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# Myriophylum Heterophylum: An Endangered Species or an Invasive Weed?

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**Title:** ***Myriophylum Heterophylum*: An Endangered Species or an Invasive Weed?**

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## Abstract:

Invasive species are of great concern throughout the world because they are displacing native species, forming monocultures, and at times resulting in enormous economic costs. What happens when a plant that is considered rare and critically imperiled poses the same threats to its native habitat? *Myriophyllum heterophyllum* (Haloragaceae) has raised this question. The historic range of this species stretches from New York and Ontario south to Florida, and South Dakota to Texas, and Mexico (Godfrey and Wooton, 1981; Taylor, 1915). Its range has grown to encompass most of the eastern half of the United States and north to Quebec and British Colombia (ARS, 1970, NatureServe). More obvious is the expansion within its former range and the development of some exceedingly abundant populations. In New York State and throughout New England this species is currently treated as an invasive (NatureServe). In contrast, Pennsylvania lists this species as critically imperiled (PNHP).

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## INTRODUCTION

Prior to recent discoveries in northeastern Pennsylvania, extant populations of *M. heterophyllum* were known only from limited sites in the western part of the state. Herbarium specimens document several historic populations in Bucks County that no longer exist due to habitat loss and alteration. Surveys in northeastern counties have revealed several vigorous populations that bring the current population number in Pennsylvania to over ten. Population sizes vary from small fragments to masses that can encompass entire lakes. With the addition of new populations and the vigorous growth at some new and old sites, the status of this species should be reevaluated and the management of some populations should be considered.

Eight species of *Myriophyllum* and two species of *Proserpinaca* (Haloragaceae) are present in Pennsylvania (Rhoads and Block, 2000). In the absence of reproductive structures it can be impossible to tell some of the species apart due to their similar and variable vegetative growth. In addition, hybrids have been found in the genus *Myriophyllum*; of interest are hybrids between *M. heterophyllum* and *M. laxum*, a southern range species, that are present in Connecticut and Florida (Moody and Les, 2002). Even sterile hybrids have the ability to thrive due to the prolific vegetative reproduction in this genus. Continuing research by Moody and Les (2002) suggest the possibility that some of the hybrids may be more aggressive than the true species.

We hypothesize that hybrids are causing the abundant populations in Pennsylvania. In order to address this hypothesis, morphological and molecular analyses of each population were performed. The internal transcribed spacer region (ITS) of the nuclear ribosomal DNA was successfully used in other research in the genus *Myriophyllum* (Moody and Les, 2002). It is a conserved non-coding region that has significant variation and bi-parental regions that are retained through vegetative reproduction. The amount of conserved variation makes this a suitable region for identifying hybridization events, and differentiation between intrageneric species (Schilling et al, 1998).

In addition to looking for hybrids, water quality was assessed. Lakes with high nutrient availability are hypothesized to increase the abundance of the plant.

## METHODS

***Sampling Method***. Samples were collected from eight sites in Pennsylvania and one site in Delaware. Some additional sites (Appendix 1) in Pennsylvania were visited but *M. heterophyllum* was not evident at the time. Numerous historic sites (Appendix 2) found in herbaria were not visited, but could potentially be added in future studies. Sufficient plant material for DNA extraction and herbarium specimens was collected. Water samples were collected at each site. Each site was evaluated for abundance on a scale of 1-4; 1 - only plants remnants found, indicating scarce plants, 2 – scattered plant clusters, 3 – abundant, large masses of plants but not encompassing the entire water body, 4 – very abundant, large masses encompassing nearly all of the water body. These values were assigned based on observation. Nine sites were included in the molecular, water, and morphological analyses. Plant material collected from two sites in Connecticut was added into the molecular analysis. One sample was a known hybrid (*M. heterophyllum* x *M. laxum*) and the other plants came from a lake where the hybrid and *M. heterophyllum* occurred. They were used for comparison with the collections

from local sites (PA and DE).

***Morphological Characteristics*.** Morphological characteristics were measured on pressed herbarium specimens. Herbarium specimens were taken from numerous herbaria, so not all specimens were coordinated with water sampling. Measurements were taken from standardized points along the stem of each individual plant (multiple plants could be found on one herbarium sheet). Vegetative characteristics were taken 10 cm from the top of the plant or from where the aerial stem meets the water. The following measurements were taken approximately at this point: leaf length, number of leaf segments, stem width, leaf arrangement, and number of leaves per node. Aerial stem measurements were taken in the middle of the aerial portion due to the variation in length. The width of aerial stem, bract arrangement, bract margin, bract length, petal length, fruit length, and fruit width were measured at this point. Not all characters were measured due to their absence and some general floral observations were noted. The measurements were compared to the species descriptions in taxonomic literature.

