Allelopathy in the Non-Native Macrophyte, Myriophyllum Spicatum and its Influence on Trophic Dynamics in Aquatic Systems

Daniel J Sullivan

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Allelopathy in the non-native macrophyte, *Myriophyllum spicatum* and its influence on trophic dynamics in aquatic systems

By

Daniel J. Sullivan

A Thesis
Submitted to the Faculty of Mississippi State University
in Partial Fulfillment of the Requirements for the Degree of Master of Science in Wildlife, Fisheries, and Aquaculture in the Department of Wildlife, Fisheries and Aquaculture

Mississippi State, Mississippi

August 14, 2015
Allelopathy in the non-native macrophyte, *Myriophyllum spicatum* and its influence on trophic dynamics in aquatic systems

By

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Non-native macrophytes structurally impact aquatic assemblages, yet little is known regarding how they influence energy pathways in freshwater ecosystems. Allelopathy in Eurasian watermilfoil- *Myriophyllum spicatum* has been shown to target basal epiphytic organisms resulting in differences in assemblage structure of colonizing epiphyton between *M. spicatum* and native *M. sibiricum*. I conducted a growth chamber experiment to investigate the hypothesis that differences in assemblage structure of colonizing epiphyton between these two macrophytes influence trophic dynamics within aquatic systems. My data suggest *M. spicatum* produces higher concentrations of allelochemicals, resulting in a more diverse epiphytic assemblage compared to *M. sibiricum*. This could result in potential transformation of trophic dynamics by decoupling carbon as it flows from primary producer to primary consumer. This work identifies a contributing mechanism responsible for *M. spicatum* invasiveness and provides new insight in its ecology and management of this non-native macrophyte.
DEDICATION

I would like to dedicate this thesis to my friends and family that have supported me along this crazy but fun journey of life.

I would like to thank the United States Army for giving me discipline and the values that require a person to never quit and keep on fighting until the war is won, or in this case my thesis is finished, even if the end may not be in sight. I would like to show special gratitude to Ms. Amy Fortener for her overwhelming support, wisdom, and for taking the time to ensure I am grammatically correct.

Lastly, my passion for hunting and fishing was sparked by my uncle, Mr. Tim Simpson, and Mr. Jeff Hughes at a young age. That spark has developed overtime into a raging fire that will never be exhausted and has driven me to pursue a career in this amazing field.
ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Dr. Eric Dibble for giving me the opportunity to conduct this research, my committee members Dr. Ervin and Dr. Rush as well as colleagues Clint Lloyd, Jason Bies, Kandis Cazenave, and Bryant Haley. I would also like to thank Mr. Richard Rezanka with the Minnesota Department of Natural Resources and Dr. John Skogerboe for their efforts in identifying and collecting the macrophytes essential for my research. Lastly, I would like to thank Dr. Getsinger and the U.S. Army Corp of Engineers’ Engineer Research and Development Center for supporting my experiments.
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CHAPTER I
INTRODUCTION

Freshwater systems around the world are being invaded by non-native macrophytes at an alarming rate. Non-native species are commonly referred to as invasive species because of their propensity to invade native systems and cause adverse effects. Invasive species can impose major ecological and economic threats to many countries around the world. The total economic cost of management and damage caused by invasive species is estimated at nearly $1.2 million annually in the United States and the global cost is an estimated $1.4 billion annually (Pimental et al., 2005; Pimental et al., 2002). The ecological impacts of invasive species may be even greater. However no direct evidence is available so far (Gurevitch & Padilla, 2004), it has been speculated that 400 of the 958 endangered plant and animal species are facing a risk of extinction due to non-native introduced species (Wilcove et al., 1998).

In many cases, introduced non-native macrophytes have been effectively changing native dominated landscapes by altering native assemblages and interactions within ecosystems. This is important in applied ecology and management of these systems because in areas where non-native macrophytes become invasive, they can produce monoculture beds reducing habitat heterogeneity within a system with the potential of decreasing biodiversity. These monoculture beds can also produce anoxic
conditions within the littoral zone of a lake that may lead to kills of fish and other aquatic organisms using these areas for cover and forage.

Non-native species often possess life history traits that allow for essential competitive advantages over native species such as higher reproductive rates, climate/temperature plasticity, and rapid growth rates (Aiken et al., 1979). The non-native macrophyte, *Myriophyllum spicatum*, has proven to be successful in North America by outcompeting many native macrophytes following introduction (Madsen et al., 1988). Competitive mechanisms that provide *M. spicatum* with competitive advantages have been thoroughly investigated (Madsen et al., 1991). One such mechanism thought to support invasion success of *M. spicatum* is allelopathy. Allelopathy is defined as biochemical interactions between all types of plants and microorganisms in terrestrial and aquatic environments (Molisch, 1937; cited in Willis, 1985). Allelopathic interactions may be responsible for unexplained patterns that provide introduced, non-native macrophytes a competitive advantage over native macrophytes and epiphyton by inhibiting growth, thereby leading to displacement of the native assemblage. Displacement or modification of firmly and loosely attached epiphyton (cyanobacteria, green algae, and diatoms; Goldsborough et al., 2005), may be detrimental for an aquatic system as these microorganisms serve as a basal resource for higher trophic organisms such as macroinvertebrates and fish (e.g., amphipods, larval fish, sunfish). However, little is known regarding the ecological implications of *M. spicatum* allelopathy on the epiphytic assemblage due to experimental difficulty documenting the natural occurrence and effects of allelopathy (Willis, 1985; Ervin & Wetzel, 2003; Gross et al., 2007).
In determining which chemicals may be responsible for allelopathic interactions, previous investigations have demonstrated *M. spicatum* is capable of producing and exuding phenolic compounds and polyphenols, more specifically hydrolysable tannins. Hydrolysable tannins; gallic acid and ellagitannins to include Tellimagrandin II, have been shown to effectively inhibit growth of surrounding primary producers (Gross et al., 1996). To better strengthen our conceptual understanding of how *M. spicatum* produces and exudes hydrolysable tannins (allelochemicals hereafter), axenic cultures of *M. spicatum* have been grown in a known growth medium to measure differences in exuded allelochemical concentration levels (Gross et al., 2000). This investigation was not capable of quantitatively measuring differences in exuded allelochemicals, but did identify the presence of exuded allelochemicals in the known growth medium. Furthermore, it was suggested there may be spatial and temporal variations in *M. spicatum* production of total phenolic compounds (TPC) and Tellimagrandin II (2000). An increase in production of both TPC and Tellimagrandin II occurred in spring, reaching maximum production levels in June as competition with native primary producers for light and nutrient resources reached a peak. Concentrations of TPC and Tellimagrandin II were also observed to correlate with anatomical location; apical meristems were measured to have increased levels of both TPC and Tellimagrandin II compared to concentrations located in lower stems.

In determining the response of epiphyton to exuded allelochemicals, concentrations of allelochemicals were tested against cultured cyanobacteria and chlorophytes in an agar diffusion assay (ADA). During trials, 5 µg of extracted Tellimagrandin II was observed to clear cyanobacteria from an area greater than 5 mm,
while 50 µg of extracted gallic and ellagic acid were required to provide the same results (Gross et al., 1996). The effect of *M. spicatum* allelopathy was determined to be less effective when measuring photosystem (PS) II activity of three cultured epiphyte species compared to planktonic species (Hilt, 2006). It is speculated that certain epiphytes might be capable of metabolizing allelochemicals exuded by *M. spicatum* which could serve to explain why *M. spicatum* allelopathy was measured to be less effective on epiphytes than planktonic species (Gross, 1999). While some epiphytes may be capable of metabolic breakdown of non-native allelochemicals to resist their effects, *M. spicatum* allelopathy does appear to inhibit growth of epiphyton by targeting the physiological processes that are essential for growth (Li et al., 2010). Inhibiting epiphyton growth could have a negative effect on aquatic foodwebs because epiphytes are an important basal dietary source for higher trophic level organisms. If allelochemicals exuded from *M. spicatum* influence a difference in the epiphyton assemblage, a possible decoupling of energy could occur by alteration of trophic dynamics (Lindeman, 1942). This could provide an explanation for a recent study that observed a decoupling of energy in the form of isotopic carbon as it was transferred from *M. spicatum* to invertebrates (e.g., chironomids, amphipods) living in the monoculture beds of *M. spicatum* (Kovalenko & Dibble, 2013). This could lead to possible increased mortality rates of both invertebrate and vertebrate species as they are forced to seek carbon sources elsewhere in a changing landscape.

The goal of my research was to improve ecological understanding of allelopathy in non-native *M. spicatum* and its potential influence on trophic dynamics within aquatic systems. The work presented here summarizes my approach and results that provide
evidence for how this non-native macrophyte becomes highly invasive and a mechanism on how allelopathy potentially influences colonizing epiphytic assemblages and energy exchange within freshwater ecosystems.

In chapter two, I discussed a simple but essential study that provides foundational knowledge of the naturally occurring epiphyton between non-native *M. spicatum* and native *M. sibiricum*. Understanding the epiphyton assemblage and how this non-native macrophyte may influence a change in the assemblage is important because epiphyton assemblages serve as a basal energy source for higher trophic organisms in aquatic systems. This study was also important because to the best of my knowledge there has been little to no work previously conducted to identify these differences in the epiphyton assemblages between non-native *M. spicatum* and native *M. sibiricum*.

To further increase our understanding of how these epiphyton assemblages may be directly influenced by a chemically-mediated defense mechanism, in chapter three, I investigated the hypothesis that differences in allelochemicals between non-native *M. spicatum* and native *M. sibiricum* influence the assemblage structure of colonizing epiphyton. I predicted that if differences in allelochemicals between non-native *M. spicatum* and native *M. sibiricum* are present, then there will be differences in the assemblage structure of colonizing epiphyton. I tested this prediction by measuring epiphyton genera richness and abundance, while measuring the concentration levels of allelochemicals; gallic acid and ellagittannins.

