How Varying Water Levels Affect Surrounding Biodiversity - Identified Through DNA Barcoding

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Abstract

Living organisms are able to adapt to their surroundings in order to create better chances of survival. This project serves to determine the correlation between water quality and biodiversity surrounding that water. By testing the phosphorus levels of different areas of the pond with nearby fertilizer water runoff and collecting grass samples from these areas, we can make any potential connections between the two. From our testing, there was little correlation between the grass growing and the water quality. Most of the grass samples were the same kind, with two samples varying in species.

Introduction

Pfister Pond:

- Man-made approximately 100 years ago, and walkways nearby were originally used as a road for horses and carts, and the pond was created to prevent flooding of the land.
- Pfister pond goes through stages of succession: Pond pioneers, submergent vegetation appearing, decaying matter raising the bottom, and the creation of a marsh.
- Human activity added excess nutrients and elements so succession rate was sped up
- Last year, a large hydro raking project was performed on the pond to remove the dead plant matter that contributed to the increasing speed of succession.
- The excess nutrients were likely runoff from a nearby field with pesticides and fertilizers.

Phosphorus:

- Allows the plant to capture and convert the sun's energy so that the process of photosynthesis can occur.
- Phosphorus deficiency will lead to distortion and abnormal coloring.
- Too much phosphorus will stunt plant growth.
- In freshwater ponds the general levels are 0.03mg/L. [5,6]

Methodology

We collected 12 samples from four different points at Pfister Pond, Tenafly Nature Center, Tenafly, New Jersey with 3 samples from each point. We chose Pfister Pond due to the proximity to a country club, Montammy Golf Club in Alpine, New Jersey. With the country club, there is a high probability of runoff from the fields, which are routinely sprayed with pesticides, to enter the pond.

Figure 1: Map of Pfister Pond, Tenafly, NJ Points A, B, C, and D are labeled accordingly

We used 500mL plastic bottles to sample water from each location on November 5th, 2022. We submerged each bottle into the pond water closest in proximity to the location where we collected the plant sample. We made sure to avoid as much debris in the water as possible to avoid any tainting of the water. The bottles were loosely capped to allow for circulation, and to prevent any extraneous reactions from taking place in the bottles after collection until testing.

To extract our plant samples we carefully dug 3-4 inches into the ground under the plant to preserve its roots. One of the challenges we faced was keeping the plants alive until we could DNA barcode. In order to do this we made sure to extract the roots of the plants, and re-potted them in plastic containers to create a small ecosphere. Water, soil, and a small piece of fruit was added to each container. The decomposers, small organisms, and inclosed space allowed for the cycling of necessary nutrients and water.

Methodology (cont.)

DNA barcoding starts with DNA Extraction. Using sterile scissors we cut off a small piece off one plant sample from each of the 4 locations. After placing these samples in a 1.5mL labeled tube we will add around 50 µL of lysis solution to each tube. The lysis solution will dissolve all the membrane bound organelles, like the nucleus. This allowed the DNA to be exposed in the cell. The next step is to grind the sample with a pestle for around two minutes which will break down the tough cell walls.

- A sterile tweezer was used to add one 3-mm disc of Whatman No.1 Chromatography paper to the solution. DNA attaches to Whatman No.1 Chromatography paper for easy separation.
- As the disc is soaking, 200 µL of wash buffer is added to a clean 1.5-mL labeled tube to make sure there are no contaminants. When the disc was done soaking a tweezer is used to transfer the disc to the second tube. Before letting the disc sit in the wash buffer for a minute, tube was mixed for about five seconds.
  - After each step, the tweezers previously used was discarded to prevent any impurities from affecting PCR.
- Lastly, a sterile pipette tip was used to drag the disc to the top of the tube(here the disc will dry for two minutes). Drying the disc was necessary because any leftover ethanol on the disc inhibits PCR. The tube will be cleaned with 30 µL of TE. For storage the disk was placed in a new tube containing 30 µL of TE. The disk was contained at -20℃ for a long term.

PCR amplification

- PCR tube containing Ready-To-Go PCR Bead was used. This set contains dehydrated Taq polymerase, nucleotides, and a buffer. After labeling the tube we will use a micropipette with a fresh tip to add 23 µL of *rbc*L primer. The beads will need 1 minute to dissolve at ambient temperature.
- After dissolving, in order to prevent premature replication of unwanted primer dimers, place the PCR tubes on ice. While it is on ice, use a micropipette to add 2 µL of DNA previously extracted directly onto the PCR tube with the primer and polymerase mixture. We will make sure no DNA is left on the tip of the pipet. Once again, store samples on ice before thermal cycling.
- To start thermal cycling, PCR tube is placed in a thermal cycler. The sample initially is cycled at 94℃ for one minute. Then the sample will be cycled 35 times and each cycle will contain 3 steps: denaturation at 94℃ for 15 seconds, annealing at 54℃ for 15 seconds, and extending at 72℃ for 30 seconds. Once the 35 cycles are complete, the sample were cycled ad infinitum at 4℃ for 30 seconds.

Analyzing PCR

- A gel-casting tray was obtained, the ends were sealed, and a well-forming comb was inserted. Then, 2% agarose solution was poured into the tray around one-third the height of the comb teeth. After waiting for the agarose gel to completely solidify(about 20 minutes), the gel was placed into the electrophoresis chamber and a 1× TBE buffer was added to cover the surface of the gel. The comb was then removed and add an additional 1× TBE buffer was added to fill the wells and cover the gel. This resulted in a smooth buffer surface. Utilizing a micropipette, 5 µL of each PCR product was transferred to a fresh 1.5-mL microcentrifuge tube. Then 2 µL of SYBR Green DNA stain was be added to each tube with 5 µL of PCR product. 2 µL of SYBR Green DNA stain was also be added to 20 µL of pBR322/*Bst*NI marker. For the final steps the gel was oriented lining the wells along the top of the gel. The gel was run for about 30 minutes at 130V. Adequate separation was achieved when the cresol red dye moved 50mm from the well. To observe the results UV transillumination was used.

