The potential of Elophila obliteralis larvae (waterlily leafcutter moth) as a biological control for the invasive aquatic plant Nymphoides peltata (yellow floating heart)

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Abstract

We tested the potential of Elophila obliteralis larvae (waterlily leafcutter moth, native to the eastern United States) as a biological control for Nymphoides peltata (yellow floating heart). N. peltata , an invasive aquatic floating-leaved plant in the United States, grows in mats creating profound shade reducing macrophyte community biodiversity below the water's surface. The larvae cut pieces from its host plant's foliage to live in and consume. It is hypothesized that the E. obliteralis larva has potential as a biological control for N. peltata ; as the density of larvae to leaf SA (surface area (cm 2)) increases, a greater decline of leaf SA occurs. To determine the ability of the larvae to control the plant, different density ratios of larvae: leaf SA (1:8, 1:4, and no larva=control) were observed over 5 weeks, measuring the progression using Leafscan app. The higher density of larvae to leaf SA resulted in more foliage consumed. The treatments had a mean percent change ± standard deviation of +0.43% ± 22.26% SD (control), -40.37% ± 29.48% SD (1:8), and -71.27% ± 21.95% SD (1:4) from week 0-5. A one-way repeated measure ANOVA found a statistically significant difference among treatment groups (F= 10.51, p=0.002), indicating a significant effect of the larvae on plant SA. Observationally, the larvae impacted regrowth of the plant. These results indicate that E. obliteralis is a potential biological control for N. peltata . Future research should be conducted.

Introduction:

Invasive aquatic plants cause great damage to the natural function of an ecosystem and harm economic interests related to human use of aquatic ecosystems (Li et al 2012). One study estimated that the loss from invasive species, including plants as well as animals, has cost the United States nearly $100 billion from 1906–1991 alone (Office of Technology Assessment of the U.S. Congress 1993). To the best of our knowledge, there is no national financial assessment on the economic impacts of *Nymphoides peltata* in the United States, however, several studies have documented the spread of the plant in Sweden, where the plant has spread dramatically. In the river Arbogaån, a single plant was placed in 1933. By 1975, it had spread to cover an area of 0.45km^2 (111 acres) (Josefsson and Andersson 2001). A single *N. peltata* plant has been shown to produce over 100 new individual plants by means of vegetative reproduction in just 12 weeks (Zhonghua et al 2007). A 2009 study estimated that the cost to control *N. peltata* in Sweden in 2006 ranged from 28–73 million SEK, which is equal to about 3.8–9.9 million USD (Gren et al 2009). Once in an environment, *N. peltata* outcompetes native and other present species for nutrients (Nault and Mikulyuk 2009). The floating leaves block nearly all penetrating sunlight, outcompeting submersed plants, and limit the diffusion of oxygen into the water, reducing habitat quality for aquatic animals (Wu and Yu 2004; Nault and Mikulyuk 2009). Overgrowth of the plant can also be harmful to boat traffic, housing areas, and recreational activities such as fishing and swimming (Carlsson and Kataria 2008).

Management of this species requires intensive efforts to mitigate and prevent their harmful impact. The most common management methods include the use of mechanical or physical control, herbicides, biological control, or a combination of these methods (Madsen 2000; Wainger et al 2018). Mechanical methods involve physically removing the plant with equipment or physically blocking the plant from spreading by manipulating the environment. Herbicides are a popular method, however, the use of chemicals can have adverse effects on non-target species in the ecosystem (Madsen 2000). Biological controls use a host-specific organism that targets the invasive plant to suppress its growth and spread (Harms and Nachtrieb 2019). The interaction between the plant and its natural enemy is the central determining factor for expanding or controlling the spread of the plant. However, using a natural enemy from where the plant originated as the biological control would cause an additional exotic species to be introduced into the already invaded area and they may not target the species as expected in the new area (Colautti et al 2004). Therefore, the ideal biological control organism is a native pest insect that has shown to cause damage. If the organism can be introduced without its own predators, populations may show rapid growth and suppress the target plant species. The use of biological controls is a self-sustaining effort that can produce a long-term solution with economic and ecological benefits (Wainger et al 2018).

