A Survey of Algal Biodiversity in Ponds of Northern New Jersey

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Abstract

Our original objective in this project was to analyze the water pollution levels in the ponds of Northern New Jersey through the examination of the algae that inhabit these ponds. After finding copepods in our samples, we refined our study to examine the invertebrate presence in the ponds in addition to algae. In order to do this, we first isolated the DNA from our samples and then used PCR to amplify the algal and invertebrate DNA. The primer used to amplify algal DNA was for the *tuf-A* gene (which encodes was for elongation factor Tu), and the diverse metazoan invertebrate (DMI) primer used to amplify invertebrate DNA encoded for the mitochondrial gene *COI* which encodes for *MT-CO1* gene. We sequenced the DNA and identified the specific species of each sample using GenBank. Our results included many different organisms such as copepods, algae, and wild turkey, among others. We hypothesized that some of our results may be due to run-off contamination from waste. After analysis, we also concluded that our sampling tactics need revision.

Introduction

- **Biodiversity** - the variety of life in the world or in a particular habitat or ecosystem.
- **Algae** – Simple, nonflowering seaweeds and single-celled plants lacking true stems, roots, leaves, and vascular tissue
  - Ubiquitous in aquatic environments
  - Can indicate levels of water pollution (Author, year)
- **Copepods** - a small or microscopic aquatic crustacean of the large class
  - Dominant members of the zooplankton in ponds
  - Major food organisms for small fish.

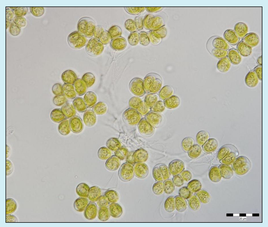

Figure 1. Picture of algal species: *Botryococcus braunii*
(Image:http://ccala.butbn.cas.cz/sites/default/files/styles/ccala_big/public/ccala_collection/14005/ccala778botryococcusbraunii002.jpg)

Figure 2. Picture of a copepod species: *Acartia tonsa*
(Image:https://scripps.ucsd.edu/zooplanktonguide/sites/default/files/Acartia_tonsa_female_thumbnail_300x300_0.jpg)

Goals

1. Identify the species of algae and/ or invertebrates in 3 Northern NJ ponds
2. Analyze the evolutionary relationship between the different species
3. Ascertain the levels of pollution in these ponds based on the species identified.

Methods

1. Sampling

a. 4 out of 9 locations were randomly selected at 3 lakes:
   - Tenafly Nature Center (40.9246° N, 73.9450° W)
   - Closter Nature Center (40.9763° N, 73.9521° W)
   - Tenakill Pond (40.9299° N, 73.9678° W)

b. 7.5- 10 mL water samples were taken at each location.
c. Each sample was preserved with isopropanol and refrigerated.

Figure 3. Map of the locations algae was collected.
(Image: Google Maps)

2. DNA Extraction

a. The samples were spun down into 10-20 mg pellets.
b. 300µL HCl lysis solution was added to each sample. Samples were crushed for 2 min, heated to 65^{0}C, and centrifuged for 1 min.
c. 150µL supernatant was removed from each tube and placed into a new tube with 3µL silica resin, incubated at 57^{0}C for 5 min, and centrifuged for 30 s.
d. The supernatant was discarded, and 500µL wash buffer was added to the pellet, and then centrifuged for 30 sec. This step was repeated twice.
e. 100µL distilled water was added to the tube and mixed. After being incubated at 57^{0}C and centrifuged, 50µL of supernatant was transferred.

3. Polymerase Chain Reaction (PCR)

a. 2µL of each sample was transferred into the tubes, with 23µL of algal primer (*tuf-A*) or diverse metazoan invertebrate (DMI) primer and PCR beads.
b. After amplification, 5µL of each sample, a DNA ladder, and a positive control, were each mixed with 2µL of loading dye, and placed in an agarose gel for gel electrophoresis.
c. Correctly amplified DNA samples were sent for sequencing by Genewiz.

4. Data Analysis

a. The DNA Subway bioinformatics platform was used to analyze sequences.
b. DNA sequences were trimmed and paired to create a consensus sequences, which were uploaded to GenBank. A nucleotide BLAST was run on GenBank to identify species with matching DNA sequences.
c. Phylogenetic trees were made.

Results

Figure 4. Gel electrophoresis results after PCR with DMI *COI* primer (target sequence = ~ 300 bp). (Photo taken by Melissa Lee)

Table 1. Location of samples and species with greatest degree of DNA matching.

