and mixed again thoroughly via vortexing. The solution was transferred via pipet to a DNeasy Mini spin column placed in a 2 mL collection tube. The sample was centrifuged at 6000 x g (8000 rpm) for 1 min. Flow-through and collection tube were discarded. The DNeasy Mini spin column was placed in a new 2 mL collection tube. 500 µl Buffer AW1 was added, and centrifuged for 1 min at 6000 x g (8000 rpm). Flow-through and collection tube were discarded. DNeasy Mini spin column was placed in a new 2 mL collection tube. 500 µl Buffer AW2 was added, and centrifuged for 3 min at 20,000 x g (14,000 rpm) to dry the DNeasy membrane. Flow-through and collection tube was discarded. DNeasy Mini spin column was removed so that the column did not come into contact with the flow-through. DNeasy Mini spin column was placed into a clean 2 mL microcentrifuge tube, and 200 µl Buffer AE was pipetted directly onto the DNeasy membrane. The sample was incubated at room temperature for 1 min and centrifuged for 1 min at 6000 x g (8000 rpm) to elute. The elution step was repeated to increase the DNA yield.

For Amplifying via PCR

Transfer 2 µl of solution from DNA extraction into a PCR tube. Place in a thermal cycler. Run PCR machine under the following protocol:

Initial step: 94°C   1 minute

35 cycles of the following profile:

- Denaturing step: 95° C -- 30 seconds
- Annealing step: 50° C -- 30 seconds
- Extending step: 72° C -- 45 seconds
- One final step to preserve the sample: 4° C -- ad infinitum

PCR Analysis

2 µl of SYBR Green dye was transferred into a clean 1.5 mL tube and 5 µl of PCR product was added. Gel electrophoresis was used to analyze the PCR product. Agarose gel was poured into the gel electrophoresis chamber and left to set for 20 minutes. After setting, 5 µl of solution was added into the wells of the agarose gel. The electrophoresis machine was run at 130 volts for 30 minutes.

PCR Product Analysis

The sample was then sent the Cold Spring Harbor Lab for further sequencing, the data sent back to researchers, and viewed through a software program called DNA Subway. This program brings together the key biological information to assemble gene models which makes it easier to analyze data and the organism.

Data Analysis

Percent Cover

Representative photos were chosen for each tile at each site. Twenty-five random points were chosen on each tile via the program Coral Point Count (CPC) and the organisms under each

point were recorded. The percentage of organisms for the site were then found and inserted into a pie graph.

Number of Individuals Over 1mm per cm²

Representative photos were chosen for each tile at each site. All of the organisms over 1mm were counted and normalized by dividing the total by the area of tile at the site. This number was then inserted into a stacked histogram as compared to sites.

Tunicates

Representative photos were chosen for each tile at each site. Colonial tunicates (Tentative *Botryllus schlosseri*) were counted and normalized by dividing the total by the area of tile at the site. This number was then inserted into a stacked histogram as compared to sites.

The total number of tunicates was normalized by dividing by the area of the tiles. These data were then put into a histogram.

Biodiversity

Historical salinity data was collected from the Stephens Institute of Technology. An average was found for all of the salinity data from the previous two years. A range was also collected for this data set. For the diversity of all the organisms the species richness was calculated and so was the Shannon entropy (see below). For the diversity of just the tunicates the species richness was calculated and so was the Shannon entropy (see below).

$$H' = -\sum_{i=1}^{R} p_i \ln p_i = -\sum_{i=1}^{R} \ln p_i^{p_i}$$

Tunicate Biodiversity

A maximum likelihood phylogenetic tree was created using DNA Subway.