***Molecular Analysis***. DNA was extracted from fresh material preserved in silica gel or from herbarium sheets. The material was extracted with the QIAGEN Dneasy® Plant Mini Kit according to their protocol. Two samples from each site were extracted. The primers "C26A" and "N-nc18S10" were used to amplify the ITS region. They were designed for use in plants (Wen and Zimer, 1996) and tend to not favor amplification of fungal contaminants as readily as other universal primers (McDade et al, 2000). The following was added to each 25 µl PCR reaction: 14.5 µl of $H_20$, 2.5 µl of 10x buffer, 2.0 µl of 25 mM $MgCl_2$, 0.8 µl of mM DNTP's, 1.0 µl of 20 µM forward primer (N18S), 1.0 µl of 20 µM reverse primer (C26A), 2.0 µl of Dimethyl sulfoxide, and 0.3 µl of Taq polymerase. The amplification was run under the following conditions: 10 minutes at 94˚C, followed by 35 cycles of a 1 minute denaturation step at 90˚C, 1:10 minute annealing step at 52˚C, and a 1:00 minute extension step at 72˚C. The final extension at 72˚ C for 10 minutes was followed by dormancy at 4˚C.

The PCR products were run on a 1% agrose gel (~1gram of agrose per 100 ml of 0.5 x TBE buffer, and 5 µl of Ethidium bromide). Bands were cut, weighed and cleaned using protocol from the QIAGEN QIAquick® Gel Extraction Kit. The final elution amount depended on the source of material. Herbarium specimens were eluted in less buffer (35µl) to increase the DNA concentration.

The sequenced samples were produced on Beckman automated sequencers (McDade et al, 2000) with the same primers used in amplification. The electropherograms of the forward strands were generally clean; when overlap occurred the reverse strands were sequenced for verification of the forward strands. The sequence chromatographs were edited in 4Peaks ™ 1.6. The edited sequences were transferred to SeqApp ™ 1.9 and aligned by eye (Gilbert, 1992). Additional sequences were downloaded from NCBI GenBank. The parental sequences from GenBank and the local samples were compared to identify areas of differentiation. Hybrids are identified based on polymorphisms at these sites where the parental sequences differ (Moody and Les, 2002). The sequences of the local samples were compared to look for polymorphisms and ambiguities.

***Water sampling and analytical methods***. Approximately 200 ml of water were collected in precleaned plastic containers. Both filtered (Whatman GFF 0.7 µm filters using syringe filtration) and unfiltered water stored in the dark at 4°C until frozen in the laboratory, generally

within 4 days of collection. Field and laboratory blanks (i.e., double-deionized water) were handled similarly to assess potential sample contamination.

In the laboratory, dissolved $NH_4^+$ and $NO_3^- + NO_2^-$ (hereafter $NO_3^-$) were measured using the indophenol and Cd-reduction methods, respectively, with an Alpkem Autoanalyzer (RFA 300; O. I. Analytical, College Station, Texas; US EPA 1983; 1992). Total nitrogen (TN=TKN + NO3–) was obtained using the Kjeldahl N method (block digestion) on unfiltered samples. Soluble reactive P (SRP) and total P (TP; persulfate digestion on unfiltered water) were determined using the ascorbic acid method with the Alpkem Autoanalyzer (US EPA 1983; 1992). Total hardness (calcium and magnesium) in the sample are titrated with disodium ethylenediamine tetraactate (Na2EDTA) using calmagite indicator, while total alkalinity measured by titrating the sample to a pH 4.5 with a dilute solution of sulfuric acid (US EPA 1983).

***Data analysis.*** A principle component analysis (PCA) performed with Canoco™ was used to explore relationships between the water quality data and abundance values. The data was centered and standardized in Canoco™.

## RESULTS

***Morphological Characteristics.*** The range of characters (Table 1) measured from herbarium specimens fall within the ranges found in taxonomic literature (Small, 1913; Godfrey and Wooton, 1981; Yu and Dong, 2002). The arrangement of the leaf and/or bract on the stem was sometimes found to be strongly alternating leading to curiosities about its identification. However, the leaves technically are alternating and have a whorled appearance due to a series of short and long nodes that cause the alternate leaf arrangement to appear whorled in most cases (England and Tolbert, 1964; Aiken, 1981). Therefore, there was no unexpected variation or indication of hybrids in any of the sampled populations.