Yellow floating heart (Pfingsten et al 2021) is a floating-leaved plant native to Eurasia, the Mediterranean, and Eastern Asian countries including China, Japan, and India (Nault and Mikulyuk 2009). It is able to grow in wetlands, lakes, ponds, swamps, damp muddy areas, and other slow-moving bodies of water including ditches and waterways at depths less than 3 meters ("Nymphoides peltata (yellow floating-heart)." n.d.). The first record of *N. peltata* in the United States was in 1882 in Winchester, Massachusetts. Into the 1890s, multiple United States Fish Commission ponds reported the plant being 'naturalized and spreading into adjacent ponds'. It was later reported to have been found in the Hudson River in 1929, with the origin thought to be escaped plants from water gardens. *N. peltata* was reported in Ohio in 1930 (Stuckey 1973), and it has since spread across the United States and Canada, currently in at least 29 states (Pfingsten et al 2021). Although this plant has not spread as efficiently in the time it has been in the United States as other plants like *Hydrilla* which has multiple ways of spreading and has cost the Florida Fish and Wildlife Conservation Commission $5 to $15 million per year between 2015 and 2019 to control it (FWC 2019), *N. peltata*'s ability and ways of spreading should not be underestimated. It can be bought over the internet and through mail order, creating an easy opportunity for it to spread (Nault and Mikulyuk 2009). Only six states have designated *N. peltata* as a noxious weed or higher (CT, ME, MA, OR, VT, WA) and is not designated as a noxious weed in the United States as a whole (USDA 2021). Since 2018, Ohio has listed *N. peltata* as invasive, meaning it cannot be sold or propagated, however this is a different designation from noxious weed (Invasive Plant Species 2018). Being categorized as a noxious weed puts government control and restrictions on moving and selling the plant, and authorities can take actions to contain or destroy the plant to further limit its spread within the country or state (USDA 2021).

N. peltata is a perennial bottom-rooted aquatic plant with long branching stolons up to 2 meters long and roots below the water's surface. Plantlets grow along nodes with floating round, heart-shaped green to yellow-green leaves growing 3–15 cm in diameter with slightly wavy margins (Nault and Mikulyuk 2009; Pfingston 2021). Each leaf has a life span from 23–43 days during the growing season from April to late October. Depending on water temperature, each node can produce two to five five-petaled yellow fringed flowers that last one day. The fruit is a beaked capsule that holds many smooth, flat, ovular seeds with winged margins (Pfingston 2021). The margins allow the seeds to float on the surface and attach to avian vectors allowing spread over large distances. *N. peltata* reproduces sexually and vegetatively through plant fragments, underwater roots, and rhizomes (Cook 1990).

Finding controls for *N. peltata* has continued to be a challenge. Physical and mechanical control of *N. peltata* has been shown largely ineffective and expensive. Due to its ability to propagate easily through plant fragments, mechanical harvesting can create increased numbers and worsen invasion (Larson 2007). Both roots and rhizomes can withstand dredging, but hand raking can be effective in very small areas. Overall, physical and mechanical controls are costly and time consuming. Herbicides have been shown to be effective in minimizing the spread of *N. peltata*, for example, Dichlobenil, a granular treatment applied at the beginning of growth, can minimize the spread when used in combination with mechanical removal of leaves. A second application may be necessary for complete eradication. Glyphosate has also been shown to control 40–50% of the plant, but results only last one season (CAPM-CEH 2004). Treatment with Florpyrauxifen-benzyl (ProcellaCOR) shows promising results due to its effectiveness in the control of other invasive aquatic plants like *Hydrilla verticillata* (Mark Warman, personal communication, April 13, 2020). Other sources point to the use of herbicides Imazapyr and Imazamox in private ponds in Oregon being effective (Harms and Nachtrieb 2019). Chemical herbicides do not only affect the target species but also to some extent the entire ecosystem. Chemicals, specifically glyphosate, have been found to decrease species richness and even be toxic to some plant species (Mudge and Houlahan 2019; Dalton and Boutin 2010).

To the best of our knowledge, there is no known biological control or agent for *N. peltata* (CAPM-CEH 2004; Nault and Mikulyuk 2009; Harms and Nachtrieb 2019; Pfingsten et al 2021). However, a previous study assessed the suitability of *N. peltata* to be a target for biological control using the Peschkin-McClay scoring system (Peschken and McClay 1992). The Peschkin-McClay scoring system gives a semi-quantitative method for assessing the potential for invasive plant species to be a target for biological control. The system looks at biological (known enemies, climate, ways of reproduction) and economic (costs associated with damages) criteria related to the plant and the probability of it being suppressed by biological control. A score of 148.5 out of 179 was given to *N. peltata*, matching scores given to other plant species that have been successfully controlled with biological controls like *Matricaria perforata* (scentless false mayweed or wild chamomile) (Harms and Nachtrieb 2019; Peschken et al 1988; McClay and De Clerck-Floate 1999). During a previous study conducted to observe the growth and development of *N. peltata* in the Netherlands, damage to the leaves was observed caused by the larvae of a native moth, *Cataclysta lemnata*. The larvae were observed to use *N. peltata* as both a food source and to build protective cases. The study found the larvae damaged the *N. peltata*, however, in the presence of other native plants, the larvae preferred the native plants over the *N. peltata* (Van der Velde 1979).

