Using DNA to assess the impact of water quality on plankton communities

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Background

Plankton- "Canaries in a Coal Mine"[1]

Plankton make up a major portion of the ocean's biomass; they act as a food source for marine life and play key roles in chemical cycling. There are two main types of plankton: phytoplankton and zooplankton. Phytoplankton are a diverse assemblage of photosynthetic organisms that cycle carbon dioxide and nutrients producing large amounts of oxygen as a byproduct. Zooplankton, by contrast, are small multicellular animals and larvae that feed on phytoplankton and transfer carbon to higher trophic levels. Both phytoplankton and zooplankton are critical to natural physical and chemical pathways, and are sensitive to environmental changes, such as fluctuations in nutrients and pollutants. Thus, monitoring plankton communities provides valuable information about water quality and ecosystem health[1,2]

DNA Metabarcoding

Traditional plankton identification involves visual classification using microscopes, which is time-consuming and requires trained taxonomists. DNA metabarcoding is a proposed solution to this problem. This technique that utilizes next-generation sequencing technologies to identify multiple species within a single sample using a short DNA sequence, or barcode (Figure 1) and has been shown to provide greater species resolution and more complete community assessments.[3]

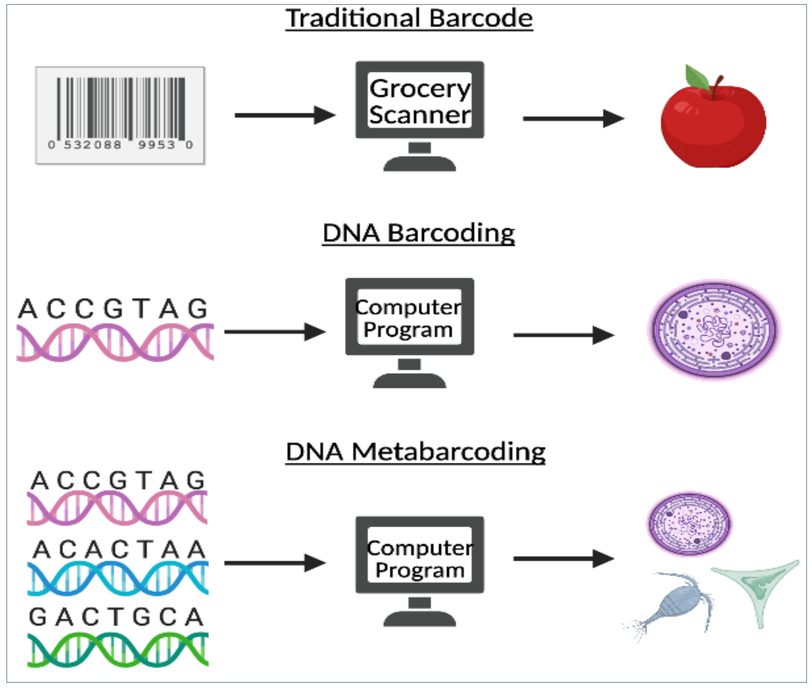

Figure 1. Diagram of traditional barcodes, DNA barcoding, and metabarcoding. Bioinformatic computational programs allow for species identification from genetic information[4].

Objectives

1) Optimize metabarcoding methods to create an efficient and cost effective metabarcoding "toolkit" for plankton bioassessment
2) Augment current plankton surveys within the reserve to create more robust baseline datasets.
3) Investigate relationships between water quality and changes in plankton communities.

Methods

Sample Collection: Currently, water samples are being collected monthly at five sites in the Guana River Estuary and four sites in the Tolomato and Matanzas River Estuaries (Figure 2). Sampling occurs alongside the GTM staff's monthly nutrient and System Wide Monitoring Program (SWMP) collections. Three replicates of 500 ml of surface water are taken at each site, in addition to two field controls each sampling day and filtered using 8-µm mixed cellulose ester (MCE) filters. Filters are preserved in Longmire's buffer and stored at -20°C until DNA extraction (Figure 3).