Materials

Sample Collection

Item	Quantity	Function
Gloves	2	Protect researchers hands
Dissection Kit	1	Separate organisms from tiles
RODI Water Bottle	1	Clean organisms
Bucket	1	Get extra water to prevent desiccation of other organisms
Line	1	Attach to bucket
Tubes with 100% Ethanol	100	Place samples into
Extra Tubes of 100% Ethanol	2	Clean tools and to refill any tubes
Tape	1	Label tubes
Sharpie	2	Write labels
Cooler	1	Preserve samples
Ice Packs	2	Preserve samples
Data Tables	4	Keep track to data
White Board	1	Label samples and sites in pictures
White Board Marker	2	Write labels on white board

DNA Isolation

Item	Quantity	Function
Nitrile Gloves	1 Box	Protect researchers hands and prevent DNA contamination
Sample	85	Extract DNA
Buffer ATL	19 mL	Clean and stabilize DNA
Proteinase K	2 mL	Break Open Nucleus
Buffer AL	20 mL	Clean and stabilize DNA
100% Ethanol	20 mL	Clean and stabilize DNA
Buffer AW1	500 mL	Clean and stabilize DNA
Buffer AW2	500 mL	Clean and stabilize DNA
Buffer AE	50 mL	Clean and stabilize DNA
DNeasy Mini Spin Column	85	Hold DNA and solutions
1.5 mL microcentrifuge tube	170	Hold DNA and solutions
2 mL microcentrifuge tube	340	Hold DNA and solutions
Incubator	1	Break up connective tissue
Centrifuge	1	Separate solutions by density
Thermomixer	1	Mix concentrates
Weighing Tray	1	Weigh concentrates
1-10 µl Pipette	2	Move solutions without contaminating
10-100 µl Pipette	2	Move solutions without contaminating
100-1000 µl Pipette	2	Move solutions without contaminating
1-10 µl Pipette Tips	5 Boxes	Move solutions without contaminating
10-100 µl Pipette Tips	5 Boxes	Move solutions without contaminating
100-1000 µl Pipette Tips	5 Boxes	Move solutions without contaminating

Amplifying via PCR

Item	Quantity	Function
PCR Tubes	85	Hold DNA
PCR Beads	85	-
Primers	-	Organize dNTPs and bind to RNA
Buffer	-	Stabilize DNA and RNA
dNTPs	-	Make copies
Polymerase	-	Copy DNA
Thermal Cycler	1	Heat up and cool DNA to make copies

PCR Analysis

Item	Quantity	Function
Agarose Concentrate		Make gel
TBE Buffer		Stabilize electricity
Gel Electrophoresis Chamber	1	Separate DNA by size of fragment
Gel Electrophoresis Tray	1	Hold agarose gel
Rubber Stopper	2	Help agarose gel set
Comb	1	Create wells to insert DNA
1-10 µl Pipette	2	Move solutions without contaminating
1-10 µl Pipette Tips	5 Boxes	Move solutions without contaminating
UV Light	1	Illuminate DNA

Results

Two sets of results were collected: one set of visual assessment and another of genetic biodiversity data.

Salinity as Compared to Biodiversity

Biodiversity

Table 01: Table displaying average salinity, salinity range, species richness, Shannon entropy, and the inverse of Simpson dominance of all organisms

Site	Avg. Salinity (psu)	Salinity Range (psu)	Hill number qD, where q=0	Hill number qD, where q=1	Hill number qD, where q=2
			Species Richness	Shannon Entropy	Inverse of Simpson Dominance
UWS	12.57143	3 to 26	5	0.826	1.7
LIC	22.37143	7 to 30	5	0.425	1.23
RH	21.14286	13 to 28	6	1.1	2.13
GK	23.71429	17 to 30	4	0.904	2.04

At the Upper West Side site there is an average salinity of 12.57143 psu, the range of salinity is 3 to 29 psu. The species richness is 5 and the Shannon entropy is 0.826, and the inverse Simpson Dominance is 1.7. At the Long Island City site there is an average salinity of 22.37143 psu, the range of salinity is 7 to 29 psu. The species richness is 5 and the Shannon entropy is 0.425 and the inverse Simpson Dominance is 1.23. At the Red Hook site there is an average salinity of 21.14286 psu, the range of salinity is 13 to 28 psu. The species richness is 6 and the Shannon entropy is 1.100 and the inverse Simpson Dominance is 2.13. At the Great Kills site there is an average salinity of 23.71429 psu, the range of salinity is 17 to 30 psu. The species richness is 4 and the Shannon entropy is 0.904 and the inverse Simpson Dominance is 2.04.

