The effect of the presence of non-native plant species along stream ecosystems on the leaf-shredding organism Tipulae (Crane fly)

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Introduction

Leaf-Shredding Organisms

Leaf-shredding organisms play a vital role in aquatic ecosystems (Meyer and O'Hop, 1983). Organisms that break down and consume organic matter, such as leaf litter, make nutrients available for other organisms within the ecosystem (McCord et al. 2006). The decomposition of leaf litter is a primary pathway of returning nutrients to the ecosystem since senesced leaves make up more than 70 percent of above ground particulate organic matter (Karberg et al. 2008). Leaf-shredding organisms (shredders) make dissolved organic carbon (DOC), phosphorus (P), and nitrogen (N) available to plants, the microbial community, and other organisms within the ecosystem (Lawson et al. 1984, Meyer and O'Hop, 1983). DOC, N, and P are necessary nutrients for the growth and survival of all organisms (Bourtis and Heckman, 2018). make dissolved organic carbon (DOC), phosphorus (P), and nitrogen (N) available to plants, the microbial community, and other organisms within the ecosystem (Lawson et al. 1984, Meyer and O'Hop, 1983). DOC, N, and P are necessary nutrients for the growth and survival of all organisms (Bourtis and Heckman, 2018).

Shredder productivity can be influenced by growth rates, size, and nutrient availability. Shredder size is directly correlated with the amount of DOC produced, since larger organisms can process significantly larger quantities of leaf litter at more rapid rates than smaller organisms of the same species (Meyer and O'Hop, 1983). The rate of leaf processing also depends on the stage of decomposition (Lawson et. al., 1984). Microbe conditioning of leaves increases nutrient availability, increases leaf-shredding rates, shredder growth rates, and favorability of leaves to shredders (Lawson et. al., 1984). Species composition of the leaf litter impacts the amount and type of nutrients produced (Meyer and O'Hop, 1983) and shredder processing rates (Karberg et al. 2008).

Changing Ecosystems in Pennsylvania

The state of Pennsylvania contains various ecosystems (forests, wetlands and grasslands) that have been experiencing land use changes since the late 1700s (Abrams, 1998). Land use changes and increased globalization have resulted in the introduction and spread of non-native species to previously undisturbed ecosystems (Dickinson et al. 2016). Historically, Pennsylvania forests were dominated by oaks (*Quercus sp*.), hickories (*Carya sp.),* and other fire-resistant tree species but are now dominated by invasive species (Abrams, 1998). Native organisms, including mammals, birds, and macroinvertebrates, have co-evolved with the native plant species and have historically competed for the same resources (Bourtis and Heckman, 2018).

Previous studies have shown that the introduction of non-native plant species can alter pre-existing ecosystem dynamics (Bourtis and Heckman, 2018). Non-native species have been known to out-compete native organisms for resources, such as sunlight or nutrients, which has the potential to decrease the survival and overall success of native species (Bourtis and Heckman, 2018). Native plant species have been found to decompose more rapidly than non-native plant species (Straigyte et al., 2009). However, few studies have been conducted to determine the effects of the presence of non-native plant species along stream ecosystems and their effects on leaf-shredding organisms.

Our study examined three native and three invasive plant species commonly found in western Pennsylvania ecosystems. Native species include *Lindera benzoin* (spicebush), *Acer rubrum* (red maple), and *Sassafras albidum* (sassafras). Invasive species include *Acer platanoides* (Norway maple), *Elaeagnus umbellata* (autumn olive), and *Fallopia japonica* (Japanese knotweed). The three invasives were brought to the United States to be used as ornamental plants but quickly became invasive due to their rapid growth rates and ability to colonize and outcompete native plants for resources (Bailey, 2013; Braatne et. al., 2007; Wychoff and Webb, 1996; Yancey, 2009).

Objectives

We aimed to determine how the presence of invasive leaf litter impacted the growth of *Tipula* larvae within stream ecosystems. We attempt to determine differences in leaves via leaf strength testing to determine if characteristics associated with leaf strength could impact leaf preference and growth rates of *Tipula* larvae. We hypothesized that (1) *Tipula* larvae would show a preference towards native leaf species over non-native leaf species when in the presence of both, (2) consuming native leaf species would contribute to more rapid *Tipula* growth rates, and (3) leaf species that demonstrate weaker average leaf strength are more likely to be preferred and result in increased growth rates when compared to leaf species that demonstrate stronger average leaf strength.

Methods

Tipulidae (crane flies)

Insects of the family *Tipulidae* function as shredders during their larval stages in aquatic ecosystems (Lawson et al. 1984; McCord et al. 2006). Macroinvertebrates are commonly thought to be a proxy for measuring stream quality; *Tipulae* larvae are known generalists that can adapt to fluctuating environmental conditions (Pritchard, 1976). Members of the *Tipulidae* family experience "adaptive growth," meaning they grow in response to their current environmental conditions—rather than being dependent upon those conditions (McCord et al. 2006). In manipulative studies, *Tipulidae* survival was found to be dependent upon food availability. Blackshaw and Petrovskii (2007) found that food scarcity is rarely experienced in pristine ecosystems since *Tipula* rely on the presence of leaf litter within their environment. Limited research has been done to determine how changing plant composition caused by the introduction of non-native plant species in ecosystems surrounding the streams inhabited by *Tipula* larvae impacts the growth and survival of the larvae as well as their ability to function as shredders.

