MONITORING OF SPHAGNUM AT A RESTORATION SITE AND POSSIBILITIES FOR RESTORATIVE ACTIVITIES

A Thesis
Presented to
The Graduate Faculty of The University of Akron

In Partial Fulfillment
for the Requirements for the Degree
Master of Science

John Anthony Miller
August, 2016
MONITORING OF SPHAGNUM AT A RESTORATION SITE AND POSSIBILITIES FOR RESTORATIVE ACTIVITIES

John Anthony Miller

Thesis

Approved: Advisor
Dr. Randall J. Mitchell

Accepted: Dean of the College
Dr. John Green

Dean of the Graduate School
Dr. Chand Midha

Date

Interim Department Chair
Dr. Stephen Weeks
ABSTRACT

The restoration of the Tamarack Bog in Bath Township, Ohio is focused on a poor fen which was ditched and partially drained in the 1960’s. This thesis documents two studies relevant to the restoration of this site- initial survey and monitoring work, and a test of local adaptation. As *Sphagnum* is an important genus in the restoration of peatlands, the baseline coverage and extent within the restoration site was measured. Also, another important moss, *Thuidium delicatulum*, was monitored because it seemed to co-occur with *Sphagnum* during informal observations. 52 monitoring plots were setup for the purpose of measuring the coverage of these two genera within the Tamarack Bog. Nearly all of the *Sphagnum* occurred in the Core Bog region, but was absent from adjacent degraded areas. The coverage of *Sphagnum* in the monitoring plots amounted to 1.3% percent in 2014 and 1.4% in 2015. *Thuidium* coverage in the monitoring plots amounted to 7.5% in 2014 and 6.4% in 2015. In the surveys for 2015, *Sphagnum* presence was associated with *Thuidium* presence. One aim of the restoration project is to increase *Sphagnum* coverage within the Tamarack Bog, and this paper provides a method for measuring such coverage and provides baseline data of coverage and extent before the restoration begins.

In peatland restoration projects, donor sites for *Sphagnum* collection are chosen based upon similar vegetation as the proposed restoration site. To test for local adaptation in *S. palustre*, I conducted two experiments using three different local populations grown in water collected from the three locations. Local adaptation would be
indicated by a significant interaction in these analyses, where the moss from each location grows best within its water source and worse within the water sources of the other two locations. The first experiment showed *Sphagnum* source and water source yielding significant effects, but there was no observable interaction between *Sphagnum* source and water source. The second experiment yielded *Sphagnum* source effects, and again, no interaction. By the definition outlined above, no local adaptation was observed in these two experiments. Therefore, for the Tamarack Bog restoration project, the donor source of *Sphagnum* does not need to be collected from one specific site.
ACKNOWLEDGEMENTS

I would like to start by acknowledging my advisor, Randy Mitchell. Randy was a great mentor, and he has helped me a great deal in learning the scientific process. Jean Marie Hartman of Rutgers University was also a great help in designing experiments. I also would like to thank my committee members, Drs. Ott and Wiley. A special thanks also to the members of Dr. Mitchell’s lab, especially Chris Chaney for editing help and experimental design help. In help with *Sphagnum* identification, I would like to thank Diane Lucas. I would like to thank the Cleveland Museum of Natural History, especially Renee Boronka, for permitting collecting locations. I would also like to give special thanks to the Ohio Biological Survey small grants program.

Also, thanks to the members of the biology department here at The University of Akron. Many undergraduate researchers provided much help in setting up experiments and field manipulations. In 2014, Katie Suhadolnik-Wellert helped a great deal on the field surveys. Ziad Shwaiki helped with the numerous trials of the cup experiment. In 2015, Rochelle King, Natasha Romanoff, Adrian Wakeen, and Michael Weber were helpful in field surveys and field collections for the greenhouse experiments. I would also like to thank Carrie Childs for helping with field surveys and field setups, as well as her support. I also would like to thank my mother, Rebecca, and my brother, Robert for their support. Many other friends and family provided support, as well.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>LIST OF TABLES</th>
<th>ix</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
<td>x</td>
</tr>
</tbody>
</table>

## CHAPTER

### I. MONITORING THE MOSS OF A WETLAND RESTORATION .................1

Introduction..............................................................................1

Role of *Sphagnum* in Peatland Restorations...........................4

Role of *Thuidium delicatulum* at the Tamarack Bog ...............5

Materials and Methods................................................................5

Restoration Site Characteristics ..............................................5

*Sphagnum* and *Thuidium* Monitoring ...................................6

Results.......................................................................................8

*Sphagnum* Monitoring ...........................................................9

*Thuidium delicatulum* Monitoring .........................................10

Discussion.................................................................................12

*Sphagnum* Monitoring ...........................................................12

*Thuidium* Monitoring ............................................................13

Annual Variation.........................................................................14

Recommendations for Future Surveys and Mapping....................15

Situation as of Spring 2016.......................................................15

Role of *Thuidium delicatulum* at the Tamarack Bog ...............16

Future Directions for the Tamarack Bog ...................................16

### II. LOCAL ADAPTATION IN *Sphagnum palustre*..........................18

Introduction................................................................................18

Tamarack Bog Restoration Site and *Sphagnum palustre* ...........19
Materials and Methods ........................................................................................................... 20

Site Characteristics ................................................................................................................ 20

Water Collection for Cup Experiment ....................................................................................... 22

Experimental Design for Cup Experiment ............................................................................... 23

Water Collection for Peat Substrate Experiment .................................................................... 23

Experimental Design for Peat Substrate Experiment ............................................................... 24

Analysis .................................................................................................................................... 26

Results ....................................................................................................................................... 26

Cup Experiment ....................................................................................................................... 26

Peat Substrate Experiment ..................................................................................................... 29

Discussion ................................................................................................................................. 32

Cup Experiment ....................................................................................................................... 32

Peat Substrate Experiment ..................................................................................................... 34

Shade Effects ........................................................................................................................... 34

Implications for Restoration .................................................................................................... 35

III. CONCLUSIONS .................................................................................................................. 37

BIBLIOGRAPHY ....................................................................................................................... 39

APPENDICES ........................................................................................................................... 43

APPENDIX A: SAMPLE GRAPH SHEET FOR SURVEYS ........................................... 44

APPENDIX B: S3 SURVEY PLOT (TOP-2014, BOTTOM-2015) ................................. 45

APPENDIX C: HISTOGRAM OF SPHAGNUM COVERAGE IN 2014 AND 2015 .......................................................................................................................... 46

APPENDIX D: HISTOGRAM OF THUIDIUM COVERAGE IN 2014 AND 2015 ...................................................................................................................................................... 47

APPENDIX E: CHI SQUARE OF SPHAGNUM PRESENCE IN 2014 BY REGION OF RESTORATION SITE .................................................................................................................. 48

APPENDIX F: CHI SQUARE OF SPHAGNUM PRESENCE IN 2014 BY THUIDIUM PRESENCE IN 2014 (2M X 2M PLOT ASSOCIATION) .......................................................... 49

APPENDIX G: CHI SQUARE OF SPHAGNUM PRESENCE IN 2015 BY REGION OF RESTORATION SITE .................................................................................................................. 50

APPENDIX H: CHI SQUARE OF SPHAGNUM PRESENCE IN 2015 BY THUIDIUM PRESENCE IN 2015 (2M X 2M PLOT ASSOCIATION) .......................................................... 51
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>8</td>
</tr>
<tr>
<td>1.2</td>
<td>9</td>
</tr>
<tr>
<td>1.3</td>
<td>12</td>
</tr>
<tr>
<td>1.4</td>
<td>12</td>
</tr>
<tr>
<td>2.1</td>
<td>27</td>
</tr>
<tr>
<td>2.2</td>
<td>30</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Approximate map of the Tamarack Bog restoration project, showing research plots, transects, approximate boundaries of the three regions, and other notable features</td>
<td>2</td>
</tr>
<tr>
<td>1.2 Scatterplot of percent coverage of <em>Sphagnum</em> in the monitoring plots</td>
<td>10</td>
</tr>
<tr>
<td>1.3 Scatterplot of percent coverage of <em>Thuidium</em> in the monitoring plots</td>
<td>11</td>
</tr>
<tr>
<td>2.1 Percent change in mass of <em>S. palustre</em> (A) and length change in <em>S. palustre</em> (B) in the cup experiment</td>
<td>28</td>
</tr>
<tr>
<td>2.2 Percent change in mass of <em>S. palustre</em> (A), length change in <em>S. palustre</em> (B), and number of capitula produced (C) in the peat substrate experiment</td>
<td>31</td>
</tr>
</tbody>
</table>
CHAPTER I
MONITORING THE MOSS OF A WETLAND RESTORATION

Introduction:

In the lower 48 United States, over 50% of original wetland coverage has been lost since the onset of European settlement, and this includes an estimated 90% loss in Ohio (Dahl 1990). The peatlands of Ohio have greatly diminished, and only 2% of the total peatland coverage in 1900 still exists today (Andreas and Knoop 1992). Since peatland ecosystems of the northern hemisphere are estimated to store 25% of the world’s soil carbon (Gorham 1991, O’Neill 2000) and peatlands can function to sequester carbon (Clymo et al 1998, Gorham 1991), loss of peatlands may contribute to an increase in atmospheric carbon levels. However, if native vegetation is restored, peatlands can regain functioning as carbon sinks (Tuittila et al 1999). Peatland conservation not only benefits scientific study, but it also can serve to preserve plant diversity and serve economical purposes. For example, peatlands serve as effective resources in the study of archaeological and paleoecological records (Buckland 1993, Jacobson et al 1991). In the northeastern United States, shrubby peatlands often host rare vascular plants (Latham 2003). A possibility for ecotourism also contributes to the necessity to preserve peatlands (Chapman et al 2003).

Located in the Bath Nature Preserve in Bath Township, Ohio, a tamarack bog (hereafter, termed “Tamarack Bog”) is quickly transitioning into a wetland forest (Miletti
et al 2005). The common practices of the 20th century led to the ditching and partial draining of the Tamarack Bog. Peatland destruction is not rare, especially in Ohio, as drainage for agriculture has been a leading cause of wetland loss in Ohio (Andreas and Knoop, 1990). Restoration efforts are now underway at the Bath Tamarack Bog to stabilize and expand the habitat, and the end goal is to restore the area back to an intact-peatland. Research to date has documented three main regions of habitat within the Tamarack Bog restoration project based on the visual appearance of the vascular plant community, microtopography, hydrology, and soil surface. The Core Bog habitat is mostly intact, the Wetland Edge habitat is mostly intact and contains some bog species, and the upland transition (‘Enhancement’) consists of marginal areas that were once bog

Figure 1.1: Approximate map of the Tamarack Bog restoration project, showing research plots, transects, approximate boundaries of the three regions, and other notable features.
but now appear to be transitioning toward more upland vegetation (Figure 1.1). The Enhancement region may possibly be restored to the point of containing bog species again.

