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Patterns of Amphipod Feeding and Phenolic Content in Apical and Middle Portions of Invasive Myriophyllum spicatum and Native Ceratophyllum demersum

Meghan Kebalka

Patterns of amphipod feeding and phenolic content in apical and middle portions of invasive *Myriophyllum spicatum* and native *Ceratophyllum demersum*

Meghan Kebalka and *LaTina Steele

Biology Department, Sacred Heart University, Fairfield, CT 06825

*Faculty mentor

Sacred Heart University
DEPARTMENT OF BIOLOGY

Abstract

Although the factors leading to successful submerged plant invasions are poorly understood, high levels of chemical feeding deterrents in invasive plant species may be partly responsible. However, chemical deterrents are not always uniformly distributed within plant tissues, and little is known about how that may affect herbivore feeding patterns in aquatic and marine systems. To examine the distribution of phenolics within the tissues of invasive *Myriophyllum spicatum* and native *Ceratophyllum demersum*, 2-cm fragments were taken from the apex and middle portions of 10 individuals of each species. A two-way ANOVA showed that phenolic content in *M. spicatum* plants was significantly higher than in *C. demersum* but detected no significant differences in phenolics between apical and middle tissues in these species. A no-choice feeding experiment showed that amphipods, the dominant herbivore at our study site, consumed significantly more middle than apical *C. demersum* tissue and ate very little *M. spicatum*. A choice feeding experiment confirmed that amphipods preferred the low-phenolic native *C. demersum* to the phenolic-rich, invasive *M. spicatum*. Although we expected apical portions of *C. demersum* to contain more phenolics than middle segments, this was not the case, suggesting that factors other than phenolics contributed to the feeding preferences we observed and that future studies should seek to identify these factors.

Introduction

Eurasian watermilfoil (*Myriophyllum spicatum*) is one of the most notorious invasive aquatic plants in the U.S. and can outcompete many native species[1]. The mechanisms leading to successful aquatic plant invasions are poorly understood, but production of allelopathic phenolic compounds by *M. spicatum* is well-documented[2] and may contribute to its successful takeover in some waterways. Phenolics have been associated with reduced herbivory on *M. spicatum*'s apical tissues by aquatic insects[2], suggesting that this species selectively protects its apical meristem, as predicted by the optimal defense theory. However, the role of chemical defenses (e.g., phenolics) in milfoil invasions has received relatively little attention, particularly in comparison to chemical deterrent production in native competitors. This study investigated the role of chemical feeding deterrents in aquatic plant invasions by comparing phenolic levels in apical and middle portions of *C. demersum* and *M. spicatum* individuals, as well as assessing amphipod feeding on the same tissues.

Hypotheses:

1. Amphipods consume more native *Ceratophyllum demersum* (coontail) than invasive *Myriophyllum spicatum* (milfoil) and more tissue from middle than apical fragments of either species when presented with a single food option
2. Amphipods prefer coontail to milfoil when offered the two plants simultaneously
3. Phenolic levels are higher in apical than in middle fragments of native coontail and invasive milfoil stems, with milfoil containing more phenolics than coontail

Methods

Amphipod Feeding Experiments

- Native coontail, invasive milfoil, and amphipods (*Hyalella azteca*) were collected from Osbourndale Pond in Derby, CT (Fig. 1)
- Experiments were conducted in 500 ml bowls containing ~250 ml of bubbled tap water in the lab under grow lights on a 12 hour light: 12 hour dark cycle
- **No-choice experiment**: five replicates each of four independent treatments (milfoil apex, milfoil middle, coontail apex, coontail middle; Fig. 2)
    - One 2-cm apex or middle fragment was taken from 10 different individuals of each species (coontail and milfoil), blotted on paper towels, and weighed
    - One fragment was placed into each experimental bowl containing 10 amphipods
    - Fragments were weighed again after one week
- **Choice experiment**: 10 replicates of each paired treatment (coontail with milfoil)
    - One pre-weighed 2-cm plant fragment from the middle portion of each species (Fig. 2) was placed into each experimental bowl containing 10 amphipods
    - Fragments were weighed again after one week

Plant Phenolic Analysis

- 2-cm fragments from the apical and middle portions of coontail and milfoil (Fig. 2) were flash frozen at -80°C, then freeze-dried, ground in liquid nitrogen, and stored at - 80°C
    - Fragments from each species were pooled by tissue location to ensure sufficient tissue for analysis (n = 5 pooled samples each of milfoil apex, milfoil middle, coontail apex, coontail middle)
    - 1 mM ascorbic acid in 70% acetone was used to extract phenolics from the tissues, and total reactive phenolics were measured using the colorimetric Folin-Denis assay

Figure 1. Plant and amphipod collection at Osbourndale Pond (Derby, CT).

Figure 2. Areas denoted as apex and middle in *Myriophyllum spicatum*.