Table 1. The range of characters for all the specimens observed from herbarium records in Pennsylvania and Delaware.

| **Character** | **Range** |
|---|---|
| Leaf Length (mm) | 8.22-47.36 |
| # Of segments per leaf | 11-24.667 |
| # Leaves per node | 4 or 5 |
| Leave arrangement | Whorled (sub opposite) |
| Distance between nodes (mm) | 2.59-8.51 |
| Submersed stem width (mm) | 0.79-5.11 |
| Bract Length (mm) | 3.2-7.97 |
| Bracteole length (mm) | 0.67-1.06 |
| Bract margin | Serrate |
| Aerial Stem Width (mm) | 0.53-3.625 |
| Distance between nodes (mm) | 2.61-14.48 |
| Flower arrangement | Whorled (alternate) |
| Flowers | Staminoide and perfect |
| Length of Fruit (mm) | 0.82-2.27 |
| Width of Fruit (mm) | 0.45-1.81 |

***Molecular Analysis***. Sequencing DNA from the samples was successful for all sites except Harris Pond. The morphological analysis was conclusive enough that continued efforts without acquiring new material were ceased. The region of interest was identical, except for a few base changes attributed to error in PCR. As a result of comparing the expected parental strands, eight sites of differentiation were found. The hybrid collected in Connecticut showed polymorphisms at each of these sites whereas the local samples and the straight species from Connecticut did not. The molecular analysis confirmed that all material collected that was sequenced in Pennsylvania and Delaware are the same species and there is no evidence in the morphological or molecular study to indicate that hybrids are present.

***Water Quality, Abundance, and Data Analysis.*** The ordination of water quality data (Table 2) and abundance data (Table 3) is illustrated in figure one.

Table 2. Water Quality Data for the sites located in Pennsylvania and Delaware

| Site | pH (SI) | Alkalinity (mg/L) | Hardness (mg/L) | Dissolved NO2 + NO3 (ug N/L) | Dissolved NH4 (ug N/L) | TKN (ug N/L) | Dissolved Ortho-P (ug P/L) | Total P (ug P/L) | Total N (ug N/L) | N/P Molar |
|---|---|---|---|---|---|---|---|---|---|---|
| Promised Land* | 6 | 5.2 | 11 | 6 | 5 | 445 | 1 | 14 | 451 | 73.6 |
| Shohola | 7 | 6.8 | 14 | 6 | 14 | 1076 | 4 | 70 | 1082 | 35.3 |
| Harris Pond | 7 | 23 | 26.4 | 6 | 86 | 1000 | 8 | 44 | 1006 | 52.2 |
| Inlet at Harvey's Lake | 7 | 24.4 | 36 | 16 | 28 | 274 | 2 | 13 | 290 | 51 |
| Lily Lake | 6 | 6.8 | 14.4 | 4 | 6 | 278 | 2 | 13 | 282 | 49.6 |
| Ditch next to Abbott's Pond | 6 | 20.8 | 129.2 | 17647 | 23 | 356 | 2 | 12 | 18003 | 3428.1 |
| Cheat River | 7 | 17.8 | 79.8 | 426 | 61 | 180 | 1 | 5 | 606 | 276.9 |
| Beaver Pond | 7 | 31.2 | 39.6 | 7 | 12 | 1595 | 4 | 36 | 1602 | 101.7 |
| Ridge Pond | 6 | 31.8 | 40 | 7 | 13 | 1242 | 5 | 50 | 1249 | 57.1 |

Relationships derived from the ordination were considered weak, and insubstantial due to the small number of variable sites. The strongest relationship observed in the analysis was between abundance and hardness, a negative relationship where increasing hardness resulted in decreasing abundance. However, without more samples the data is unsubstantial.

Table 2. Water Quality Data for the sites located in Pennsylvania and Delaware

| Site | Abundance |
|---|---|
| Promised Land Lower Lake | 4 |
| Shohola | 4 |
| Harris Pond | 4 |
| Inlet at Harvey's Lake | 3 |
| Lily Lake | 3 |
| Ditch next to Abbott's Pond | 1 |
| Cheat River | 2 |
| Beaver Pond | 4 |
| Ridge Pond | 4 |

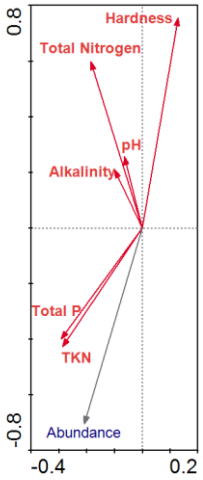


Figure 1. PCA with abundance and water quality data sets.

