Examining the Biodiversity of Plankton in the Hudson River

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Abstract

Copepods, a class of metazoan organisms, occupy a crucial role in the water ecosystem, as they are both predator and prey to a variety of organisms. Because of their importance to the ecosystem and their abundance, the purpose of this study was to investigate the biodiversity of copepod species within the Hudson River (specifically the polyhaline zone). A 1977 study surveyed the copepod species found in the Hudson River at that time. Since biodiversity can change in the course of decades, the goal was to verify and update the catalog of species found. A total of 9 water samples were collected from three different locations along the NJ side of the Hudson River: Englewood Boat Basin, Alpine Boat Basin and Hazard's Ramp. After DNA extraction, a PCR was performed with diverse metazoan invertebrate (DMI) primers for the cytochrome c oxidase subunit i (*COI*) gene. After running gel electrophoresis to determine the PCR success, samples were sequenced by GeneWiz. Using the DNA subway website and software, the gene sequences were identified and phylogenetic trees were generated. None of the samples collected contained copepods, so very little can be said about the state of copepod biodiversity in the Hudson estuary. However, two species of comb jellies native to the Western Atlantic were present at most sample sites. Since these species mainly prey on copepods, it can be inferred that the copepod population in the Hudson River is normal.

Introduction

Important Terms:

- **Biodiversity:** variety of life in a particular habitat or ecosystem
- **Zooplankton:** microscopic organisms that inhabit the water and are ubiquitous in aquatic environments
- **Copepods:** subclass of zooplankton composed of microscopic shrimp; an important part of the marine food chain as the primary food source for larval fish
- **Polyhaline Zone**: region with 18-30 ppt (estuarine) salinity

Table 1. Estuarine copepod species found, NY Bight Study (Malone, 1977)

Estuarine Copepod Species
Eurytemora affinis
Eurytemora americana
Eurytemora herdmani
Acartia clausi
Acartia tonsa
Pseudodiaptomus coronatus
Oithona brevicornis
Oithona similis
Tortanus discaudatus
Paracalanus crassirostris

- As an estuary, the Hudson River is an area that should have an abundance of different species of zooplankton.
- Knowledge of copepods in the Hudson River area is limited; our study aims to broaden this knowledge.

Figure 1. Close-up of an *Oithona similis* copepod
Image: http://www.plantbio.com/contents/upload-images/z022oithona%20brevicornis%E2%99%80.jpg

Research Goals

1. To identify species of copepods found at sampling points in the Hudson River Estuary in the polyhaline zone;
2. To compare the species found with those in the 1977 study;
3. To analyze the genetic relationship between species found.

Hypothesis: The species of copepods we will find will be similar to the identified species in the 1977 study.

Methods

Sampling & Preparation:

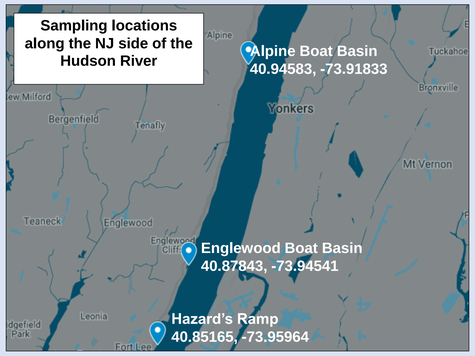

Figure 2. Map of sampling locations along Hudson River and GPS coordinates (Image: Google Maps)

1. Three ~40 mL water samples were collected per location using three Educational Insights GeoSafari Plankton Nets.
   - Alpine Boat Basin **(RSS-002,004)**
   - Hazard's Ramp **(RSS-005,006,007)**
   - Englewood Boat Basin **(RSS-008,009,010)**
2. 10 mL of isopropyl alcohol was added. Samples were spun down using a centrifuge to form pellets.
3. Just prior to DNA extraction, the liquid was pipetted out, and the tubes left open to evaporate any remaining isopropyl alcohol.

DNA Extraction

1. **LYSING THE CELLS:**
   - 300 µL of guanidine HCl solution was added to each sample pellet and mechanically ground
   - Samples were heated for 10 min at 65°C and centrifuged for 1 min at 13.3 rpm.
2. **BINDING THE DNA:**
   - 50 µL of supernatant was mixed with 3 µL of silica resin to bind the DNA.
   - Samples were heated for 5 min at 57°C and centrifuged for 30 sec.
3. **REMOVING CELL DEBRIS/ CONTAMINANTS:**
   - The supernatant poured out. 500 µL of wash buffer added to pellet, and the sample was resuspended by pipetting and centrifuged for 30 sec.
   - The removal of the supernatant, washing, and centrifuging was repeated, and then supernatant was completely removed.
4. **UNBINDING SILICA FROM DNA:**
   - 100 µL of dH_2O was added to unbind the silica from the DNA.
   - Sample was heated for 5 min at 57°C and centrifuged for 30 sec.
   - 50 µL of supernatant were transferred to a new tube ,and the DNA samples were stored on ice.