Research on peatland restoration techniques primarily focuses on two main methods of peat harvesting. Block-cutting of peatlands is carried out through construction of drainage trenches consisting of the construction of many trenches draining into one or two major ditches, followed by extraction of peat by hand (Price et al 2003, Lavoie and Rochefort 1996). A newer process, vacuum harvesting of peatlands is cutting surface vegetation and mechanically collecting the peat deposited beneath the surface (LaRose et al 1997). In both circumstances, the surface layer of peat, defined as the acrotelm, and the surface vegetation is removed, leaving the catotelm (the anoxic lower peat layers) exposed (Heathwaite et al 1993). The drainage and harvesting practices change the hydrological and physical conditions of the peatland (Price 1996), and when left alone after peat harvesting, the *Sphagnum* recolonization of bogs surveyed in Quebec was low (Lavoie and Rochefort, In Lavoie and Rochefort 1996). The situation at the Tamarack Bog does not present as many problems as a vacuum harvested peatland because at the Tamarack Bog, the peat and surface vegetation has remained more or less untouched. The ditches at the Tamarack Bog seem to resemble the trenches of a block-cut peatland. However, only two major ditches were constructed at the tamarack bog, as opposed to the numerous trenches constructed in a block-cut design draining into the major ditches. Furthermore, the acrotelm was not removed other than in the ditch itself. Therefore, the *Sphagnum* at this site does not have to contend with the barren conditions of harvested peatlands. Research exploring *Sphagnum* regeneration has mainly focused
on sites recovering from vacuum harvesting (Rochefort et al, 1997; Pouliot et al 2012). More research is needed on sites having been drained but left alone, in terms of peat harvesting. The Tamarack Bog restoration provides such an opportunity.

Role of *Sphagnum* in Peatland Restorations

Restoring hydrology, attaining an appropriate *Sphagnum* coverage, and controlling invasive species are some of the actions necessary in order to accomplish a successful peatland restoration (Gorham and Rochefort 2003). This paper focuses on one of the above listed goals, the reestablishment of *Sphagnum*. Reestablishment of *Sphagnum* at the Tamarack Bog is important because *Sphagnum* is a major component of such restorations (Rochefort 2000), and *Sphagnum* is also an important ecosystem engineer (Jones et al 1994, van Breemen 1995).

To monitor the extent of *Sphagnum* coverage at this restoration site, I developed a method for surveying the moss in the field. With the data collected, the following questions were investigated about *Sphagnum* at the Tamarack Bog: (1) What is the initial coverage of *Sphagnum* within the Tamarack Bog before restorations? (2) Does the extent of *Sphagnum* coverage vary among habitat type (Core, Wetland Edge, or Enhancement) within the restoration project? If *Sphagnum* coverage within the bog does not expand, then the mitigation and management agreement between the Ohio EPA and the township requires actions to facilitate *Sphagnum* expansion within the bog. One goal of the restoration project is to see *Sphagnum* expand into the wetland edge (and possibly the enhancement region) of the tamarack bog and to see an overall increase in *Sphagnum* coverage. Restoration methods could include spreading of diaspores from donor peatland
sites, spreading diasporas from *Sphagnum* collected at the Tamarack Bog, and exploring nurse-plant interactions.

**Role of Thuidium delicatulum at the Tamarack Bog**

A general walk through of the bog led me to believe there may be an association between *T. delicatulum* and *Sphagnum*. *Sphagnum* seemed to be more often present when *T. delicatulum* was present. For this reason, *T. delicatulum* was also included in the study. If such an association does exist, the relationship should be investigated further. Much like the observations for *Sphagnum* mentioned above, *T. delicatulum* coverage will be tracked within the tamarack bog restoration site. An association between *T. delicatulum* and *Sphagnum* could prove useful if we need to take future actions to facilitate *Sphagnum* expansion. Focusing on *T. delicatulum*, the following questions were investigated: (3) What is the initial coverage of *T. delicatulum* within the tamarack bog? (4) Does *T. delicatulum* have an association with *Sphagnum*?

**Materials and Methods:**

The restoration design of the Tamarack Bog project at the Bath Nature Preserve divides the site into three types of habitat designated Core Bog, Wetland Edge, and Enhancement. The hope is for the overall wetland habitat to expand and for the Core Bog habitat to expand with the raising of the water level and removal of invasive species.

**Restoration Site Characteristics**

The Core Bog region contains most of the site’s *Sphagnum* and all of the site’s tamarack trees (*Larix laricina*), along with a dense thicket of shrubs consisting of *Alnus incana*, *Ilex verticillata*, and *Vaccinium corymbosum*. This region also has a highly interspersed microtopography with areas of open water, moss lawns (consisting of
Sphagnum and T. delicatulum), and hummocks. The Wetland Edge region contains wetland plants, such as Carex lacustris, Leersia oryzoides, Polygonum sagittatum, and Symplocarpus foetidus. In the Wetland Edge region, there is less microtopographic interspersion than in the Core Bog region. There are some mounded areas, but there are few pools. The Enhancement region represents a transition from wetland to upland. Hence, wetland plants are present alongside some upland plants. Wetland plants include Carex lacustris, Fraxinus pennsylvanica, Glyceria striata, and Symplocarpus foetidus, while some of the notable upland species are Acer saccharum, Carya ovata, Circaea lutetiana, and Pyrus sp. The microtopography of the enhancement region is minimal.

Sphagnum and Thuidium Monitoring

I monitored the extent of Sphagnum and Thuidium coverage each spring for 2 years (2014, 2015). At the Tamarack Bog, 52 moss monitoring plots were setup to track Sphagnum coverage throughout the near 14-acre restoration area. The monitoring plots are 2m X 2m. There are 12 monitoring plots in the Core region of the bog, 16 plots in the Wetland Edge region, and 16 plots in the Enhancement region. Eight additional plots are setup at the end of already established monitoring transects; 6 in the Wetland Edge region and 2 in the Enhancement region. All plots are marked with PVC pipes (1/2” diameter and 3’ in length) inserted ~2’ into the soil in one corner of each plot. The moss monitoring plots were placed at already existing vegetation plots used annually. When setting up the 2m X 2m plot, meter sticks were placed around the four constituent 1m X 1m portions of the plot using the PVC pipe as one of the corners (see Appendix B), and I mapped the moss cover on a sheet of paper (see Appendix A). In each plot, I carefully inspected the soil surface to find any specimens of the two genera of interest. If either
genus was found, I mapped the spatial coverage of moss onto the grid paper to facilitate year to year comparisons and evaluate growth and survival. I did not map other species of moss or vascular plants. I used the meter sticks and the grid paper as a guide to scale the coverage area in the plot to the coverage area mapped on the paper. The genera were noted on the map paper with “T” representing *Thuidium* and “S” representing *Sphagnum*. When drawing the maps, the coverage was measured, and hence drawn, to the nearest 1 cm. In addition, leaf litter coverage estimates were also noted. Standardized photo images of the plots and the surrounding vegetation were also taken for use in tracking the progress of the restoration project over the next 10 years. The plot images were taken from the south side of the plot facing north, unless otherwise noted on the maps. The images of the surrounding vegetation were taken while standing at the corner PVC pipe. Four images were taken from each plot (one image each was taken from the corner PVC pipe while facing northeast, northwest, southeast, and southwest).

The monitoring was conducted once a year for two years, and plans are to continue monitoring each spring for 10 years. The weather played a role in both years. In 2014, the maps were completed between late March and late May. The rainfall and snowmelt of 2014 prevented mapping for a considerable time. In 2015, the surveys were completed between mid-April to mid-May. In 2015, the snow did not melt off immediately, so the maps could not be completed until beginning in April. I conducted the surveys when ice and snow cover was absent, flooding was minimal, and before the abundant shrubs and other vegetation in the area leafed out.

All plants of *Thuidium* were the same species, *Thuidium delicatum* (Hedw.) Schimp., but I could not always distinguish *Sphagnum* spp. in these plots, so the surveys
include only genus-level identification. Identifying *Sphagnum* species requires the identification of microscopic features, such as leaf cross-section, stem cross-section, and orientation of chlorophyllose cells. However, to assemble a list of *Sphagnum* species present at the restoration site, I consulted with several regional experts; in particular Diane Lucas (moss consultant for the Cleveland Museum of Natural History), and Jim Toppin (active member of the Ohio Moss and Lichen Society). With their help and after my own intense surveys of the Tamarack Bog, I have identified the following species as being present: *S. palustre* L., *S. girgensohnii* (Russow), and *S. squarrosum* (Crome). These identifications were made using Crum (1984). Jim Toppin also found *S. centrale* (C.E.O. Jensen) and *S. fimbriatum* (Wilson).

**Results:**

Table 1.1: 2014 Survey Data

<table>
<thead>
<tr>
<th>Region</th>
<th>Plots with <em>Sphagnum</em>/Total Plots</th>
<th>Coverage of <em>Sphagnum</em>/Region</th>
<th>Plots with <em>Thuidium</em>/Total Plots</th>
<th>Coverage of <em>Thuidium</em>/Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core</td>
<td>10/12</td>
<td>5.0% + 2.7%</td>
<td>12/12</td>
<td>24.5% +/- 4.2%</td>
</tr>
<tr>
<td>Wetland Edge</td>
<td>1/22</td>
<td>0.3%</td>
<td>21/22</td>
<td>4.1% +/- 0.8%</td>
</tr>
<tr>
<td>Enhancement</td>
<td>0/18</td>
<td>0%</td>
<td>9/18</td>
<td>0.4% +/- 0.2%</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>11/52</td>
<td>1.3% +/- 0.7%</td>
<td>42/52</td>
<td>7.5% +/- 1.6%</td>
</tr>
</tbody>
</table>
Table 1.2: 2015 Survey Data

<table>
<thead>
<tr>
<th>Region</th>
<th>Plots with Sphagnum/Total Plots</th>
<th>Coverage of Sphagnum/Region +/-</th>
<th>Plots with Thuidium/Total Plots</th>
<th>Coverage of Thuidium/Region +/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core</td>
<td>9/12</td>
<td>4.6% +/- 2.1%</td>
<td>11/12</td>
<td>19.2% +/- 4.8%</td>
</tr>
<tr>
<td>Wetland Edge</td>
<td>1/22</td>
<td>0.8%</td>
<td>21/22</td>
<td>4.5% +/- 0.8%</td>
</tr>
<tr>
<td>Enhancement</td>
<td>0/18</td>
<td>0%</td>
<td>5/18</td>
<td>0.2% +/- 0.1%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>10/52</td>
<td>1.4% +/- 0.6%</td>
<td>37/52</td>
<td>6.4% +/- 1.5%</td>
</tr>
</tbody>
</table>

*Sphagnum* Monitoring

In spring 2014, *Sphagnum* was found in 11 of the 52 monitoring plots; 10 of these were in the Core Bog area. In spring 2015, *Sphagnum* was found in only 10 (9 being in the core bog) monitoring plots. A frequency scatterplot of the coverage percentages for both years is found in Figure 1.2. Compared to 2014 data, *Sphagnum* was lost from two monitoring plots. In both instances, 2014 data had a few shoots of *Sphagnum* showing up in the corner of a plot, but by 2015, it was no longer in that corner. The coverages of each of these plots was 0.25% and 0.20% in 2014. In addition, there was one monitoring plot that had no *Sphagnum* coverage in 2014, but had 0.75% coverage in 2015. This patch of *Sphagnum* was most likely missed in 2014. There was one Core Bog plot which had no *Sphagnum* in both years.

