Survey of algae diversity in man-made tide-pools on the Harlem River in Randall's Island

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ABSTRACT

Biodiversity is the variety of species within a habitat and it is an important indicator of the health of an ecosystem. Biodiversity has been adversely affected by global warming and the rapid urbanization of coastal areas. Companies like ECOncrete™ have been engineering cement using more eco-friendly materials to create structures better suited to help support healthy coastal ecosystems. One prime site for such tide pools is the shore of Randall's island, located at the crosspoint between The Bronx, Queens and Manhattan in NYC. In our study we explore the algal biodiversity in the eco-friendly tide-pools found on Randall's Island along the Harlem River. We collected specimens from each pool three times during the year and compared the different species found in each pool using DNA barcoding. We found that the standard PCR protocol we used was not as effective for the amplification of our algae samples for DNA barcoding. The analysis of the few samples that were successfully amplified identified Ulva Prolifera as one of the species in the tide pools. Future work will focus on optimizing DNA extraction and PCR amplification of algae samples and investigating variables affecting tide pool biodiversity.

INTRODUCTION

Coastal areas are losing biodiversity in part because global warming is negatively affecting coastal zones by causing sea levels to rise and increasing the intensity and frequency of storms. Many people live in coastal zones,[1, 2] and artificial shore lines are being constructed to protect these areas. The concrete used to build these structures can negatively impact the surrounding ecosystem leading to attempts to engineer more environmentally-friendly materials that support biodiversity. [3] It is of great importance to elucidate if and how these man-made structures benefit the coastline ecosystems. We are particularly interested in the growth and diversity of macroalgae as these organisms are vital for a healthy coastal ecosystem.

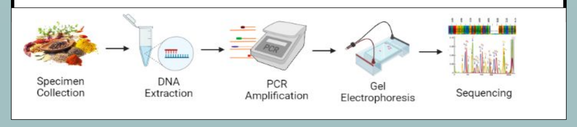
As part of the engineered protective coastal structures, communities are adding man-made tide pools.[4] Tide pools are small pools of water in the intertidal zone, which is the land located between high tide and low tide lines. On natural shorelines, tide pools are created when pools of water collect between rocks after the tide has receded. These tide pools can have diverse ecosystems that benefit the biodiversity of shorelines.[5] A keystone species of this tide pool ecosystem is macroalgae,[6] which is the algae visible with the naked eye. These algae species can be differentiated with proper training and microscopy.[7] Alternatively, DNA barcoding allows scientists who lack expertise and/or microscopy equipment to accurately identify species.

We are studying man-made tide pools on Randall's Island, located between the Harlem River and East River off the coast of Manhattan (**Figure 1**). These tide pools are composed of a bio-enhancing mixture of concrete, called ECOncrete ™, which is molded into shapes that are purported to support biodiversity.[8] We believe that the ECOncrete™ tide pools on the edge of Randall's Island retaining wall are ideal models to study biodiversity in artificial tide pools.

Our group is interested in how environmental engineering can mitigate the effects of global warming and urbanization on coastal environments. Although it has been shown that ECOncrete™ supports algal recruitment,[3] more should be learned about how man-made tide pools support biodiversity. Our goal is to characterize the algae in the Randall's Island ECOncrete™ tide pools.

MATERIALS & METHODS

Sample collection: Algae samples were collected on: November 19, January 28, and March 24, 2023 at low tide from tide pools. For each morphologically-distinct algae in each tide pool, samples were collected in vials filled with water from the corresponding pool. The samples were stored at RT until they were processed for DNA extraction.
DNA extraction and purification: DNA extraction was performed using Thermo Scientific™ GeneJET Plant Genomic DNA Purification Kit.
DNA barcoding and data analysis: DNA was amplified using Promega GoTaq™ G2 Green Master Mix, using TufA_F/TufA_R primers specific for algae using the DNALC protocol. The PCR product was run on a 1.5% agarose in 1X TBE gel stained with SYBR green and sent for sequencing to GENEWIZ/AZENTA Life Sciences. Sequencing data was analyzed using DNA Subway and BLASTN.



RESULTS

Figure 1: Location of the ECOncrete™ Tide Pools. Map of the location of the man-made tide pools along the Harlem River on Randall's Island between the 125th Street Bridge and the Randall's Island Ferry dock.

Figure 2: Tide Pool at low and at high tide. In the figure, representative tide pools are shown at low (left) and high tide (right). Samples were collected during low tide on three different dates during the year. In the image it is possible to see the varied surface on the inside of the pool meant to support biodiversity.

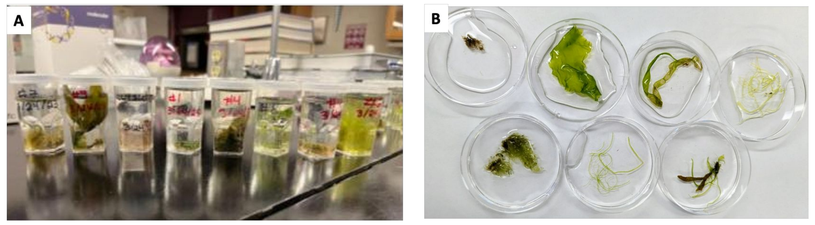


Figure 3: Algae samples. A. Vials containing individual algae samples. **B.** Examples of the more common algae found in various tide pools.