Additionally in chapter four, I investigated the hypothesis that differences in the assemblage structure of colonizing epiphyton between non-native *M. spicatum* and native *M. sibiricum* influence trophic dynamics in aquatic systems. I predicted that if differences
in the assemblage structure of colonizing epiphyton between non-native *M. spicatum* and native *M. sibiricum* are present, then trophic dynamics in aquatic systems will be negatively affected by the decoupling of energy as it flows from primary producer to secondary consumer. To test this prediction, I measured epiphyton genera richness and abundance with concentration levels of allelochemicals: gallic acid and ellagitannins. Furthermore, I measured trophic dynamics by using stable isotopes: carbon and nitrogen.

In closing (chapter five), I synthesized my results and discussed further implications and future research. This chapter provides alternative hypotheses and sets out to explain why we as ecologists need to continue investigating allelopathy of non-native species and how it may influence aquatic systems. Further investigation of allelopathic interactions may offer invaluable insight into understanding and management of invasive plant species and the aquatic system it is found growing.
References


Molisch, H. 1937. Der Einflusseiner Pflanze auf die andere—Allelopathie. Fischer, Jena, Germany.


CHAPTER II
COMPARISON OF EPIPHYTON ASSEMBLAGES BETWEEN NON-NATIVE 
MYRIOPHYLLUM SPICATUM AND NATIVE MYRIOPHYLLUM 
SIBIRICUM IN A CONTROLLED ENVIRONMENT

Introduction

Submerged macrophytes have a structural impact in both lentic and lotic systems. The structural complexity of macrophytes provide habitat for a variety of aquatic organisms within each system (Dibble et al., 1996). Macrophyte habitat is important for ecological interactions and influences many predator-prey relationships (Savino & Stein, 1982; Carpenter & Lodge, 1986). However, when a non-native macrophyte is introduced, habitat within a system may become less optimal as a result of successful invasion. The success of invasion can lead to the displacement of native macrophyte species as the introduced species reproduces and disperses (Madsen et al., 1991).

In previous years, there has been increasing focus on studying the effects of introduced species due to their invasiveness; however, many of these studies often only investigated the effects on macro-assemblages (e.g., macro-invertebrates, fish). While determining the effects of introduced macrophytes on macro-assemblages is important, our knowledge of how introduced macrophytes impact micro-assemblages such as epiphytic algae is limited. Ecologists investigating the impacts of introduced species in aquatic systems should also diligently work to determine the effects on micro-
assemblages that are frequently recognized to serve as a basal resource for higher trophic organisms.

A micro-assemblage that is often overlooked because of time and limited expertise is the epiphyton assemblage. Epiphyton are firmly and loosely attached cyanobacteria, green algae, and diatoms (Goldsborough et al., 2005). Epiphyton serve as a primary basal resource for higher trophic organisms. Because these organisms live in close proximity to macrophytes, the assemblages may be positively or negatively influenced by the introduction of a non-native species.

My goal in this study was to further our understanding of how the epiphyton assemblage may differ on an introduced non-native macrophyte, in comparison to a native macrophyte. Understanding how the composition of the epiphyton assemblage is influenced by a non-native macrophyte compared to a native macrophyte is important because of the significant role epiphyton serve as a basal resource of energy for higher trophic organisms. To accomplish this, I investigated the differences of epiphyton assemblages between introduced non-native macrophyte, *Myriophyllum spicatum* and native macrophyte, *Myriophyllum sibiricum*. To my knowledge, this is the first study to investigate these differences.

**Methods**

**Macrophyte Collection and Cultivation**

*M. spicatum* was identified and collected from Emily Lake in north central Minnesota whereas *M. sibiricum* was collected from Red Cedar Lake, Wisconsin. Collected macrophytes were genetically confirmed to be *M. spicatum* and *M. sibiricum* by AquaGen located at Grand Valley State University, Michigan. Macrophytes were
shipped directly to the experimental site at the U.S. Army Corp of Engineers’ Environmental Laboratory overnight.

Once received, macrophytes were inspected for grazers and prepared for cultivation by trimming macrophyte segments with apical meristems to lengths of 20 cm. Four individual 20 cm apical meristem segments were grown in a 750 mL container with Blackkow topsoil amended with Osmocote (1.85 g L⁻¹).

The study consisted of two experimental treatments: 1. *M. spicatum* and 2. *M. sibiricum*. Treatments were triplicated in randomly assigned 48L aquaria containing Smart and Barko growth medium (1985) in an environmental growth chamber. The temperature was programmed for 22°C with a photoperiod of 16:8 (light: dark) to replicate mid-summer growing conditions. To account for lake effect of the epiphyton assemblage, a 750 mL water sample was collected from each aquarium on day 7, following macrophyte acclimation. The water sample was collected near the apical meristems and the water was transferred to one, 48L aquarium for epiphyton homogenization. By day 21, macrophytes had reached the water’s surface and a 750 mL water sample from the homogenized epiphyton aquarium was reintroduced into each experimental aquarium. The experiment ended on day 49 as macrophytes were beginning to senesce.

**Epiphyton Collection**

Epiphyton were collected following the methods outlined by Aloi (1990). A sterile 5 cm Perspex tube sampler was used to capture both firmly and loosely attached epiphyton. The tube sampler was placed over a randomly selected individual apical meristem from each triplicated treatment and removed using sterilized scissors.
Individual apical meristems were transferred to 50 mL collection vials and shaken vigorously for 30 seconds to remove attached epiphyton. Apical meristems were then removed from their appropriate collection vial and inspected under a dissecting scope for the absence of epiphyton. Meristems that still contained epiphyton were lightly brushed with a sterile brush and the loosened epiphyton were transferred into the collection vial. Each vial containing epiphyton was standardized to 40 mL using reverse osmosis water and 4 drops of 70% EtOH (1 to 10 mL ratio). Collection vials were packaged and shipped overnight to Algal Analysis, LLC (Missoula, Montana) for analysis.

**Analysis**

Epiphyton samples were identified to genera and phyla. Epiphyton genera richness was calculated and differences in epiphyton diversity across triplicated treatments were determined using the Shannon-Wiener Diversity Index. A Bray-Curtis non-metric multidimensional scaling (NMDS) ordination was used to visually depict similarities in the density of epiphyton genera among treatments. Non-metric multidimensional scaling is an indirect gradient analysis maximizing rank-order correlations between distance measures and distance in ordination space. Due to small sample size and distribution not meeting normality, total epiphyton richness and phyla richness was analyzed using the non-parametric Kruskal-Wallis (KW) test to determine difference in variation in rank order between treatments. All statistical analyses were performed in statistical package R (R Core Team, 2014). Due to limited sample size and because this work aims to identify potential patterns, significance was determined at an alpha level of 0.1.
Results

A total of 20 epiphyton genera from four different phyla were identified from apical meristems collected from both treatments (Table 2.1). Of the nine genera identified belonging to phylum Cyanophyta, seven genera were found on *M. spicatum* whereas only five genera were found on *M. sibiricum*. Only Cyanophyta genera *Oscillatoria*, *Anabaena*, and *Calothrix* were present in both treatments. A total of nine Chlorophyta genera were identified across both triplicated treatments. Eight genera were found on apical meristems of *M. spicatum* whereas only three genera were identified on *M. sibiricum*. Chlorophyta genera *Spirogyra* and *Ankistrodesmus* were present in both treatments with *Spirogyra* having the highest density of all epiphyton genera. An additional genus *Tribonema* belonging to phylum, Xanthophyta was identified in both treatments. Epiphyton assemblages showed grouping for different treatments with treatment *M. sibiricum* having the tighter grouping in the NMDS ordination (Stress: 0.0000; $R^2$ Axis 1: 0.6349 Axis 2: 0.0656; Figure 3.3). *M. sibiricum* grouped more closely than *M. spicatum*, indicating more similarity of the collected epiphyton. *M. sibiricum* showed grouping on axis 1 (Figure 2.1) in the positive direction (0.34, 0.24, 0.52, respectively) with an influence from *Rivularia* and *Nostoc* (0.71, 0.62, respectively). *M. spicatum* grouped on axis 1 in the negative direction (-0.36, -0.37,-0.138, respectively) with a strong influence from *Anabaena* (-0.69).

The results of the Shannon-Weiner Diversity Index ($H'$) revealed slightly greater epiphyton diversity for *M. spicatum* treatment than *M. sibiricum* (Table 2.2) but the two did not statistically differ (KW, $x^2= 2.33$, df = 1, p = 0.1266). The results of the Kruskal-Wallis test for total epiphyton richness revealed *M. spicatum* was significantly greater
than *M. sibiricum* (*KW, χ^2 = 4.35, df = 1, p < 0.05*). Neither, Cyanophyta or Chlorophyta richness were found to be different between *M. spicatum* and *M. sibiricum* (*KW, χ^2 = 0.45, df = 1, p = 0.500, χ^2 = 1.76, df = 1, p = 0.184, respectively).

**Discussion**

My goal was to elucidate differences in the epiphyton assemblage between non-native *M. spicatum* and native *M. sibiricum*. Although the results of my study provide statistical evidence for differences in epiphyton richness between the two macrophytes, the remainder of my results do not provide evidence for differences in phyla richness or diversity likely due to limited sample size. The data, however, suggest there might be differences in the structure of epiphyton assemblages that should be further explored. The structure of epiphyton assemblages is thought to be dependent on a multitude of factors relating to the morphological and biochemical features of the host macrophyte and surrounding environment.