## DISCUSSION

No conclusions have been found to explain why some populations show high abundance and others have relatively low abundance. There was no indication that higher nutrients caused an increase in the abundance of the plants. The molecular and morphological analyses have shown no indication of hybrids in any of the local sites. The additional exploration of water quality had no conclusive results, however, it has provided some interesting relationships to pursue and develop in future research.

In similar situations, water quality has been found to explain some aspects of vigorous behavior in *Myriophyllum spicatum*, an invasive species found throughout the United States and Canada. Nitrogen was identified as the limiting factor of *M. spicatum*. When grown in controlled environments with low concentrations of nitrogen, the production of autofragments increases significantly. Autofragments are "self-initiated abscissions that begin with root formation on the upper portions of the stem." (Smith et al., 2002) Autofragments may be a tool for migrating to more favorable environments but they are attributed to more abundant growth (Smith et al., 2002). The management recommendations and results cannot be used for or compared to *M. heterophyllum* due to differences in sampling methods. Smith et al. (2002) looked at sediment nutrients, which was not relative to the nutrient levels measured in the water column in this research. Despite the inability to compare results, the factors they explored should be carried over to further research with *M. heterophyllum*.

As far as delisting the species, the decision should be approached carefully as there are many unknowns remaining. We don't know which populations are native, and we don't know how the populations spread through the state. It is currently assumed that the populations in western and perhaps southeastern Pennsylvania are native. If this were the case the northeastern populations would be considered invasive and humans are the most likely the mechanism of dispersal to those areas. There are no historic records of their presence in the northeast and the climate/climatic history is more similar to New York and other New England states that treat *M. heterophyllum* as an invasive species.

The designation could be preserved to protect the theorized native populations, leaving special exceptions for the northeastern populations. Without further research this seems like the best option. The plant is apparently secure throughout the state but without any conclusive results to why the plant is aggressive the plant should retain its status. Several reasons for remaining critically imperiled include the possibility of cyclic populations, response to nutrients that maybe cyclic in availability, and the questionable absence of two 'historic' populations at Presque Isle. Without more knowledge and conclusive data the plants status should be left as is and populations can dealt with on a case-by-case basis.

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## APPENDIX A: KNOWN POPULATIONS OF MYRIOPHYLLUM HETEROPHYLLUM IN PENNSYLVANIA AND DELEWARE

| Location | County | Quad | Observation upon visit, or abundance rating |
|---|---|---|---|
| Ridge Pond, PA | Erie | Erie North | 4 |
| Cheat River, PA | Fayette | Morgantown North | 2 |
| Inlet of Harvey's Lake, PA | Luzerne | Harvey's Lake | 3 |
| Lily Lake, PA | Luzerne | Nanticoke | 3 |
| Shohola, PA | Pike | Shohola | 4 |
| Harris Pond, PA | Luzerne | Sweet Valley | 4 |
| Beaver Pond, PA | Erie | Erie North | 4 |
| Promised Land Upper Lake, PA | Pike | Promised Land | 2 |
| Promised Land Lower Lake, PA | Pike | Promised Land | 4 |
| Ditch by Abbott's Pond, DE | Sussex | Milford | 1 |
| Niagara Pond, PA | Erie | Erie North | Could not find |
| Long Pond, PA | Erie | Erie North | Could not find |
| Pond # 4, PA | Carbon | Hickory Run | Misidentification, water body dried up |
| Deer Lake, PA | Greene | Carmichaels | Not accessible w/o permission, no herbarium specimens |

## APPENDIX B: HISTORIC POPULATIONS IN PENNSYLVANIA FOUND IN RESEARCHING HERBARIUM SPECIMENS

Appendix. 2. Historic populations in Pennsylvania found in researching herbarium specimens.

| Location | County | Quad |
|---|---|---|
| Tullytown, PA | Bucks | Trenton West |
| Tohickon Creek, PA | Bucks | Lumberville |
| Schuylkill River, PA | Philadelphia | Philadelphia |
| Penn Valley, PA | Bucks | Trenton West |