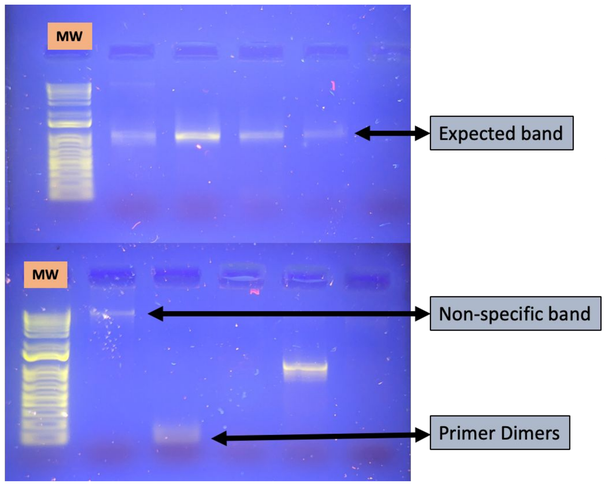


Figure 4: Gel Electrophoresis of PCR for DNA barcoding. In the top gels it is possible to see the appropriate band expected from the PCR reaction. In the lower gel it is possible to see an example of the non specific bands and primer dimers for samples that did not amplify correctly.

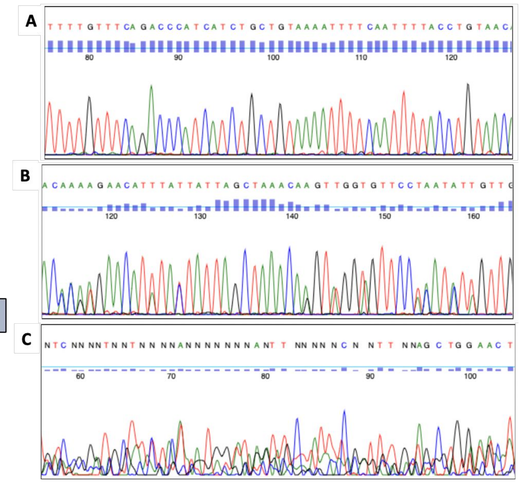


Figure 5: Examples of successful and unsuccessful DNA Sequences from barcoding PCR. A. Example of successful sequencing for DNA barcoding of an algae sample. The peaks are strong and clear. **B.** In this sequence, the peaks are strong, but in many of the cases it shows a double peak, which potentially means that more than one species was present in the sample. **C.** This is an example of one of the sequences that did not yield useful results.

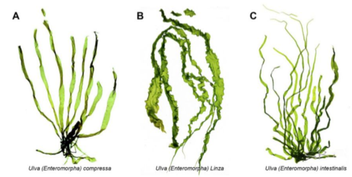


Figure 6: Images of Ulva species (from https://encyclopedia.pub/entry/20755)

DISCUSSION

We observed that the diversity of macroscopic algae in the tide pools varied by their location. More algae and more morphologically diverse algae were found in tide pools further away from the bridge and the dock. To ensure we collected all the possible species we gathered all morphologically unique algae specimens in each pool even if they were similar across the various pools. We planned to reduce the number of samples that needed to be tested by determining which algae looked similar or unique by observing their morphology in the lab, but at the same time process a few of the commonly seen samples of algae to determine whether there was any genetic difference. Unfortunately we encountered some technical challenges both with the DNA extraction and DNA barcoding PCR protocol. The parameters for DNA extraction needed to be optimized to obtain a higher DNA concentration per sample. We obviated the initial issues by using larger pieces of algae. However, for future extractions we plan to add small amounts of sand to help the pestle better crush the algae, as we think the mucilaginous nature of the samples was impeding the breaking down of the cells and the release of DNA.

To get samples for DNA barcoding we used a standard PCR using primers for the TufA gene, specific for algae. However, the initial attempt resulted in no visible band in our gels, suggesting that PCR also needed optimization. We ran the PCR at a lower temperature to facilitate primers annealing. Albeit not completely successful, the change resulted in some samples producing clean bands upon gel electrophoresis. The successful PCR samples were sent for sequencing. The sequences were then analyzed using DNA Subway. A good number of samples did not produce usable sequences stressing the importance of optimizing the PCR reaction. The working sequences were then analyzed by BLAST. Interestingly, the analysis indicated that the samples were all one species of algae, Ulva Prolifera. This possibly means that the morphologically different algae could simply represent different stages of the same species.

FUTURE DIRECTIONS

1. To further investigate algae biodiversity in tide pools, the first step will be to optimize DNA extraction, adding small concentrations of sand in our collection tube to better break down the algae cells.
2. Optimize PCR protocol through lowering the annealing temperature and adjusting the concentration of Mg^{+} in the PCR mix will produce a better product for barcoding.
3. After optimization, variables affecting biodiversity, such as distance from human activity, vertical height above the low tide line, and water quality, can be investigated. In addition, as more algae growth is expected with warmer temperatures, it might be interesting to collect more samples over the summer months.
4. Finding and exploring algae databases to examine algae morphology to complement barcoding data.

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