To investigate this, we contacted the Aquatic Invasive Species (AIS) Project Coordinator at the Cleveland Metroparks Mark Warman. He commented that observations of a *N. peltata* population had leaf damage caused by a native moth larva, *Elophila obliteralis*, more commonly known as a waterlily leafcutter moth (Mark Warman, personal communication, September 9, 2019).

Elophila obliteralis is a moth native to eastern North America. Adults lay small, white, domelike eggs on the edges of their host plant, often in ribbons (Dyar 1906). The larva is semi-aquatic and found in both still and fast-moving freshwater. It has gained the nickname “the sandwich man” due to its habit of living in between two pieces of leaves it cuts from its host plant. The pupa is formed in silk cocoons in between two leaf pieces cut by the larva. No information is known for the development time of the moth’s life cycle. *E. obliteralis* larvae most commonly feed on water lilies and have been shown to consume other invasive aquatic plants including water lettuce, salvinia, hygrophila, and hydrilla (Habeck 2008).

This experiment was designed to test the potential of *Elophila obliteralis* larvae as a biological control for *Nymphoides peltata*. We hypothesized that the *Elophila obliteralis* larvae will show potential for being a biological control for *Nymphoides peltata* and the greater the density of *E. obliteralis* larvae to *N. peltata* leaf surface area (cm^2) the greater the decline of *N. peltata* leaf surface area. In conducting this experiment, the results will indicate whether the larvae are able to control the growth of the plant through observations of the changes in leaf surface area over time. By testing various densities of larvae to leaf surface area of the plant (cm^2) over time, we measured the relationship between larval density and leaf area. Based on these data, assessments can be made about the potential of *E. obliteralis* as a potential biological control of *N. peltata.*

Methods:

N. peltata plants were collected from Mentor Lagoons Nature Preserve & Marina, Mentor, Ohio, (41.7224972 N, -81.3409806 W) from a population that had not been previously treated with herbicide or other means of control (June 2020). Plants were removed by hand and transported in large plastic tubs with sediment and water from the lagoon. Extra sediment was also collected from the site for a substrate in the tanks. Plants were separated and cleaned in freshwater. Only the healthiest plants were used, and any with flowers or seed pods were excluded. Additional plants and plant debris was collected and disposed of properly to prevent spreading of the species. To create the mesocosm, a 3 cm natural sediment substrate was added to the bottom of the tanks (Aqueon Glass Aquaria, Size 5½ gallon) and freshwater was used to fill them halfway. After the sediment settled, one plant with similar leaf area was added to each tank and was left to acclimate until the larvae were introduced. Plants were kept at constant conditions indoors at room temperature and 12 hours of light daily using white light banks. Water was added when necessary to keep the water level constant.

E. obliteralis larvae were collected from three locations: Mentor Lagoons Nature Preserve & Marina, Mentor, Ohio; Drake Road Wetlands, Strongsville, Ohio; Bay Village High School pond, Bay Village, Ohio. Before adding larvae, leaf surface area was measured (cm^2) using the Leafscan app by taking a picture of the leaf against reference points. Three treatments were established: 1 larva for every 8 cm^2 of leaf surface area (1:8 density/low density), 1 larva for every 4 cm^2 of leaf surface area (1:4 density/high density), and no larvae (control). The calculated number of larvae needed for each tank, based on surface area, was added within 24 hours of collection. Each treatment was replicated 5 times using a total of 15 tanks. Plant surface area was measured weekly for 5 weeks. Measurements were stopped at 5 weeks due to moths emerging during the last day of week 5.

A one-way repeated measure ANOVA and mean comparisons for all pairs using the Tukey-Kramer HSD test will be used to determine statistical significance.

Results:

A negative relationship is found between the leaf surface area (cm^2) and the number of larvae. Over the 5-week period, *N. peltata* leaf surface area declines in both the 1:8 and 1:4 density treatments but does not decline in the control group.

