Investigation of Biodiversity of Marine Invertebrate Animals and Estuary Health in City Island, NY

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Abstract

This study investigated the biodiversity of marine invertebrates collected in City Island, New York. There are many marine invertebrates that have not been identified to the species level. In this project we used two separate identification methods, iNaturalist and DNA barcoding not only to gain insight on the biodiversity of invertebrates but to also compare and see which method of identification is better. 81 specimens were collected and their pictures were uploaded to citizen science platform iNaturalist for identification. 36 of the specimens were used for DNA barcoding through isolation of the DNA, PCR, and gel electrophoresis. The resulting data contributed to understanding marine invertebrates and provided insight on when different methods of identification should be used.

Introduction

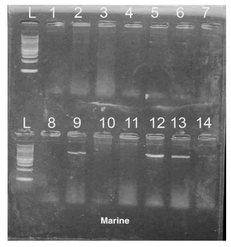
- Marine invertebrates are animals without backbones found in salt water. They include, in particular: mollusks, worms, and crabs. Marine invertebrates make up over 92% of marine animals. They are essential to ocean ecosystems because they transfer energy through food webs, recycle nutrients, build habitats, and maintain water quality.
- There are about 2.2 million species of marine invertebrates and other marine organisms in the world, and it is estimated that about 90% of these species (about 2.0 million) are yet to be discovered.
- Our school is located near City Island, a small island located in the Long Island Sound. City island is situated in an estuarine environment that supports very diverse marine life. Estuaries serve as important breeding, feeding, and nursery grounds for animals, making them ideal locations to study biodiversity of marine invertebrates.
- Methods to investigate marine biodiversity, in particular, include a classic identification based on visual characteristics, as well as DNA barcoding, which is based on the analysis of DNA sequences.
- To better understand the biodiversity of marine invertebrates in this ecosystem, DNA barcoding was used to identify species using short, standardized segments of DNA.
- In addition to DNA analysis, the app iNaturalist was used to support identification by comparing findings with database based on photographs, using AI and and the help of the experts in the field.
- The objective of this project was to study the biodiversity of marine invertebrates in City Island, NY. Additionally, there was a goal to gain insight into which method of species identification is better: iNaturalist or DNA barcoding.
- Our hypothesis was that DNA barcoding will be more reliable for species level identification than iNaturalist.

Figure 1: Map of city Island Beach, NY

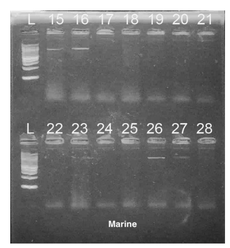
Figure 2: Soft shell clam, *Mya arenaria*

Methods

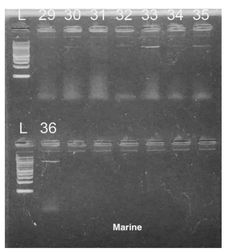
1. Sample collection: 81 specimens were collected using quadrant sampling of 3 habitats in City Island Beach, NY, on September 29, 2025. The habitats were the sandy beach, wet grasses, and rocky areas.
2. Photos were taken of each specimen. These photos were uploaded to iNaturalist for identification.
3. DNA extraction: The DNA was isolated using the Qiagen Dneasy Blood and Tissue Kit.
4. PCR was used to amplify the DNA sequences
5. Gel Electrophoresis was used to separate the DNA and visualize the PCR results. Then DNA was sent to Azenta for sequencing.
6. The sequences were analyzed in DNA Subway and edited, and then species were identified using BLAST database.



Specimens 1-14



Specimens 15-28



Specimens 29-36

Figure 3: Gel Electrophoresis Results

- The gel electrophoresis showed that for some specimens we either lost DNA during extraction or had unsuccessful PCR reaction (no DNA band size 658bp showed on gel electrophoresis).
- Specimens that failed to show bands on the gel electrophoresis likely suffered from DNA degradation, low extraction yields, or the presence of PCR inhibitors (common in marine samples).
- 10 specimens (9, 12, 13, 15, 16, 26, 27, 33, 35, and 36) showed clear (even though sometimes thin) bands from the gel electrophoresis. This indicates high-quality DNA extraction for those samples and a successful PCR reaction.
- DNA sequences for 8 of these 10 specimens were compared to other sequences on BLAST and DNA Subway.