Results

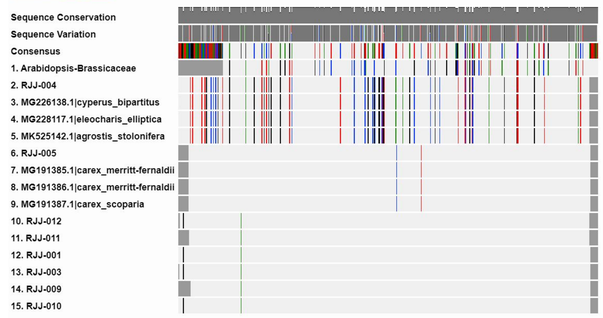

Figure 2: Sequence alignment viewer comparing samples to potential species

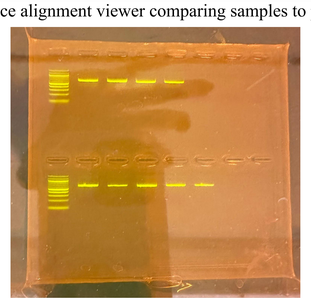

Figure 3: PCR indicator - Note: Sample RJJ-002 was not included, and RJJ-006, RJJ-007, and RJJ-008 are not showing

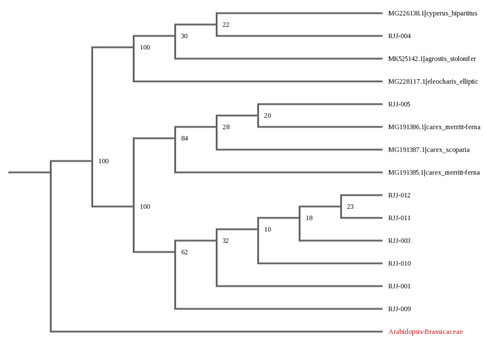

Figure 4: Phylogenetic tree comparing the samples with potential species

Conclusion

- Based on the results, there is little correlation between water quality and species of an area of a pond. In the phylogenetic tree, most of the samples except for RJJ-004 and RJJ-005 are branching off the same section, meaning that they are closely related by species. This indicates that there is either little correlation between water quality and type of species or this type of grass is highly adaptable and doesn't need high quality nutrients to survive.
- Despite collecting 12 samples, with 3 from each of the 4 locations, we only got results for 8 of the samples. These were a result of samples being unusable or the PCR being unsuccessful. Sample RJJ-002 was unsuccessful because the plant was no longer extractable at the time of PCR. Samples RJJ-006, RJJ-007, RJJ-008 were all due to an unsuccessful PCR.
- Using the phylogenetic tree, sample RJJ-004 is closely related to *cyperus-bipartitus*, *eleocharis-elliptica*, and *agrostis-stolonifera*, whereas sample RJJ-005 is closely related to *carex-merritt-fernaldii (TJD-706)*, *carex-merritt-fernaldii (TJD-708)*, and *carex-scoparia*. Samples RJJ-001, RJJ-003, RJJ-009, RJJ-010, RJJ-011, and RJJ-012 are closely related to each other, and are likely either *carex-norvegica*, *carex-vesicaria*, or *carex-saxatilis*. Meaning, of the samples from around the different areas of the pond, similar or the same species of plants are growing.
- *Agrostis-stolonifera* is a perennial bunchgrass with prostrate stems. Also known as creeping bent or common bent, it is native to Europe, Asia, and North Africa, and could have been introduced to North America, but some research suggests that it was native. It is common in New England, growing in sites prone to seasonal or temporary flooding. It often grows in anthropogenic regions, brackish or salt marshes, meadows and fields, shores of rivers or lakes, or edges of wetlands. [1]
- *Carex-norvegica* or *carex-media* is a closed-headed sedge with origination in Maine, but is common in northern and western North America. It often grows in alpine or subalpine zones, cliffs, balds, or ledges and usually in wetlands but occasionally in non-wetlands. [2]
- *Carex-scoparia* is a pointed broom sedge with differing heads, being either crowded or spread out, stiff or arching. There are two varieties in New England, one being common throughout New England and the other in eastern Maine. It often grows in anthropogenic regions, meadows and fields, edges of wetlands. It grows in Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island, and Vermont. [3]
- **Phosphate:** At point A on Pfister pond phosphate levels were 0.2 ppm. At point B on the pond, phosphate levels were 0.2 ppm. At point C on the pont phosphate levels were 0.2ppm. At point D on the pond phosphate levels were 2.0ppm. This significant increase in ppm for point D was most likely due to the notable number 0of leaves in this area of the pond. Dead plant matter such as leaves are known to be significant contributors to increases in water phosphate levels. When plants die, decomposers like bacteria convert the organic phosphates of the leaves into bioavailable phosphates. This simply means that the phosphate that the water contains is in a form that plants can absorb through water. In this way phosphates are constantly being recycled throughout this ecosystem. Additional phosphate introduced to this pond through human behavior could be detrimental to this ecosystem.

Figure 5: Point D at Pfister Pond

Acknowledgements

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References

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