In both years, regions differed significantly in the presence of *Sphagnum* (Contingency analysis for both years: Chi Square p<0.0001) (Appendices E and G). In
both years, the *Sphagnum* was found only in the Core Bog region, except for one of the plots at the end of the transects. This transect plot, being located within the Wetland Edge region, was the only other plot outside of the Core Bog region which housed *Sphagnum* in 2014 and 2015. Referring to Figure 1.1, this plot is at the end of “T2”, which lies within the outlined area of *Sphagnum* presence. The average coverage of the *Sphagnum* in the 11 plots in 2014 was 6%. The average coverage of the *Sphagnum* in the 10 plots in 2015 was 7%. Overall, there was no change in percentage of *Sphagnum* coverage from 2014 to 2015 (paired t-test, p=0.7, Appendix I).

![Sphagnum Surveys](image)

Figure 1.2: Scatterplot of percent coverage of *Sphagnum* in the monitoring plots (2014, 2015).

*Thuidium delicatulum* Monitoring

*Thuidium delicatulum* was found in 42 of the 52 monitoring plots in 2014; however, it was only found in 37 monitoring plots in 2015. The average coverage of *T. delicatulum* in the 52 monitoring plots in 2014 was 7.5% +/- 1.6%. The average coverage of the *T. delicatulum* in the 52 monitoring plots in 2015 was 6.4% +/- 1.5%. The highest percent coverage found in a plot was 51% in both years (Figure 1.3 shows...
the frequency scatterplot). Five plots that contained *Thuidium* in 2014 had none in 2015; three of these were in the Enhancement region.

To determine if there was an association between *Thuidium delicatulum* presence and *Sphagnum* spp presence, I used a contingency analysis and chi square test. In both years, *Sphagnum* and *T. delicatulum* tended to be associated with each other in the same 2m X 2m plot. Furthermore, in both years, *Sphagnum* was never present when *Thuidium* was absent. In 2014 (Table 1.3), this association was nonsignificant (Fisher’s exact test p=0.07), and in 2015 (Table 1.4), the association was significant (Fisher’s exact test p=0.022). As a follow-up, I did an analysis observing the presence of *Sphagnum* and *Thuidium* within the same patch. Here, I defined a patch as being a distinct spatial area isolated from another patch of moss. For *Sphagnum* and *Thuidium* to show up in the same patch, therefore, the patches would need to be touching each other (Growing side by side) or be found growing within the same patch. In both years, analysis showed that *Sphagnum* presence is greater when *Thuidium* presence is lower (Appendices Z and AA).

![Figure 1.3: Scatterplot of percent coverage of *Thuidium* in the monitoring plots (2014, 2015).](image)

\[y = 0.846x + 0.0542; \quad R^2 = 0.8448\]
Table 1.3: Contingency Table Results of *Thuidium* and *Sphagnum* Association in 2m X 2m Plots (2014)

<table>
<thead>
<tr>
<th></th>
<th><em>Thuidium</em> Present</th>
<th><em>Thuidium</em> Absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sphagnum</em> Present</td>
<td>11</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td><em>Sphagnum</em> Absent</td>
<td>31</td>
<td>10</td>
<td>41</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>42</strong></td>
<td><strong>10</strong></td>
<td><strong>52</strong></td>
</tr>
</tbody>
</table>

Table 1.4: Contingency Table Results of *Thuidium* and *Sphagnum* Association in 2m X 2m Plots (2015)

<table>
<thead>
<tr>
<th></th>
<th><em>Thuidium</em> Present</th>
<th><em>Thuidium</em> Absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sphagnum</em> Present</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td><em>Sphagnum</em> Absent</td>
<td>27</td>
<td>15</td>
<td>42</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>37</strong></td>
<td><strong>15</strong></td>
<td><strong>52</strong></td>
</tr>
</tbody>
</table>

Discussion:

At the Tamarack Bog, *Sphagnum* occurs almost exclusively in the Core Bog region, with the exception being one plot in the Wetland Edge. *Thuidium delicatulum*, however, is present in all regions of the restoration site.

*Sphagnum* Monitoring

In the plots where *Sphagnum* is present, its coverage is low - mean coverage for the 11 plots containing *Sphagnum* is around 6%, but across all 52 plots throughout the bog, the coverage of *Sphagnum* is just over 1%. For a successful peatland restoration, *Sphagnum* should be the dominant vegetation (Rochefort 2000). Looking at repeatability of the monitoring process, there was no significant difference between measurements of *Sphagnum* between 2014 and 2015. This seems to indicate that this is an effective and repeatable method for tracking *Sphagnum* coverage. Within the Core Bog region, the two species which seem to have the most coverage are *S. palustre* and *S. squarrosum*
(personal observation). This region of the bog seems to have the most microtopographic interspersion (personal observation), as many hummocks have formed on the base of shrubs and cinnamon ferns (*Osmunda cinnamomea*). There are some pools scattered throughout the Core region. The *Sphagnum* does not seem to grow in the pools. In fact, one of the two plots which lost *Sphagnum* coverage from 2014 to 2015, originally had *Sphagnum* growing in a pool. The one Wetland Edge monitoring plot containing *Sphagnum* is located in the southern part of the restoration site. This area of the Wetland Edge region has small *Sphagnum* populations scattered in it. Overall, the *Sphagnum* of the Tamarack Bog is mainly found in the Core Bog region, and the percent coverage is low.

Thuidium Monitoring

*Thuidium delicatulum* and *Sphagnum* were positively associated at a scale of 2m X 2m in the 2015 data, and showed the same trend (although non-significant) for 2014. In fact, over both years, *Sphagnum* was only found in plots containing *T. delicatulum*. *T. delicatulum* was found in all regions of the restoration site. Although *T. delicatulum* is abundant throughout the restoration site, transitioning from the Core and Wetland Edge regions toward the Enhancement region leads to a drop in overall *T. delicatulum* presence and percent coverage (Tables 1.1 and 1.2). In terms of repeatability of the survey and mapping method, *T. delicatulum* did show a significant difference in percent coverage between 2014 and 2015 (Tables 1.1 and 1.2). One potential reason could be over-identification in 2014. In some occasions, other mosses may have been mistakenly mapped as *T. delicatulum*. When walking through the restoration site before the mapping and surveying method was developed, *T. delicatulum* and *Sphagnum* were sometimes
mistaken for each other. Another moss, *Aulacomnium palustre* is also present within the Wetland Edge and Enhancement regions. This moss was sometimes mistaken for *T. delicatulum* as well, especially during dry periods, as the pinnate branches of *T. delicatulum* may shrivel toward the stem. When the pinnate branches of *T. delicatulum* shrivel, some populations looked similar to *A. palustre*. Four plots, in particular, had numerous clumps of *T. delicatulum* that had dried and turned a brown color. When found, the dried *T. delicatulum* was not included in the maps because it was not green and was assumed to be dead. Another reason for the differences in *Thuidium* coverages could be the seasonal variation in observation date between 2014 and 2015. One problem with seasonal variation is the coverage of graminoids in the plot. I noticed that *T. delicatulum* was found growing underneath some graminoid species, so it may be difficult to get an accurate measurement later in the spring due to being overshadowed by sedges.

**Annual Variation**

Annual variation may also play a role in the mapping process. In Ohio, some years may see snow coverage into April, and in other years, the snow may be melted off by March. Surveys should be completed before the end of March so that there is less herbaceous and shrub vegetation to contend with, and measurements would be more precise. In 2014, snowmelt was coupled with higher rainfall, so often times, the monitoring plots had standing water that may have decreased detection of mosses (Appendix S). In 2015, there was considerably less snowmelt and a milder winter than in 2014, which accounted for the shorter time span of the 2015 surveys before plant emergence and shrub leafing in the spring. For future scoring of these plots, I
recommend conducting all of the surveying and mapping by the end of March in order to avoid these problems. This task, however, is highly weather-dependent.

Recommendations for Future Surveys and Mapping

In order to reduce errors associated with monitoring plot setup, PVC pipes will be installed at every corner of the monitoring plots. As stated in the methods section, the plots are situated with one PVC pipe at only one of the four corners of each plot. From this, meter sticks are placed to make a 2m X 2m grid. Without clearly marked corners, the placement of the meter sticks may change slightly from year to year, introducing variability to the results (Appendix T). Installing PVC pipes at every corner (achieved for over half of the plots as of February 2016) will allow for more permanent positions of the monitoring plots for the decade long monitoring process. Also, more PVC pipes will allow for better digital tracking of changes as there will now be four permanent markers within the images collected on an annual basis.

Situation as of Spring 2016

As a key part of the overall restoration project, an AgriDrain was installed in the main outlet of the bog, and in the fall of 2015, a 6” ‘stoplog’ was placed to begin restoring the water level. This could impact the measurements for 2016. As such, the data collected from 2014 and 2015 provide baseline data for the tamarack bog before any restoration practices were undertaken. Once again, the Core Bog region contained nearly all of the project site’s Sphagnum coverage (with the exception being one of the Wetland Edge monitoring plots). Surveying and mapping each spring for the next 10 years will allow us to track if Sphagnum begins to establish in the Wetland Edge or Enhancement
region. With the onset of the new monitoring system in 2016, the annual variation due to observer bias and meter stick placement should be reduced.

Role of *Thuidium delicatulum* at the Tamarack Bog

The finding of an association between *Sphagnum* and *T. delicatulum* presents some background information for future studies. One possibility is that *T. delicatulum* could be a nurse-plant in facilitating *Sphagnum* growth. As a follow-up, greenhouse studies and field introduction studies should be conducted to investigate this possibility further. *Sphagnum* has been found to benefit from nurse-plant interactions, in particular with *Polytrichum strictum* providing favorable microclimate conditions in a restoration setting (Groenveld et al 2007). Another possibility is that *T. delicatulum* could have encroached upon the niche that *Sphagnum* filled in the Tamarack Bog after the bog was ditched and drained. Field studies and greenhouse experiments should be setup to investigate this as well. The patch association study provides possible evidence for competition, as *Sphagnum* is significantly more likely to be found in a patch without *T. delicatulum*. Mapping the mosses could also provide evidence for competition or encroachment because as the hydrology is restored, the *Sphagnum* may begin to dominate the bryophyte coverage within the restoration site.

