Assessing the Filtration Efficacy of Free-Floating Aquatic Plants and Identifying Self-Purification Potential Through DNA Barcoding

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Abstract

With a recently growing base of study in the use of aquatic and semi-aquatic plants in the filtration of wastewater to provide clean, sustainable water, the possibility of utilizing aquatic free-floating plants such as duckweed and water spangles, presented itself as a definite opportunity for expansion through DNA barcoding and experimentation. We went to the Tenafly Nature Center to collect duckweed samples from Pfister's Pond and purchased water spangles from a plant vendor. At Overpeck County Park, surrounding a freshwater creek fed by the Hackensack River, in addition to the Tenafly Nature Center, we took aquatic and semi-aquatic plant samples to identify the potential for self-purification within the two locations studied through DNA barcoding processes at Cold Spring Harbor. At both locations, we also took water samples to test through controlled experimentation, growing duckweed, and later water spangles, in the separated water samples from both sources and testing for concentration of nitrates and phosphates before and after a week or living within the water utilizing a LaMotte Nitrate-N/Phosphate Low Range Test Kit . With immensely promising results in controlled experimentation and opportunities for self-purification within the Hackensack River and Tenafly Nature Center identified through barcoding, natural purification methods stand as an encouraging, location-specific method of naturally cleaning and controlling a man made pollution crisis.

Introduction

- **The Hackensack River Area's** polluted waters recently proven to contain dozens of dangerous contaminants[5]
- Desperate need for rehabilitation - **Superfund** site
- **Plant-based water filtration** has been placed in the research spotlight as a sustainable method of purifying water, low-cost solution to a high-risk problem.[2]
- **Duckweed** has the ability to absorb chemicals, rapidly reproduce, its wide distribution throughout North America, and global use in countries like Palestine and Yemen.[4]
- **Water spangles**, an extensively researched floating fern has been noted for its nitrate uptake
- This filtration operates off of the **free-floating plants'** need for nitrogen and phosphate to grow, allowing purification.[1]
- We set out to answer the following questions:
  - Can natural filtration be applied to the local pollution crisis of the Hackensack River area?
  - What free-floating plants are viable for filtration use?
  - Do filtration-capable plants live within the two subject locations and can their growth be fostered to encourage self-purification?
- Our goal is to **explore the extent of duckweed/water spangle filtration capability** and with these findings to create discussion regarding the possibilities of **applying such valuable knowledge in the face of pollution**, locally, with the Hackensack River pollution crisis, and beyond.

Fig. 1 *Lemna minor,* Duckweed
(Image: www.fs.fed.us/duckweed)

Fig. 2 *Salvinia minima,* Water Spangles
(Image: www.nathistoc.bio.uci.edu/plants/Ferns/Salvinia%20minima)

Methodology

1. **Sampling**
   a. Aquatic and semi-aquatic species (including duckweed) collected for genetic identification of purification potential at both locations
      i. 7 samples from Pfister's Pond at Tenafly Nature Center (40.9229°N, -73.94789°W)
      ii. 5 from Overpeck Creek at Overpeck County Park (40.8650°N, -74.0000°W)
   b. 3+ L water were collected at both locations to provide ample water supply for experimentation.
   c. Samples of duckweed and water spangles were purchased from an aquatic plant supplier to ensure **species specificity for experimentation**

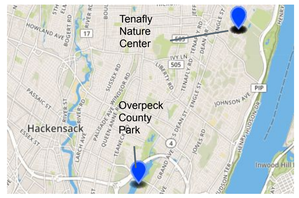


Fig. 3 Map of collection locations
(Image: www.sampledb.dnalc.org)

2. **DNA Barcoding Processes** (Key Steps)

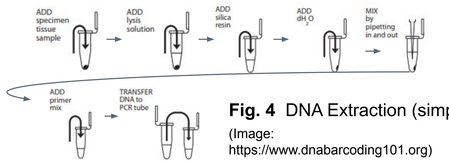
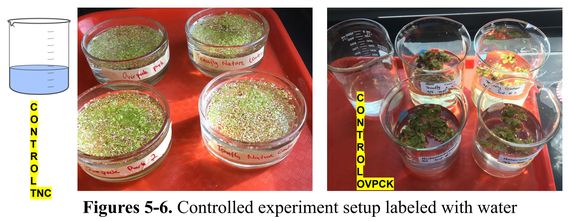


Fig. 4 DNA Extraction (simp.)
(Image: https://www.dnabarcoding101.org)

3. **Polymerase Chain Reaction (PCR) and Data Analysis**
   a. Once we have finished the final centrifuge, we began the PCR amplification, we started by taking only 2 microliters of the available 50 microliters of DNA.
   b. Once the DNA was in the tube, we added **rbcL primer** which reproduced one section of the strand of DNA, after which we amplified in thermal cycler, stored, and ran it through a gel electrophoresis to verify amplification.
   c. The sequencing was done using **GeneWiz**, and after the sequences were downloaded, the species were determined by using the **DNA subway** platform and **BLAST searching**.

