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DNA barcoding reveals a new morphotype of the sugar kelp, Saccharina latissima

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Introduction

Phytoplankton are a mixed group of aquatic unicellular photo-autotrophic organisms which are separated by cell size into micro-phytoplankton, nano-phytoplankton and pico-phytoplankton (Vaulot, 2015). Phytoplankton blooms follow predictable annual cycles in the Gulf of Maine, characterized by a large spring bloom and a smaller bloom in the fall. Marine phytoplankton form the foundation of primary production in Gulf of Maine waters, and thus community changes in composition, and abundance could have cascading effects on our coastal ecosystems.

We set out to monitor the community composition, diversity, and abundance of the spring micro-phytoplankton bloom, at a Friends of Casco Bay water quality monitoring site, in South Portland, Maine. The Gulf of Maine is experiencing warming faster than 99% of the Global oceans (Pershing et al., 2015). This type of monitoring can aid in our understanding of what this warming trend may mean for our waters. Additionally, harmful algae blooms (HAB's) can have significant socio-economic impacts on the fishing and tourist industry (Dias et al, 2015). This makes monitoring for toxic species such as *Alexandrium sp*. in Casco Bay necessary.

Methods and Materials

Figure 3. DNA extraction

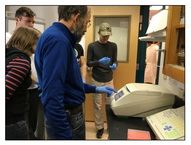


Figure 4. PCR

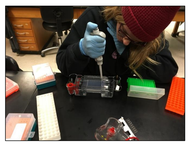


Figure 5. Electrophporesis

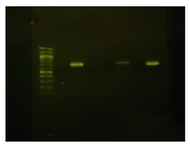


Figure 6. Gel transillumination

1. Ocean Approved provided a **specimen of the narrow-bladed skinny kelp** to the SMCC Introductory Seatime class.
1. We performed **DNA extraction** of the skinny kelp specimen using the Mobio PowerSoil® DNA Isolation Kit.
1. We **amplified the DNA with Polymerase Chain Reaction (PCR)** using forward (GAZF2) and reverse (GAZR2) primers. This step used a PCR thermocycler to subject the samples to high temperatures to break DNA strands and trigger DNA replication and amplification.
1. Analyzed PCR products using **Gel Electrophoresis** as a quality control test to confirm that the PCR worked. In this step we compared the PCR amplicons and control samples to a reference DNA ladder to see if the COI base sequence was successfully amplified by the PCR step.
1. We selected our best sample and sent it to the Mount Desert Island Biological Laboratory for **DNA sequencing**.
1. We used **FinchTV** software to look at and edit our DNA sequence.
1. Using **MEGA 6** software we conducted a **BLAST search** on the National Center for Biotechnology Information (NCBI) for similar sequences to compare our kelp DNA with other kelp DNA to see if there were any similarities. Using the MEGA 6 software we compiled a **multiple alignment** and created a **phylogenetic tree.**

(photo C. Yarish et al, UCONN)

Figure 1. Skinny kelp- **n**arrow-blade *Saccharina sp.*

(Photo Ocean Approved)

Figure 2. Wide-blade *Saccharina latissima*

Results

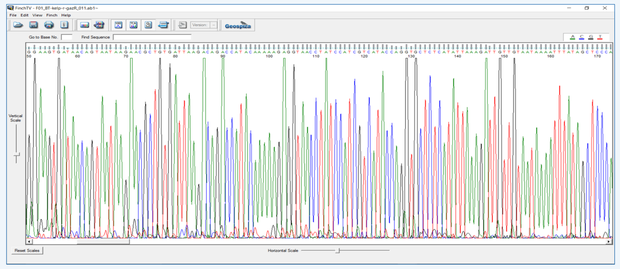


Figure 7. **DNA Barcode Sequence.**
As shown on Figure 7, we used Finch TV to plot the sequence which showed very distinct peaks indicating that it was a reliable sequence. The first 50 pairs of the sequence were not clear data so we cut them from the DNA barcode sequence and evaluated base pairs 50-650 approximately.

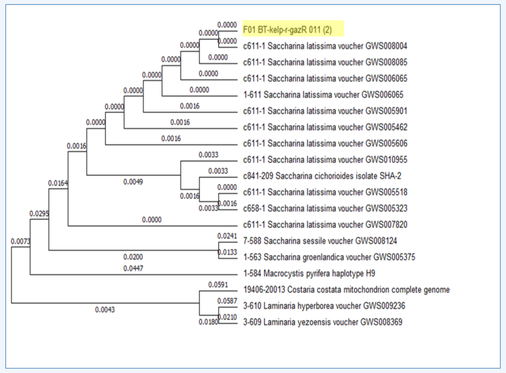


Figure 8. Phylogenetic Tree
The phylogenetic tree was created after running a BLAST to compare our kelp sequence (highlighted in yellow) with other sequences in the NCBI database. The numbers on the branches represent the percentage of base pairs that are different.

Discussion and Conclusions

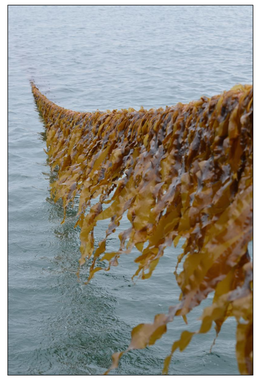
A phylogenetic tree is a graphical representation of relationships between taxonomic groups. A phylogenetic tree is created by analyzing the similarities and differences in DNA sequences. The length of each branch is a measure of the evolutionary distance from the ancestral sequence at the node. Species or sequences with short branches from a node are closely related, while those with longer branches are more distantly related.

Figure 8 shows our sample sequence groups with a clade of closely related *Saccharina latissima.* The very small differences in the DNA sequences led us to conclude that our sample kelp is not a novel species, but a **morphotype** of *Saccharina latissima.*

Paul Dobbins of Ocean Approved indicated that this unique phenotype may be caused by a difference in environment such as sheltered or high wave action habitats (personal communication, April 7, 2017).

Further Studies

Other researchers have suggested that wave exposure may influence the development of kelp morphotypes, and may be an example of phenotypic plasticity, or the ability to adjust its morphotype throughout its life cycle (Fowler-Walker et al.,2006). To further investigate why the two varieties look different, an experiment could be conducted growing *Saccharina latissima* in different wave environments, and changing the wave exposure during an individuals life cycle. Being able to control the morphotype of this species would have great impacts on the aquaculture industry in the Gulf of Maine



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P. Dobbins, personal communication, April 7, 2017

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