Leaf strength testing

For the leaf strength tests, we took each leaf species and measured the tear force after soaking for a set amount of days. Leaves were soaked for four days, eight days, and fifteen days. The strength tests were conducted over a three-week period. A custom-made apparatus was used for this experiment (see Figure 1). Native and invasive leaves were soaked in separate containers to allow for easier identification of leaves of similar morphologies. Whole leaves were removed from the soaking containers and clipped to the: clothespins on the apparatus; one clip attached to the tip of the leaf and one attached to the leaf near the leaf stem. Sand was deposited into a plastic cup on the other side of the apparatus connected by a pully system. The sand was deposited via a funnel until the weight of it caused the leaf to break or tear. The sand was then poured into a separate cup to be weighed on a scale. To calculate the total weight applied to induce tearing, the weight of the sand was added to the combined weight of the clothes pin, string, and receptacle cup. We repeated these steps for each test.

Invertebrate Growth

We compared the growth of crane fly larva (*Tipula spp*.) who were experimentally fed *A. rubrum, L. benzoin, S. albidum, A. platanoides, E. umbellata, and F. japonica*. We used senescent leaf litter gathered from the woods surrounding Chatham University's Eden Hall Campus in Gibsonia, PA. *Tipula* were collected from Montour Run on the Eden Hall campus by examining submerged leaf litter and removing larva by hand. We chose to work with the genus Tipula because they are native to Pennsylvania and considered model shredders (Moline et al. 2008). We utilized a recirculating aquaculture system with aquatic housing units to store and feed the *Tipula* larvae. A handful of submerged leaves were obtained from Montour Run, and placed in a mesh bag inside the tank system establish a natural microbiome in the housing unit. Twenty-six *Tipula* were housed individually in 2.8-liter tanks. Fry screens were sealed in each tank to prevent larva from being suctioned out and into the system. Prior to being added to the tank system, each larva was blotted dry on a paper towel and weighed to the nearest 0.001 gram. Each organism was randomly assigned a leaf species and fed ad libitum on a diet of a single leaf species (Moline, 2008). Small ceramic tiles were used to keep leaf litter submerged and accessible to the larvae. *Tipula* larvae were subsequently blotted dry and weighed on a weekly basis for five weeks.

Feeding Preference Assays

Preference assays were conducted to determine how *Tipulae* shredding behavior is influenced by the presence of invasive leaf litter. Two tanks containing forty-five were filled with two gallons of non-chlorinated water, lined with leaf litter, and housed with air stone aquarium pumps. Invasive leaves were placed on the left side and native leaves on the right side of each tank. In order to observe preference of specific leaf species, leaf packs with individual species were submerged and isolated using ceramic tiles. At the time of observation, a plastic divider was placed in the center of the tank to prevent larva from moving to a different side of the tank. Species specific leaf packs were examined for the presence of larva and aligned in their tank positions on a dissection plate until the end of the observation. We recorded the number of *Tipulae* present per leaf pack and placed them in separate containers until counts were completed. Observations were conducted twice a week for one month.

Statistical Analyses

All statistical analyses were conducted in R. test function for Welch's Two Sample t-tests was used to determine mean differences, confidence intervals, and t-values. The pastecs package was used to verify the results of t-tests and provided summary statistics (Grosjean et al. 2018). The ggplot2 package was utilized to create figures and make within and between group comparisons Wickham et al. (2016).

Results

Preference assays revealed significant differences between the average number of *Tipulae* observed per leaf species based on Welch's t-test of means ($n = 89$, $t = 2.071$, $df = 37$, $p = 0.045$) (Figure 2). The mean number of individuals found on invasive species per trial was 8.416 (Figure 2). The mean number of individuals found on native species per trial was 5.583 (Figure 2). Collective mean preference for invasive and native leaf litter did not differ significantly ($n = 336$, $t = 1.521$, $df = 2$, $p = 0.2292$) (Figure 3). The total mean number of individuals observed on invasive (67.33) and native (44.67) were not significantly different ($t = 1.521$, $df = 3$, $p = 0.2292$) (Figure 3). The total number of *Tipluae* found on invasive and native leaf packs were 202 (41 on *E. umbellate*, 85 on *F. japonica*, and 76 on *A. platanoides*) and 134 (35 on *A. rubrum*, 57 on *S. albidum*, and 42 on *L. Benzoin*) (Figures 2 and 3), respectively.

