

## SUMMARY:

- Eastern oysters (*Crassostrea virginica*) are a keystone species, from filtering water to providing habitat protection with their reefs.
- After being decimated in the mid 20th century, there are efforts being made by groups such as Billion Oyster Project to bring oyster populations back to New York Harbor.
- Aquatic environmental DNA could potentially be used to monitor oyster restoration.
- I aim to develop an assay to detect eastern oyster eDNA.

## BACKGROUND:

Oysters are important contributors to our ecosystem. They clean our waters from toxins and pollutants, with adult oysters being able to filter up to 50 gallons of water a day. Oysters reefs also provide habitats to marine life and attract prey that those creatures rely on. Finally, they help protect shorelines from violent storms, rising tides, and prevent erosion.



Figure 1. Oysters live in salty coastal waters, clustering on rocky surfaces. They fuse together as they grow, forming reefs. (1)

Eastern oysters (*Crassostrea virginica*) is the species name of oysters that live alongside the East coast.

Environmental DNA (eDNA) is DNA that is collected from environmental samples such as water sources, soil, or air, instead of an individual organism. As organisms constantly interact with the environment, their DNA is ejected and accumulated in the surroundings.



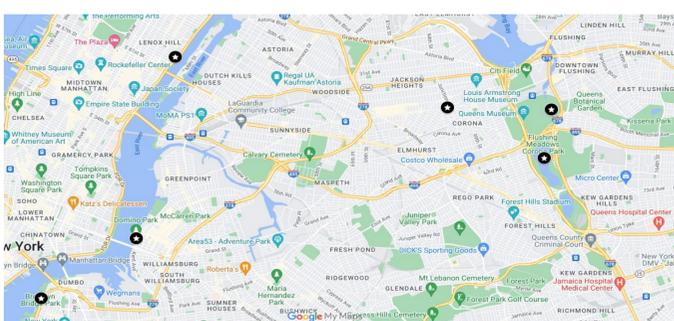
Figure 2. Billion Oyster Project has restored oysters at 15 reef sites across the five boroughs called "field stations". (2)

Billion Oyster Project is a nonprofit organization founded in 2014 that is on a mission to restore one billion oysters throughout New York harbor by 2035. As of 2020, Billion Oyster Project has restored 30 million oysters into the harbor. Having more oysters may be contributing to why New York Harbor is currently the cleanest it has been in over 100 years.

## METHODS :

### Collecting water samples & oysters

Figure 3. I collected 1L of water samples from three locations on the East River: 63rd ST, Domino Park, and Brooklyn Bridge Park. I collected water from fresh water samples: laboratory tap water, Meadow Lake and Fountain of the Planets. I bought eastern oysters from New York and Florida at my local fish market.



### Filtering & extracting DNA

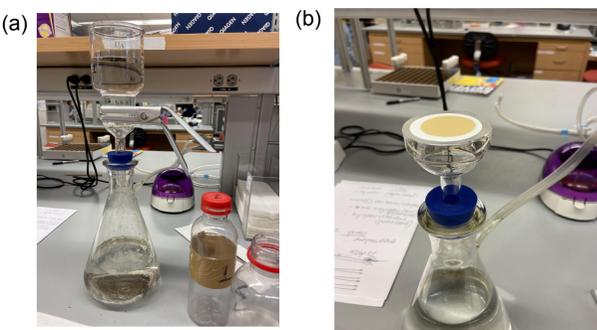


Figure 4. Each of the water samples was filtered using vacuum filtration with a 0.45µm pore size nitrocellulose filter. DNA was extracted from the samples with DNeasy PowerSoil Pro Kit following manufacturer's protocol. The tap water, Meadow Lake and Fountain of the Planets water samples were the negative controls while the fish market oysters were positive controls.



## PCR, Gel Electrophoresis & DNA Sequencing

Figure 5. Primers were designed that target a 250 bp segment of *C. virginica* mitochondrial 16s rRNA gene.

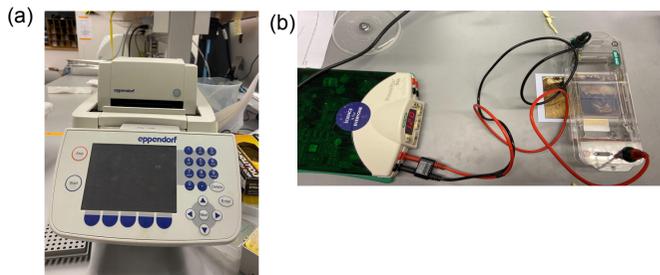
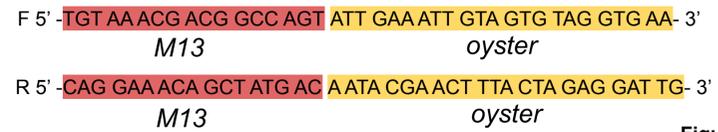


Figure 6. PCR was used to amplify a 250 bp segment of 16s rRNA gene using 200nM of M13 tailed oyster primers. Thermal cycling parameters were 95C 5m; (95C 20s, 52C 20s, 72C 20s) x 40; 72C 1m. Gel electrophoresis was performed to visualize if the expected size product was present. The remainder of each sample was sent to GENEWIZ for Sanger sequencing.