Future Directions for the Tamarack Bog

Looking at the baseline data for *Sphagnum* and *T. delicatulum* coverage being outlined here, we can begin to design restoration activities. If the *Sphagnum* population does not recover, we should begin exploring options for reestablishment. For the tamarack bog restoration project, we should pay close attention to the following topics: (1) encouraging *Sphagnum* coverage increase by seeding vegetation fragments collected
from donor sites, (2) exploring if the Wetland Edge and the Enhancement regions are suitable for *Sphagnum* growth, (3) exploring if *T. delicatulum* can provide further information on the ecology of the bryophytes of the Tamarack Bog (i.e. nurse-plant and/or niche studies), (4) using repeated digital images to determine if the vegetation is changing, and (5) exploring the state of the tamarack population within the restoration site.
CHAPTER II

LOCAL ADAPTATION IN *SPHAGNUM PALUSTRE*

**Introduction:**

There have been many studies demonstrating local adaption of different plant traits in response to the growing environment (Linhart and Grant 1996). When local plant populations and foreign plant populations of the same species are grown together, the local populations usually perform better than the foreign plants (Leimu and Fischer 2008), and such cases provide evidence for local adaptation (Kawecki and Ebert 2004).

Peatland restoration relies on reestablishment of appropriate vegetation, including particularly *Sphagnum* moss. Donor sites for *Sphagnum* reestablishment are typically chosen because they have a similar plant species composition as the proposed restoration site (Quinty and Rochefort 2003), and because they are nearby (Gorham and Rochefort 2003). These decisions capitalize on the fact that *Sphagnum* often performs better when grown in pH conditions most similar to the collection site (Sstad et al 1999). Because the effects of local adaptation can enable nearly a 50% higher fitness in the local populations (Hereford 2009), the principles of local adaptation may be useful in the restoration of peatlands.

*Sphagnum* spp. often occur along chemical and physical gradients (Andrus 1986) and exhibit local adaptation in response to these gradients. pH is one important gradient determining the species distribution of bryophytes in peatland habitats (Vitt and Chee
Populations of *S. fuscum* (Schimp.) Klingr. differed significantly in their performance along a pH gradient, and also in response to another important gradient, microtopographic conditions (Gunnarsson et al 2007). Also, local adaptation to a nitrogen gradient may play a role in the response of *Sphagnum* (Granath et al 2009). These strong responses show that *Sphagnum* spp. may be locally adapted to their environmental conditions, and that this might be important to consider during restorations.

**Tamarack Bog Restoration Site and *Sphagnum palustre***

At the Tamarack Bog in Bath Township, Ohio, *Sphagnum* coverage is low (<10% cover) and is only found in a central area designated as the Core Bog region (Chapter 1). In accordance with the goals of the restoration project, if the *Sphagnum* population does not begin to recover, restoration methods should be explored and enacted (Unpublished Army Corps of Engineers mitigation documents). To better focus the plans for restoration, I wanted to investigate whether or not *Sphagnum* is locally adapted to its water source.

One of the more common species of *Sphagnum* at this restoration site is *Sphagnum palustre* (personal observation). *S. palustre* can be found across a gradient of different pH conditions, ranging from around 3.9 to around 7 and even sometimes in slightly basic conditions (Andrus 1986, Hajkova and Hajek 2007). Different local populations of *S. palustre* in northern, central, and southern Italy have also shown strong genetic differentiation, including two nearby (within 50 miles) populations (Terracciano et al 2012). Since a pH gradient is important in the variation of habitats within peatlands
(Rydin and Jeglum 2013), it would be beneficial to see if the different populations of *S. palustre* have differing localized optima for their pH preferences.

More research on local adaptation is necessary in order to examine what ecological factors play a role in the process (Kawecki and Ebert 2004). To determine if water source plays a major role in local adaptation of *S. palustre*, I tested three populations of *Sphagnum*. To determine if *S. palustre* specimens are locally adapted, I grew plants from three different source populations in water from each location. If there is local adaptation, each *S. palustre* population will perform best in water from its own source and will perform worse in water from other sources. Answering this question has ramifications for restoration projects. An answer providing evidence for local adaptation will allow project managers to focus donor collections from a site with similar water conditions as the restoration site. An answer providing no evidence for local adaptation will allow for the collection of donor material being made in broader environmental conditions and possibly, help prevent overexploitation of locations within certain pH ranges.

**Materials and Methods:**

I chose three source populations for this study; they all contained *S. palustre* but were likely to provide differing environments for *Sphagnum* growth in terms of the pH, light, and other factors.

**Site Characteristics**

The first location was the Tamarack Bog in Bath Township, Ohio. This location is currently being restored, as two ditches were constructed in the 1960’s and have greatly changed the hydrology and allowed invasive species to establish (Miletti et al
This site is a poor fen with substantial groundwater inflow and a circumneutral pH (6.5-7; Mezentseva 2015). The site is dominated by shrubs and trees, providing shaded conditions. Water levels are within ~10cm of the peat surface throughout the year (Miletti et al 2005). The second location was a red maple (*Acer rubrum*) swamp located in Mentor, Ohio (part of the Mentor Marsh complex). *S. palustre* at this location was growing on hummocks built up around dead and living *A. rubrum* tree roots. Also at this site was an encroaching population of *Phragmites australis*, which, when combined with the red maples provided mainly shaded conditions. The pH of the water at this site has not been reported previously (see below). At Mentor Marsh, the swamp habitat had flooded conditions throughout the year, although toward the fall and winter months, the water level drops (personal observation). The third location is an acidic kettle bog (Singer Lake) located near Green, Ohio. This location is a floating mat dominated by *Sphagnum* spp. and leatherleaf (*Chamaedaphne calyculata*) with some poison sumac (*Toxicodendron vernix*) providing sparse shade. The *S. palustre* was collected from a hummock with sunlight throughout the day. The floating mat at Singer Lake is the only location in this experiment surrounded by a lag, and water levels appear to be very steady throughout the year.

To investigate whether or not the *S. palustre* is locally adapted to its water source, I performed two common garden experiments. In the first experiment, the moss was grown directly in water from different sources, and in the second experiment, the moss was grown on a peat substrate and watered with water from those same sources. The same three collecting sites were used in both experiments.
Water Collection for Cup Experiment

To test for local adaptation, *Sphagnum palustre* and source water were collected from each of the three northeastern Ohio locations described above between June 24th and July 15th 2015. The moss was collected and identified in the field using a microscope and key (Crum 1984). Identification of species was also confirmed when the collected moss was brought into the lab. All mosses from each site were collected from a single hummock, and the water was collected from the nearest pool (within one meter of the hummock). One gallon of water was collected from each location and then stored in the refrigerator. pH was measured 24 hours after storage in the refrigerator with a temperature-compensated pH meter (VWR Symphony b10p). After measuring pH, the water samples were returned to the refrigerator until the experiment began. The water samples were also measured for Nitrate, Sulfate, and Chloride concentration using Dionex Liquid Chromatography. The *Sphagnum* was stored in a gallon-sized plastic baggie and was also placed in the refrigerator until the beginning of the experiment (depending on the location between 1 to 3 weeks). The experiment was conducted for thirty days beginning in July 2015. The measured pH of the gallon of water collected at the Tamarack Bog location was 7.24. The calcium, nitrate, sulfate, and chloride concentrations were measured at 0.8mM, 0.24mM, 0.16mM, and 0.06mM, respectively. The measured pH of the gallon of water collected at the Mentor Marsh location was 6.27. The calcium, nitrate, sulfate, and chloride concentrations were measured at 1.6mM, 0.32mM, 1.01mM, and 2.20mM, respectively. The measured pH of the gallon of water collected at the Singer Lake location was measured at 5.70. The calcium, nitrate, sulfate, and chloride concentrations were measured at 0.1mM, 0mM, 0.07mM, and 0.10mM,
respectively. These three locations provided a slightly basic water source, a slightly acidic water source, and an acidic water source.

Experimental Design for Cup Experiment

One method of testing local specialization, the cup experiment, was modeled after Ingerpuu and Vellak (2013). *S. palustre* from each location was grown in clear plastic cups (radius=5.5cm, height=6.5cm) for 30 days in a greenhouse. The experiment was designed as a full factorial of the three water source treatments and the three *S. palustre* source locations with 9 replicates for a total n=81. For each cup, five shoots of *S. palustre*, with capitulum intact, from the same location were each cut to 1.5cm length, and their combined mass was measured. The cups were initially watered with 15mL of water from the appropriate source, and a fill line was marked on each cup and then filled to the mark every Monday, Wednesday, and Friday. The cups were placed on trays and placed in a shaded area of the greenhouse, as earlier trials had shown that direct sunlight in these conditions led to desiccation. Then, two additional trays were placed on top of the cups in order to provide more shading. At the end of the experiment, the length of each shoot and the mass of the combined shoots were measured. Through the experimental time period, two of the collected water sources had populations of duckweed (*Lemna minor*). Each time a duckweed was found in the cups, it was removed. The design yielded three trays worth of cups that were rotated on a biweekly basis. Each tray contained 3 replicates of each treatment.

Water Collection for Peat Substrate Experiment

The same three locations were used for the peat substrate experiment as in the cup experiment. Water and moss were collected in October 2015. The method for collecting
was the same as the cup experiment. I did not mark the hummock locations in July of 2015, so it is likely that the *S. palustre* from the peat substrate experiment was not collected from the same hummock as the *S. palustre* in the cup experiment. Once again, water was collected and placed into the refrigerator for 24 hours before obtaining pH measurements. Ten gallons of water were collected from each location. The average pH readings obtained were: Tamarack Bog 6.95 +/- 0.06, Mentor Marsh 6.80 +/- 0.08, and Singer Lake 5.85 +/- 0.02 (N=2 for each location). The measurements of nitrate, sulfate, and chloride concentrations for Tamarack Bog were 0.14mM, 0.18mM, and 0.13mM, respectively. The measurements of nitrate, sulfate, and chloride concentrations for Mentor Marsh were 0.12mM, 0.85mM, and 3.40mM, respectively. The measurements of nitrate, sulfate, and chloride concentrations for Singer Lake were 0.001mM, 0.006mM, and 0.40mM, respectively. For this experiment, the water was stored in opaque tubs in the greenhouse near the experimental setups.