In determining mechanisms that impact structure of epiphyton assemblages, it has been shown that macrophyte biomass may directly influence epiphyton abundance and biomass (Karosienė & Kasperovičienė, 2012). An increase in macrophyte biomass may bring about an increase in epiphyton abundance and biomass. Conversely, as macrophyte biomass decreases, epiphyton abundance and biomass decreases. While macrophytes used in my study share similar morphological characteristics, the increase of finely dissected leaflets found on *M. spicatum* may lead to an increase in surface of *M. spicatum* apical meristems. Differences in the apical meristem surface area between *M. spicatum* and *M. sibiricum* could explain the slight increase in epiphyton richness that was observed on *M. spicatum*. 
It has also been hypothesized that allelopathic interactions of non-native macrophytes may serve as a novel weapon (Callaway & Ridenour, 2004) that can influence the structure of local epiphyton assemblages. As competition for light and nutrient resources occurs between non-native macrophytes and epiphyton, macrophytes are suspected to exude allelochemicals responsible for decreasing resource competition. The presence of epiphyton incapable of metabolizing or resisting non-native allelochemicals may be reduced, causing a decrease in overall diversity and richness. While allelopathic interactions may be actively occurring, the results from my experiment do not directly support this hypothesis as epiphyton diversity and richness was found to be slightly greater in *M. spicatum* treatments than *M. sibiricum* treatments. If the non-native macrophyte was effectively targeting epiphyton with exuded allelochemicals, I suspect epiphyton would be reduced when compared to the native macrophyte.

An alternative hypothesis that may explain differences in the epiphyton assemblage is exploitation efficiency of available nutrients from the growth medium. *M. spicatum* may possibly utilize fewer nutrients to grow thereby elevating availability of nutrients for epiphyton growth. This would inherently reduce resource competition, thus increasing the presence of epiphyton. Alternately, competition for nutrients in *M. sibiricum* may be greater if *M. sibiricum* requires more nutrients for similar growth thus leading to a reduction in available nutrients for epiphyton. This hypothesis may support the neutral substrate hypothesis (Blindow, 1987) which postulates that structuring of epiphytic assemblage is unaffected by the identity of the host macrophyte and is only affected by the surrounding environmental conditions or limnological characteristics.
Although limited, this study is, to the best of my knowledge, one of the first to compare epiphyton assemblages inhabiting *M. spicatum* and *M. sibiricum*. This study provides an insight into the micro-assemblage that is often overlooked but is an important basal resource for higher trophic organisms in aquatic systems. Future studies should focus on determining how subtle changes in morphological and environmental characteristics may have an effect on epiphyton assemblages. Furthermore, conducting research to increase our understanding of the nutrient requirements for physiological development of each macrophyte and how it influences competition with epiphyton assemblages is essential. Through increasing our knowledge of the macrophytes and their associated micro-assemblages, we can better manage our lentic and lotic systems.
### Tables and Figures

Table 2.1  Epiphyton identified to phyla and genera.

<table>
<thead>
<tr>
<th>Phyla</th>
<th>Genera</th>
<th>Treatment</th>
<th>M. spicatum</th>
<th>M. sibiricum</th>
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<tbody>
<tr>
<td>Cyanophyta</td>
<td><em>Aphanothece</em></td>
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<td><em>Chroococcus</em></td>
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<td><em>Oscillatoria</em></td>
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<td><em>Anabaena</em></td>
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<td><em>Calothrix</em></td>
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<td><em>Leptolyngbya</em></td>
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<td>+</td>
</tr>
<tr>
<td>Xanthophyta</td>
<td><em>Tribonema</em></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Bacillariophyta</td>
<td><em>Diatoma</em></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Notes: Epiphyton identified to phyla and genera collected from 5 cm apical meristems oftriplicated treatments of native *M. sibiricum* and non-native *M. spicatum*. 
Table 2.2  Epiphyton assemblage metrics.

<table>
<thead>
<tr>
<th>Replicate</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>$M. \text{ spicatum}$</td>
<td>$M. \text{ sibiricum}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diversity ($H'$)</td>
<td>Richness</td>
<td>Evenness ($J$)</td>
</tr>
<tr>
<td>1</td>
<td>1.184</td>
<td>6</td>
<td>0.661</td>
</tr>
<tr>
<td>2</td>
<td>1.045</td>
<td>7</td>
<td>0.537</td>
</tr>
<tr>
<td>3</td>
<td>1.222</td>
<td>11</td>
<td>0.510</td>
</tr>
</tbody>
</table>

Notes: Epiphyton assemblage metrics for native $M. \text{ sibiricum}$ and non-native $M. \text{ spicatum}$. Differences in the epiphyton assemblage attached to 5 cm apical meristems were determined using the Shannon-Diversity ($H'$) index, genera richness, genera evenness ($J$).

Figure 2.1  NMDS ordination.

Notes: NMDS ordination of the density of epiphyton genera sampled from non-native $M. \text{ spicatum}$ and native $M. \text{ sibiricum}$.
References


CHAPTER III

ALLELOPATHY AND ASSEMBLAGE COMPOSITION OF EPIPHYTON BETWEEN

MYRIOPHYLLUM SPICATUM AND MYRIOPHYLLUM SIBIRICUM

Introduction

In freshwater ecosystems, native macrophytes play an important role in the physical and chemical processes within a system (Dibble et al. 1996; Wetzel, 2001). The structural nature of macrophytes provides essential habitat that influences a diverse littoral assemblage. However, when non-native species are introduced, they have been observed to change the landscape through mechanistic processes that can reduce accessibility to native macrophyte habitat. The diminution of native habitat may transform the diversity of the native assemblage.

The introduced non-native species, *Myriophyllum spicatum*, has been effective in altering landscapes in many aquatic systems across North America because of its life history strategies. Previous studies investigating *M. spicatum*, have identified that it uses multiple competitive mechanisms that negatively impact native species (Aiken et al., 1979; Madsen et al., 1988; Madsen et al., 1991). One mechanism that may be used against native primary producers during competition is allelopathy. Allelopathy is defined as biochemical interactions between plants and micro-organisms in terrestrial and aquatic environments that can have beneficial or detrimental effects (Molisch, 1937; cited in Willis, 1985). Allelopathic interactions may be responsible for unexplained patterns
that provide introduced, non-native macrophytes a competitive advantage over native macrophytes and epiphyton, defined as firmly and loosely attached cyanobacteria, green algae, and diatoms (Goldsborough et al., 2005), by inhibiting growth and thereby leading to the displacement or modification of the native assemblage. However, due to difficulty capturing the natural occurrence of allelopathic interactions, little is known regarding the effects of allelopathy on littoral biota as a result of this mechanism.

Manipulative experiments have investigated the effects of *M. spicatum* allelopathy through the production of allelopathic biochemicals (allelochemicals) (Gross et al. 1996; Gross, 1999; Hilt & Nicklisch, 2002). These studies provide evidence that allelochemicals produced by *M. spicatum* can effectively inhibit growth and survival of other macrophytes and epiphyton (Gross, 1999; Nakai et al., 2000; Li et al., 2010). A study by Gross et al. (1996) investigated the release of allelochemicals from *M. spicatum* and identified three main inhibitory allelochemicals: Tellimagrandin II, gallic acid, and ellagic acid. While each of the allelochemicals were effective in inhibiting growth of epiphyton, Tellimagrandin II was observed to have the greatest effect of clearing cultured epiphyton on an agar assay (Gross et al. 1996). Marko et al. (2008) chemically profiled both non-native *M. spicatum* and native *M. sibiricum* by investigating the chemical composition of both macrophytes. They identified that *M. spicatum* and *M. sibiricum* produced allelochemicals including Tellimagrandin II known to be effective against cultured epiphyton, however, *M. spicatum* produced nearly twice the amount of Tellimagrandin II and other allelochemicals compared to *M. sibiricum*. Marko et al. (2008) concluded increased production of allelochemicals by *M. spicatum* may serve as a
defense mechanism related to seasonal variations in phytoplankton or as a competitive mechanism against native macrophytes during introduction.

While past studies have increased our understanding of non-native allelopathy and its effect on cultured epiphyton, little is known about the natural differences in the assemblage composition of epiphyton between *M. spicatum* and *M. sibiricum* as a result of allelopathic interactions. Understanding the effect of allelopathy on the assemblage composition of epiphyton is critical because of the significant role epiphyton serve as a basal resource in aquatic foodwebs. Transformation of the epiphyton assemblage may alter the flow of energy through the trophic levels as higher trophic organisms are forced to seek their basal resource elsewhere within the aquatic environment.

To determine the effect of allelopathy on the epiphyton assemblage, I investigated the hypothesis that differences in concentration levels of two putative allelochemicals (gallic acid and ellagitannins) between non-native *M. spicatum* and native *M. sibiricum* influence the assemblage structure of colonizing epiphyton. By correlating the differences in allelochemical concentrations between non-native *M. spicatum* and native *M. sibiricum* with associated epiphyton richness and diversity, I provide an ecological perspective of the differences in natural epiphyton assemblages related to non-native allelopathic interactions.

**Methods**

**Macrophyte Collection**

*M. spicatum* was identified and collected from the western littoral zone of Emily Lake, MN. *M. sibiricum* was collected from Red Cedar Lake, WI, northwest of Faust Island. Species confirmation of *M. spicatum* and *M. sibiricum* by genetic analysis was
performed by Aqua Gen at Grand Valley State University, MI. Collected plants were placed on ice and shipped overnight to the experimental site at the U.S. Army Corp of Engineers’ Research and Development Center (ERDC) in Vicksburg, MS.

**Macrophyte Cultivation**

Macrophytes were standardized using the top 20 cm segment comprising of the apical meristem for the following treatments: 1. *M. spicatum* (No choice), 2. *M. spicatum* (Choice) and *M. sibiricum* (Choice), and 3. *M. sibiricum* (No choice). Each treatment was triplicated using four individual macrophyte segments in four 750 mL plastic cups containing Blackkow topsoil amended with Osmocote (1.85 g-L). To prevent suspension of sediment, a one inch layer of sand was added to the surface of the Blackkow top soil in each 750 mL plastic cup. Treatments were randomly assigned to nine, 48L aquaria filled with Smart and Barko nutrient water in a controlled environment growth chamber (1985). For replication of summer growing conditions, the temperature was programmed at 22º C with a photoperiod of 16:8 hrs (light: dark).

