Viability of eDNA in a Time Series After Collection

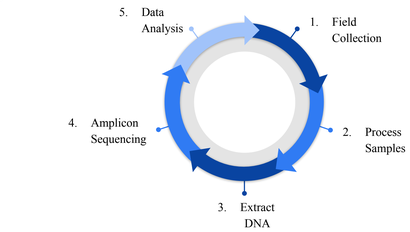
Authors: Neil Sarkar[1] and Shubh Khanna[2]
Mentors: Seth Cunningham[3] and Michael Tessler[3]
[1]Stuyvesant High School; [2]Hunter College High School; [3] Sackler Institute for Comparative Genomics, American Museum of Natural History

URBAN BARCODE RESEARCH PROGRAM

Abstract

Metabarcoding using environmental DNA (eDNA) is making rapid advances when it comes to surveying species in a geographic region. We aimed to find out if time or temperature impacted the quality of eDNA samples in storage. Our hypothesis was that there would be a decrease in concentration as time went on but the decrease would not be drastic. We took samples from the Croton Reservoir and extracted DNA on the same day, as well as 4, 8, and 16 days after sampling. We stored samples at room temperature (and -20 ℃ for a second 16 day sample). This allowed us to correlate DNA concentration/quality over time for these eDNA samples. Our results show that as time went on, the DNA concentration slightly decreased. However, the decrease is not dramatic enough to entail leaving the field to extract the DNA. We also found that freezing does not affect DNA concentration significantly. Furthermore, we used the trnL and 16S genes to do metabarcoding of our samples to look at plant and arthropod species composition differences. Using ordination visualizations, we found little species composition level differences between samples collected at different time points, indicating storage is not an issue. This is significant because it means that researchers do not need to grapple with the issue of collecting samples and immediately leaving the field to extract the DNA. As a result, this study paves the path for future eDNA research and explorations with definitive understandings of how DNA concentration changes while in the laboratory.

Introduction



It is critical to spend **maximum** time in the field and preserve DNA for later extraction

The Problem:
After getting an environmental sample, scientists try to extract eDNA as soon as possible. This takes time away from surveying in the field, and can mean a loss of valuable data that could be collected with more field time.

Objectives

1. Establish how long scientists are able to store eDNA samples without losing DNA concentration or quality.
2. We aim to test the effect of time and the quality and analysis of eDNA samples recorded through metabarcoding.

Methods

Collection: A set of samples was collected from each of three water sources (Figure 1): Hudson River, Croton River, and New Croton Reservoir, one of NYC's sources of tap water.
Extraction: Each of the samples was filtered and the product stored at room temperature in lysis buffer from the Qiagen DNeasy PowerWater DNA extraction kit. DNA extraction was completed for one sample from each of the three sites on the same day, as well as 4, 8, and 16 days after sampling.
Sequencing: We quantified the DNA concentration from each sample. We conducted PCR reactions for plant trnL and arthropod specific 16S. The samples were then prepared for metabaroding by cleaning the PCR materials and then sequencing at GeneWiz using a MiSeq. We then used R to interpret the NGS data, and assess the differences amongst samples containing eDNA under varying periods of time.
Analysis: Species composition was compared using ordination via the metaMDS in the R package Vegan.

Figure 1: Sampling Sites

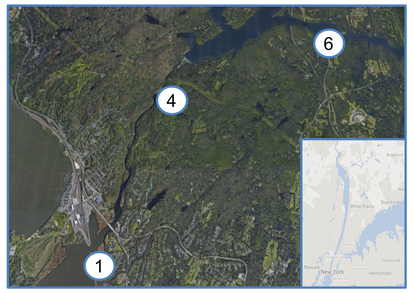


Figure 2: DNA Concentration as a Result of Collection Time

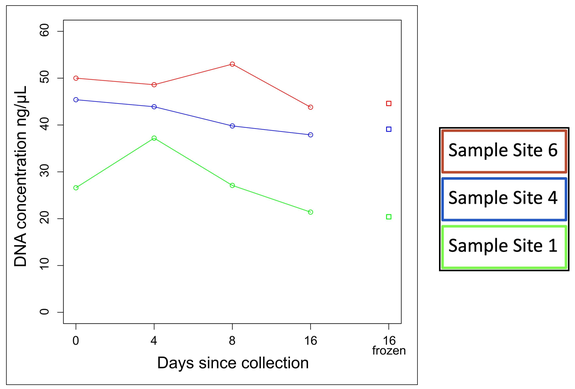
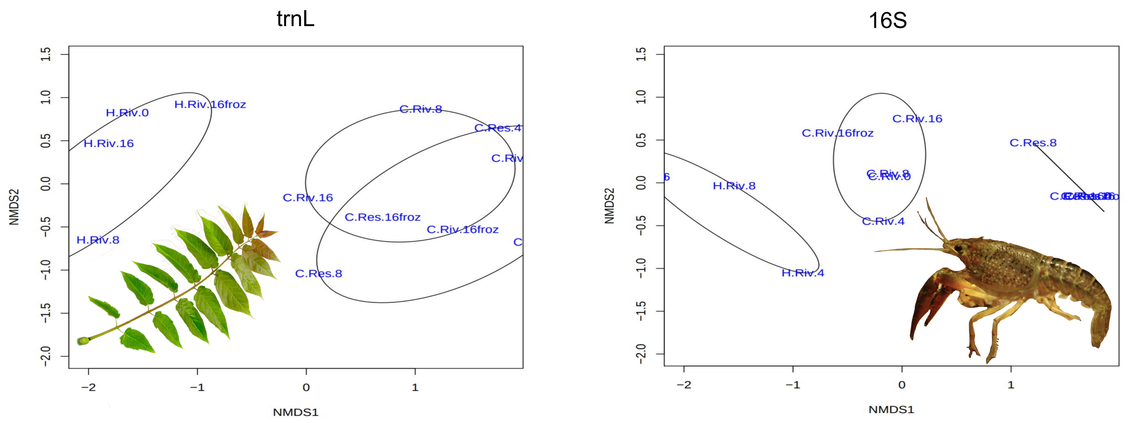


Figure 3 and 4: Comparisons of Samples Over TIme



Results

1. Time series collection has minimal effect on DNA concentration (Figure 2)
2. The decrease is not dramatic enough to entail leaving the field to extract the DNA
3. Freezing does not affect DNA concentration
4. Figures 3 and 4 show that generally the samples are closely clustered together for each location
5. We found a variety of plants and arthropods, some of which were invasive, like the Tree-of-Heaven (Ailanthus altissima), while others were native, such as the spiny-cheek crayfish (Orconectes limosus)

Key Takeaways

The results obtained in this study are significant because they highlight that DNA concentration minimally changes over time. This means that researchers do not need to grapple with the issue of collecting samples and immediately leaving the field to extract the DNA.

Future Work

This study paves the path for future eDNA research and explorations with definitive understandings of how DNA concentration changes while in the laboratory. We can examine the relationships between other factors and DNA concentration.

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Acknowledgments

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