The control replicates show a minimal mean percent change in leaf surface area, + 0.43% ± 22.26% SD, from the initial leaf surface area to the end of the five weeks. The percent change is variable within the replicates themselves, showing growth fluctuations in individual plants. The mean percent change is higher towards the beginning of the experiment and lower towards the end (Fig. 1a).

The mean percent change in leaf surface area of the 1:8 density is -40.37% ± 29.48% SD. Most replicates experience a relatively steady decline in leaf surface area over the five weeks. Most replicates experience a steady decline, with the exception of Replicate 2 (Fig. 1b).

The 1:4 density has a mean percent change of -71.27% ± 21.95% SD. Replicates experience a steady decline. Most notably, Replicate 3 reached a percent change of -98.91% of leaf surface area by week 4 (Fig. 1c). The 1:4 density had a mean percent change decline that was higher when compared to the 1:8 density percent change at the end of 5 weeks (-71.27% and – 40.37% respectively, Fig. 1b and c).

Figure 2 shows the comparisons between the mean percent change between the control, 1:8 density, and 1:4 density replicates. A one-way repeated measure ANOVA and mean comparisons for all pairs using Tukey-Kramer HSD test showed a significant difference between all groups ($F = 10.51$, $p = 0.002$), indicating a significant effect of the larvae on plant surface area.

Discussion:

These results support the hypothesis that the higher the density of larvae to leaf surface area (cm^2), the quicker the overall decline in leaf surface area. The results also point to the higher the density (1:4 density) of larvae to leaf surface area, the higher the efficacy the larvae have in controlling the leaf growth of the plant. We observed that the mean percent change of the control replicates increased in surface area starting the experiment and then started to decrease towards the end. This is thought to be due to the natural increase and decrease in leaf surface area fluctuations as leaves die off and new leaves replace them, however a longer experimentation time can indicate a more concrete answer.

These results indicate *E. obliteralis* larvae could potentially be a successful biological control for *N. peltata*. Prior research on a biological control for *N. peltata* is limited. The results, however, show that *E. obliteralis* larvae are able to consume large quantities of the *N. peltata* foliage which is similar to the observations made in the study conducted using a native aquatic moth larva in the Netherlands (Van der Velde 1979). We observed that the moths have the ability to contain lone populations of *N. peltata* through the consumption of the leaves, showing it has potential to be a biological control. Additionally, we observed that as the leaves were consumed by the larvae the plants, especially in the 1:4 density, were unable to produce enough new leaves to offset the loss. Often new shoots that were produced ended up dying as the plants were weakened by the loss of leaves. The size of the leaves produced during the five weeks was also affected; as the experiment continued, we observed that the leaves produced were smaller than the leaves produced before adding the larvae although no data were collected on the leaf size of the new leaves to compare to the previous.

The larvae not only had an effect on the leaves, but also on overall plant health. One downfall of using the larvae as a biological control for the *N. peltata* is that it only targets the foliage of the plant and not the rhizomes, the underground stems that allow the plant to reproduce vegetatively quickly and store nutrients for future growth (Pfingsten et al 2021). However, towards the end of experimentation in the 1:4 density replicates, we observed that the ends of the rhizomes started to deteriorate as the plant focused on maintaining the health of the current nodes and less on continuing to spread out and grow. Currently, we have no quantitative data to support that the larvae impact the rhizomes as well, so continued study is needed to further understand this relationship and determine if there is one.

Conclusion:

The hypothesis that the greater the density of *E. obliteralis* larvae to *N. peltata* leaf surface area (cm^2) the quicker the overall decline of *N. peltata* leaf surface area was supported by these data.

An unexpected source of error that could have occurred in the experiment is uncontrolled mortality among some of the larvae before the end of the trial. Losing larvae will skew the density mid-trial, leading to a less successful outcome of the larvae controlling the growth of the plant foliage. Plants also had limited room to grow in tanks, possibly causing some plant’s growth to stall. Improvements to this experimental design would be to look at the overall impact the larvae have on the plant, not just the leaves. The results will yield a better understanding of the future of *E. obliteralis* as a biological control for *N. peltata*.

Repeated mesocosm studies to understand the preferences of the larvae are important to verify the results presented here. Future testing and experimentation is needed to further determine how the larvae will interact with other macrophytes in the ecosystems that *N. peltata* is present in and how native predators to the moths could be a barrier to their success in controlling populations. Additional testing is also necessary to determine the ideal density of larvae and identify what happens to the larvae when the entire food source has been consumed. If the results from these experiments prove the larvae to be successful, the biological control can be used alone or in an Integrated Pest Management Program. This would incorporate using the biological control at the beginning of treatment and fewer herbicides later to ensure the removal of the invasive aquatic plant and restoration of infested wetlands.