Percent Cover

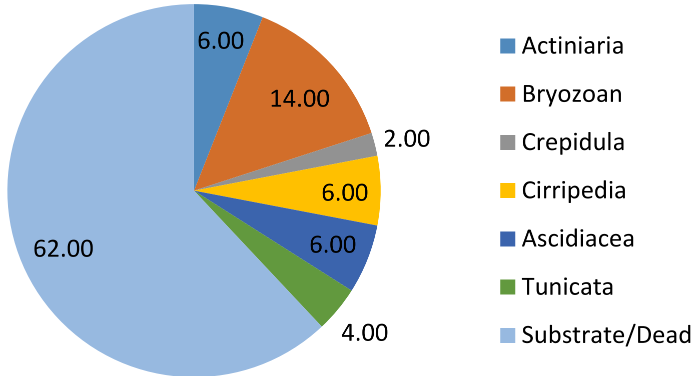

Figure 06: Pie chart displaying the percent cover of substrate and macroinvertebrates on settling tiles in Upper West Side

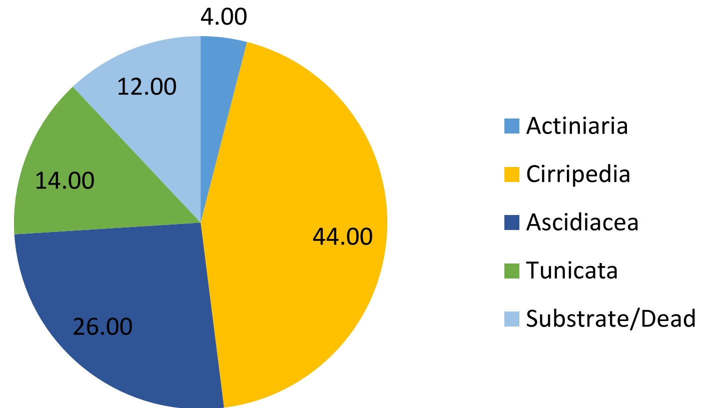

Figure 07: Pie chart displaying the percent cover of substrate and macroinvertebrates on settling tiles in Long Island City

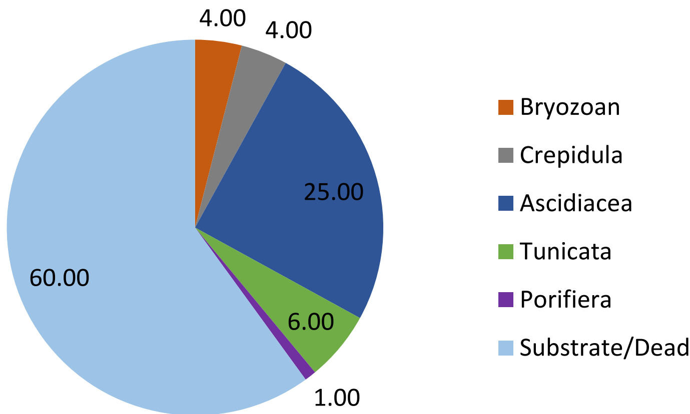

Figure 08: Pie chart displaying the percent cover of substrate and macroinvertebrates on settling tiles in Red Hook

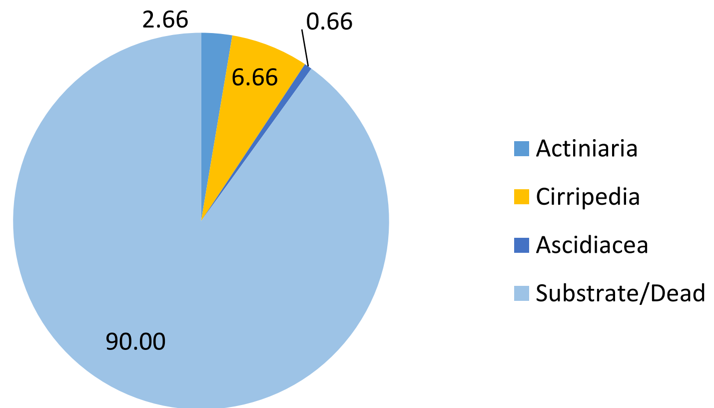

Figure 09: Pie chart displaying the percent cover of substrate and macroinvertebrates on settling tiles in Great Kills