**Experimental Design for Peat Substrate Experiment**

In this experiment, the *S. palustre* was grown on a peat substrate for 60 days. A full factorial of the three *S. palustre* locations and four water treatments (the above three water treatments and also reverse osmosis water) was setup in the greenhouse. Commercially harvested peat (Mosser Lee) was soaked in reverse osmosis water for three days prior to planting. After soaking, peat was placed into greenhouse pots (6cm length X 6 cm width X 8cm height). The pots were planted with *S. palustre* fragments cut to 0.5cm length. Only the top 10cm of the *Sphagnum* shoots were used in producing fragments because fragments from farther down on the shoot produce lower regeneration potentials in some species (Campeau and Rochefort 1996). In order to obtain another
measurement for *S. palustre* success, all capitula were cut off of the fragments and were discarded. 8 fragments lacking capitula were planted in each pot. The fragments were scattered across the peat surface and were not placed into the peat. Six pots were placed in one clear, shoe-box sized container (Length-34.6cm, Width-21.0cm, Height-12.4cm). Existing research (Bugnon et al 1997, Price 1997, Price et al 1998, Rochefort et al 2003) and trials in the greenhouse indicate that maintenance of a moist microenvironment and high humidity is important in the establishment of *Sphagnum*. To maintain a humid environment, one set (four shoe-box containers) was contained within a larger, 110L clear plastic container. Five such large containers were used. Each shoe-box container was watered with one of the four different water treatments. In each shoe-box container, 2 pots of each *Sphagnum* location were growing (hence, 6 pots in each shoe-box container). These containers were watered on a biweekly basis (Tuesdays and Fridays). To randomize across any microhabitat variations in the greenhouse, the sets of containers were rotated on a weekly basis. If herbaceous plants were found growing in the pots, they were carefully removed. The setup is shown in Appendix Y.

At the end of the experiment, I measured *S. palustre* success in this experiment by observing length change, mass change, and number of capitula growing at the end of the experiment. To obtain length measurements, a plastic ruler was placed into the peat, and the longest shoot was measured from the peat surface to the head of the capitulum. Capitulum counts were also obtained at this time. The mass was also measured at this time. At the beginning of the experiment, initial mass was calculated by first measuring the mass of the pot with the substrate. Then, the moss was cut and the fragments were planted on the substrate. The mass was once again measured. To obtain the initial mass,
the mass of the pot and substrate was subtracted from the mass of the pot, substrate, and moss. Each pot was set aside for 5 seconds to drain some excess water. Then, the weight was obtained. This same procedure was followed at the end of the experiment to obtain the final mass. Each pot was weighed by first, being placed on a petri dish, in order to avoid standing water on the scale. Then, the petri dish with the pot was placed on the scale. The percent change in mass was then obtained by dividing the change in mass by the initial mass.

Analysis

I used JMP Pro 12 to run statistical tests on the above experiments. In each experiment, a multivariate analysis of variance (MANOVA) was conducted to observe effects caused by water location, *Sphagnum* location, and the interaction of these two categories. Then, to better understand the variation of the MANOVA output, I conducted full factorial ANOVAs for each response variable. In the cup experiment, if shoots were missing, the mass was consequently measured for the 4 shoots and divided by 4. The length change also was divided by 4.

**Results:**

The two experiments do not provide evidence for local adaptation. Both the cup experiment and the peat substrate experiment showed no effect caused by the interaction of *Sphagnum* source and water source.

**Cup Experiment**

The MANOVA output of the cup experiment showed a significant effect being caused by *Sphagnum* source and water source. However, there was no observed effect caused by the interaction of *Sphagnum* source and water source. Therefore, the cup
experiment did not provide evidence for local adaptation, as defined in the introduction. A post-hoc contrast of water source showed the water from Singer Lake being different than Mentor Marsh and Tamarack Bog (p<0.0001 for both contrasts) (Appendix K). A post-hoc contrast of Sphagnum source showed the moss from the Tamarack Bog being different than Singer Lake (p=0.0004) and Mentor Marsh (p<0.0001) (Appendix L).

Table 2.1: MANOVA Output of Cup Experiment (DF of Denominator is 72)

<table>
<thead>
<tr>
<th>Source</th>
<th>Approx F</th>
<th>DF</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sphagnum Source</em></td>
<td>10.443</td>
<td>2</td>
<td><strong>0.0001</strong>*</td>
</tr>
<tr>
<td>Water Source</td>
<td>15.586</td>
<td>2</td>
<td><strong>0.0001</strong>*</td>
</tr>
<tr>
<td><em>Sphagnum X Water Source</em></td>
<td>1.232</td>
<td>4</td>
<td>0.3</td>
</tr>
</tbody>
</table>

In order to evaluate the components of the overall response tested in the MANOVA, I inspected the full factorial ANOVAs for each trait. The interaction between Sphagnum and water source did not have a significant effect (p=0.3). Percent change in mass varied significantly with Sphagnum source and water source (p<0.0001 for each; Appendix M). The Tukey’s comparison of Sphagnum location showed the moss collected from the Tamarack Bog to be significantly lower in percentage gain in mass than the mosses collected from Mentor Marsh and Singer Lake. Mentor Marsh and Singer Lake mosses were indistinguishable in terms of percentage gain in mass. The Tukey’s comparison of water location showed the water from Mentor Marsh and Tamarack Bog to be indistinguishable. Both locations produced significantly lower percentage gains in mass than the water collected from Singer Lake (Appendix M).
The full factorial ANOVA of length change showed significant effects caused by water source (p<0.0001), Sphagnum source (p<0.0001), and the interaction of water and Sphagnum source (p=0.003) (Appendix N). Referring to figure 2.1B, plants in the Singer Lake water source performed best, overall. Also, the interaction effect can be observed in the Singer Lake water and the Tamarack Bog water. Two moss sources (Mentor Marsh and Singer Lake) performed significantly better than the moss source from the Tamarack Bog. This occurred in both the water source of Singer Lake and Tamarack Bog; however, this did not occur in the Mentor Marsh water source.

![Cup Experiment (A) and Cup Experiment (B)](image)

Figure 2.1: Percent change in mass of *S. palustre* (A) and length change in *S. palustre* (B) in the cup experiment (Error bars represent SE).

One major finding with the full factorial analysis of the length change is the Singer Lake water source performing significantly better than Mentor Marsh and
Tamarack Bog. Also, the Mentor Marsh water source performed significantly better than Tamarack Bog water source (Appendix N). This variation can be explained as follows. The Tamarack Bog and Mentor Marsh water sources caused the moss to turn grey-black in color. In some instances, shoots were lost (in three of the 27 cups watered with the Tamarack Bog water source, I could only find 4 of the 5 shoots to measure at the end of the experiment. In the Mentor Marsh water source, shoots were lost in four of the 27 cups. In Singer Lake, no shoots were lost.). The *Sphagnum* in these two water sources turned black, and in some cases, the mosses were severely frail and broken apart. The moss grown in Tamarack Bog water was more brittle and less vegetative than the moss grown in Mentor Marsh water. Furthermore, seven of the 27 Tamarack Bog cups contained shoots that had lost capitula at the end of the experiment, and only one of the 27 Mentor Marsh cups experienced a capitulum loss. The finding of the *Sphagnum* dying in the Tamarack Bog and Mentor Marsh waters led to the development of the other greenhouse experiment (that of growing on a peat substrate) because there is a seemingly healthy community of *Sphagnum* spp. at the Tamarack Bog and Mentor Marsh.

**Peat Substrate Experiment**

The MANOVA output of the peat substrate experiment showed a significant effect being caused by *Sphagnum* source. However, there was no observed effect caused by the interaction of *Sphagnum* source and water source. Therefore, the peat substrate experiment also did not provide evidence for local adaptation. Furthermore, no effect was caused by water source in this experiment. A post-hoc contrast of *Sphagnum* source showed the performance of moss collected from Singer Lake being different than when collected from Mentor Marsh and Tamarack Bog (p<0.0001 and p=0.0001, respectively)
(Appendix O). Also, the moss from Mentor Marsh was significantly different than the moss from the Tamarack Bog (p=0.02).

Table 2.2: MANOVA Output of Peat Substrate Experiment (DF of Denominator is 216).

<table>
<thead>
<tr>
<th></th>
<th>Pillai’s Trace</th>
<th>Approx F</th>
<th>Numerator DF</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sphagnum Source</strong></td>
<td>0.292</td>
<td>9.244</td>
<td>4</td>
<td>0.0001*</td>
</tr>
<tr>
<td><strong>Water Source</strong></td>
<td>0.099</td>
<td>1.870</td>
<td>6</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Sphagnum X Water Source</strong></td>
<td>0.061</td>
<td>0.562</td>
<td>12</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Once again, full factorial analyses were conducted for each response variable to better understand the effect of *Sphagnum* source. The number of capitula produced was higher in *Sphagnum* originating from Mentor Marsh and Tamarack Bog than in the *Sphagnum* originating from Singer Lake (Appendix P). The percent gain in mass was higher in Tamarack Bog when compared to Singer Lake, but Mentor Marsh was indistinguishable from both Tamarack Bog and Singer Lake (Appendix Q). The final length (cm) was not significantly affected by the treatments (Appendix R).
Figure 2.2: Percent change in mass of *S. palustre* (A), length change in *S. palustre* (B), and number of capitula produced (C) in the peat substrate experiment (Error bars represent SE)
Discussion:

The results of the two experiments do not provide evidence for local adaptation. Referring back to the introduction, I defined local adaptation as each *Sphagnum* source growing best within the water collected from that source (i.e. Mentor Marsh moss should grow best in Mentor Marsh water and grow worse in Singer Lake and Tamarack Bog water). Using this definition, there is no support for local adaptation. The results do indicate the *Sphagnum* sources being phenotypically different, however. The results are also indicative of water source differences.

Cup Experiment

One important finding was the Tamarack Bog and Mentor Marsh water sources killed *S. palustre* when it was grown directly in the water. The loss of the shoots was most likely due to the fragility of the moss when subjected to this water treatment, and eventually, the fragile shoots broke apart into unobservable pieces. At other times, though, shoots were found that had been broken apart into observable pieces, so this leads me to believe that some shoots may have disintegrated by the time of the final measurements. *Sphagnum* from all locations were harmed by the Tamarack Bog and Mentor Marsh water sources, in terms of color change, death, and weight loss (see figure 2.2). One possible explanation involves pH and calcium levels. When pH and calcium levels were both increased, some species of *Sphagnum* were unable to survive (Clymo 1973). In this experiment, the pH of the Tamarack Bog source was the highest measured pH of the three locations with Mentor Marsh being the second highest. The measured calcium levels were highest at Mentor Marsh and second highest at Tamarack Bog. The
higher pH and calcium levels could be the explanation for the dying off of the moss in the
two respective water sources.

