Searching for genetic similarities and differences between wild and farmed populations of Eastern Oysters (*Crassostrea virginica*)

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Abstract: The environment is constantly being polluted and harmed, oysters are important to an environment such as ours because they act as natural filters and try to maintain the water cleanliness. Over the past few decades the wild oyster populations have been in a rapid decline, one of the solutions to this problem was to farm oysters and then put them in places like the estuary. However it is believed that the wild oysters genetic code may be different from that of the farmed oyster. It was also believed that the difference (if any) allows the wild oysters to better cope with the harsh environment. In search for these differences 15 oysters were collected. 5 were wild eastern oysters and the other 10 were farmed oysters. After the oysters were obtained their tissue samples were taken. Once the samples were taken the DNA was isolated. Afterwards the DNA was amplified by PCR. To see which PCR samples worked gel-electrophoresis had to be done.

Introduction: New York city is home to numerous rivers, harbors and other waterways. Unfortunately they have been in decline for a very long time. However there is a possibility for reparations thanks to the oyster. Oysters are fantastic natural filters. An adult oyster can filter up to 50 gallons of water a day. Our mission is to restore the oyster population in order to clean up New York's waterways. The subject of our experiment is the Eastern Oyster; *Crassostrea virginica.* This oyster is native to the eastern seaboard and gulf of mexico. Due to bacterial contamination, over-harvesting, pollution and sewage overflows only a sparse number of wild Eastern oysters (Crassostrea virginica) can still be found in the waters of New York City Harbor and coastal wetlands. Major restoration efforts have been made to genetically restore oysters to the Hudson River Estuary (HRE). This work has shown the potential for positive outcomes. In our scientific investigation, when trying to collect initial suggestions for samples, we discovered there was little scientific data to compare or validate the genetic diversity between the farm-raised oysters and the native Eastern oyster in these regions. The Eastern oyster mtDNA phylogenetic pattern across independently evolving species provides strong evidence for vicariant biogeographic processes in initiating intraspecific population structure (Blackwell 2011). Our group is trying to see if there are any genetic differences between farmed oysters and wild oysters. We are looking for any genetic mutations in wild or farmed oysters that could be advantageous to their survival and performance. We hope to find a difference that we could use to help these resilient little mollusks better cope with the harsh water conditions of the estuary and better filter it.

Methods & Materials: Our first step was to collected the fifteen samples. The samples were collected from three different sources; Fisher's Island (farmed), Muscongus Bay (farmed) and Soundview (wild). We labeled and our samples and then proceeded to remove a piece of tissue from each sample. With the tissue samples (first round), we added 300 µl of guanidine

hydrochloride solution, and ground the sample with a plastic pestle for a minute. For the other samples (second round), we added proteinase K to dissolve the tissue. After this all the samples were placed in a bath

- permanent marker
- paper cup
- 1. 5 ml microcentrifuge tubes
- micropipettes and tips (10-1000)
- microcentrifuge
- container w crushed ice
- microcentrifuge tube
- microcentrifuge adapters
- vortexer
- thermal cycler
- small razors or blades

After isolating the DNA we continued to amplify the samples by PCR. A Polymerase Chain Reaction (PCR) is a process which increases the amount of DNA by splicing the helix duplicating a half and joining the two halves together. The piece of machinery used for this is a thermal cycler which heats and cools the samples so that the polymerase enzyme the DNA is able to duplicate.

Sample Number	Time Obtained	Location	DNA Obtained	DNA Sequence
ZMB-001	3/22/14	Soundview	P	No Sequence
ZMB-002	3/22/14	Soundview	N	N/A
ZMB-003	3/22/14	Soundview	N	N/A
ZMB-004	3/22/14	Soundview	P	No Sequence
ZMB-005	3/22/14	Soundview	N	N/A
ZMB-006	3/22/14	Muscongus	N	N/A
ZMB-007	3/22/14	Muscongus	N	N/A
ZMB-008	3/22/14	Muscongus	N	N/A
ZMB-009	3/22/14	Muscongus	N	Ant DNA

ZMB-010	3/22/14	Muscongus	N	N/A
ZMB-011	3/22/14	Fisher's Island	P	Crassostrea Virginica
ZMB-012	3/22/14	Fisher's Island	N	N/A
ZMB-013	3/22/14	Fisher's Island	N	N/A
ZMB-014	3/22/14	Fisher's Island	P	Crassostrea Virginica
ZMB-015	3/22/14	Fisher's Island	N	N/A

Analysis: Unfortunately, many of the DNA samples turned up negative during the gel electrophoresis test. The first round of gel electrophoresis turned up mostly negative and we theorized that the oysters had too high Zinc levels that inhibited the PCR. For the second round of testing we used new protocols to break down Zinc in order for the PCR to properly process the DNA. This time, more samples tested positive but there were still a lot of samples that registered negative. This indicates that the problem with the first round of samples may have been zinc and other causes. If the negation of samples continued after Zinc levels were reduced then the original zinc levels may have only partially contributed to the problem.

Conclusion: Because of factors and variables out of our control such as the problems in high zinc levels, cross-contamination, and human error, there was not enough positive DNA to compare for the experiment. However, this research will continue over the span of the next 2 years. The vital DNA research and sequencing on the wild and farm-raised eastern oyster (Crassostrea virginica) is still a work in progress, yet we expect to present again a continued version of our findings at the next Urban Barcode Project (UBP) Symposium in the American Museum of Natural History. We hope to present this project again which we attain new discoveries, and results of whether our hypothesis can be verified or refuted.

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