The species that were found were actiniaria (anemone), ascidiacea (sea squirt), bryozoan (bryozoana), crepidula (slipper snails), cirripedia (barnacle), porifiera (sponge), tunicate (colonial tunicate).

In the Upper West Side 62% of the settling tiles was blank tile, dead organisms, or non-invertebrate organisms. The macroinvertebrates that were covering the tile were bryozoans, anemones, barnacles, sea squirts, colonial tunicates, and slipper snails. The bryozoans covered 14%, anemones covered 6% of the tile, barnacles covered 6%, sea squirts covered 6%, colonial tunicates covered 4%, and slipper snails covered 2%.

In Long Island City, 12% of the settling tiles was blank tile, dead organisms, or non-invertebrate organisms. The macroinvertebrates that were covering the tile were barnacles, sea squirts, colonial tunicates and anemones. The barnacles covered 44%, sea squirts covered 26% of the tile, tunicates covered 14%, and anemones covered 4%.

In Red Hook, 60% of the settling tiles was blank tile, dead organisms, or non-invertebrate organisms. The macroinvertebrates that were covering the tile were sea squirts, colonial tunicates, bryozoans, slipper snails, and sponges. The sea squirts covered 25%, tunicates covered 6% of the tile, bryozoans covered 4%, anemones covered 4%, and slipper snails covered 1%.

In Great Kills, 90% of the settling tiles was blank tile, dead organisms, or non-invertebrate organisms. The macroinvertebrates that were covering the tile were sea barnacles, anemones, and sea squirts. The barnacles covered 6.66%, anemones covered 2.66% of the tile, and sea squirts covered 0.66%.

Number of Individuals Over 1mm per cm^2

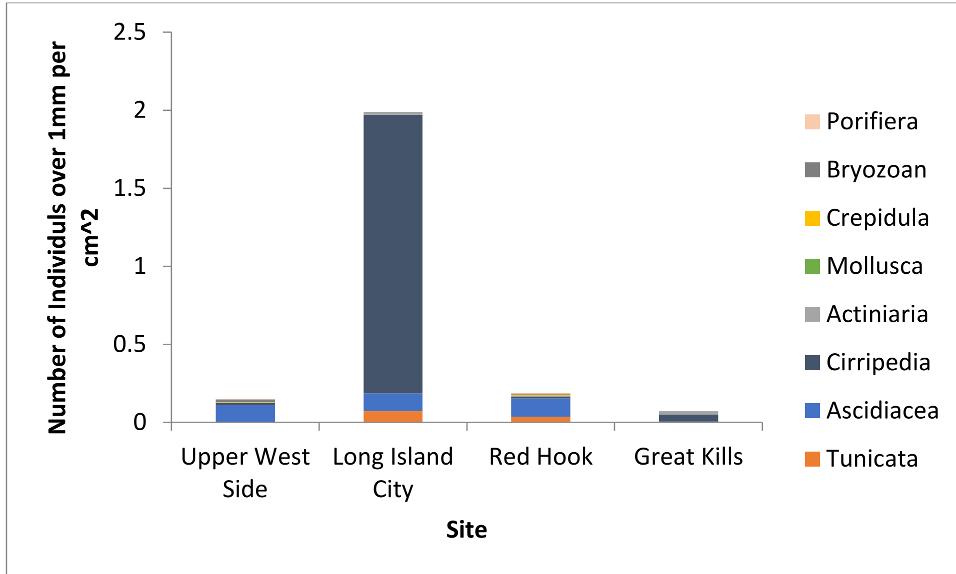

Figure 10: Graph comparing the number of individual macroinvertebrates over 1mm per cm^2 to the site at which they were found