Declarations

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Project design was conducted by Mark J. Warman, Rachael E. Tomasko, Seth J. Jennrich, and Kathleen R. Gorbach. Material preparation was performed by Mark J. Warman, Rachael E. Tomasko, and Seth J. Jennrich. Data collection was performed by Rachael E. Tomasko. Data analysis was performed by Rachael E. Tomasko, Seth J. Jennrich, and Kathleen R. Gorbach. The first draft of the manuscript was written by Rachael E. Tomasko and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

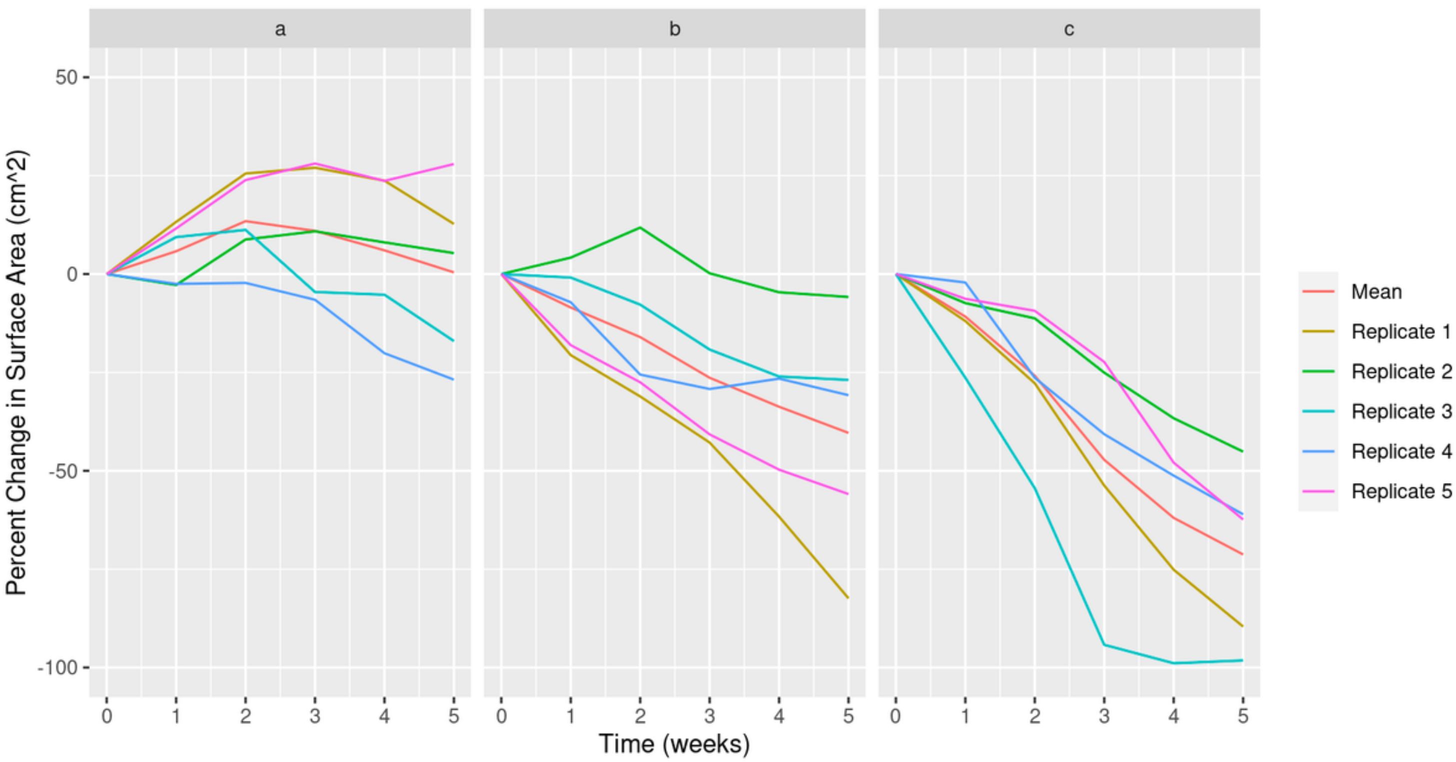

Figure 1

(a) percent change of leaf surface area (cm^2) in *N. peltata* over time for replicates in the control treatment (b) percent change of leaf surface area (cm^2) in *N. peltata* over time for replicates in the 1:8 density treatment (c) percent change of leaf surface area (cm^2) in *N. peltata* over time for each replicate in the 1:4 density treatment