Macrophytes and their associated epiphyton were provided an acclimation period of 7 days before a 750 mL water sample was collected from each aquarium near the apical meristems of the plants. The 750 mL water samples were transferred to one, 48L aquarium to culture mixed epiphyton. By day 21, macrophytes had reached the water surface and a 750 mL mixed epiphyton sample was transferred from the culture aquarium into each experimental aquarium for homogenization of epiphyton to account for lake effect. The experiment ended prior to plant senescence on day 49 and apical meristems were removed for epiphyton and allelochemical collection.
**Epiphyton Collection**

Following the methods outlined by Aloi (1990) for the removal of epiphyton, one-5 cm apical meristem segment was removed from each replicated treatment using a Perspex tube sampler 5 cm in length. The Perspex tube sampler has been proven to efficiently capture both firmly and loosely attached epiphyton during collection. The collected apical meristems were transferred to individual 40 mL collection vials. Vials containing the apical meristems were filled with 30 mL of reverse osmotic (RO) water and shaken vigorously for 30 seconds to remove epiphyton. Following the shaking process, apical meristems were removed and inspected under a dissecting scope to confirm the removal of epiphyton from the surface. Epiphyton not removed by the shake method, was removed by gently brushing the surface of the apical meristem leaflets. The absence of epiphyton was confirmed using a dissecting scope and epiphyton samples were preserved using 70% etoh (1 drop to 10 mL water). Epiphyton samples were shipped overnight to Algal Analysis, LLC (Missoula, Montana) for analysis.

**Allelochemical Collection**

Apical meristems collected for epiphyton removal were used for analysis of allelochemical concentrations within apical meristem tissue. Using a 5 cm Perspex tube sampler, an individual apical stem measuring 5 cm in length was collected at random from each triplicated treatment. The collected apical meristem segments were rinsed with sterile reverse osmotic (RO) water to remove attached epiphyton and placed into individually, sterilized 10 mL vials. Water samples were also collected at time of apical meristem collection to capture exuded allelochemicals. Here, water samples from each triplicated treatment were collected individually at a standardized location. Water
samples were collected from the center of each aquaria, directly below the surface water using individual, sterilized 5 mL syringes. Macrophyte and water samples were transferred to the College of Forest Resources at Mississippi State University for processing, where macrophyte and water samples were freeze dried for 96 hours. They were then shipped directly to Clemson University’s Multi-User Analytical Laboratory (MUAl) for allelochemical analysis.

**Analysis**

Genera and Phyla richness was calculated by determining the total number of individual genera and phyla attached the surface of each 5 cm apical meristem. Evenness was calculated using Pielou’s Evenness Index ($J$). Differences in epiphyton diversity across treatments were determined using the Shannon-Wiener diversity index. The Shannon-Wiener diversity index;

$$H' = - \sum_{i=1}^{s} p_i \ln p_i$$  \hspace{1cm} (2.1)

where $H'$ is the species diversity index, $s$ is the number of species, and $p_i$ is the proportion of individuals of each species belonging to the $i^{\text{th}}$ species of the total number of individuals. Due to small sample size and data not being normally distributed, differences in total epiphyton richness, phyla richness, diversity, and evenness across treatments were analyzed using non-parametric Kruskal-Wallis (KW) test.

To standardize allelochemical data received from Clemson MUAl, data was first multiplied by three for standardization of concentration levels in total micrograms per 5 cm apical meristem segment. Concentration levels from the water samples were below
analytical detection limits. Concentration levels of each individual allelochemical were analyzed using a one-way analysis of variance (ANOVA). Normality and homogeneity of variance were analyzed with the Shapiro and Bartlett test, respectively. A post-hoc Tukey HSD test was performed using function TukeyHSD in package multcomp to determine means that significantly differ across treatments. Epiphyton and allelochemical data were analyzed using statistical package R (R Core Team, 2014) and significance was determined at an alpha level of 0.1 due to limited sample size.

Paleontological statistics software (PAST, 2001) was used for non-metric multidimensional scaling (NMDS) and canonical correspondence analysis (CCA). NMDS ordination based on Bray-Curtis similarities was used to visually depict similarities in the density of epiphyton genera between treatments. NMDS is an indirect gradient analysis maximizing rank-order correlations between distance measures and distance in ordination space. Allelochemicals were combined with density composition of epiphyton genera and analyzed with CCA. CCA is a direct gradient analysis that was used to ordinate epiphyton genera density based on allelochemical variables.

Results

A total of 25 genera belonging to four phyla were identified across treatments (Table 3.1). Of the 25 epiphyton genera identified, only 9 genera were found in all treatments. Genera *Oscillatoria, Anabaena,* and *Calothrix* of phylum Cyanophyta with genera *Spirogyra* and *Ankistrodesmus* of phylum Chlorophyta were present across treatments. Additionally, genus *Diatoma* of phylum Bacillariophyta and genus *Tribonema* belonging to phylum Xanthophyta was found in all treatments. *Anabaena* (50.94%) dominated treatment *M. spicatum (No choice)* while *M. sibiricum (No choice)*
was dominated by *Tribonema* (67.83%). In the mixed treatment, *Heteroleibleinia* (27.30%) dominated *M. spicatum* (Choice), while *Gleotrichia* (24.80%) dominated *M. sibiricum* (Choice).

Epiphyton assemblages showed grouping of different treatments, with treatment *M. spicatum* (No choice) having the tightest grouping in the NMDS ordination (Stress: 0.2221; R² Axis 1: 0.2768 Axis 2: 0.1227; Figure 3.1). *M. spicatum* (No choice) grouped more closely than the other treatments, indicating more similarity of the collected epiphyton. *M. spicatum* (No choice) grouped on axis 1 in the negative direction (-0.01, -0.12, -0.12, respectively) with a strong influence from *Anabaena* (-0.33). *M. spicatum* (Choice) showed some grouping on axis 2 in positive direction (0.12, 0.01, and 0.22, respectively). This group was influenced by *Heteroleibleinia* and *Homeothrix* (0.46, 0.39, respectively). *M. sibiricum* (No choice) showed slight grouping on axis 1 in the positive direction with an influence from *Rivularia* and *Tribonema* (0.48, 0.75, respectively) while *M. sibiricum* (Choice) showed spread distribution in both the positive and negative directions on both axes.

Results of the Shannon-Wiener diversity index revealed slightly greater diversity overall in both *M. spicatum* treatments than *M. sibiricum* treatments (Table 3.2), however, yielded no significant difference in epiphyton diversity between treatments (KW, $\chi^2 = 2.59$, df = 3, p = 0.4593). While total epiphyton richness appeared to vary between treatments, no significant difference was found in total epiphyton richness (KW, $\chi^2 = 5.07$, df = 3, p = 0.17) across treatments. When investigating richness separated by phyla, both *Cyanophyta* and *Chlorophyta* genera richness also showed no significant
difference across treatments ($KW, x^2 = 3.71, df = 3, p = 0.29, x^2 = 5.07, df = 3, p = 0.11$, respectively).

When determining differences in allelochemicals across treatments, gallic acid was found to be nearly six times greater in apical meristems of *M. spicatum* (No choice) when compared to *M. sibiricum* (No choice) with pooled means of 208.55 and 34.85 µg per 5 cm apical meristem, respectively. A one-way ANOVA yielded significant differences among treatments in the concentration levels of gallic acid ($F_{3, 7} = 6.411, P < 0.05$). A post hoc Tukey test (Table 3.3) showed that concentrations of gallic acid in *M. spicatum* (No choice) significantly differed from *M. spicatum* (Choice) and *M. sibiricum* (No choice) at $p < 0.05$. However, *M. spicatum* (No choice) was not significantly different than *M. sibiricum* (Choice). When comparing concentration levels of ellagitannins, ANOVA results yielded no significant differences across treatments ($F_{3, 7} = 2.151, P = 0.182$). A post hoc Tukey test comparing the means of ellagitannins revealed *M. spicatum* (No choice) when compared to *M. sibiricum* (No choice) had the lowest p-value at 0.188.

The results from the CCA indicate axis 1 had an eigenvalue of 0.48865 and explained 92.01% of the variability in the ordination of treatments and epiphyton genera in relation to allelochemical parameters, whereas axis 2 had an eigenvalue of 0.04241 and explained 7.986% of the variance in the ordination of treatments and epiphyton genera in relation to allelochemicals (Figure 3.7). Gallic acid and ellagitannins correlated with treatments and epiphyton genera ordinations on axis 1 in the positive direction (0.65 and 0.37, respectively). For axis 2, gallic acid and ellagitannins negatively correlated with the ordination of treatments and epiphyton genera (-0.75 and -0.84, respectively).
**Discussion**

This study makes an effort to elucidate the differences in the assemblage composition of epiphyton and allelochemical concentrations between non-native *M. spicatum* and native *M. sibiricum*. Understanding the structure of epiphyton assemblages within their environment can be of great difficulty due to the complexity of their inter-relationships between macrophyte and epiphyton.

Although I was not capable of capturing significant differences because of limited sample size, this study shows there may be differences in the distribution of epiphyton between non-native *M. spicatum* and native *M. sibiricum* that should be further investigated. One explanation for differences in distribution of epiphyton that I did not measure for is macrophyte cover (i.e., biovolume and percent cover). Macrophyte cover has been shown to have a direct relationship resulting in higher abundances of epiphyton. Conversely, as macrophyte cover increases, epiphyton abundance is thought to increase. Inversely, as macrophyte cover decreases, epiphyton abundance decreases. However this may not always be true because as *M. spicatum* macrophyte cover increases, self-shading can occur, thereby reducing the availability of light for epiphyton, thus influencing a decrease in epiphyton abundance. The macrophyte cover-epiphyton abundance relationship may be dependent on scale and only explainable at the individual apical meristem level.