In the Upper West Side site there were 0.109792 Sea squirt per cm^2, 0.015069475 Cirripedia per cm^2, 0.002152782 Mollusca per cm^2, 0.002152782 Crepidula per cm^2, 0.017222257 Bryozoan per cm^2, and 0.002152782 Porifiera per cm^2. In the Long Island City site there were 0.071041809 Tunicata per cm^2, 0.115173841 Sea squirt per cm^2, 1.784656347 Crepidula per cm^2, 0.015069475 Actiniaria per cm^2, and 0.002152782 Bryozoan per cm^2. In the Red Hook site there were 0.033726919 Tunicata per cm^2, 0.119838203 Sea squirt per cm^2, 0.009328722 Cirripedia per cm^2, 0.009328722 Actiniaria per cm^2, 0.005023158 Crepidula per cm^2, 0.005740752 Bryozoan per cm^2, and 0.004305564 Porifiera per cm^2. In the Great Kills site there were 0.005023158 Tunicata per cm^2, 0.001435188 Sea squirt per cm^2, 0.045208424 Cirripedia per cm^2, and 0.018657445 Actiniaria per cm^2.

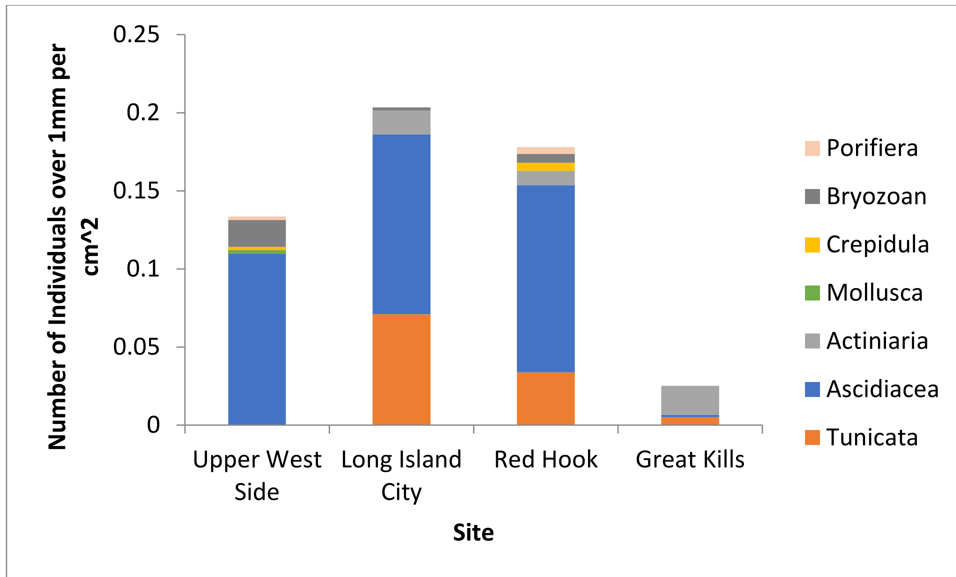

Figure 11: Graph comparing the number of individual macroinvertebrates over 1mm per cm^2 to the site at which they were found, excluding barnacles

In order to take a closer look at the sites as compared to each other, barnacles were excluded from this stacked histogram.