Of the three water treatments in the cup experiment, the Singer Lake water source
was the only source that produced an increase in length and an increase in mass of
*Sphagnum*. The other two water sources produced decreases in mass. One possible
explanation is the differences in the pH. The Singer Lake water source was the lowest
pH of the three (5.70). The pH reading for Singer Lake is the closest of the three
locations to the mean pH measured for *S. palustre* across six different sites (Andrus
1986). Another possible explanation is the state of the collected water. Anecdotally, the
Singer Lake water had less suspended particles than the Mentor Marsh water and
Tamarack Bog water. To investigate this, in January 2015, I ran a trial measuring growth
in water collected directly from the field, without filtration, and I ran a trial in March
2015 measuring growth in water after being filtered with gravity filtration using a
Whatman No. 1 filter paper. In both trials, the *S. palustre* growing in the Tamarack Bog
water turned the same black color and had significantly less growth. I concluded that
suspended particles were not responsible for the blackening response. For this reason, I
did not filter the water in the cup experiment.

The moss collected from Mentor Marsh and Singer Lake outperformed the moss
collected from the Tamarack Bog. In fact, the Tamarack Bog moss ended up losing, on
average, 20% of its mass. This is a troublesome finding. It could be indicative of an
unhealthy *S. palustre* population at the Tamarack Bog. Even when grown in the water
from Singer Lake and Mentor Marsh, this moss still lost mass.
Peat Substrate Experiment

Unlike in the cup experiment, in the peat substrate experiment, the collection site of *S. palustre* was the only significant influence on plant growth. The *S. palustre* from Mentor Marsh and Tamarack Bog produced more capitula, and they likewise produced higher percent increase of mass than Singer Lake. This is promising, as capitulum development has been used to measure the regeneration success of *Sphagnum* spp. (Campeau and Rochefort 1996). No significant effects were observed on the length change of the moss in this experiment.

Interestingly, the water treatments had no effect on the moss growth in the peat substrate experiment. When the moss was grown directly in the water of the Tamarack Bog in the cup experiment, the moss was killed. However, when grown on a substrate and watered with the Tamarack Bog source, the mosses grew at the same rate as the other water sources. Another interesting contrast between the two experiments was the behavior of the moss collected from the Tamarack Bog. In the cup experiment, the *S. palustre* of the Tamarack Bog had the least growth in terms of mass gain and length gain. When grown on a peat substrate, the *S. palustre* of the Tamarack Bog had the highest percent change in mass gain.

Shade Effects

As mentioned in the materials and methods section, the shade of the three locations differed considerably. At the Tamarack Bog and Mentor Marsh, many shrubs and trees dominated the overstory, which would enable these two areas to be denoted as “Shaded”. At Singer Lake, it is a floating mat of *Sphagnum* spp., and the shrubs have sparse coverage and are stunted in growth. Singer Lake could then be considered as
“Unshaded”. In the cup experiment, moss from shaded conditions grew a mean of 0.28 cm and a mean of -7.72% gain in mass. When the moss was collected from unshaded conditions, the mean length change was 0.33 cm with a mean increase in mass of 2.25%.

In the peat substrate experiment, the mean number of capitula formed on shaded plants (from Tamarack Bog and Mentor Marsh) was 9, and the mean number of capitula in unshaded (Singer Lake) conditions was 7. The final length of shaded plants was 2.1 cm, and the final length of unshaded plants was 1.9. The percentage of mass change was higher when collected from the shaded environment (mean increase of 48%) than when collected from the unshaded environment (mean increase of 35%). When subjected to shade, some species of Sphagnum experience significant reductions in biomass growth (Hayward and Clymo 1983, Bonnett et al. 2010). In my experiment, the moss taken from the shaded conditions grew better than when collected from unshaded conditions. There is a possibility of the shaded mosses being acclimated to the shaded conditions. The greenhouse conditions were setup to provide shade because without extra shade, the previous trials caused the Sphagnum to bleach. Since the greenhouse was setup to provide extra shade, acclimation of the shaded plants could be the reason that the shaded plants seemed to grow better than the unshaded plants.

Implications for Restoration

The results of the greenhouse experiments provide information for bogs and fens undergoing restoration. First, the S. palustre in this experiment showed no evidence of local adaptation. This could be an important finding. It could allow for collections being made from a wide array of donor sites, as opposed to specificity with similar vegetation.
and water conditions. A field study would be helpful in exploring this idea. Secondly, the *S. palustre* collected from Mentor Marsh and Tamarack Bog formed a significantly higher number of capitula than the moss collected from Singer Lake. This provides evidence for vegetative reproduction (Cronberg 1992) because the main stem has another branch which begins forming a capitulum and, essentially, a new *Sphagnum* shoot.

Looking back at the results and the literature, the moss of Mentor Marsh may be better enabled to grow at the Tamarack Bog than the moss of Singer Lake. The environmental conditions of Tamarack Bog are closer to Mentor Marsh than they are to Singer Lake, with respect to shade and water pH.
CHAPTER III
CONCLUSIONS

Reviewing the baseline measurements, the coverage of the *Sphagnum* spp. at the Tamarack Bog restoration project is around 1.3% and mainly has a presence within the Core Bog plots. There is an association between region of the bog and *Sphagnum* presence. In fact, as of the 2015 data, 9 of the 10 monitoring plots where *Sphagnum* is present are found in the Core Bog region. Taking into account only the Core Bog region, the coverage of *Sphagnum* is around 5%. If the coverage does not increase, then restorative methods for increasing *Sphagnum* coverage may be needed.

One future direction should be the investigation of *T. delicatulum* and its relationship with *Sphagnum* spp. at the Tamarack Bog. The 2015 data indicated an association between *T. delicatulum* and *Sphagnum* spp. One possible explanation for this association could be that *Thuidium delicatulum* is a potential nurse-plant. Another explanation could be that *T. delicatulum* filled a new niche, made possible by the alteration of the environment brought about from the ditching and partial drainage of the wetland. Investigating this interaction would help us understand the ecology of these two bryophyte genera at the Tamarack Bog.

If *Sphagnum* spp. introduction does become a necessary action, then we now have some background information on *S. palustre*. The source waters did not have an impact on the growth of *S. palustre* when grown on a peat substrate. When grown directly in the
collected source waters, the water source did have an effect on *Sphagnum* growth. The Tamarack Bog and Mentor Marsh water sources killed the *Sphagnum*. This could be the reason why *Sphagnum* is rarely found growing in the pools of the Tamarack Bog. One finding that is useful for restoration projects was the difference among the three *Sphagnum* locations. *Sphagnum* locations had differing rates of captiulum production. The mosses of Mentor Marsh and the Tamarack Bog produced more capitula than Singer Lake, and the same held true for percent gain of mass. The results may indicate that the mosses from Mentor Marsh and Tamarack Bog are quicker in vegetative reproduction because in this experiment, they placed more resources into producing new capitula than in elongation. Referring back to the literature, it may be best to use moss from Mentor Marsh in the reestablishment of the *Sphagnum* population at the Tamarack Bog because the pH and shaded conditions of Mentor Marsh are a closer representation to the Tamarack Bog habitat than the conditions found at Singer Lake.
BIBLIOGRAPHY


41


APPENDIX A

SAMPLE GRAPH SHEET FOR SURVEYS
APPENDIX B

S3 SE SURVEY PLOT (TOP-2014, BOTTOM-2015)
APPENDIX C

HISTOGRAM OF SPHAGNUM COVERAGE IN 2014 AND 2015
APPENDIX D

HISTOGRAM OF *THUIDIUM* COVERAGE IN 2014 AND 2015

*Thuidium delicatulum* Coverage

Number of Plots

Percent Coverage

2014

2015
APPENDIX E

CHI SQUARE OF SPHAGNUM PRESENCE IN 2014 BY REGION OF RESTORATION SITE

Contingency Table

<table>
<thead>
<tr>
<th></th>
<th>Sphagnum Present (OBS/EXP)</th>
<th>Sphagnum Absent (OBS/EXP)</th>
<th>TOTAL (OBS/EXP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core</td>
<td>10/19.2</td>
<td>2/3.9</td>
<td>12/23.1</td>
</tr>
<tr>
<td>Wetland Edge</td>
<td>1/1.9</td>
<td>21/40.4</td>
<td>22/42.3</td>
</tr>
<tr>
<td>Enhancement</td>
<td>0/0</td>
<td>18/34.6</td>
<td>18/34.6</td>
</tr>
<tr>
<td>Total (OBS/EXP)</td>
<td>11/21.1</td>
<td>41/78.9</td>
<td>52</td>
</tr>
</tbody>
</table>

N=52

DF=2

\[ \text{Chi}^2 = 36.28 \quad p < 0.0001 \]

*NOTE: Over 20% of the data cells have expected values less than 5.
APPENDIX F

CHI SQUARE OF *SPHAGNUM* PRESENCE IN 2014 BY *THUIDIUM* PRESENCE IN 2014 (2M X 2M PLOT ASSOCIATION)

<table>
<thead>
<tr>
<th></th>
<th><em>Sphagnum</em> Present (OBS/EXP)</th>
<th><em>Sphagnum</em> Absent (OBS/EXP)</th>
<th>TOTAL (OBS/EXP)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thuidium</em> Present</td>
<td>11/21.2</td>
<td>31/59.6</td>
<td>42/80.8</td>
</tr>
<tr>
<td><em>Thuidium</em> Absent</td>
<td>0/0</td>
<td>10/19.2</td>
<td>10/19.2</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>11/21.2</strong></td>
<td><strong>41/78.8</strong></td>
<td><strong>52</strong></td>
</tr>
</tbody>
</table>

N= 52
DF= 1
Chi²= 3.32  p=0.07

Fisher’s Exact: For *Sphagnum* presence being greater with a greater *Thuidium* presence: p=0.07.
APPENDIX G

CHI SQUARE SPHAGNUM PRESENCE IN 2015 BY REGION OF RESTORATION SITE

<table>
<thead>
<tr>
<th>SITE</th>
<th>Sphagnum Present (OBS/EXP)</th>
<th>Sphagnum Absent (OBS/EXP)</th>
<th>TOTAL (OBS/EXP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core</td>
<td>9/17.3</td>
<td>3/5.8</td>
<td>12/23.1</td>
</tr>
<tr>
<td>Wetland Edge</td>
<td>1/1.9</td>
<td>21/40.4</td>
<td>22/42.3</td>
</tr>
<tr>
<td>Enhancement</td>
<td>0/0</td>
<td>18/34.6</td>
<td>18/34.6</td>
</tr>
<tr>
<td>Total</td>
<td>10/19.2</td>
<td>42/80.8</td>
<td>52</td>
</tr>
</tbody>
</table>

N=52

DF=2

Chi²=31.37  p<0.0001
APPENDIX H

CHI SQUARE OF SPHAGNUM PRESENCE IN 2015 BY THUIDIUM PRESENCE IN 2015 (2M X 2M PLOT ASSOCIATION)