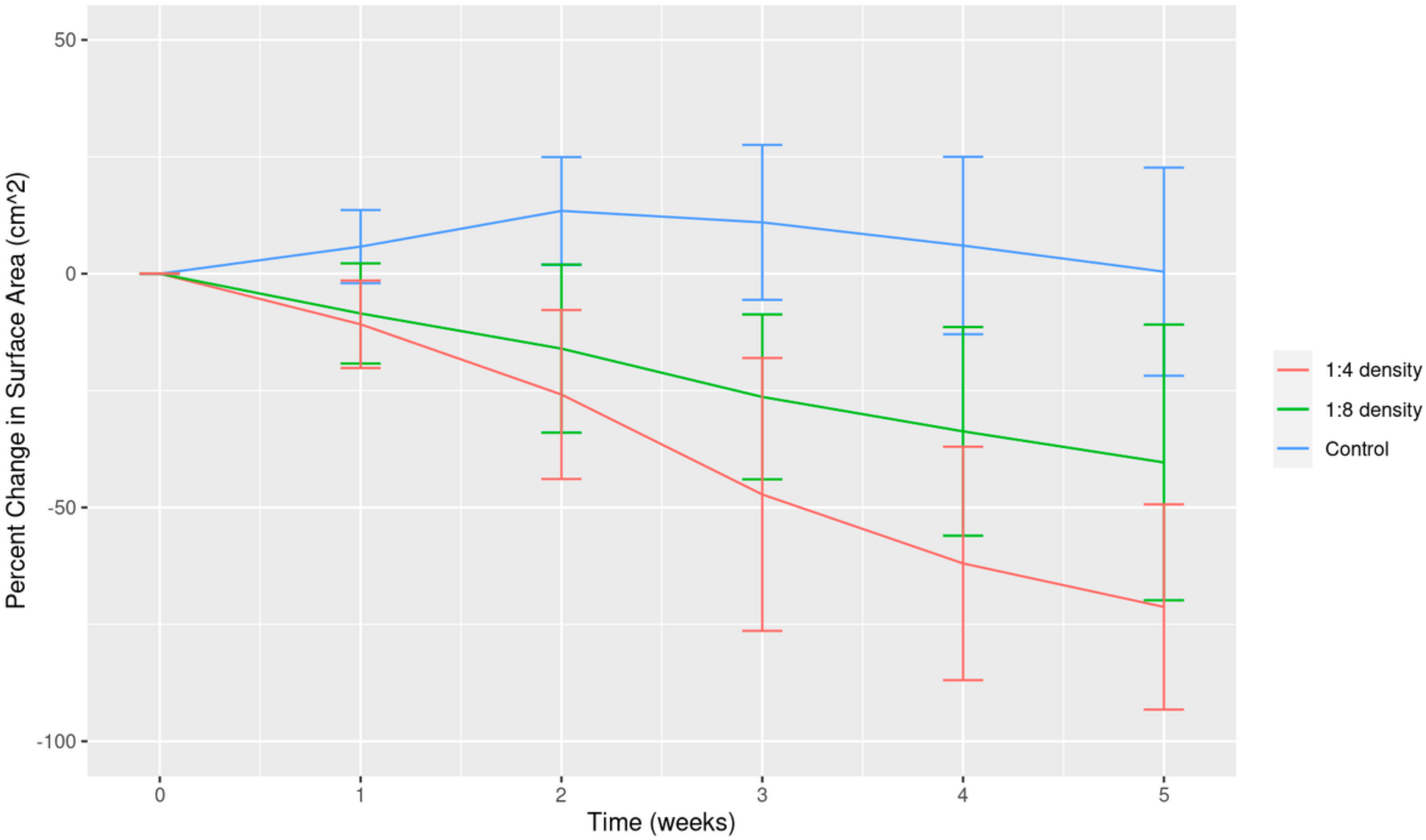

Figure 2

percent change of leaf surface area (cm^2) in *N. peltata* over 5 weeks for each larval density, comparing means from all three treatments. Error bars denote standard deviation. One-way repeated measure ANOVA with Tukey-Kramer HSD test found a statistically significant difference between all treatment groups ($F= 10.51$, $p=0.002$)