Polymerase Chain Reaction & Gel Electrophoresis

1. **DNA AMPLIFICATION (PCR):**
   - 5 µL of each (defrosted) sample was added to 10.5 µL diverse metazoan invertebrate (DMI) primers for the cytochrome c oxidase subunit i (*COI*) gene and 12.5 µL *Taq*/ dNTP (nucleotide) mix, and placed in the thermal cycler for 3.5 hrs.
2. **GEL ELECTROPHORESIS:**
   - 2 µL of each amplified DNA sample was mixed with loading dye and loaded into a 2% agarose gel with a positive control and DNA ladder.
   - The gel was electrophoresed 30 minutes at 130 V.
   - Samples that produced the target bands (~300 bp) were sent to GeneWiz for sequencing in both the forward and reverse directions.

DNA Sequencing, Editing, & Analysis

1. The sequenced DNA was uploaded into the DNA Subway platform, where the sequences were trimmed, paired, and edited for consensus.
2. Sequences were then matched to those in BLAST and GenBank to identify species.

Results

Figure 3. Gel electrophoresis results after PCR with DMI *COI* primer (target sequence = ~300 bp). (Photo taken by Melissa Lee)

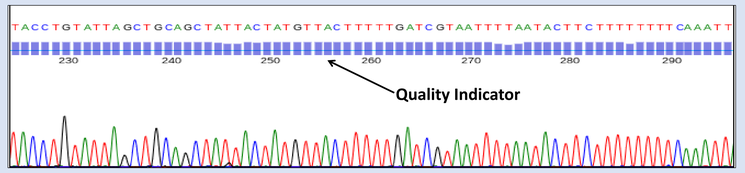

Figure 4. DNA sequencing results from the Sanger sequencing method used by GeneWiz (Sample RSS-008) (Image: DNA Subway)

- DNA sequencing results from Englewood Boat Basin **(RSS-008, 009, 010)** had the best quality sequences, but all sequences were good quality.

Table 2. Species found in 3 sampling locations

Location	Sample	Closely related species
Alpine Boat Basin	RSS-002, 004	*Pleurobrachia pileus, Mnemiopsis leidyi*
Hazard's Ramp	RSS-005, 006, 007	*Mnemiopsis leidyi*
Englewood Boat Basin	RSS-008, 009, 010	*Mnemiopsis leidyi, Pleurobrachia bachei, Pleurobrachia pileus Beroe ovata* (mitochondrial partial COI gene)

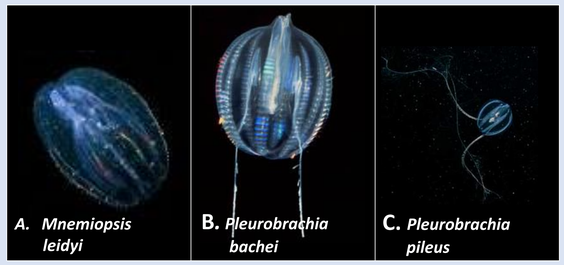

Figure 5. Comb jelly species found in samples.
Image A: https://ocean.si.edu/oceanphotos/sea-walnut-mnemiopsis-leidyi
Image B: http://jellieszone.com/ctenophores/pleurobrachia/
Image C: http://www.habitas.org.uk/marinelife/species.asp?item=E50

Results (Continued)

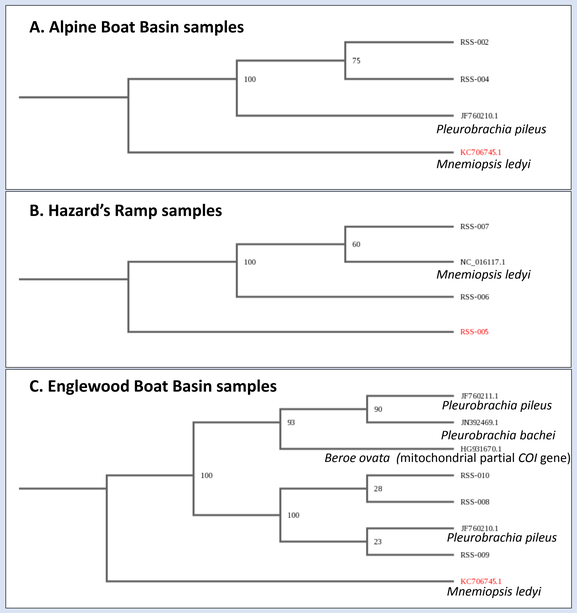

Figure 6. Maximum Likelihood (ML) phylogenetic trees of analyzed samples (Images: DNA Subway)

- The phylogenetic trees that were generated show the evolutionary relationship between the samples and GenBank sequences.
- Most of the DNA samples are very closely related to the database sequence for various comb jellies, so the water samples from the 3 locations tested most likely contained comb jellies.
- No copepod species were identified.

Discussion/ Conclusion

- Because no copepods were identified, we could not compare present day copepod diversity in the Hudson River with that found in the 1977 study.
- Comb jellies mainly prey on copepods and their presence may indicate that the population of copepods is normal within the polyhaline zone Hudson River estuary.

References

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