Another present, but counteractive force that I suspect influences the distribution of epiphyton is the production of allelochemicals in the apical meristems that are responsible for increasing macrophyte cover. As *M. spicatum* grows, it has been shown to invest more energy into producing allelochemicals in apical meristems for defense
against other primary producers (i.e., epiphyton) during resource competition (Marko et al., 2008). My study shows substantive support for previous work that has shown *M. spicatum* produces gallic acid and ellagitannins which are known to serve as primary allelochemicals during allelopathic interactions. Further, my study validates previous studies that have identified *M. spicatum* produces greater concentrations of allelochemicals in apical meristem tissue compared to *M. sibiricum* (Marko et al., 2008). Although concentrations of exuded allelochemicals from the collected water samples were below detection limits, it is possible the allelochemicals I detected in the macrophyte tissue are still being released (Gross, 1999). It is likely that as allelochemicals are released through diffusion across the outer membranes of the macrophyte tissue to where epiphyton live, direct or indirect displacement of epiphyton incapable of metabolizing or resisting the effects of the allelochemicals occurs leaving only co-evolved epiphyton in place. This could explain the results of the CCA that showed limited effect of allelochemicals from in the macrophyte tissue on only a few epiphyton genera.

Additionally, although I did not test for differences of condensed tannins in *M. spicatum* and *M. sibiricum*, greater concentrations of condensed tannins were identified during analysis of allelochemicals in *M. spicatum* compared to *M. sibiricum* (Mean, 538.57 and 130.75 µg per 5 cm apical meristem, respectively). While there have been increasing investigations focused on determining the use of condensed tannins by terrestrial plants as a deterrent of herbivory, little is known about the effects of condensed tannins when released from submerged macrophytes. If the macrophyte uses condensed tannins similarly to terrestrial plants, the condensed tannins could possibly have an
indirect effect on epiphytic species present on the surface of the macrophyte as they diffuse across the outer membrane.

As a result of these different counteractive forces, the distribution of epiphyton may be oscillating in nature through a series of ebbs and flows which may explain why my study did not find significant differences in the epiphyton assemblage between *M. spicatum* and *M. sibiricum*. It is possible that I collected epiphyton and allelochemicals from the apical meristems of *M. spicatum* at a point of stability. Hypothetically, if I had collected the apical meristem at point A (Figure 3.3), I may have found that while the area of the apical meristem was increasing, epiphyton growth may have been reduced because of high investment of energy in the production and release of allelochemicals. However, I may have collected epiphyton and allelochemicals at point B, where the apical meristem reaches a stable state and area has reached its max potential. Energy invested into the production and release of allelochemicals may be reduced and stability in epiphyton growth may occur. If I had collected at point C, area, allelochemicals, and epiphyton would be greatly reduced because of macrophyte senescence or because of a shift in allocation of energy to new branching apical meristems.

Further experiments should be performed to explore the response of area, allelochemical production, and epiphyton growth in relation to individual apical meristem growth. I alternatively hypothesize that if a range was experimentally determined for maximum growth potential of apical meristems, differences in the growth (i.e., time and length) of individual apical meristems may yield different area, allelochemical production, and epiphyton responses until reaching a stable state prior to senescence. By knowing the interactions between macrophyte-allelochemicals-epiphyton at the meristem
scale, our ecological knowledge of the interactions taking place at the individual level
that may explain the holistic differences at the population and assemblage scale.

Tables and Figures

Table 3.1  Epiphyton genera by phyla identified across replicated treatment.

<table>
<thead>
<tr>
<th>Epiphyton</th>
<th>Treatment</th>
<th>M. spicatum (No Choice)</th>
<th>M. spicatum (Choice)</th>
<th>M. sibiricum (Choice)</th>
<th>M. sibiricum (No Choice)</th>
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<td><em>Gomphosphaeria</em></td>
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<td><em>Heteroleileinia</em></td>
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<td><em>Rivularia</em></td>
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<td><em>Homeothrix</em></td>
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<td><em>Zygnema</em></td>
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<td><em>Cladophora</em></td>
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<td><em>Stigeoclonium</em></td>
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<td><em>Scenedesmus</em></td>
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<td>Xanthophyta</td>
<td><em>Tribonema</em></td>
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<tr>
<td>Bacillariophyta</td>
<td><em>Diatoma</em></td>
<td>+</td>
<td>+</td>
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Table 3.2  Epiphyton assemblage metrics.

<table>
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<th>( M. \text{spicatum} ) (No choice)</th>
<th>( M. \text{spicatum} ) (Choice)</th>
<th>( M. \text{sibiricum} ) (Choice)</th>
<th>( M. \text{sibiricum} ) (No choice)</th>
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<td>Diversity (H') Richness Evenness</td>
<td>Diversity (H') Richness Evenness</td>
<td>Diversity (H') Richness Evenness</td>
<td>Diversity (H') Richness Evenness</td>
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Table 3.3 Allelochemical and Condensed tannin data.

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<th>Allelochemicals and Condensed Tannins</th>
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<td>Gallic acid</td>
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<tr>
<td>M. spicatum (No choice)</td>
<td>208.55 ± 22.30</td>
</tr>
<tr>
<td>M. spicatum (Choice)</td>
<td>46.35 ± 46.01</td>
</tr>
<tr>
<td>M. sibiricum (Choice)</td>
<td>126.65 ± 45.06</td>
</tr>
<tr>
<td>M. sibiricum (No choice)</td>
<td>34.85 ± 10.16</td>
</tr>
</tbody>
</table>

Notes: Allelochemical and Condensed tannin data (Mean ± SE) in Total µgs per 5 cm apical meristem collected from each treatment.

Table 3.4 Results of post hoc Tukey HSD test.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Estimate</th>
<th>T-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. sibiricum (No choice) - M. sibiricum (Choice)</td>
<td>-91.80</td>
<td>-2.102</td>
<td>0.2400</td>
</tr>
<tr>
<td>M. spicatum (Choice) - M. sibiricum (Choice)</td>
<td>-80.30</td>
<td>-1.645</td>
<td>0.4142</td>
</tr>
<tr>
<td>M. spicatum (No choice) - M. sibiricum (Choice)</td>
<td>81.90</td>
<td>1.876</td>
<td>0.3171</td>
</tr>
<tr>
<td>M. spicatum (Choice) - M. sibiricum (No choice)</td>
<td>11.50</td>
<td>0.235</td>
<td>0.9950</td>
</tr>
<tr>
<td>M. spicatum (No choice) - M. sibiricum (No choice)</td>
<td>173.70</td>
<td>3.978</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>M. spicatum (No choice) - M. spicatum (Choice)</td>
<td>162.20</td>
<td>3.323</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Notes: Results of post hoc Tukey HSD test showing multiple comparisons of means for gallic acid. Bold text indicates which treatments significantly differ in gallic acid concentrations.
Table 3.5  Results of post hoc Tukey HSD test showing multiple comparisons of means for ellagitannins.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Estimate</th>
<th>T-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. sibiricum (No choice) - M. sibiricum (Choice)</td>
<td>-279.228</td>
<td>-3.702</td>
<td>0.40778</td>
</tr>
<tr>
<td>M. spicatum (Choice) - M. sibiricum (Choice)</td>
<td>-270.614</td>
<td>-3.209</td>
<td>0.70941</td>
</tr>
<tr>
<td>M. spicatum (No choice) - M. sibiricum (Choice)</td>
<td>128.594</td>
<td>1.705</td>
<td>0.91711</td>
</tr>
<tr>
<td>M. spicatum (Choice) - M. sibiricum (No choice)</td>
<td>8.614</td>
<td>0.102</td>
<td>0.97648</td>
</tr>
<tr>
<td>M. spicatum (No choice) - M. sibiricum (No choice)</td>
<td>407.822</td>
<td>5.407</td>
<td>0.18785</td>
</tr>
<tr>
<td>M. spicatum (No choice) - M. spicatum (Choice)</td>
<td>399.208</td>
<td>4.734</td>
<td>0.41134</td>
</tr>
</tbody>
</table>

Figure 3.1  NMDS ordination.
Notes: NMDS ordination of the density of epiphyton genera sampled from three treatments; 1: M. spicatum (No choice), 2: M. spicatum (Choice), M. sibiricum (No choice), and 3: M. sibiricum (Choice).
Figure 3.2  Canonical correspondence analysis.

Notes: Canonical correspondence analysis of treatments and density of epiphyton genera correlated with allelochemicals.
Figure 3.3  The responses of macrophyte area, allelochemical production, epiphyton growth.

Notes: The responses of macrophyte area, allelochemical production, epiphyton growth to the potential growth of an individual apical meristem from *M. spicatum*. Point A represents the growth process, where the apical meristem is increasing in area and the macrophyte is investing more energy into allelochemical production causing a reduction in epiphyton growth. Point B represents the point where the apical meristem reaches stability, causing a reduction in the production of allelochemicals, and increasing stability in the colonizing epiphyton assemblage. Point C, represents the point in which the apical meristem senesces or reallocates energy to a new apical meristem, causing a negative response in area, allelochemical production, and epiphyton growth.
References


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CHAPTER IV

THE EFFECT OF *MYRIOPHYLLUM SPICATUM* ON TROPHIC DYNAMICS IN AQUATIC SYSTEMS

Introduction

In the United States, a non-native invasive macrophyte, *Myriophyllum spicatum*, is known to impact premier fisheries by altering habitat structure and foraging capabilities of certain fish species (e.g., *Lepomis* spp. *Micropterus* spp.). *M. spicatum* possesses a variety of life history mechanisms that allow it to gain a competitive advantage over native macrophytes (Aiken et al., 1979; Madsen et al., 1988; Madsen et al., 1991). It has been hypothesized that *M. spicatum* uses allelopathy as a defense mechanism for a competitive advantage that has far reaching ecological implications that extend beyond primary producers but little is currently known about this (Willis, 1985; Ervin & Wetzel 2003; Gross et al., 2007).