Results

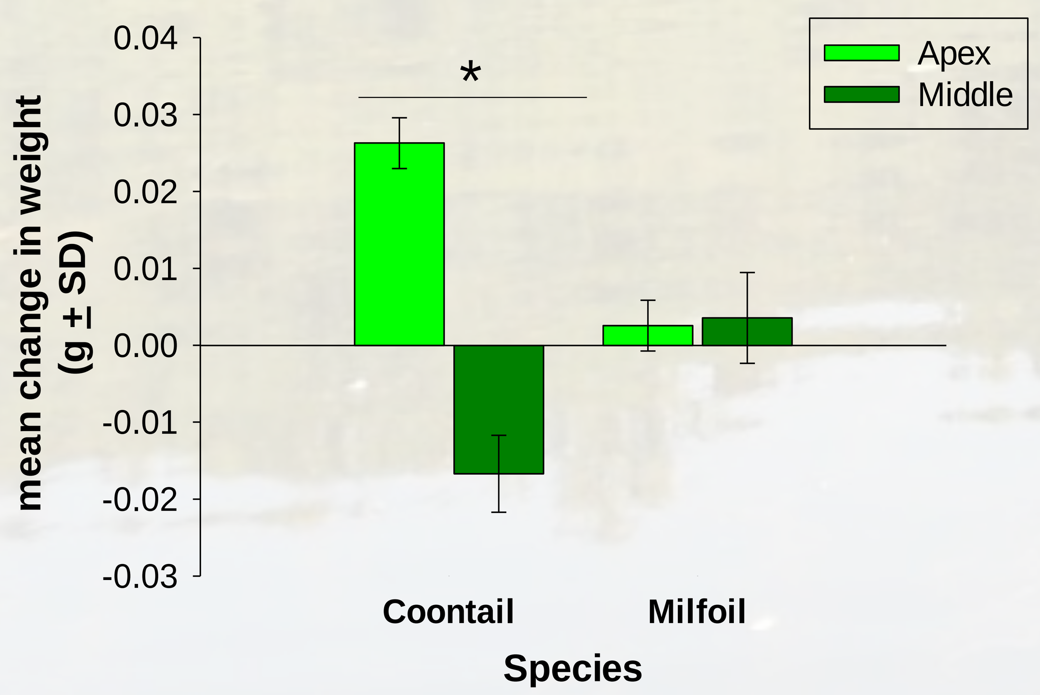

Figure 3. Mean change in weight (g$\pm$ 1 SD) of native *Ceratophyllum demersum* (coontail) and invasive *Myriophyllum spicatum* (milfoil) fragments from apical and middle portions of the stem after exposure to 10 amphipods for one week in a no-choice feeding experiment. Two-way ANOVA detected no significant difference in growth between species, but there was a difference in growth among tissue locations, as well a **significant interaction between species and tissue location** (Two-way ANOVA: Tissue Location $F_{1,16}$=111.786, p<0.001; Species $F_{1,16}$=0.769 p=0.393; Interaction: $F_{1,16}$=122.751, p<0.001). **Apex fragments grew significantly more than middle fragments in coontail** (Independent samples t-test with Bonferroni correction: t_9=16.136, p<0.001).

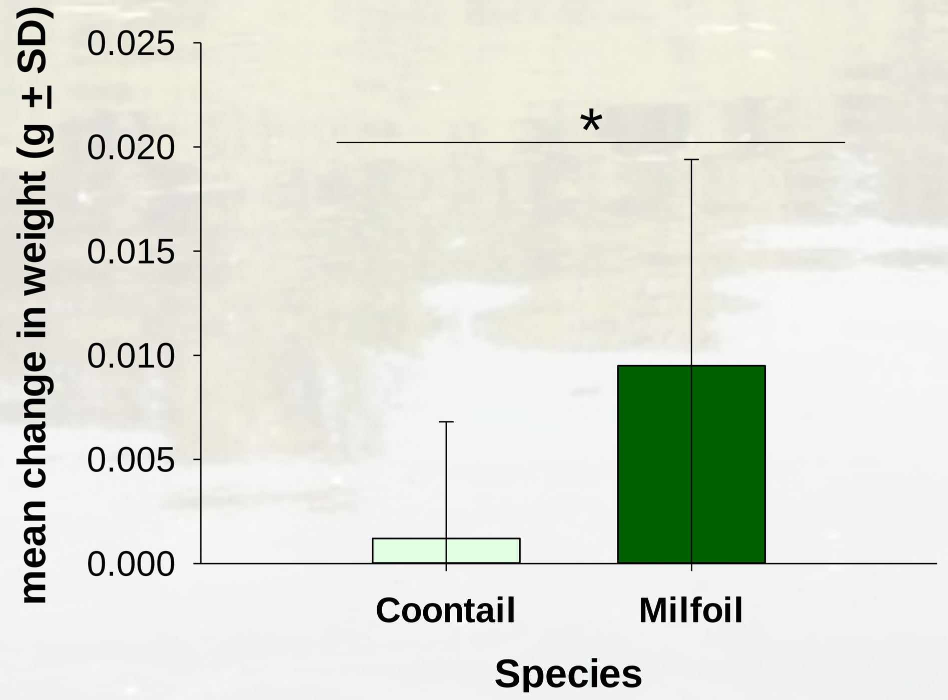

Figure 4. Mean change in weight (g$\pm$ 1 SD) of native *Ceratophyllum demersum* (coontail) and invasive *Myriophyllum spicatum* (milfoil) fragments after exposure to 10 amphipods for one week in a choice feeding experiment. **Invasive milfoil grew significantly more than native coontail** (Paired t-test: t_9=2.536, p=0.032).