Location	Samples	Species with greatest degree of DNA matching
Tenafly Nature Center (TNC)	PXS-013	Algae *(Roya obtusa)*
	PXS-015	Wild Turkey *(Meleagris gallopavo)* & Rotifer *(Keratella cochlearis)*
	PXS-016	-
Closter Nature Center (CNC)	PXS-021	(omitted)
	PXS-022	Rotifer *(Keratella cochlearis)*
	PXS-024	
Tenakill Pond (TP)	PXS-017	-
	PXS-019	Wild Turkey *(Meleagris gallopavo)*
	PXS-020	Copepod *(Cyclops strenuus)*

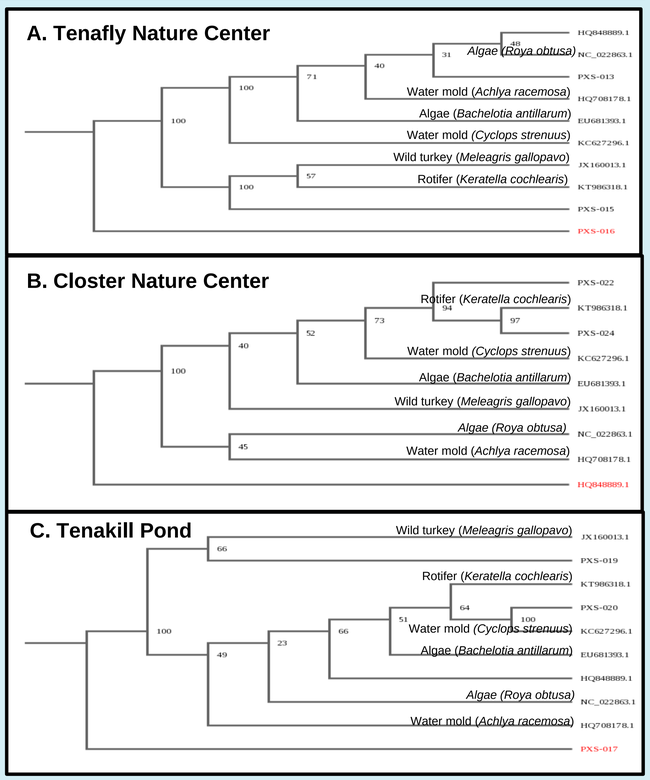

Figure 5 A, B and C. Phylogenetic trees for the DNA samples from each location and species with matching DNA.

Results (cont.)

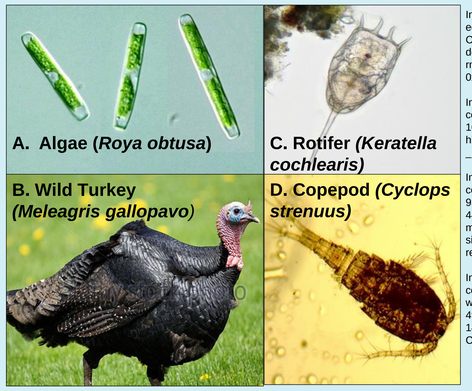

Image A (http://cfb.unh.edu/phycokey/Choices/Charophyceae/Desmids/desmid_unicells/placoderms/ROYA/Roya_02_500x372_sp.jpg?)

Image B (http://www.discoverlife.org/IM/I_MWS/1045/320/Keratella_cochlearis_forma_robusta,I_MWS104508.jpg)

Image C (http://l7.alamy.com/zooms/846b246d879341e58c797d35af2deb44/wild-turkey-meleagris-gallopavo-silvestris-have-been-reintroduced-anfyeg.jpg)

Image D (https://classconnection.s3.amazonaws.com/345/flashcards/4496345/jpg/buchanka-142F20ECA524EDDE8CC.jpg)

Figure 6 A, B, C, and D. Organisms with DNA matching sample DNA.

- Although the primer used was targeted for a segment of the invertebrate *COI* gene, the short length of the DNA sequence produced DNA matches with several organisms (not all invertebrates)
- A possible explanation for the presence of wild turkey DNA in the samples may be due to contamination of the water (via runoff) from the feces or feathers from wild turkeys living in the nearby woods.

Discussion / Conclusion

- Our results suggest a diverse community of organisms inhabiting the areas around and in the ponds of Northern New Jersey.

FUTURE RESEARCH

- Samples were taken in late November, but sampling in the warmer months could increase the quantity and quality of the DNA from the organisms found in the water sample..
- Possible future research would include improving collection time and methods so that only algae are sampled since they are an indicator species for water quality.
- Improved, and more specific, sampling of one type of organism would allow us to use a longer primer during PCR and produce more accurate identifications of matched species.

References

"Algae Can Function as Indicators of Water Pollution." WALPA RSS. N.p., n.d. Web. 11 Oct. 2016.
Cold Spring Harbor Laboratory DNA Learning Center. "Using DNA Barcodes to Identify and Classify Living Things." (n.d.): n. pag. Web.
"Environmental Pollution." Biology Online Help, Biology Topics & Biology Tutor. Tutor Vista, n.d. Web. 07 Oct. 2016.
Hebert, P. D. N., A. Cywinska, S. L. Ball, and J. R. Dewaard. "Biological Identifications through DNA Barcodes." Proceedings of the Royal Society B: Biological Sciences 270.1512 (2003): 313-21. Web. 14 Oct. 2016.
"National Drinking Water Database." EWG Tap Water Database 2009. Environmental Working Group, n.d. Web. 07 Oct. 2016.
Sen, Bulent, Mehmet Tahir Alp, Feray Sobmez, Mehmet Ali Turan Kocer, and Ozgur Canpolat. "Relationship of Algae to Water Pollution and Waste Water Treatment." Intechopen. In Tech, 16 Jan. 2013. Web. 11 Oct. 2016.

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