Further, studies investigating *M. spicatum* allelopathy have provided evidence that allelochemicals produced by *M. spicatum* may be effective herbicidal, algicidal, and anti-microbial agents that work against native macrophytes as well as epiphyton attached to macrophyte stems and leaves which are important basal resources in aquatic systems (Gross, 1999; Nakai et al., 2000; Li et al., 2010). A recent study investigating the effects of *M. spicatum* on trophic structure and energy flow provided evidence of a decoupling of energy from primary producer to secondary consumer in four Minnesota lakes using...
stable isotopes, carbon and nitrogen (Kovalenko & Dibble, 2013). They showed that invertebrates inhabiting monoculture beds of *M. spicatum* represented the *M. spicatum* associated stable isotope values, however, fish captured in and around the same monoculture beds of *M. spicatum* were not representative of the associated stable isotope values. This decoupling of energy may be explained by the effects allelochemicals exuded by *M. spicatum* on epiphyton assemblages during periods of competition for light and nutrient resources.

One such effect of allelopathy could be the colonization of different epiphyton assemblages upon *M. spicatum* relative to those on native macrophytes such as *M. sibiricum*. These differing assemblages could possibly prove to be less optimal forage species for native herbivorous grazers (MacArthur & Pianka, 1966) causing a disruption in trophic dynamics to the primary consumer, ultimately having an effect on higher trophic organisms (Lindeman, 1942).

In recent years, stable isotope analysis (SIA) has become an integral tool used in ecology to provide information regarding foraging relationships in food webs, niche breadth and overlap, and resource partitioning from the individual to the assemblage level (Kling et al., 1992; Newsom et al., 2012; Kovalenko & Dibble, 2013). Stable isotope $^{15}\text{N}$ has been shown to become heavier through fractionation as it is assimilated by higher trophic organisms (DeNiro & Epstein, 1980; Fry, 2006) thus making $\delta^{15}\text{N}$ a good indicator of a species’ trophic position. Unlike $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ does not become heavier as it shifts to higher trophic positions. Thus $\delta^{13}\text{C}$ is commonly used as an indicator of basal resources and food for higher trophic organisms (DeNiro & Epstein, 1978).
Using stable isotope analysis, the goal of this study was to increase our theoretical and applied understanding of how a non-native macrophyte may influence trophic dynamics in an aquatic system through modification of the epiphyton assemblage. This is necessary to investigate because the modification of the epiphyton assemblage could cause a disconnection in the transfer of energy to higher levels leading to lower survivability of higher trophic organisms in systems with broad monoculture beds of these non-native macrophytes. Thus, I hypothesized that differences in colonizing epiphyton between non-native *M. spicatum* and native *M. sibiricum* may alter energy exchange from macrophyte to the primary consumer. Thus I would expect to see a decoupling in energy flow within monoculture beds of *M. spicatum*.

**Methods**

**Macrophyte Collection**

Non-native *M. spicatum* was collected from Lake Emily in north central Minnesota southeast of the town Emily, Minnesota. Native *M. sibiricum* was collected from Red Cedar Lake, WI next to Faust Island (Figure 4.1). To ensure collected macrophytes were *M. spicatum* and *M. sibiricum*, genetic analysis was conducted by AquaGen at Grand Valley State University, Michigan. Macrophytes were separated into bags, placed into coolers, and shipped overnight directly from the collection site to the growth chamber facility at U.S. Army Corps of Engineers’ Research and Development Center (USAERDC) in Vicksburg, Mississippi.
Mesocosms

This experiment was conducted using mesocosms within the growth chamber facility at USAERDC. Mesocosms provided an opportunity to replicate natural conditions within a controlled environment in order to manipulate and test ecological mechanisms that may negatively or positively influence interactions observed in nature. Mesocosms allowed for control of both water and ambient conditions. Further, it provided an opportunity to test trophic structure through strict control of species presence through food web reconstruction.

Macrophyte Cultivation

Macrophytes were standardized using the top 20 cm segment comprising of the apical meristem for the following treatments: 1. *M. spicatum* (*spicatum*, hereafter), 2. *M. spicatum* and *M. sibiricum* (*spicatum* mixed, *sibiricum* mixed, or mixed hereafter) and 3. *M. sibiricum* (*sibiricum*, hereafter). Each treatment was triplicated using four individual macrophyte segments in four 750 mL plastic cups containing Blackkow topsoil amended with Osmocote (1.85 g-L). To prevent suspension of sediment, a one inch layer of sand was added to the surface of the Blackkow top soil in each 750 mL plastic cup. Treatments were randomly assigned to nine 48L aquaria filled with Smart and Barko (1985) nutrient water in a controlled environment growth chamber. Aeration was provided by air stones in each chamber to prevent hypoxic conditions. For replication of optimal summer growing conditions, ambient temperature was programmed at 22º C with a photoperiod of 16:8 hrs (light: dark).
For cultivation, macrophytes and their associated epiphyton were provided an acclimation period of 7 days (Figure 4.2) before a 750 mL water sample was collected from each aquarium within 5 cm of apical meristems of the macrophytes. The 750 mL water samples were transferred to one, 48L aquarium to culture mixed epiphyton. By day 21, macrophytes had reached the water surface and to account for lake effect a 750 mL mixed epiphyton sample was transferred from the culture aquarium into each experimental aquaria for homogenization. The experiment ended prior to macrophyte senescence on day 49.

**Invertebrate Introduction**

Newly hatched Chironomids and Amphipods were purchased from Sachs Systems Aquaculture (St. Augustine, Florida, USA) for introduction of lower trophic levels in replicated treatments. Chironomids and Amphipods were used because they have been shown to feed on epiphytic algae and are an important dietary source for upper trophic level organisms, specifically young of year and omnivorous fish (Jaschinski et al., 2011). To model realistic conditions, target densities of Chironomids and Amphipods for each tank was extrapolated from reported densities collected from a one m² area in a lake at varying depths (Wetzel, 2001). It has been reported that Chironomids and Amphipods can reach densities up to 279 and 72 m² per day in lakes, respectively (Wetzel, 2001). Approximately, 100 Chironomids and Amphipods were introduced into each tank on day 21. Ten individual Chironomids and Amphipods were retained from introduction to provide a baseline isotopic value to ensure differences in assimilation of energy from the Chironomids and Amphipods introduced in treatments.
Sample Collection

Epiphyton were collected using procedures outlined by Aloi (1990). Briefly, twelve-5 cm apical meristem segments were removed from each replicated treatment using sterilized scissors and a Perspex tube sampler 5 cm in length. Apical meristems were transferred to individual 40 mL collection vials filled with 30 mL of reverse osmotic (RO) water. Vials were shaken vigorously by hand for 30 seconds to remove epiphyton from the surface of the apical meristems. Apical meristems were then removed from their vials and inspected under dissecting scope to confirm the absence of epiphyton on the apical meristem surface. Epiphyton not successfully removed by shaking were removed by gently brushing the surface of the apical meristem leaflets and placed into their associated vial. For macrophyte tissue, twelve additional 5 cm apical meristems were collected from each treatment for analysis of the ratios for stable isotopes, $^{15}$N and $^{13}$C. Apical meristems were rinsed to remove attached epiphyton and the absence of epiphyton on the surface was confirmed using a dissecting scope. Clean apical meristems were then placed into individual, sterilized 10 mL vials for analysis.

To recapture introduced Chironomids and Amphipods, growth medium and soil material from each replicated treatment were processed through continuous rinsing in 90 µm sieves. Macrophyte material was carefully removed separately from each tank and sorted to identify and collect organisms attached to their surface. Due to the small size of individual Chironomids and Amphipods, samples were pooled for each treatment to meet analytical weight requirements between 400 and 600 µg. All samples were placed on ice and transported back to Mississippi State University. All samples except epiphyton were vacuum filtered through a 0.1 µm Whatman glass filter paper to remove excess water.
Following filtration, all samples (including epiphyton) were transferred to sterilized vials and freeze dried for 96 hours before shipment to the Chemical Tracers Laboratory at Great Lakes Institute for Environmental Research (GLIER) at the University of Windsor, Canada.

**Analysis**

All stable isotope samples were analyzed using isotope mass spectroscopy at the Chemical Tracers Laboratory. To ensure homogeneity, freeze dried samples were ground to a powder using a ball mill (Spex SamplePrep 8000 Ball Mill/Mixer). Samples were weighed between 400 and 600 µg and encapsulated in tin capsules. Following encapsulation, samples were run through an Elemental Analyzer-Isotope Ratio Mass Spectrometer (CosTech and Thermo Delta V, respectively). Isotope values were expressed in standard notation as a ratio between the sample and appropriate standard (air for $^{15}$N and PeeDee Belemnite for $^{13}$C) in parts per thousand (‰) using the following equation:

$$\delta X^{\circ\circ} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$ \hspace{1cm} (4.1)

For quality control purposes, standards (air for $^{15}$N and PeeDee Belemnite for $^{13}$C) were run after every 13th sample and the 13th sample were triplicated to ensure analytical precision based on the standard deviation of an NIST standard (1577c Bovine Liver). Standard deviation of 11 standards analyzed for $\delta^{15}$N was 0.19 ‰ and for $\delta^{13}$C was 0.03‰. Differences in $\delta^{13}$C and $\delta^{15}$N values between treatments were assessed using linear models with $\delta^{13}$C and $\delta^{15}$N values as the response variable and isotope source as response variable. A Post-Hoc Generalized Linear Hypothesis Tests (GLHT) set with a
Tukey contrast was performed when linear models were significant in order to identify. Stable isotope Bayesian ellipses were used to estimate isotopic niche breadth and space. Size-corrected standard ellipse areas (SEAc) representing isotopic niche spaces were calculated from the residual δ^{13}C and δ^{15}N values for each isotopic source (Jackson et al., 2011). Differences in isotope values among macrophytes, epiphyton, and Amphipods were evaluated by comparing the overlap of standard ellipses among treatments. All statistical tests were performed using statistical program R (R Core Team, 2014) and because the goal of this research was to provide foundational support for further exploration, results were considered statistically significant at P <0.1.

