

An Optimized DNA Metabarcoding Toolkit for Monitoring Plankton Communities in the GTM Research Reserve

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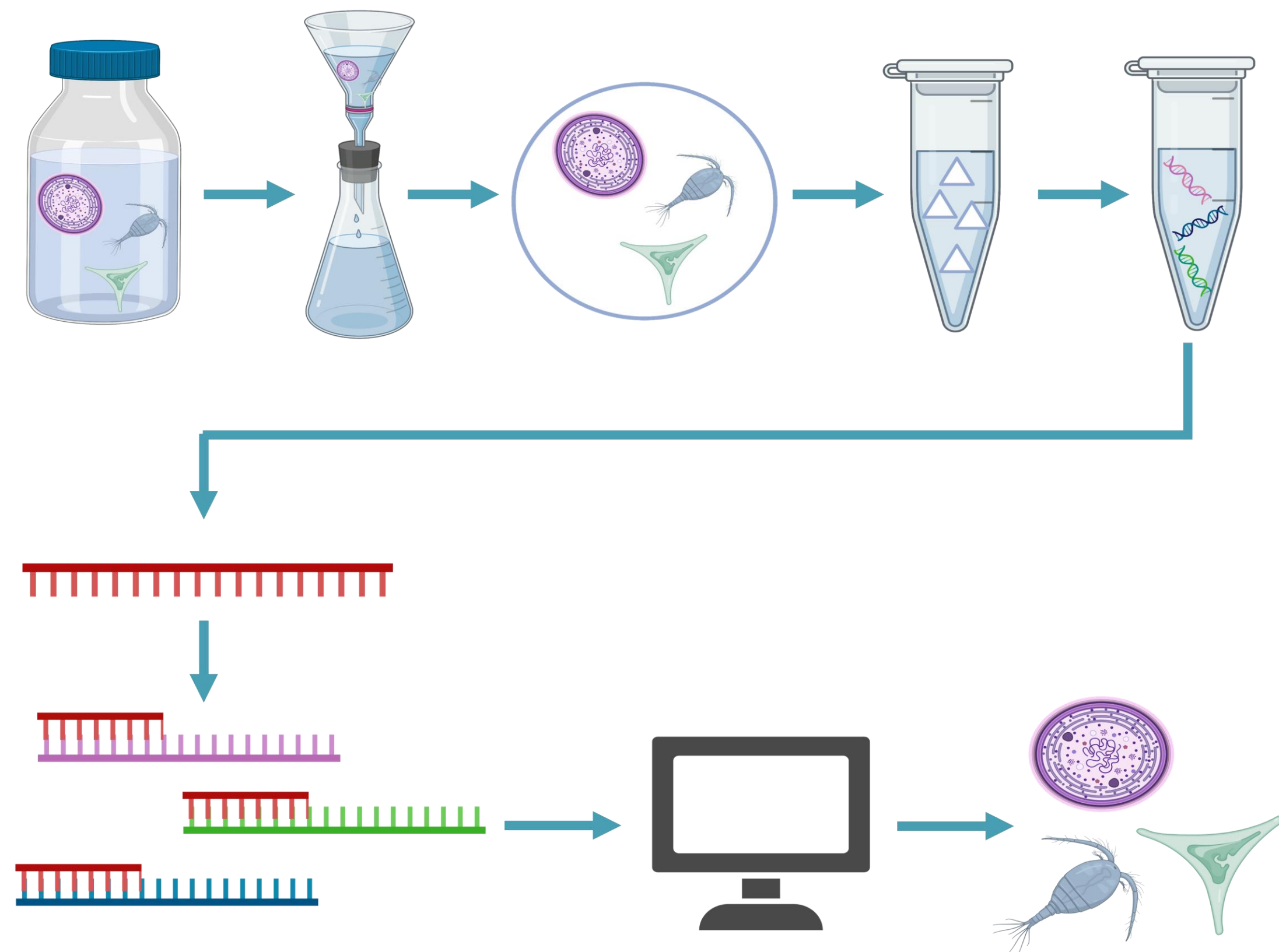


BACKGROUND

Plankton communities are key components of estuarine ecosystems and serve as bioindicators of environmental changes. DNA metabarcoding is a tool that can efficiently expand upon traditional plankton monitoring methods.

OBJECTIVE

Optimize a DNA metabarcoding protocol for monitoring plankton communities in the Guana Tolomato Matanzas (GTM) National Estuarine Research Reserve.