## RESULTS:

### PCR & Gel Electrophoresis

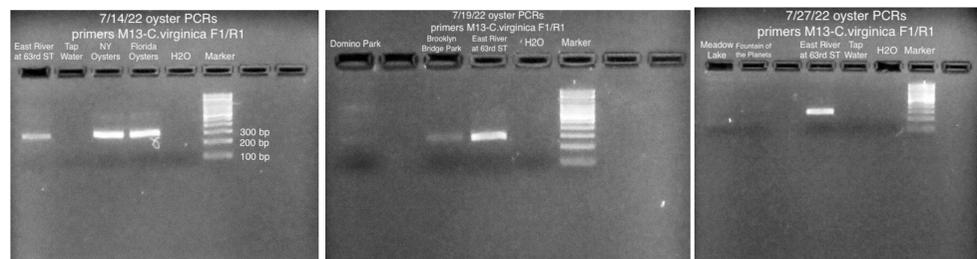


Figure 7. After gel electrophoresis, the gel was put under a UV illuminator, where amplified eDNA can be seen. If oyster eDNA amplified, a band at approximately 300 bp was expected. In the water samples collected from East River at 63rd ST, Brooklyn Bridge Park, and Domino Park, oyster eDNA was detected. However, in samples from Meadow Lake and Fountain of the Planets, there was no eDNA, which makes sense since oysters do not live in freshwater. The tap water negative control was also negative.

### DNA Sequencing

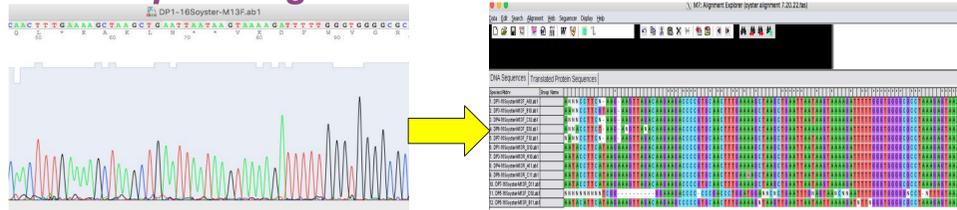


Figure 8. eDNA sequences from the samples were aligned in the software, MEGA7, using MUSCLE. A consensus sequence was constructed that combined aspects of the forward and reverse sequences.

### BLASTing Consensus Sequence

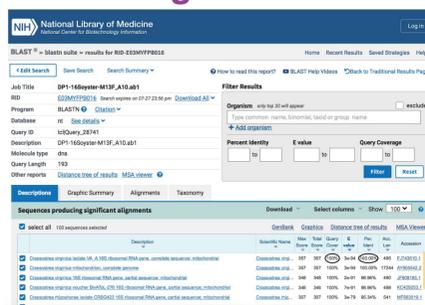


Figure 9. I ran the consensus sequence under the program, BLAST, to compare the nucleotide sequence of the amplified eDNA to all GenBank records. The only 100% matches were to *C. virginica*.

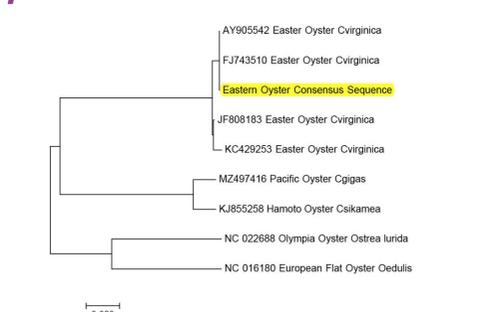


Figure 10. A Neighbor Joining Tree was used to compare my sequence to 16s records of *C. virginica* and other oyster species. The *C. virginica* records clustered together and was distinct from those of other oyster species.

## CONCLUSIONS:

The results demonstrate that eastern oyster eDNA can be detected in New York Harbor using our assay. Potential future directions include mapping the presence of oyster eDNA in and around New York Harbor, and developing a quantitative assay that could be used to determine whether the amount of oyster eDNA detected correlates with how many oysters are nearby.

## ACKNOWLEDGEMENTS:

Thank you to Mark for not only guiding me with this project along the way, but actively giving me feedback that will help me improve as a scientist. Another thank you to RockEdu for welcoming me back into the program a second year.

## REFERENCES:

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2. <https://www.billionoysterproject.org/reefs>
3. Underwater Naturalist *The Oyster: Culinary Delight and Ecosystem Engineer* Spring 2022 Vol.36 No.1 by American Littoral Society Coastal Conservation