**Results**

Mean δ^{13}C value of *spicatum* (-15.78 ± 1.67 SD) was slightly more depleted than *sibiricum* (-13.00 ± 0.84 SD), however, when both macrophytes are grown together, there appears to be no difference in their δ^{13}C ratios (Table 4.1). For δ^{15}N, *spicatum* and *sibiricum* were not different when grown alone, but in the mixed treatment, nitrogen was greater in *spicatum* mixed than *sibiricum* mixed (Z = -2.37, df = 1, P <0.08). The δ^{13}C and δ^{15}N values of epiphyton and Chironomids were not statistically different across treatments. The δ^{13}C values of Chironomids do not reflect a source accounted within my sampling structure, likely soil. Amphipods collected from *spicatum* were significantly more depleted of δ^{13}C in *spicatum* than *sibiricum* (Z = -2.94, df = 1, P<0.01). The δ^{15}N values of Amphipods did not differ among treatments. C:N ratios did not vary between Amphipods and Chironomids across treatments. However, C:N ratios of macrophyte tissue collected from *spicatum* was significantly greater than tissue collected from *sibiricum*. C:N ratios also were found to vary for epiphyton; epiphyton associated with
spicatum were significantly greater than both spicatum mixed and sibiricum mixed (Z = -2.33, df = 1, P<0.08 and Z = 2.40, df = 1, P<0.08, respectively).

Size corrected standard area ellipses (SEAc) showed that spicatum and sibiricum when grown alone have separated niche spaces with similar size niche breadths (Table 4.2, Figure 4.3). However, when both macrophytes were grown together, the niche breadth of sibiricum mixed encompassed the niche space of both, spicatum and sibiricum. The niche breadth of spicatum mixed overlapped the niche space of both sibiricum mixed and spicatum while completely encompassing the niche space of sibiricum. Epiphyton associated with spicatum had a narrow niche breadth and were shown to have overlapping niche space with epiphyton associated with sibiricum which had greater niche breadth (Table 4.2). Epiphyton collected from spicatum mixed had slightly greater niche breadth and overlapped niche space with epiphyton from spicatum and sibiricum.

For the primary consumers, niche breadth of Amphipods collected from spicatum was similar to the niche breadth of Amphipods collected from sibiricum (Table 4.3). The ellipse of Amphipods from spicatum did not overlap with the ellipse of epiphyton, however, the Amphipod ellipse from sibiricum partially overlapped the epiphyton ellipse indicating shared niche space. The ellipse of Amphipods from the mixed treatment had greater overlapped with epiphyton collected from sibiricum and spicatum. Chironomids did not differ across treatments for both δ13C and δ15N values.

Sample-size corrected standard area ellipses (SEAc) for the spicatum treatment indicated that spicatum had the largest niche breadth while epiphyton attached to the surface of spicatum had a smaller niche breadth. Ellipses for the mixed treatment indicated overlapping of niche space for spicatum and sibiricum when grown together,
however, they do not appear to share niche space with their associated epiphyton or Chironomids and Amphipods. Niche breadth of *spicatum* was greater than *sibiricum* and the other sources. Epiphyton associated with *spicatum* had overlapping niche space with both Chironomids and Amphipods while niche space of epiphyton associated with *sibiricum* only overlapped with Amphipods. Both *spicatum* epiphyton and *sibiricum* epiphyton ellipses overlapped each other indicating epiphyton from both species shared niche space. The *sibiricum* treatment had the smallest niche breadth and its niche space did not overlap with other sources in the treatment. However, epiphyton were shown to have a niche breadth that overlapped with Amphipods but not Chironomids. Ellipses of Chironomids and Amphipods did not overlap indicating different niche spaces.

**Discussion**

The results of this study indicate that *M. spicatum* and *M. sibiricum* may indirectly alter energy exchange in an aquatic system through their associated epiphyton. When grown independent of one another, ellipses of *M. spicatum* and *M. sibiricum* do not overlap, indicating they do not share niche space. The location of the ellipses indicate that both macrophytes may be using soil for nitrogen and receiving carbon through the production of CO$_2$. However, when both macrophytes were grown together, their niche breadth increased and overlapped suggesting competition for nutrient resources. *M. spicatum* niche breadth was four times larger in size in comparison to *M. sibiricum* suggesting that *M. spicatum* could shift from using soil nutrients, to exploiting nutrients from both soil and water. Such a shift provides some insight into how *M. spicatum* can become invasive into novel systems.
The results of stable isotope analysis for epiphyton, provide further support for previous results in Chapters 2 and 3, indicating there are likely differences in the colonizing epiphyton assemblages between *M. spicatum* and *M. sibiricum*. Epiphyton across treatments used similar niche space indicating epiphyton are possibly receiving nutrients from the water instead of the soil supporting their associated macrophytes. However, their ellipses were shown to differ in size across treatments indicating different niche breadths. The difference in niche breadth may be representative of a different epiphyton assemblage as each assemblage could differ in their niche breadth. In the mixed treatment, niche breadth of epiphyton associated with *M. spicatum* appear to similarly respond with epiphyton from *M. spicatum* grown alone, shifting towards increasing depletion of $\delta^{13}C$ but slightly greater enrichment of $\delta^{15}N$. However, epiphyton sampled from *M. sibiricum* were shown to be more enriched in both $\delta^{13}C$ and $\delta^{15}N$ compared to *M. spicatum*.

Although I had a small sample size of Chironomids, niche breadth and space of Chironomids do not appear to change across treatments. Results suggest that neither macrophyte nor epiphyton are a carbon source for Chironomids which could explain the separation of niche space. It is possible the Chironomids are receiving their carbon and nitrogen by feeding upon epipelon from the soil providing an explanation for why Chironomids share similar $\delta^{15}N$ values with both, *M. spicatum* and *M. sibiricum*, but not their associated epiphyton.

Isotope ellipses for Amphipods among treatments were shown to be separated with minimal overlapping. Amphipods from *M. spicatum* and *M. sibiricum* appear to have similar size niche breadth. Stable isotope $\delta^{15}N$ values indicate that Amphipods are
processing nitrogen similarly, but Amphipods from *M. spicatum* were more depleted of δ¹³C than Amphipods from *M. sibiricum* and the mixed treatment. δ¹³C values of Amphipods from *M. spicatum* indicate they may be disconnected from *M. spicatum* and associated epiphyton. The disconnection of carbon indicates Amphipods were not capable of foraging on epiphyton from *M. spicatum* or not capable of assimilating the material into their tissues. The disconnection of carbon within Amphipods from *M. spicatum* does not directly support previous work (Kovalenko & Dibble, 2013) indicating invertebrates reflected *M. spicatum* δ¹³C values, but does provide an alternative insight as to why fish collected from monoculture beds of *M. spicatum* may not reflect the δ¹³C values of this macrophyte. Specifically, if fish are foraging on invertebrates that reflect similar foraging patterns and isotopic values of the Chironomids and Amphipods in my study, then a disconnection in energy flow from macrophyte to secondary consumer could occur. The disconnection of carbon does not occur for Amphipods collected from *M. sibiricum*. In the *M. sibiricum* treatment, Amphipods are likely influenced by the epiphyton which have been shown to have greater carbon and enriched nitrogen values, indicating they are likely a better food source.

The effect of introduced non-native species on trophic dynamics should be further explored through experimental and field-based studies. Continued research is needed to increase our understanding of how an introduced non-native species can become invasive and may alter energy exchange from lower to higher trophic levels. By understanding these ecological relationships, we can set forth to better manage our natural systems and justify our efforts in preventing the spread of species that can negatively affect them.
### Tables and Figures

Table 4.1  Isotopic ratios (Mean ± SD) for $\delta^{13}$C and $\delta^{15}$N collected from each source sample across treatments.

<table>
<thead>
<tr>
<th>Source</th>
<th>$\delta^{13}$C ± SD</th>
<th>$\delta^{15}$N ± SD</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macrophyte</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spicatum</td>
<td>-15.78 ± 1.67</td>
<td>5.68 ± 1.93</td>
<td>6</td>
</tr>
<tr>
<td>spicatum mixed</td>
<td>-15.83 ± 4.51</td>
<td>1.87 ± 6.04</td>
<td>6</td>
</tr>
<tr>
<td>sibiricum mixed</td>
<td>-15.03 ± 3.04</td>
<td>6.45 ± 3.43</td>
<td>6</td>
</tr>
<tr>
<td>sibiricum</td>
<td>-13.00 ± 0.84</td>
<td>6.49 ± 0.78</td>
<td>6</td>
</tr>
<tr>
<td><strong>Epiphyton</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spicatum</td>
<td>-22.76 ± 2.74</td>
<td>1.47 ± 0.72</td>
<td>5*</td>
</tr>
<tr>
<td>spicatum mixed</td>
<td>-24.16 ± 3.44</td>
<td>0.87 ± 2.30</td>
<td>4*</td>
</tr>
<tr>
<td>sibiricum mixed</td>
<td>-23.32 ± 1.81</td>
<td>3.93 ± 3.90</td>
<td>4*</td>
</tr>
<tr>
<td>sibiricum</td>
<td>-21.97 ± 2.06</td>
<td>1.37 ± 2.96</td>
<td>6*</td>
</tr>
<tr>
<td><strong>Chironomids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spicatum</td>
<td>-27.82 ± 2.04</td>
<td>5.57 ± 2.48</td>
<td>3*</td>
</tr>
<tr>
<td>sibiricum</td>
<td>-27.82 ± 1.24</td>
<td>6.78 ± 1.42</td>
<td>2*</td>
</tr>
<tr>
<td>mixed</td>
<td>-28.09 ± 1.68</td>
<td>4.33 ± 4.03</td>
<td>2*</td>
</tr>
<tr>
<td>culture</td>
<td>-25.11</td>
<td>5.44</td>
<td>1</td>
</tr>
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<td><strong>Amphipods</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spicatum</td>
<td>-25.93 ± 0.81</td>
<td>3.23 ± 0.98</td>
<td>6*</td>
</tr>
<tr>
<td>sibiricum</td>
<td>-24.09 ± 1.04</td>
<td>3.12 ± 0.67</td>
<td>6*</td>
</tr>
<tr>
<td>mixed</td>
<td>-24.64 ± 0.78</td>
<td>3.02 ± 1.07</td>
<td>6*</td>
</tr>
<tr>
<td>culture</td>
<td>-24.19</td>
<td>3.54</td>
<td>1</td>
</tr>
</tbody>
</table>

Notes: * indicates pooled samples.
Table 4.2  Proportion of standard ellipse area overlap between basal resources and primary consumers in Treatment 1: *spicatum*.