4. **Controlled Experimentation**
   a. Set an **experiment** to measure the nitrate and phosphate concentration of the water samples collected at TNC and OPCK **before (control)** and **after one week** of in-water duckweed and water spangle living.
   b. Using the LaMotte Low Range Nitrate/Phosphate Comparator we recorded the concentrations before and after experimentation
   c. We then analyzed concentration change to properly assess the filtration efficacy of both plants



Figures 5-6. Controlled experiment setup labeled with water origins (duckweed left, water spangles right,)

Results

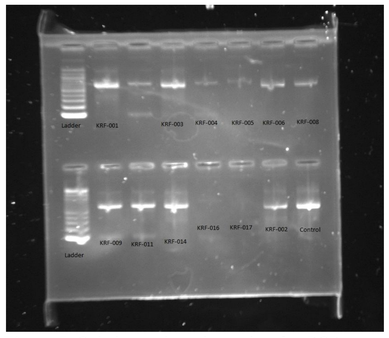


Figure 7. Gel electrophoresis results after PCR

Table 1. Sample code number, correlating sample species and location

Sample #	Sample Species	Sample Location
KRF-001	***Lemna minor***	Tenafly Nature Center
KRF-002	***Lemna minor***	Overpeck Creek
KRF-003	*Phragmites australis*	Overpeck Creek
KRF-004	*Solidago virgaurea*	Overpeck Creek
KRF-005	*Ageratina altissima*	Overpeck Creek
KRF-006	*Celastrus paniculatus*	Overpeck Creek
KRF-008	*Trifolium repens*	Tenafly Nature Center
KRF-009	*Ligustrum vulgare*	Tenafly Nature Center
KRF-011	*Calliciadium hadanianum*	Tenafly Nature Center
KRF-014	*Prunella vulgaris*	Tenafly Nature Center

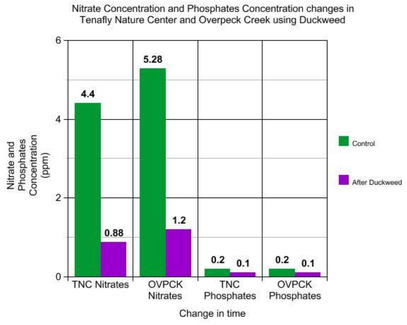


Figure 8. Changes in Nitrate and Phosphate Concentration from sample water in Tenafly Nature Center and Overpeck Creek using Duckweed

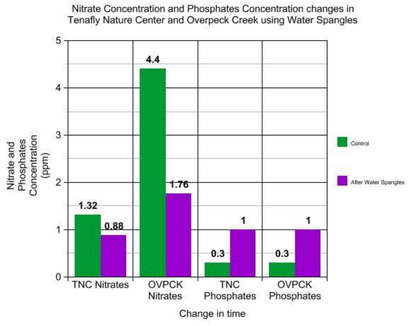


Figure 9. Changes in Nitrate and Phosphate Concentration from sample water in Tenafly Nature Center and Overpeck Creek using Water spangles

Results (Continued)

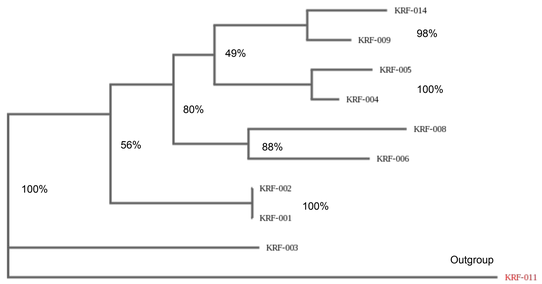


Figure 10. Phylogenetic tree for the DNA samples from each location and species with matching DNA

- **Several species identified** (i.e. common reed, white clover, branch moss) have been studied for their dependence upon nitrate/phosphate water content or **filtration ability**
- Duckweed allowed a **80% reduction** of nitrate concentration and approximately **50%** in phosphate in TNC water and an approximate 77% nitrate reduction in OVPCK as well as about **50%** in phosphates.
- Water spangles enacted **34.4%** decrease in nitrate concentration and was unable to filter phosphates resulting in a **200%** phosphate increase in the final sample due to evaporation
- Water spangles also allowed an approximate **60%** decrease in nitrate concentration of OVPCK and a 100% increase for phosphates

Discussion / Conclusion

- Our results suggest a **significant** decrease in nitrate concentration after in-water habitation for both species and **phosphate reduction for duckweed** as well
- We found an **inability to filter phosphates** in water spangles as illuminated by an increasing phosphate concentration and decreasing water volume due to evaporation
- DNA barcoding also illuminated **existing purification potential** within the two subject areas, suggesting the possibility of **fostering naturally filtering plants** to approach water pollution
- For future research, the **specific application** of these findings still remains unknown, we would like to test ecological effects of species introduction or promotion
- We would also like to test various other plants that grow locally that may be applied to filtration.

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REFERENCES CONTINUED ON PAPER

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