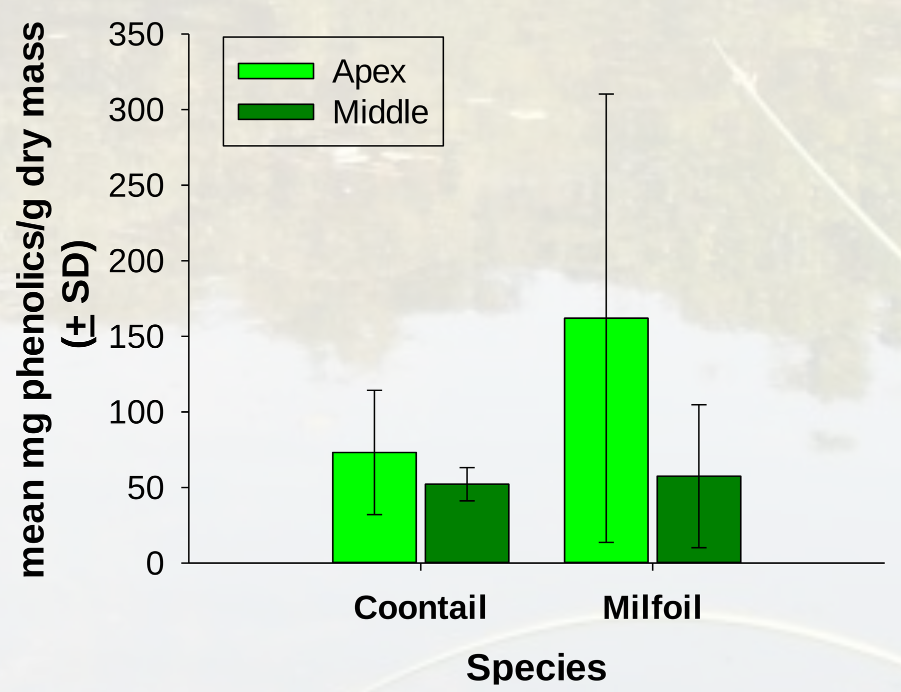

Figure 5. Mean mg phenolics g dry mass^{-1} ($\pm$ 1 SD) in samples from the apex and middle sections of native *Ceratophyllum demersum* (coontail) and invasive *Myriophylum spicatum* (milfoil) stems. **Phenolic content was significantly higher in invasive *M. spicatum* than in native *C. demersum*** (Two-way ANOVA $F_{1,16}$=22.947, p<0.001). There was no significant difference in phenolic levels in apical and middle sections of these species (Two-way ANOVA $F_{1,16}$=1.243, p=0.281).

Discussion & Conclusions

Amphipod Feeding Experiments

Amphipods consumed significantly more middle than apical tissue from coontail in the no-choice feeding experiment (Fig. 3), as we predicted based on previous studies[2]. However, amphipods consumed similar amounts of apical and middle tissues from milfoil. We did not detect a significant difference in amphipod consumption of the two plant species, possibly due to a significant interaction between species and tissue location (Fig. 3). In the choice experiment, amphipods consumed more native coontail than invasive milfoil (Fig. 4), consistent with our hypothesis.

Plant Phenolics

Phenolic levels were higher in milfoil tissues than in coontail tissues, and phenolic levels appear to be higher in apical tissue of both species than in middle tissue[2], although the difference was not statistically significant (Fig. 5). A larger sample size than our n of 5 may be necessary to detect a significant difference in phenolics among tissue locations, since phenolic levels were highly variable (Fig. 5).

Conclusions

The high levels of phenolics in invasive milfoil tissues compared to those of native coontail (Fig. 5), coupled with amphipods' feeding patterns (Fig. 3 & 4), suggest that chemical feeding deterrents may increase grazing pressure on native coontail in the presence of non-native milfoil. This shift in grazing pressure may allow the non-native milfoil to gain a foothold when it is introduced to areas dominated by coontail. Although milfoil can outcompete and eliminate native species in some cases[1], It is unlikely that it will extirpate coontail, given that coontail's apical meristem, which is critical for regrowth, is resistant to herbivory (Fig. 4). It is unclear from our results if such resistance is due to phenolics (Fig. 5) or other chemical or physical defenses.

Literature Cited

1. Zhu B, Georgian SE. 2014. Plant Ecology 7(6):499-508.
2. Fornoff F, Gross EM. 2014. Oecologia 175:173-185

Acknowledgements

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