<table>
<thead>
<tr>
<th></th>
<th>Sphagnum Present (OBS/EXP)</th>
<th>Sphagnum Absent (OBS/EXP)</th>
<th>TOTAL (OBS/EXP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thuidium Present</td>
<td>10/19.2</td>
<td>27/51.9</td>
<td>37/71.1</td>
</tr>
<tr>
<td>Thuidium Absent</td>
<td>0/0</td>
<td>15/28.9</td>
<td>15/28.9</td>
</tr>
<tr>
<td>TOTAL</td>
<td>10/19.2</td>
<td>42/80.8</td>
<td>52</td>
</tr>
</tbody>
</table>

N= 52
DF= 1
\( \chi^2 = 5.02 \)  p=0.03

Fisher’s Exact: For Sphagnum presence being greater with a greater Thuidium presence:  p=0.02.
APPENDIX I

PAIRED T-TEST OF 2015 % SPHAGNUM COVERAGE AND 2014 % SPHAGNUM

COVERAGE

N: 52

Mean of Sphagnum Percent Coverage in 2015: 1.38

Mean of Sphagnum Percent Coverage in 2014: 1.28

Difference: 0.10

Confidence Interval of Mean difference: -0.502 < X < 0.704

t-Stat: 0.3376

DF: 51

p-value: 0.74
APPENDIX J

PAIRED T-TEST OF 2015 % THUIDIUM COVERAGE AND 2014 % THUIDIUM COVERAGE

N: 52

Mean of Thuidium Percent Coverage in 2015: 6.40

Mean of Thuidium Percent Coverage in 2014: 7.50

Difference: -1.10

Confidence Interval of Mean difference: -2.404 < X < 0.203

t-Stat: -1.6944

DF: 51

p-value: 0.096
APPENDIX K
MANOVA CONTRASTS OF WATER SOURCE (CUP EXPERIMENT)

F-Test Contrast Mentor Marsh from Tamarack Bog

<table>
<thead>
<tr>
<th>Value</th>
<th>F</th>
<th>Numerator DF</th>
<th>Denominator DF</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0011</td>
<td>0.077</td>
<td>1</td>
<td>72</td>
<td>0.8</td>
</tr>
</tbody>
</table>

F-Test Contrast Mentor Marsh from Singer Lake

<table>
<thead>
<tr>
<th>Value</th>
<th>F</th>
<th>Numerator DF</th>
<th>Denominator DF</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.433</td>
<td>15.586</td>
<td>1</td>
<td>72</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

F-Test Contrast Singer Lake from Tamarack Bog

<table>
<thead>
<tr>
<th>Value</th>
<th>F</th>
<th>Numerator DF</th>
<th>Denominator DF</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.343</td>
<td>24.680</td>
<td>1</td>
<td>72</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>
APPENDIX L
MANOVA CONTRASTS OF SPHAGNUM SOURCE (CUP EXPERIMENT)

F-Test Contrast Mentor Marsh from Tamarack Bog

<table>
<thead>
<tr>
<th>Value</th>
<th>F</th>
<th>Numerator DF</th>
<th>Denominator DF</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.243</td>
<td>17.479</td>
<td>1</td>
<td>72</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

F-Test Contrast Mentor Marsh from Singer Lake

<table>
<thead>
<tr>
<th>Value</th>
<th>F</th>
<th>Numerator DF</th>
<th>Denominator DF</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0034</td>
<td>0.242</td>
<td>1</td>
<td>72</td>
<td>0.6</td>
</tr>
</tbody>
</table>

F-Test Contrast Singer Lake from Tamarack Bog

<table>
<thead>
<tr>
<th>Value</th>
<th>F</th>
<th>Numerator DF</th>
<th>Denominator DF</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.189</td>
<td>13.607</td>
<td>1</td>
<td>72</td>
<td>0.0004*</td>
</tr>
</tbody>
</table>
## APPENDIX M

FULL FACTORIAL ANOVA OF % MASS CHANGE (CUP EXPERIMENT)

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model</strong></td>
<td>8</td>
<td>30095.03</td>
<td>3761.88</td>
<td>7.215</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td><strong>Error</strong></td>
<td>72</td>
<td>37539.71</td>
<td>521.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>80</td>
<td>67634.74</td>
<td></td>
<td></td>
<td>P&lt;0.0001*</td>
</tr>
</tbody>
</table>

### Effects

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>SS</th>
<th>F Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water Source</strong></td>
<td>2</td>
<td>1.6507</td>
<td>15.8299</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td><strong>Sphagnum Source</strong></td>
<td>2</td>
<td>1.0986</td>
<td>10.5358</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td><strong>Water X Sphagnum Source</strong></td>
<td>4</td>
<td>0.2602</td>
<td>1.2475</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Tukey’s Connecting Letter:

### Sphagnum Source

<table>
<thead>
<tr>
<th>Sphagnum Source</th>
<th>Least Squares Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mentor Marsh</td>
<td>A 5.33</td>
</tr>
<tr>
<td>Tamarack Bog</td>
<td>B -20.77</td>
</tr>
<tr>
<td>Singer Lake</td>
<td>A 2.25</td>
</tr>
</tbody>
</table>

### Water Source

<table>
<thead>
<tr>
<th>Water Source</th>
<th>Least Squares Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mentor Marsh</td>
<td>A -13.57</td>
</tr>
<tr>
<td>Tamarack Bog</td>
<td>A -15.38</td>
</tr>
<tr>
<td>Singer Lake</td>
<td>B 15.77</td>
</tr>
</tbody>
</table>
APPENDIX N

FULL FACTORIAL ANOVA OF LENGTH CHANGE (CUP EXPERIMENT)

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>8</td>
<td>1.950</td>
<td>0.2438</td>
<td>3.394</td>
</tr>
<tr>
<td>Error</td>
<td>72</td>
<td>1.116</td>
<td>0.0155</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>80</td>
<td>3.066</td>
<td></td>
<td>P&lt;0.0001</td>
</tr>
</tbody>
</table>

Effects

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>SS</th>
<th>F Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Source</td>
<td>2</td>
<td>1.3410</td>
<td>43.2483</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Sphagnum Source</td>
<td>2</td>
<td>0.3313</td>
<td>10.6853</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Water X Sphagnum Source</td>
<td>4</td>
<td>0.2778</td>
<td>4.4797</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

Tukey’s Connecting Letter

*Sphagnum Source*  
Least Squares Mean

Mentor Marsh  A  0.36  
Tamarack Bog  B  0.21  
Singer Lake  A  0.33  

*Water Source*  
Least Squares Mean

Mentor Marsh  A  0.25  
Tamarack Bog  B  0.17  
Singer Lake  C  0.47  

57
APPENDIX O

MANOVA CONTRASTS OF SPHAGNUM SOURCE (PEAT SUBSTRATE)

F-Test Contrast Mentor Marsh from Singer Lake

<table>
<thead>
<tr>
<th>Value</th>
<th>F</th>
<th>Numerator DF</th>
<th>Denominator DF</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.295</td>
<td>15.785</td>
<td>2</td>
<td>107</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

F-Test Contrast Mentor Marsh from Tamarack Bog

<table>
<thead>
<tr>
<th>Value</th>
<th>F</th>
<th>Numerator DF</th>
<th>Denominator DF</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.076</td>
<td>4.079</td>
<td>2</td>
<td>107</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

F-Test Contrast Tamarack Bog from Singer Lake

<table>
<thead>
<tr>
<th>Value</th>
<th>F</th>
<th>Numerator DF</th>
<th>Denominator DF</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.183</td>
<td>9.775</td>
<td>2</td>
<td>107</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>
APPENDIX P

FULL FACTORIAL ANOVA OF NUMBER OF CAPITULA (PEAT SUBSTRATE EXPERIMENT)

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>11</td>
<td>190.80</td>
<td>17.346</td>
<td>3.394</td>
</tr>
<tr>
<td>Error</td>
<td>108</td>
<td>552.00</td>
<td>5.111</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>119</td>
<td>742.80</td>
<td></td>
<td>P=0.0005</td>
</tr>
</tbody>
</table>

Effects

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>SS</th>
<th>F Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Source</td>
<td>3</td>
<td>30.067</td>
<td>1.961</td>
<td>0.12</td>
</tr>
<tr>
<td>Sphagnum Source</td>
<td>2</td>
<td>132.950</td>
<td>13.006</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Water X Sphagnum Source</td>
<td>6</td>
<td>27.783</td>
<td>0.906</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Tukey’s Connecting Letter

Sphagnum Source | Least Squares Mean

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mentor Marsh</td>
<td>A</td>
</tr>
<tr>
<td>Tamarack Bog</td>
<td>A</td>
</tr>
<tr>
<td>Singer Lake</td>
<td>B</td>
</tr>
</tbody>
</table>
APPENDIX Q

FULL FACTORIAL ANOVA OF % MASS CHANGE (PEAT SUBSTRATE EXPERIMENT)

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>11</td>
<td>9321.04</td>
<td>847.37</td>
<td>1.504</td>
</tr>
<tr>
<td>Error</td>
<td>108</td>
<td>60866.85</td>
<td>563.58</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>119</td>
<td>70187.89</td>
<td></td>
<td>P=0.14</td>
</tr>
</tbody>
</table>

Effects

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>SS</th>
<th>F Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Location</td>
<td>3</td>
<td>2411.1</td>
<td>1.4260</td>
<td>0.24</td>
</tr>
<tr>
<td>Sphagnum Location</td>
<td>2</td>
<td>6344.7</td>
<td>5.6289</td>
<td>0.005*</td>
</tr>
<tr>
<td>Water X Sphagnum Location</td>
<td>6</td>
<td>565.2</td>
<td>0.1671</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Tukey’s Connect Letter:

**Sphagnum Location**  
Least Squares Mean

- Mentor Marsh: AB 43.98
- Tamarack Bog: A 52.37
- Singer Lake: B 34.57
### APPENDIX R

**FULL FACTORIAL ANOVA OF FINAL LENGTH (PEAT SUBSTRATE EXPERIMENT)**

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model</strong></td>
<td>11</td>
<td>2.11</td>
<td>0.19</td>
<td>0.669</td>
</tr>
<tr>
<td><strong>Error</strong></td>
<td>108</td>
<td>31.04</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>119</td>
<td>33.15</td>
<td></td>
<td><strong>P=0.77</strong></td>
</tr>
</tbody>
</table>

#### Effects

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>SS</th>
<th>F Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water Location</strong></td>
<td>3</td>
<td>0.2697</td>
<td>0.3128</td>
<td>0.82</td>
</tr>
<tr>
<td><strong>Sphagnum Location</strong></td>
<td>2</td>
<td>0.9603</td>
<td>1.6707</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Water X Sphagnum Location</strong></td>
<td>6</td>
<td>0.8832</td>
<td>0.5122</td>
<td>0.80</td>
</tr>
</tbody>
</table>
APPENDIX S

MONITORING PLOTS DURING WET CONDITIONS
APPENDIX T

PLOT SHOWING YEARLY DIFFERENCES IN METER STICK SETUP AND VEGETATION

(Top-2014, Bottom-2015)
APPENDIX U

WRITEUP ON THE SURVEYING AND MAPPING PROCESS

1. Locate the plot.

2. Fill out the information (Plot, Date, Observers).

3. Place meter sticks down, and form a 2mX2m grid (4 meter sticks in the middle, with 8 meter sticks making up the edges).

