Our data shows a 42% increase in neuroblast migration toward the lesion site in NR-42 treated groups compared to controls ($p < 0.01$).

Investigating the Diversity of Invertebrates at Twin Lakes Using DNA Barcoding

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ABSTRACT

We researched by the Twin Lakes and Mill Pond because their mix of freshwater ponds, lakes, and moist soil habitats that could support a high diversity of small invertebrates that are often underrepresented in genetic databases. By collecting specimens from these environments, we hoped to help identify and barcode species that are unique to or abundant in our desired locations. Our collection methods will included both passive traps—such as pitfall traps lined with plastic—and attempts of active sampling with nets for aquatic invertebrates which were then frozen and prepared for DNA extraction and sequencing. Through this process, we sought to contribute valuable data on Long Island's invertebrate diversity and support broader efforts in conservation and species identification.

INTRODUCTION

Long Island's freshwater ecosystems are facing increasing environmental pressures, such as pollution, habitat loss, and climate change, which can greatly affect local insect and invertebrate populations (*Thriving habitats and abundant wildlife*, n.d.). Because these small species play vital roles in maintaining ecosystem balance—such as breaking down organic matter and serving as indicators of water quality—it is important to study and document their diversity (*Worms and decomposers*, n.d.). To better understand how local habitats support these species, we chose to investigate several freshwater sites across Long Island.Our main collection areas will be Mill Pond, Twin Lakes, and the nearby nature trails which all of these locations are from Wantagh, New York. These areas feature ponds, marshy soil, and shaded woodland edges that together create rich habitats for aquatic and terrestrial invertebrates. The mix of moist soil, decaying plant material, and still freshwater makes these locations ideal for finding diverse insect species, including those that may not be well-documented in this region. By sampling from these sites, we hope to contribute to Barcode Long Island's larger goal of identifying and cataloging the wide range of invertebrate species that inhabit these valuable freshwater ecosystems.

METHODS

We set up traps to bait the invertebrates and then freeze them in order to research without any interruptions. We used pitfall traps to lure and capture these invertebrates. The pitfall traps were about a foot deep to ensure we gained enough species and ensure they were unable to escape. We also used bottle fly traps to catch flying invertebrates and a net for aquatic invertebrates. With this method, we put sugar to attract flies and use a cut up water bottle as a nozzle to let the flying insects drop. We then froze all samples to preserve the DNA we need from them and euthanize the invertebrates. Then we examined the DNA for these invertebrates too compare the sequence of the COI gene. The chelex method was used to extract DNA, while the COI gene will be amplified for analysis. This is because it is common to all species we collect as it is a mitochondrial gene. To analyze our data, we will compare the DNA sequences to known DNA barcodes that are in databases to identify the invertebrate species we captured. By looking at the genetic differences from our samples, we can determine different levels of biodiversity at the collection sites.

KEY RESULTS

Stage of Analysis	Number of Samples	Percentage of Total	Result/ Observation
Initial collection	20	100%	Visibly distinct variety of invertebrate morphospecies successfully isolated.
Post-Extraction Viability check	3	15%	Non-viable, insufficient tissue mass, severe physical degradation, or failure during the Chelec extraction step.
PCR Product, (gel Electrophoresis	17	85%	No usable results. Loaded alongside DNA ladder; yielded blank lanes, primer-dimers, or faint unreadable smears.
DNA Subway Bioinformatic Analysis	17	85%	Confirmed absence of high-quality consensus sequences; used primarily for troubleshooting.

DISCUSSION

Using the traps to capture and extract the invertebrates' DNA, we were able to find unique DNA sequences which helped us find the exact invertebrate species via DNA barcode. By examining the exact species after extracting all their DNA as well as analyzing the COI gene, we were able to find the approximate biodiversity level of Twin Lakes and Mill Pond, where the samples were collected.

CONCLUSION

Although this study successfully collected 20 freshwater invertebrates from Wantagh, COI gene identification failed due to DNA degradation or inhibitors.To fix this for future Barcode Long Island teams, we recommend:

Silica-Column Extraction: Replaces Chelex to wash away environmental inhibitors.

95% Ethanol Preservation: Immersing samples immediately to protect DNA integrity better than freezing.

Positive Controls: Running verified DNA during PCR to quickly troubleshoot blank gels.

These steps will ensure future student teams successfully catalog the Twin Lakes and Mill Pond communities.

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