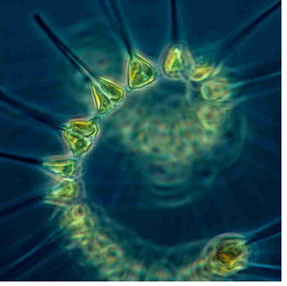
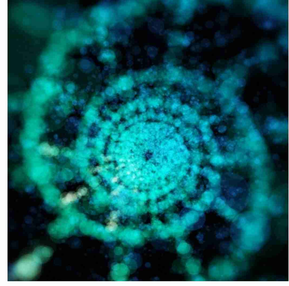


DNA Sequencing from Home: Using a Portable DNA Sequencer to Monitor Eukaryotic Microorganisms in the Indian River Lagoon

Heidi J.T. Pagán, Tatiana Ferrer, and Greg O'Corry-Crowe



Abstract

As part of the **Florida Center for Coastal and Human Health**, **18S eukaryotic community profiling** was performed on water samples from Indian River Lagoon sites over the past two years. The portable handheld **MinION device** allowed us to minimize time in the lab after preparing the sample by then running the DNA sequencer at home. **Taxonomic classification from this rapid response, long read technology identified several toxin producing species from harmful algal blooms**, including *Aureoumbra lagunensis*, *Pseudo-nitzschia*, and *Pyrodinium bahemense*, as well as a predator species of *Microcystis aeruginosa*. Variation in seasonal and geographic distribution of these and other species were observed and may reveal species' interactions driving bloom formation and duration.

Harmful Algal Blooms

Harmful algal bloom events in the Indian River Lagoon are recognized by extensive growth of a particular species, but a diverse microorganism community may be driving their rise and fall.

Predator, prey, and competitor species can all impact HAB development and longevity.

MinION Sequencing

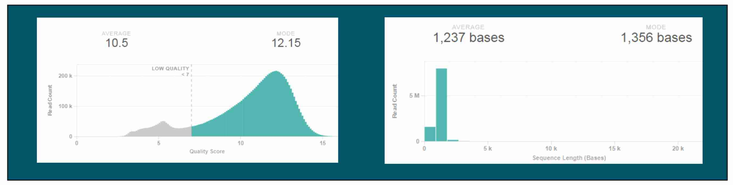
The MinION device is a single-molecule, nanopore DNA sequencer which has been adapted for minimal lab setups with real-time data acquisition in field or hospital settings.

Low Entry Cost = **Quickly Evolving**
In House Sequencing = **Faster Results**
Minimal Lab Setup = **Potential Field Use**
Single Molecule = **Longer Reads**

Error Rate ≠ Less Precise

Data Analysis

- ✓ Amplify and barcode **full-length 18S rRNA gene** (~1200 bp)
- ✓ Run 10 sites per flowcell on MinION with one **full site replicate**
- ✓ Run *ONT Guppy* basecaller on raw fast5 reads
- ✓ Run *ONT EPI2ME* to remove low quality reads and split into barcodes
- ✓ Run *USEARCH* to remove short reads (<1000 bp)
- ✓ Run *USEARCH* to trim start/end 8mers and primer regions
- ✓ Run *Centrifuge* for taxonomic classification against SILVA database
- ✓ Use *Pavian* metagenomics data explorer for interactive graphic displays

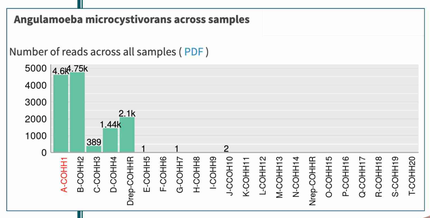


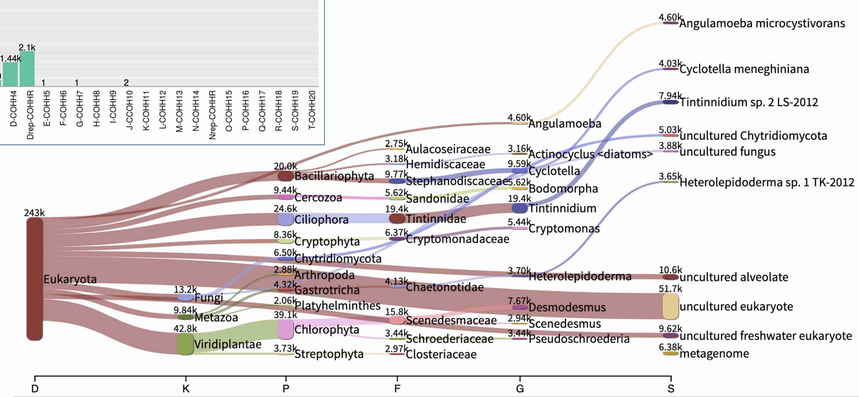
MinION Runs	Total Reads	Avg Quality	Avg Length	Successful Reads	% Success
Fall Sites 1-10	7,547,640	8.94	1,201	**6,519,974**	86.4%
Fall Sites 11-20	11,830,954	8.81	1,245	**9,947,710**	84.1%
Spring Sites 1-10	8,510,900	10.29	1,210	**7,312,518**	85.9%
Spring Sites 11-20	9,754,026	10.48	1,237	**8,554,065**	87.7%

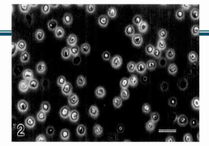
Initial Findings

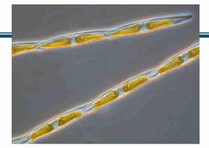
*"They had an amoeboflagellate (1–3 flagella) and a cyst stage (Fig. 8B) and **fed voraciously on Microcystis**, resulting in the **clearance of Microcystis cultures in a matter of days**."*

J. Van Wichelen et al. 2016









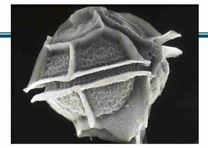


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Image Credits
*Phytoplankton spiral, and phytoplankton mixed: secchidisk.org (*Richard Kirby)
Pseudo-nitzschia: Dr. Rozalind Jester, Florida SouthWestern State College
Pyrodinium: CSIRO, CC BY 3.0, https://commons.wikimedia.org/w/index.php?curid=35488006
Aureoumbra: DeYoe *et al.* 2008