Tunicates

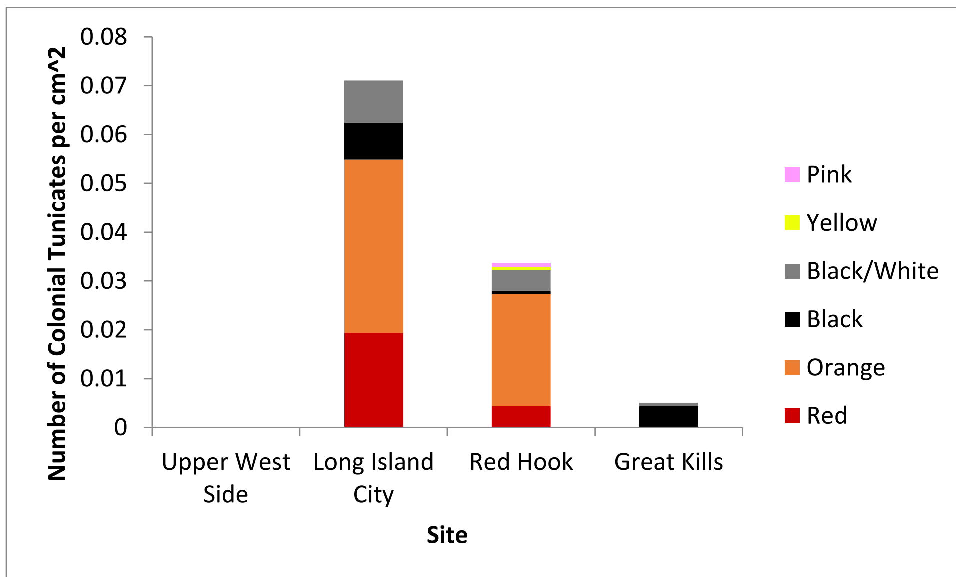

Figure 12: Graph comparing the different morphologies of Tentative *Botryllus schlosseri* per cm^2 to the site at which they were found

At the Upper West Side site there were no Tentative *Botryllus schlosseri* of any morphologies. At the Long Island City site there were 0.019375039 red morphologies per cm^2, 0.035520904 orange morphologies per cm^2, 0.007534737 black morphologies per cm^2, and 0.008611128 black with white morphologies per cm^2. At the Red Hook site there were 0.004305564 red morphologies per cm^2, 0.022963009 orange morphologies per cm^2, 0.000717594 black morphologies per cm^2, 0.004305564 black with white morphologies per cm^2, 0.000717594 yellow morphologies per cm^2, and 0.000717594 pink morphologies per cm^2. At the Great Kills site there were 0.004305564 black morphologies per cm^2 and 0.000717594 black with white morphologies per cm^2.

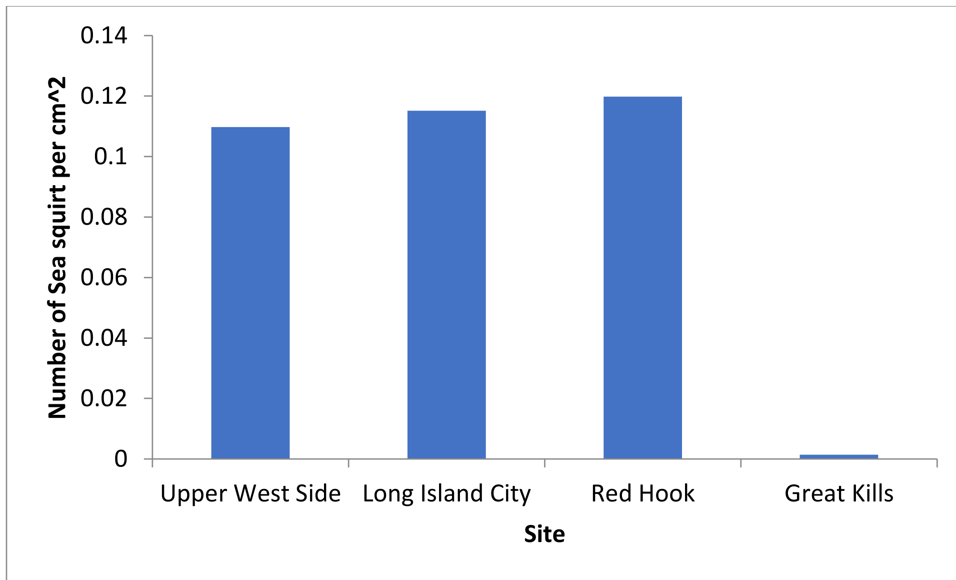

Figure 13: Graph comparing the number of sea squirt per cm^2 to the site at which they were found

At the Upper West Side site there were 0.109791886 sea squirt per cm^2. At the Long Island City Site there were 0.115173841 sea squirt per cm^2.At the Red Hook site there were 0.119838203 sea squirt per cm^2. At the Great Kills site there were 0.001435188 sea squirt per cm^2.