DNA Extraction: DNA is extracted from ½ of each filter, with the other half remaining achieved at -20°C . Extractions are performed using an Omega E.Z.N.A. Tissue DNA Kit (Omega Bio-tek Inc., USA) , followed by a clean-up step with a OneStep PCR Inhibitor Removal kit (Zymo Research Inc., USA). To avoid contamination, all extractions are conducted in a sterile laminar flow hood in a PCR free area, and all equipment is cleaned with 10% bleach and UV sterilization

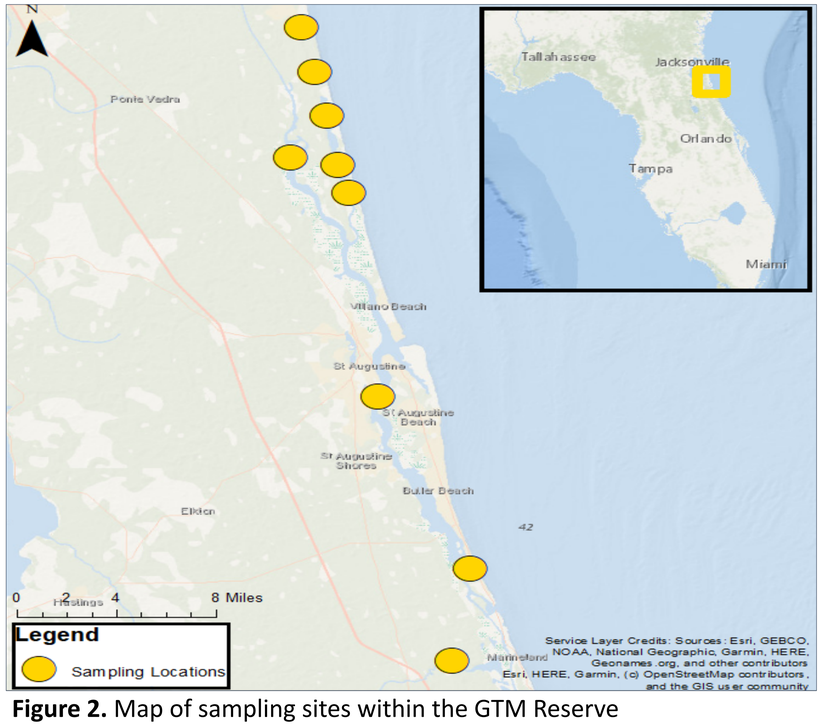

Figure 2. Map of sampling sites within the GTM Reserve

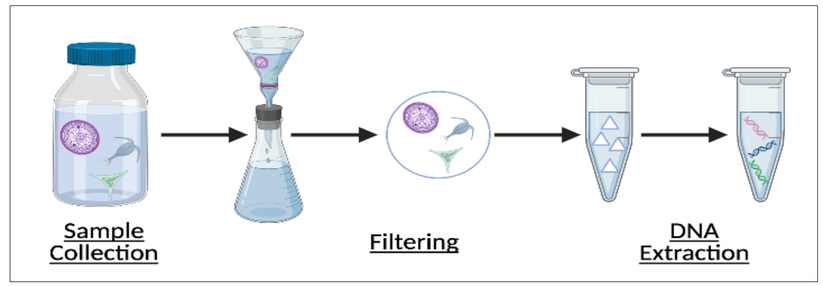

Figure 3. Flowchart of collection, filtration, and DNA extraction steps[4].

Next Steps

Primer Optimization: Three primer sets have been identified for testing including the 16S rRNA, 18S rRNA, and CO1 regions (Table 1). These have been chosen to target a wide array of phytoplankton, zooplankton, and cyanobacteria.
PCR and Sequencing: Sequencing libraries will be prepared following a two-step PCR method[5] and performed in duplicate. Final libraries for each marker will be sequenced separately using an Illumina MiSeq and 600 V3 (2x300) kits.
Data Analysis: Sequences will be trimmed, filtered, and queried against public databases to assign samples to the lowest possible taxonomic level. Species composition results and diversity metrics will be compared with water quality parameters to identify potential relationships.

Table 1. Proposed primer pairs for metabarcoding.

Primer Pair	Target Taxa	DNA Region	Fragment Size	Reference
341F/805R	Phytoplankton/ Cyanobacteria	16S rRNA	400	Herlemann et al. 2011
1391F/EukB	Zooplankton/ Phytoplankton	18S rRNA (V9)	150	Stoeck et al. 2010
mlCOIintF/ HCO2198	Zooplankton	COI	313	Leray et al. 2013

References

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3. Siegenthaler A, et al. (2019) DNA metabarcoding unveils multiscale trophic variation in a widespread coastal opportunist. *Molec. Ecol.* 28, 232-249.
4. Images created with BioRender.com.
5. Kumar G, et al. (in review) One size does not fit all: Tuning eDNA protocols for high and low turbidity sampling. *Env. DNA.*

Acknowledgements

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