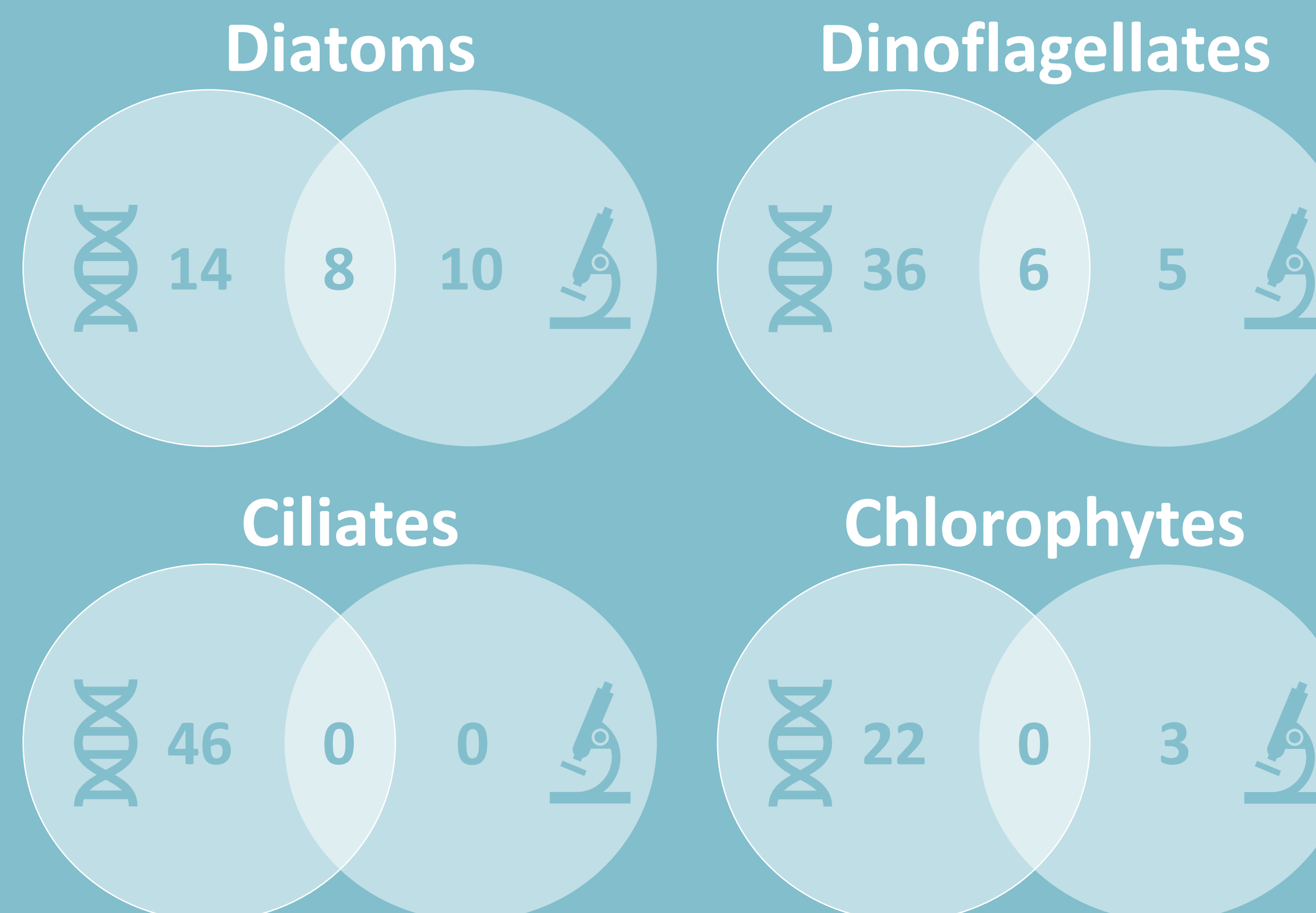
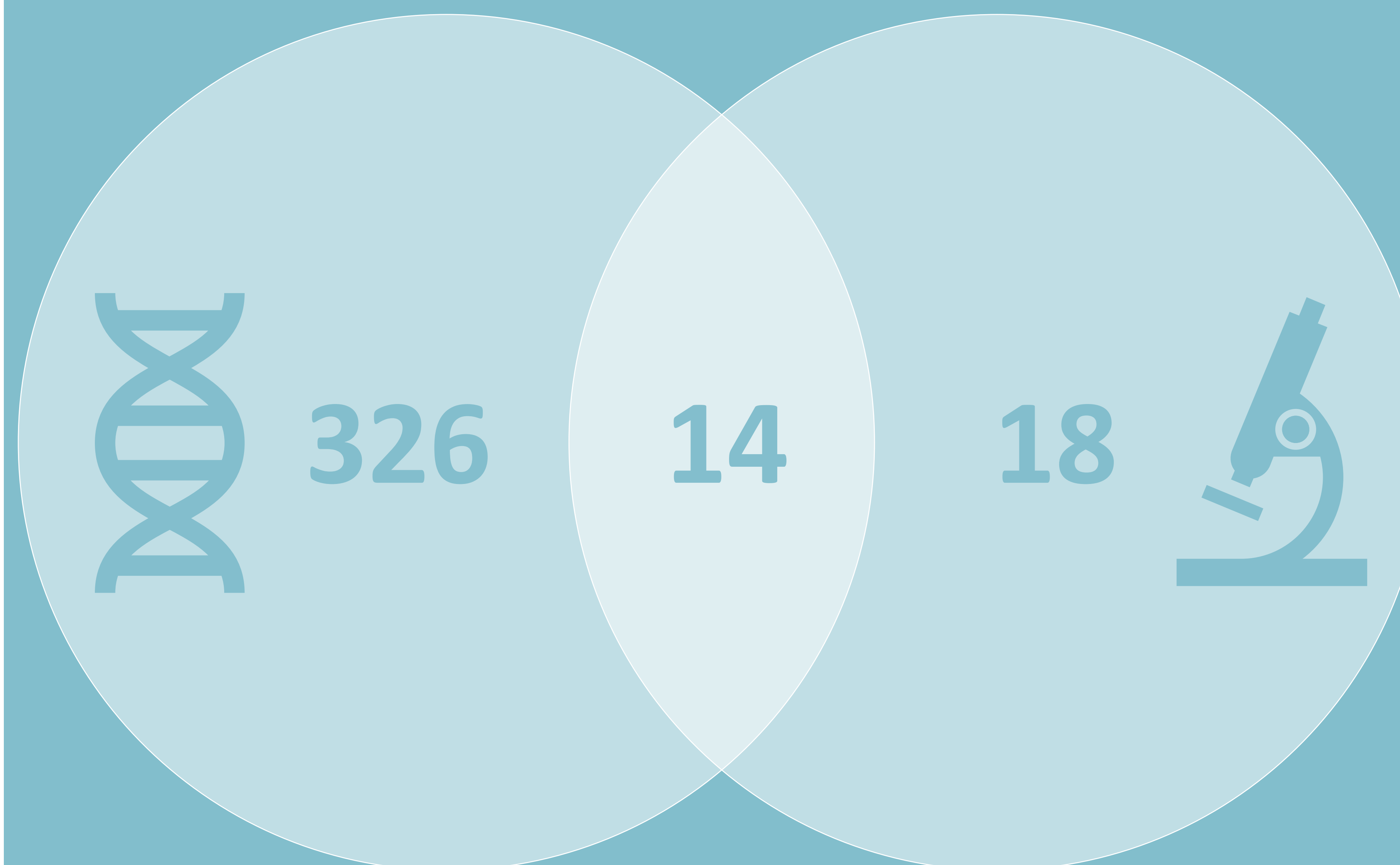