Tunicate Biodiversity

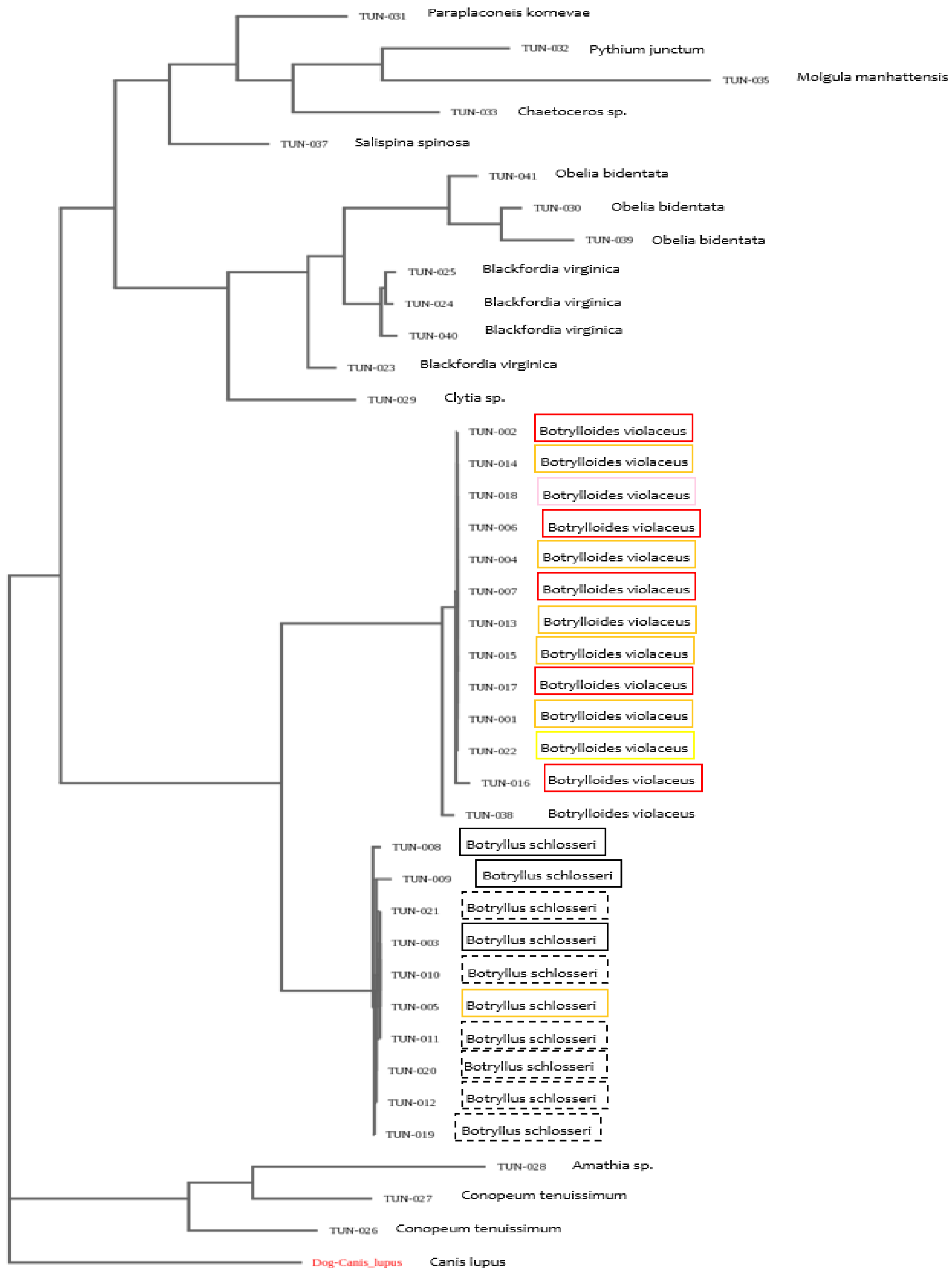

Figure 14: Maximum likelihood phylogenetic tree of all sequenced organisms with the species names to the right. The outgroup is *Canis lupis* (Dog). Colored boxes represent the color of the colonial tunicate (red, pink, orange, yellow, black, black dashed (black with white zooids). Organism TUN-038 has no color because it was sequenced as an sea squirt.

The genetic data sequenced yielded 4 main clusters of organisms. There were 5 organisms that were sea squirt (all being different species), 8 organisms that were jellyfish (3 different species), 3 organisms that were bryozoans (2 different species), 23 organisms that were colonial tunicates (2 different species). It appeared that the species *Botryllus schlosseri* had black and black with white zooids morphologies, with few exceptions. *Botrylloides violaceus* had red, pink, orange, and yellow morphologies.

Discussion

Salinity as Compared to Biodiversity

The purpose of this study was to compare biodiversity to salinity in many aspects (species richness, species evenness, and the number of individuals in a community). This study does suggest that environments that have a wide range of salinities have a greater number of organisms, but not necessarily a higher Shannon entropy (Table 01). My hypothesis was not supported by the data. It was found that there was no notable relationship between salinity and biodiversity. Species richness was similar across sites (UWS: 5; LIC: 5; RH: 6; GK: 4 (Table 01)), despite the fact that salinity ranges were very different and average salinity was fairly similar (Table 01). An explanation for this is that the range of chosen sites in New York Harbor is simply too small and dynamic to have distinct differences in biodiversity. My null hypothesis is also supported by the percent cover graphs. There was a high percent of dead/substrate at most sites (UWS: 62; LIC: 12; RH: 60; GK: 90 (Figures 06-09)). There were slight differences in the distribution of species, but it was fairly uniform across all sites (Figures 06-09). There was also no notable difference in the number of species in UWS, RH, and GK (Figures 10-11). LIC, however, was vastly different

