Barcoding Green Algae from Freshwater Ponds and Lakes

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Abstract

Algae is a unicellular or multicellular plant-based organism whose biological importance is that of a primary producer. Kuoikova, Chien, Lewis, and Karol (2010) state there are 14000 species of algae already known, but more species are discovered each year and some are difficult to identify. This research aimed to barcode algae to find differences between species in freshwater with different nutrient levels. Algae was collected at Argyle, Southards, and Hawley. A peristaltic pump and sterivex filter from EMD Millipore were used to filter the water. DNA Extraction, PCR of rbcl gene, and gel electrophoresis were conducted. Overall, 9 out of 20 samples resulted in a positive result but only 2 samples had quality sequences for identification as *Lemna minor* which grows in high levels of nitrogen and phosphate because as they grow, they absorb nutrients from water and provide food for fish.

Introduction

- Algae are plant based organisms that convert CO_2 into O_2 and algae are the largest converter of the two substances. Algae vary in color including green, brown and red due to pigments in the cells.
- Algae are at the bottom of the food web and because of this they effect the whole ecosystem. If the algae were to die off, almost all consumers in the body of water would be affected.
- Algae affect humans on a daily basis by removing CO_2 from the atmosphere. Algae can convert greenhouse gasses into oxygen which could slow the effects of Global Warming.
- The purpose of the experiment was to see how humans can affect the biodiversity of algae.
- It was hypothesized that Southards Pond would have the most diverse algae population as it is stocked with fish which could introduce new types of algae into the environment.

Methods

Algae Sample Collection

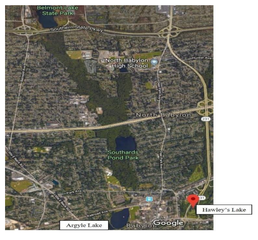

Figure 1.
- Southards Pond
  Stocked with fish
- Argyle Lake
  Salt water intrusion
- Hawley's Pond
  Near major highway

Figure 2. Water was collected then an EMD Millipore peristaltic pump assembly and sterivex filter unit were used to filter algae from the water.

DNA Barcoding

- DNA Extraction
- PCR of the rbcl gene
- Gel electrophoresis to see if the gene (648bp) was copied
- Positive results were sent to a lab for Sanger sequencing.
- DNA Subway was used for data analysis.

Results

Table: Metadata and sequence data for algae samples.

Sample ID	pH	Nitrate (ppm)	Dissolved Oxygen (ppm)	Phosphate (ppm)	Location	Bit Score	E Value	# Mismatches	Scientific Name
PLT-001	6	5	4	2	Argyle	Low Quality Sequence			
PLT-002	6	5	4	2	Argyle	Low Quality Sequence			
PLT-005	6	5	4	2	Argyle	Low Quality Sequence			
PLT-007	6	5	4	2	Argyle	Low Quality Sequence			
PLT-011	6	5	4	1	Southards	Low Quality Sequence			
PLT-017	5	5	4	1	Hawley	Low Quality Sequence			
PLT-019	5	5	4	1	Hawley	928	0	0	*Lemna minor*
PLT-020	5	5	4	1	Hawley	960	0	0	*Lemna minor*

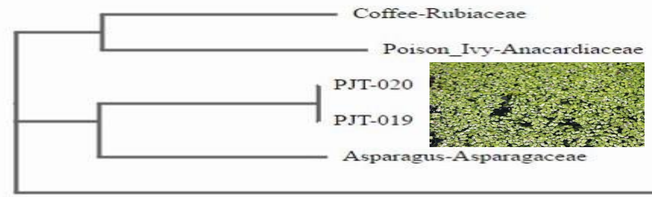

Figure 5. Phylogenetic tree shows that samples PJT-019 and PJT-020 are the same species and that the algae collected is closer in relation to asparagus when compared to other representative plants.

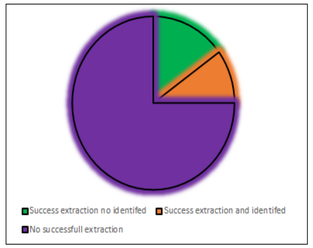

Figure 3. Out of 20 collected samples, 9 were sent for sequencing but only two were quality sequences and identified as *Lemna minor*. Both samples came from Hawley's Lake.

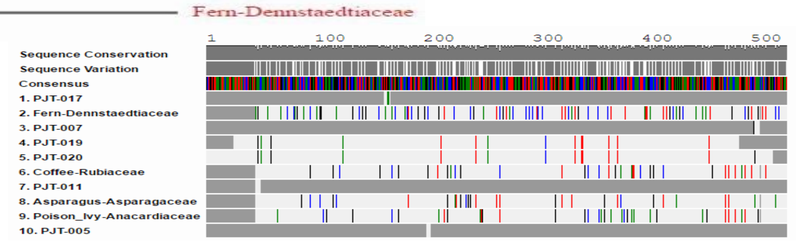

Figure 4. Barcode of PJT samples as compared to representative plants shows that PJT-019 and PJT-020 are identical.

Discussion and Conclusions

- One of the two samples of algae that were identified were taken from a scraped rock and the other was a loose water sample. Both samples were identified as *Lemna minor*.
- *Lemna minor* grow best in water with high levels of nitrogen and phosphate. As they grow, they absorb these nutrients from the water and provide food for herbivorous fish of which Hawley has different levels of fish.
- Our hypothesis that Southards Pond would yield the most diverse algae due to it being stocked with fish could ultimately not be tested due to low quality sequences.
- Understanding the biodiversity of algae in freshwater is important because algae provides a healthy and stable food web base in this environment. The biodiversity of algae also ties into human health as algae are a food source for fish which can provide nourishment to people.
- The results do not support the biodiversity of the pond as all identified samples were of one species.
- Further research in the field of barcoding would include other freshwater organisms in these lakes and ponds such as fish because they play a large part in the ecosystem and because fish have a larger impact on human health as fish are a direct food source for humans.

References

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