METHODS

Water samples were collected at both clear (Ft. Matanzas) and turbid (Lake Middle) sites for metabarcoding and microscopy analysis:

1. DNA was extracted using commercial kits
2. The 18S V9 rRNA region was amplified and sequenced following a two-step PCR protocol and Illumina MiSeq.
3. Amplicon sequence variants (ASVs; unique sequences) were determined using an in-house bioinformatics pipeline.
4. ASVs were assigned to the lowest possible taxonomic level using the PR2 database.
5. Taxa were compared between metabarcoding and microscopy.

DNA metabarcoding toolkit improves plankton monitoring in estuaries



Total number of eukaryote genera identified with metabarcoding and microscopy

RESULTS

Metabarcoding taxa include:

- Potentially harmful diatoms (Chaetoceros & Pseudo-nitzschia)
- Potentially harmful dinoflagellates (Karenia, Prorocentrum, etc.)
- Newly classified groups (Nibbleridia)
- Plant taxa (Streptophyta)
- Red algae (Rhodophyta)
- Taxa which are difficult to ID with light microscopy (Tubulinea)
- Indicator ciliates (Tintinnopsis)
- Several metazoans

Summary of taxonomic coverage

	Metabarcoding		Microscopy		
	3133 ASVs		57 IDs		
	Rank	#	%	#	%
supergroup		2743	87.6	53	93.0
	division	2629	83.9	53	93.0
subdivision		2386	76.2	49	86.0
	class	1879	60.0	38	66.7
order		1566	50.0	37	64.9
	family	1336	42.6	34	59.6
genus		958	30.6	34	59.6
	species	153	4.9	2	3.5

NEXT STEPS

- Complete analysis of ASVs from the 16S primer region (bacteria, archaea, and photoautotrophic eukaryotes).
- Use this toolkit to sequence plankton from 10 sites across the GTM over the course of a year and synthesize these data with water quality.