(Figures 10-11). This could be attributed to the fact that Long Island City has one of larger ranges in salinity (Table 01).

Tunicate Biodiversity

One thing that we noticed was that at all sites had sea squirt (Figures 10-11,13). We also noticed that there where different color morphologies of colonial tunicates (Figure 12). When we genetically sequenced the sea squirt there were 5 different species of tunicates, with no relationship to salinity (Figure 14). When sequenced, some organisms that were thought to be sea squirts, turned out to be bryozoans or jellyfish: there were 2 species or bryozoans and 3 species of jellyfish (Figure 14). This data shows that there is cryptic biodiversity and that there is likely a large piece of the picture that is cryptic. The hypothesis made about colonial tunicate diversity was supported. *Botryllus schlosseri* and *Botrylloides violaceus* are different species, however, within the species there are different color morphologies.

Conclusion

Overall, this project suggests that environments that have a wide range of salinities have a greater number of species, but not necessarily a higher Shannon entropy. This supports the hypothesis that there is a positive relationship between salinity and the number of individuals in a community. It does not, however, support the hypothesis that there is a positive relationship between salinity and overall biodiversity (including; species richness, species evenness, and the number of individuals in a community). Taking a look at cryptic biodiversity is important to get the whole picture of what is living in a community and scientists should consider reevaluate how we look at biodiversity in general, namely adding DNA sequencing. Though there does not appear to be a clear correlation between salinity and biodiversity in this study, that doesn't mean that there

is not one on a larger scale. Some suggestions I have for future research on this topic are to look at salinity as compared to biodiversity on a larger scale, use a bigger sample size, and deploy tiles farther from shore. Something that I would also be interested in pursuing further would be the question of ‘do *Botryllus schlosseri* and *Botrylloides violaceus* pass down color morphologies to their off spring?’.

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