<table>
<thead>
<tr>
<th>Source</th>
<th><em>spicatum</em></th>
<th>epiphyton</th>
<th>Amphipods</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>spicatum</em></td>
<td>—</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>epiphyton</td>
<td>0</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>Amphipods</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 4.3  Proportion of standard ellipse area overlap between basal resources and primary consumers in Treatment 3: *sibiricum*.

<table>
<thead>
<tr>
<th>Source</th>
<th><em>sibiricum</em></th>
<th>epiphyton</th>
<th>Amphipods</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>sibiricum</em></td>
<td>—</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>epiphyton</td>
<td>0</td>
<td>—</td>
<td>0.01</td>
</tr>
<tr>
<td>Amphipods</td>
<td>0</td>
<td>0.06</td>
<td>—</td>
</tr>
</tbody>
</table>
Table 4.4 Proportion of standard ellipse area overlap for basal resources and primary consumers in Treatment 2: mixed.

<table>
<thead>
<tr>
<th>Source</th>
<th>spicatum</th>
<th>sibiricum</th>
<th>spicatum epiphyton</th>
<th>sibiricum epiphyton</th>
<th>Amphipods</th>
</tr>
</thead>
<tbody>
<tr>
<td>spicatum</td>
<td>—</td>
<td>0.14</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>sibiricum</td>
<td>0.23</td>
<td>—</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>spicatum epiphyton</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>0.09</td>
<td>0.02</td>
</tr>
<tr>
<td>sibiricum epiphyton</td>
<td>0</td>
<td>0</td>
<td>0.19</td>
<td>—</td>
<td>0.06</td>
</tr>
<tr>
<td>Amphipods</td>
<td>0</td>
<td>0</td>
<td>0.33</td>
<td>0.53</td>
<td>—</td>
</tr>
</tbody>
</table>
Table 4.5  Size-corrected standard ellipse areas (SEAc).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>SEAc</th>
<th>n</th>
<th>SEAc</th>
<th>n</th>
<th>SEAc</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>spicatum</em></td>
<td>6</td>
<td>6.39</td>
<td>5</td>
<td>3.20</td>
<td>6</td>
<td>1.52</td>
</tr>
<tr>
<td>mixed</td>
<td>6</td>
<td>31.52</td>
<td>4</td>
<td>17.56</td>
<td>6</td>
<td>1.32</td>
</tr>
<tr>
<td><em>sibiricum</em> mixed</td>
<td>6</td>
<td>19.32</td>
<td>4</td>
<td>7.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.31</td>
<td>6</td>
<td>11.73</td>
<td>6</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Notes: Size-corrected standard ellipse areas (SEAc) calculated from residual values of basal resource and primary consumer $\delta^{13}$C and $\delta^{15}$N linear models among treatments.

Table 4.6  Mean, min, and max values for $\delta^{13}$C and $\delta^{15}$N values.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$\delta^{13}$C values</th>
<th>$\delta^{15}$N values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Min, Max</td>
</tr>
<tr>
<td><em>spicatum</em></td>
<td>-27.82</td>
<td>-30.01, -25.97</td>
</tr>
<tr>
<td>mixed</td>
<td>-28.09</td>
<td>-29.28, -26.90</td>
</tr>
<tr>
<td><em>sibiricum</em></td>
<td>-27.82</td>
<td>-28.69, -26.94</td>
</tr>
</tbody>
</table>

Notes: Mean, min, and max values for $\delta^{13}$C and $\delta^{15}$N values collected from Chironomids across each treatment. Values were used to make overlap areas.
Figure 4.1  Map of locations where macrophytes were collected.

Notes: Map of locations where macrophytes were collected identified by the white circle on each map. *M. spicatum* was collected from the western littoral zone of Emily Lake, MN. *M. sibiricum* was collected from the south-western littoral zone of Faust Island, Red Cedar Lake, WI.

Figure 4.2  Timeline identifying important steps by day through the duration of the experiment.
Figure 4.3  Bayesian sample-size corrected standard ellipses (SEAc) created using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

Notes: Bayesian sample-size corrected standard ellipses (SEAc) created using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for macrophyte tissue, epiphyton, Amphipods, and Chironomids sampled from the three treatments: *spicatum* (A), *sibiricum* (B), and mixed (C).
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widths among and within communities: SIBER-stable isotope Bayesian ellipses in


CHAPTER V
SUMMARY

This study is, to the best of my knowledge, the first to demonstrate differences in epiphyton genera between *M. spicatum* and *M. sibiricum*. As mentioned previously, epiphyton play an important role as a basal resource in the trophic structure of aquatic systems. Their role was evident in Chapter 4 as Amphipods were shown to not forage on epiphyton from *M. spicatum*, but did utilize epiphyton attached to *M. sibiricum*. This is important because Amphipods are a common invertebrate primary consumer in aquatic systems that are used as a source of energy for a multitude of fish. Therefore, if Amphipods are similar to other invertebrate primary consumers in their selection of forage, and if fish in monoculture beds of *M. spicatum* are foraging on these invertebrates, the results of my study would explain why fish may not represent the associated $\delta^{13}$C values of *M. spicatum* or epiphyton attached to the surface of *M. spicatum*. Thus, if *M. spicatum* is successfully introduced and displaces native littoral species that are indirectly responsible for higher trophic energy exchange through colonization of epiphyton, efforts to manage populations of fish and other aquatic organisms may be difficult because of the loss of basal energy resources.

Further, epiphyton colonies may still be influenced by the allelochemicals produced by both macrophytes, even though I was not capable of quantifying exuded allelochemicals in the water. My results provide support for previous research (See 67
Chapters 1, 2, and 3) indicating concentrations of allelochemicals in apical meristems of *M. spicatum* are relatively greater than those in apical meristems of *M. sibiricum*. The effects of these allelochemicals may be scale dependent and variation likely occurs temporally (See Chapter 3). This is important to recognize when designing future experiments investigating the effects of allelochemicals on assemblage composition of primary producers upon or around the focal species or when testing hypotheses such as the novel weapons hypothesis. Depending on how and when you sample may result in different outcomes that may or may not reflect the ecological health of the system.

An expansion of niche breadth was shown in Chapter 4 for both macrophytes. When the macrophytes grow separated from another, macrophytes appear to use soil for their nitrogen, but when introduced together, a switch occurs and they begin to take up nutrients from both the soil and water. While this expansion occurs for both macrophytes, *M. spicatum*’s niche expansion was nearly three times greater than *M. sibiricum*, which possibly provides an explanation as to why *M. spicatum* can become invasive by outcompeting *M. sibiricum* and other macrophytes following introduction. However, the mechanism responsible for the expansion of both plants is still unknown. One possible explanation may be that this expansion occurs when allelochemicals or other secondary metabolites released from one plant come into contact with another causing competition for resources they would otherwise not use if separate from one another.

Further, *M. spicatum* concentrations of condensed tannins were also identified to be greater in *M. spicatum* than *M. sibiricum* which may be used similarly by terrestrial plants to prevent herbivory, providing *M. spicatum* another mechanism to reduce direct effects on its growing capacity. This may also explain why Amphipods from the *M.*
spicatum treatment were not capable of foraging upon epiphyton attached to M. spicatum. This could indirectly cause a decoupling of energy to occur from primary producer to secondary consumer in monoculture beds of M. spicatum if, in fact, condensed tannins produced by M. spicatum prevent grazers from foraging.

Understanding these mechanisms are important to applied ecology, specifically in management of invasive plants because they provide insight into what may positively or negatively influence a system. With this knowledge, managers can make better decisions when developing plans of action for managing of the natural resources within their systems. The results of my study provide managers information regarding epiphyton assemblages and how they differ between a non-native and native macrophyte of the same genus. Additionally, my results provide an insight into how these differing epiphyton assemblages serve as a trophic foundation for higher trophic levels and how these epiphyton assemblages may alter energy flow to these higher trophic levels which is important to know when managing a freshwater ecosystem.

To further increase our understanding of macrophyte-epiphyton relationships through mechanistic approaches, I suggest a causal pathway diagram (Figure 5.1) that may be used to guide an investigator’s study through indication of different macrophyte-epiphyton relationships and how they may have an effect on each other. Each arrow in the diagram provides a possible pathway leading to an interaction that may or may not be competitive in nature. Future investigation of these pathways may provide critical information regarding the mechanisms that may further explain the invasiveness of M. spicatum or how it may affect energy exchange through its influence on epiphyton.
Tables and Figures

Figure 5.1  Suggested directions of investigating causal pathways between *M. spicatum* and its associated epiphyton.

Figure 5.2  Canonical correspondence analysis.

Notes: Canonical correspondence analysis from Chapter III of treatments and density of epiphyton genera correlated with allelochemicals.