Results

Table 1: Marine Invertebrate Species with a Research Grade on iNaturalist (with number of specimens)

Number	INaturalist identification	Scientific Name	How Many of Each Found
1	Atlantic Ribbed Mussel	*Geukensia demissa*	12
2	Asian Shore Crab	*Hemigrapsus sanguineus*	4
3	Soft-shelled Clam	*Mya arenaria*	20
4	Eastern Mudsnail	*Ilyanassa obsoleta*	14
5	Eastern Oyster Drill	*Urosalpinx cinerea*	2
6	Common Periwinkle	*Littorina littorea*	1
7	Flat Periwinkle	*Littorina obtusata*	1
8	European Green Crab	*Carcinus maenas*	1
9	Threeline Mudsnail	*Ilyanassa trivittata*	1
10	Eastern Oyster	*Crassostrea virginica*	1

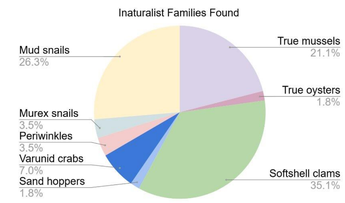


Figure 4: Families Successfully Identified on iNaturalist

- Of the 81 specimens collected, 57 of these specimens received a research grade on iNaturalist for species identification, and they represent ten species (see table 1).
- 71 specimens were narrowed down to the species level, 1 was narrowed down to suborder, 2 were narrowed down to the genus level, 4 were narrowed down to the family level, 1 was narrowed down to class, and 2 were narrowed down to animals.
- Figure 4 shows the eight families that the specimens were categorized into according to iNaturalist.
- We were unable to receive research grade on iNaturalist for the following species: Clam worms (Ragworms), Eastern mudsnails (Nassa Mudsnails), and Soft-shelled Clams (Softshell Clams).
- Two invasive species were identified, those being the Asian Shore Crab and the European Green Crab.
- The 8 specimen that received BLAST results on DNA Subway were each identified as Clam Worms (*Alitta succinea)*

Table 2: Marine Invertebrate Species without a Research Grade on iNaturalist (with number of specimens)

Number	iNaturalist Identification	Scientific Name	How Many of Each
1	Clam Worm	*Alitta succinea*	10
2	Eastern Mudsnail	*Ilyanassa trivittata*	1
3	Soft-shelled Clam	*Mya arenaria*	2

Discussion

- We found that iNaturalist is efficient for the identification of some of the species, but not of the others. Successful species identification (research grade) was found for 57 specimens out of 81 in total, 70% of collected specimens. For some specimens (13, 16%) our observation received species identification on iNaturalist but didn't yield a research grade.
- And, finally, for some other (11, 14%) species iNaturalist was unable to suggest an identification for it. We propose that in cases when iNaturalist identification is inconclusive, DNA barcoding would be a better method for species identification.
- The DNA Barcoding was useful when it came to identifying 8 Clam worms, all of which had not received a research grade on iNaturalist and couldn't be narrowed down to the species level. Part of this was due to their external morphology, which can be unclear and ambiguous for citizen scientists to suggest an identification.
- The Asian Shore Crab (*Hemigrapsus sanguineus*) and the European Green Crab (*Carcinus maenas*) both are examples of invasive species. Invasive species can be harmful to the environment because they can outcompete native species for shelter and other resources, possibly leading to a situation where newly arrived species replace native species.
- Overall, image-based identification using iNaturalist successfully classified the majority of collected marine invertebrates to the species level (70%), but its reliability decreased for morphologically similar or underrepresented taxa, highlighting the need for DNA barcoding as a complementary method to achieve accurate and comprehensive taxonomic identification.
- In the future, we could re-do this experiment with a larger sample size to get a clearer idea of the biodiversity. We would also improve DNA extraction and preservation to receive better quality sequences. Finally, we could compare the biodiversity of marine invertebrates in City Island to other estuaries in New York.

Acknowledgements

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Figure 5: European Green Crab, *Carcinus maenas*

Figure 6: Family Nereidae

Figure 7: Eastern Oyster Drill, *Urosalpinx cinerea*

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Figure 8: Flat Periwinkle, *Littorina obtusata*

Figure 9: Atlantic Ribbed Mussel, *Geukensia demissa*

Figure 10: Asian Shore Crab, *Hemigrapsus sanguineus*