4. Survey the area for *Sphagnum*. *Note:* When surveying for moss, it is necessary to look carefully under graminoids and leaves.

5. If *Sphagnum* is found, use the meter sticks to obtain measurements of coverage area and plot on the mapping paper.
   a. In order to do this:
      i. Use meter sticks as X and Y axes.
      ii. Orient yourself to the correct direction (top of the mapping paper is North).
      iii. On the mapping paper, translate the measurements for the X axis onto the grid (Make dash marks at the beginning of where moss is and at the end of where the moss is).
      iv. On the mapping paper, translate the measurements for the Y axis onto the grid (Same dash mark system as Step iii).
v. Connect the dash marks within the grid, and shape the mapped moss to the shape of the moss within the plot.

vi. Mark this area with an “S”.

6. Repeat steps 3 and 4, only doing this with respect to *Thuidium delicatulum* and marking the mapped area with a “T”.

7. Take note of leaf litter coverage. This is a visual estimate of the entire plot.

8. Write down any notes (i.e. weather, trampling, debris from construction).

9. To obtain percent coverage:
   
a. Use Microsoft Excel.

   i. Count up the number of squares covered by a moss (To the nearest ¼ of a square).

   ii. Put this number into excel.

   iii. Plug in a formula to calculate percent coverage.

   1. Number of squares divided by 4. 4 is obtained from the following:

   a. The number of squares is multiplied by 100 to get total area in cm\(^2\). Each square on the grid paper is 10cm X 10cm.

   b. The overall plot size is 4 m\(^2\), or 40 000cm\(^2\).

   c. To obtain a percentage, the product in part a is then divided by 40 000, and then multiplied by 100.
d. Hence, overall, to obtain percent coverage, the number of squares is divided by 4.

10. General notes:

   a. If brown *T. delicatulum* is found without any branches being green, this was considered dead, and was not mapped within the study.

   b. When *Sphagnum* and *T. delicatulum* were found in the same clump (as in Appendix A), both *Sphagnum* and *T. delicatulum* received the amount of squares in that area.
I conducted many trials growing *Sphagnum*, both in the field and in the lab. First off, I will discuss field manipulations. I first attempted to grow *Sphagnum* in the field by introducing cut moss onto a bare peat surface during the spring of 2014. I used scissors to cut *Sphagnum* into fragments, including the capitula. When spreading the moss, I added straw on top to help maintain a high humidity environment. None of the moss survived in the field. The introduction areas were flooded, and the moss was washed away within the first week. I ran another trial in Spring 2015, similar to this trial. I spread *Sphagnum* fragments in 5 different places in the bog. However, once again, the moss was flooded away.

After this first experiment, I tried my first attempt at artificial hummocks. This experiment was conducted in the late spring of 2014. To construct the artificial hummock, I clumped commercial peat into burlap, and I tied off the burlap. I used two 432 cubic inch bales of fibric moss from Mosser Lee to construct the 6 hummocks. I presoaked the burlap hummock in reverse osmosis water before placing it into the field. After placing the hummock, I used surrounding muck on top of the hummocks as a substrate. Then, I planted cut *Sphagnum* on top. After two weeks, all of the hummocks had been flattened, most likely due to flooding of the hummock and washing out of the
commercial peat structure. Interestingly, as of fall 2015, one of the six artificial hummock locations has a small *Sphagnum* population growing there. This particular location is bordering the Wetland Edge and Core Bog region. The hummock flattened and is roughly even with the surrounding surface.

A third attempt was made to grow *Sphagnum* at the field site. This attempt involved artificial hummocks as well. Small wicker baskets were placed in the field, and commercial peat was placed atop to make a mound to mimic a hummock. Then, burlap was wrapped across the mound and anchored into the soil using lawn pins. *Sphagnum* was planted on the hummock as in a transect across the hummock. 10 shoots were placed going across the hummock. Then, straw was placed on top. Two hummocks were constructed in each of the three regions of the Tamarack Bog restoration site. The hummocks in the Enhancement and Wetland Edge regions remained intact. However, the *Sphagnum* planted on these hummocks all dried out. Meanwhile, the hummocks placed in the Core Bog region were once again dismantled, most likely due to water flooding the peat away from the wicker basket. With the numerous setbacks from the above trials, I was unable to include a field manipulation in my studies.
APPENDIX W

GROWING SPHAGNUM (LAB SETTINGS)

I originally attempted to grow Sphagnum in the same plastic shoe-box sized containers listed in the methods section. The first attempt, I tried to grow Sphagnum in an incubator set to 50 degrees Fahrenheit and on a 14-hour light and 10-hour dark cycle. In this experiment, no lid was used. The moss was planted on a fibric peat substrate (Mosser Lee). However, the Sphagnum dried out apparently due to the constant air circulation and lack of a lid to maintain humidity. Also, the incubator stopped working, and the temperature rose above 80 degrees Fahrenheit.

Then, I attempted growth in the same containers in the lab under grow lamps. In this experiment, I wrapped plastic wrap over top of the shoe-box container in order to maintain humidity. At first, the Sphagnum was growing. However, within two months, once the Sphagnum reached the plastic wrap, it began to raise the plastic wrap and allow air inside, which caused drying out again. I also tried this manipulation in the greenhouse, and the same results were observed.

I read the publication by Ingerpuu and Vellak (2013), and I attempted to grow Sphagnum in cups. I conducted the experiment and obtained my first results on Sphagnum growth in length and weight. Similar results as were found within the cup experiment of the thesis were found in this experiment, with the Tamarack Bog water
turning the *Sphagnum* black and killing the moss. I thought the reason could be related to the amount of the suspended particles in the Tamarack Bog water. For this reason, I reran the experiment after filtering the water sources. The collected water sources were filtered using Whatman No. 1 filter paper. Once again, the results obtained saw the moss die and turn black in the Tamarack Bog water, although, it seemed to take a few days longer than when the water sources were not filtered. The two preceding trials were conducted using only water from Singer Lake and Tamarack Bog. Then, I applied for collecting permits for new locations, one of which was Mentor Marsh. I also obtained collecting permits for two other locations, Geneva Swamp and Koelliker Fen. However, I could not find *S. palustre* at these locations. Hence, the cup experiment was conducted using moss and water sources from Tamarack Bog, Singer Lake, and Mentor Marsh.

I further attempted to grow *Sphagnum* on a peat substrate. I used commercially harvested peat which was purchased from a peat supplier (Mosser Lee). This substrate was fibric *Sphagnum* peat, and it was soaked for three days prior to planting. At first, I grew three containers in the greenhouse and placed the lids of the opaque containers atop in order to maintain a humid environment. Once the moss reached the lid, however, it was prevented from growing further. Then, I removed the lids and placed another shoe-box container on top, so the moss had more room to grow. Some treatments, I used tape to seal the cracks between the containers (One treatment was taping around the cracks. Another treatment was taping one of the containers to make a type of gasket for the containers to seal.). A third treatment, I just placed the container on top without sealing
it. All three treatments performed well (although no statistical test was run on these growth treatments.

For the peat substrate experiment performed in the thesis, I wanted to devise a way to contain all of the containers within one environment (as opposed to separately sealing each with its own container lid. In response to this, I thought of getting a larger rigid plastic container and placing it over top of the four shoe-box containers. This allowed for the four separate water treatments to be contained within the same humid environment.
APPENDIX X

FUTURE RESEARCH QUESTIONS

1. Does *Thuidium delicatulum* function as a nurse-plant for *Sphagnum* at the Tamarack Bog?
   
   a. Experiment one- Plant vegetation fragments of *Sphagnum* on bare substrates and on substrates that have a *T. delicatulum* cover. Does *Sphagnum* grow better in either condition?
   
   b. Experiment two- Do the above experiment, only this time, use spores collected from *Sphagnum*, instead of vegetation.

2. Is *Thuidium delicatulum* filling in a niche that was created when the bog was partially drained?
   
   a. Experiment three- Manipulate different factors and grow *T. delicatulum* and *Sphagnum* in a full factorial. Such factors include: water pH, humidity, soil substrate (pH and mineral content), and microtopography.

3. Does the *S. palustre* from Mentor Marsh grow best when introduced into the field?
   
   a. Experiment 4- Plant *S. palustre* from different locations and see if Mentor Marsh performs the best due to the findings of higher capitula formation.
APPENDIX Y

EXPERIMENTAL SETUP FOR PEAT SUBSTRATE EXPERIMENT
APPENDIX Z

CHI SQUARE OF SPHAGNUM PRESENCE IN 2014 BY THUIDIUM PRESENCE IN 2014 (PATCH ASSOCIATION)

<table>
<thead>
<tr>
<th></th>
<th>Sphagnum Present (OBS/EXP)</th>
<th>Sphagnum Absent (OBS/EXP)</th>
<th>TOTAL (OBS/EXP)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thuidium Present</strong></td>
<td>11/16.4</td>
<td>45/67.2</td>
<td>56/83.6</td>
</tr>
<tr>
<td><strong>Thuidium Absent</strong></td>
<td>11/16.4</td>
<td>0/0</td>
<td>11/16.4</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>22/32.8</td>
<td>45/67.2</td>
<td>67</td>
</tr>
</tbody>
</table>

N= 67  
DF= 1  
Chi$^2$= 29.34  p<0.0001

Fisher’s Exact: For Sphagnum presence being greater with Thuidium absence:  
p<0.0001.
APPENDIX AA

CHI SQUARE OF SPHAGNUM PRESENCE IN 2015 BY THUIDIUM PRESENCE IN 2015 (PATCH ASSOCIATION)

<table>
<thead>
<tr>
<th></th>
<th>Sphagnum Present (OBS/EXP)</th>
<th>Sphagnum Absent (OBS/EXP)</th>
<th>TOTAL (OBS/EXP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thuidium Present</td>
<td>8/9.4</td>
<td>45/52.9</td>
<td>53/62.3</td>
</tr>
<tr>
<td>Thuidium Absent</td>
<td>32/37.7</td>
<td>0/0</td>
<td>32/37.7</td>
</tr>
<tr>
<td>TOTAL</td>
<td>40/47.1</td>
<td>45/52.9</td>
<td>85</td>
</tr>
</tbody>
</table>

N= 85
DF= 1
Chi²= 72.56  p<0.0001

Fisher’s Exact: For Sphagnum presence being greater with Thuidium absences: p<0.0001.