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Phenolic levels of invasive *Myriophyllum spicatum* and native *Elodea canadensis* at different temperatures

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Abstract

Eurasian watermilfoil (*Myriophyllum spicatum)* is an invasive aquatic plant that may dominate lakes and waterways throughout the U.S. It is known to crowd out native plants and create dense mats that interfere with recreational activity. Milfoil also produces allelopathic chemicals, such as phenolics, that may give the invasive plant a competitive advantage over native species like *Elodea canadensis*. In a laboratory competition experiment, we tested the effects of competition and temperature on growth and phenolic levels in both *Myriophyllum spicatum* and *Elodea canadensis*. The experiment consisted of six replicates each of six treatments (*M. spicatum* alone, *E. canadensis* alone, and the two species grown together, with each combination of plants grown at ambient temperature and with the temperature increased by 2°C using individual aquarium heaters). After three weeks, the length of each plant fragment was measured, the number of branches on each fragment was counted, each fragment was weighed, and mean growth per fragment was calculated as the change in weight. Plant fragments were frozen for phenolic analysis at the end of the experiment. In general, *M. spicatum* showed more growth and produced more phenolics than *E. canadensis* in all treatments. At higher temperatures *E. canadensis* produced fewer phenolics than at lower temperatures. Based on these results, it is possible that rising temperatures due to climate change will increase invasive milfoil's competitive advantage over native *E. canadensis* by shifting herbivory to the less chemically defended native plant.

Introduction

- Eurasian watermilfoil (*Myriophyllum spicatum*) is a common invasive plant in the U.S. with the ability to outcompete many native species[1].
- Milfoil produces allelopathic phenolic compounds[2], which may contribute to its successful takeover of some waterways.
- Although phenolics are well-known to reduce herbivory, the role of chemical deterrents (e.g., phenolics) in successful invasions remains poorly understood, especially in the face of rapidly rising temperatures.
- This experiment examined the potential effect of climate change on competitive interactions between invasive *M. spicatum* (milfoil) and native *Elodea canadensis* by comparing growth and phenolic compound production in the two species at ambient temperatures and at a temperature 2°C higher, in line with the minimum temperature increases predicted by most climate change models.

Results

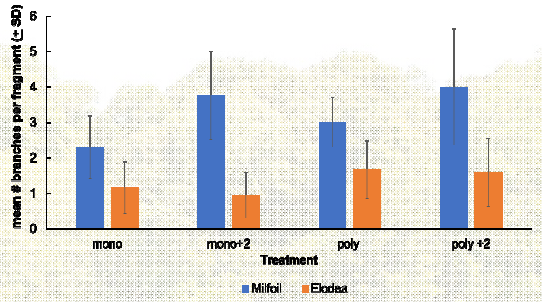

Figure 3 (above). Mean number of branches per *Myriophyllum spicatum* (milfoil) and *Elodea canadensis* fragment (± 1 SD) grown in monoculture, monoculture with a 2°C increase in temperature, polyculture, and polyculture with a 2°C increase in temperature. There was no significant effect of treatment on the number of branches (2-way ANOVA $F_{3,40}$= 2.285, p = 0.094), but milfoil fragments branched significantly more than *Elodea* fragments (2-way ANOVA $F_{1,40}$= 43.813, p < 0.001).

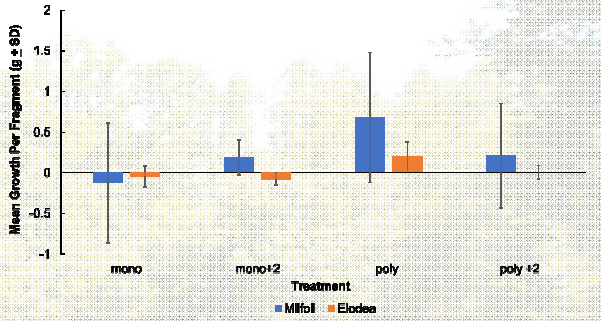

Figure 4 (above). Mean growth per fragment (g ± 1 SD) in competition experiment tanks containing *Myriophyllum spicatum* (milfoil) and *Elodea canadensis* grown in monoculture, monoculture with a 2°C increase in temperature, polyculture, and polyculture with a 2°C increase in temperature. There was no significant effect of either treatment (2-way ANOVA $F_{3,40}$= 2.781, p = 0.053) or species on growth per fragment (2-way ANOVA $F_{1,40}$= 2.672, p = 0.110), although milfoil growth was generally higher than *Elodea* growth.

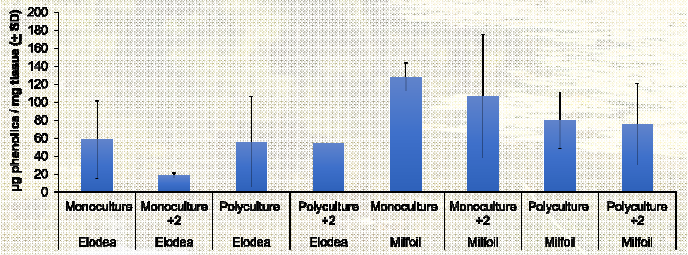

Figure 5 (left). Phenolic concentration (mean µg phenolics / mg tissue ± 1 SD) in competition experiment tanks containing fragments of *Myriophyllum spicatum* and *Elodea canadensis* grown in monoculture, monoculture with a 2°C increase in temperature, polyculture, and polyculture with a 2°C increase in temperature (One-way ANOVA $F_{7,31}$= 14.744, p < 0.001).

Methods

- Invasive milfoil (*Myriophllum spicatum*) and native *Elodea canadensis* were collected from Osbourndale Pond in Derby, CT for use in a lab competition experiment.
- The experiment consisted of the following treatments grown at ambient temperature and at a temperature 2°C above ambient (six replicates each): milfoil in monoculture, *Elodea* in monoculture, a polyculture of both species (Fig. 1).
- Plant fragments (10 cm each) were weighed and placed in 2-gallon tanks containing 1 inch of sand, aerated tap water, a thermometer, an air stone, and an aquarium heater (4 fragments per tank).
- At the end of the experiment, fragments were weighed, the number of branches was counted, and plant tissue samples were frozen for later phenolic analysis.
- A colorimetric assay (Folin-Denis assay) was used to measure total reactive phenolic concentrations in the plant tissue (Fig. 2).

Figure 1. Competition experiment tanks.

Figure 2. Folin-Denis assay for total reactive phenolics.

Conclusions

- Invasive milfoil appeared to outperform native *Elodea* in terms of both weight and number of branches (Figs. 3 & 4).
- Phenolic production was lower in *Elodea* than in milfoil in all treatments (Fig. 5).
- A small increase in temperature, consistent with predictions of even the most conservative climate change models, reduced phenolic production in native *Elodea* but not milfoil (Fig. 5).
- As temperature continues to increase with global climate change, the rate at which milfoil outcompetes and overgrows *Elodea* may increase if the lower production of phenolics in *Elodea* at higher temperatures causes herbivory on the native species to increase.

Literature Cited

1. Zhu B, Georgian SE. 2014. Interactions between invasive Eurasian watermilfoil and native water stargrass in Cayuga Lake, NY, USA. Plant Ecology 7(6):499-508.
2. Gross EM, Meyer H, and Schilling G. 1996. Release and ecological impact of algicidal hydrolysable polyphenols in *Myriophyllum spicatum*. Phytochemistry 41: 133-138

Acknowledgements

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