Diet did not have on growth rates over the course of 5 weeks (n = 24, t = 0.31679, df = 20, p = 0.7546) (Figure 4). The mean growth rates for individuals fed native (0.342 g) and native (0.313 g) were not significantly different (Figure 4). There was not a significant difference in the total growth of individuals fed invasive or native leaf litter (t = 22.91, df = 2, p = 0.087) (Figure 5). The mean growth totals for an invasive-fed and native-fed Tipulae were 0.667g and 0.270g (Figure 5).

The leaf strength tests revealed there was not a significant difference between the force required to tear native and invasive leaves (t = 0.258, df = 22, p = 0.799) (Figure 6). The mean force to tear for invasive species was 313.5 g and 303.1 g for native species (Figure 6). The average force to tear for all leaf species was 308.3 g (Figure 6). The average force to tear increased with the number of days soaked but there was not a significant relationship between leaf strength and soak time (Figure 7).

Discussion

Though we did not see a significant difference in leaf degradation rates over the course of our study, a literature review shows trends in leaf degradation that were not observed in our study. Discrepancies between our results and that literature may have been due to an insufficient study duration, or a lack of enough replicates. Leaf litter often varies in chemical composition and physical properties, which have been shown to influence consumer feeding preferences (Gessner et al. 2010). Leaves may be nutrient rich, with structures that facilitate non-energy intensive utilization of carbon, while others may be lacking nutrients and have higher concentrations of organic compounds like lignin, which make the leaf resistant to degradation (Gessner et al. 2010). Invasive plants species often produce leaf litter with higher decomposition rates than their co-occurring natives due to a nutrient investment strategy that favors increased biomass of leaves over a more complex leaf structure. Invasive species will allocate more nitrogen to photosynthesis and growth, while reducing the energy input needed to build more robust cell walls (Penuelas et al. 2010). These leaf characteristics could be a driving force behind *Tipula* feeding behavior observed in the preference assays. When fed mixed leaf litter, stream detritivores fed preferentially on leaves with easily utilized carbon, while rates of decomposition fell among recalcitrant leaf species (Gessner et al. 2010).

Author's Contributions

M.S., S.H, and J.K compiled and J.K. formatted databases, performed data analysis, consulted data base structure, created R codes, and conducted statistical analyses. S.B., J.K., and M.S. wrote manuscript outline. S.B. wrote the introduction. S.H., J.K., and L.M. wrote methods. J.K. designed figures, wrote results and author's contributions sections. S.B., L.M, and M.S. wrote discussion sections. S.B., S.H., J.K., and, M.S., edited manuscript. J.K., L.M, J.M., and M.S. helped with project logistics and organization. S.B., J.K., S.H., J.M., H.M., L.M., and M.S. performed a literature search. S.B., J.M., H.M., M.S., and L.S. established microbiome for aquaculture system. S.B., S.H., J.K., J.M., L.M., H.M., and M.S. participated in initial specimen acquisition. J.M., M.S., L.M, and S.H. conducted subsequent specimen acquisitions. S.B., J.K, J.M., L.M., H.M., and M.S. assisted in set up of aquaculture system. J.M., M.S., and S.M. prepared growth mesocosm. M.S., J.M., L.M., J.K., and H.M. contributed to animal husbandry. H.M. designed initial laboratory schedule. J.K. and L.M. designed contributed to methods for growth rates. M.S., J.M., L.M., S.B., and H.M. conducted initial growth measurements. L.M., M.S., and J.M. conducted subsequent weekly growth measurements. S.H. designed and conducted initial leaf strength tests. S.H. and L.M. conducted subsequent leaf strength testing.

J.K. designed preference assays. J.K. and L.M. conducted initial and subsequent preference assays.

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Figure Captions

Fig. 1. Image of experimental apparatus used to conduct leaf strength tests. Leaves were clipped with clothes pins at the apex and base. The second pin was connected to a weighted pulley system. Sand was placed into the funnel until leaves were ripped in half. The weight of the same was recorded in grams.

Fig. 2. Species-specific counts for *Tipulae* preference assay. Boxplot corresponds to the number of individuals found per leaf species during preference counts. The mean of each observation, IQRs, and standard error of means are displayed for each leaf species. Overlapping standard errors between groups represent non-significant differences. Outliers are represented by black dots. The average number of individuals per leaf species were significantly different across all treatments ($p = 0.045$).

Fig. 3. Total number of *Tipulae* observed on invasive and native leaf litter. Boxplots correspond to the number of individuals found on invasive and native leaf packs during preference assays. The means, IQRs, and standard error of means are displayed for each group. Overlapping standard errors suggest invasion status does not have a significant impact of feeding preferences ($p = 0.2292$).

Fig. 4. Average growth rates per diet. Boxplots corresponds to the average growth rates experienced by *Tipulae* fed species-specific diets for 5 weeks. The mean of each observation, IQRs, and standard error of means are displayed for each leaf species. Overlapping standard errors between groups represent non-significant differences. Outliers are represented by black dots. Differences between diets did not have a significant effect on average growth rates ($p = 0.754$).

Fig. 5. Collective growth of *Tipulae* fed invasive or native leaf litter. Boxplots represent averages of the total growth of *Tipulae* reared on invasive and native leaf litter. The averages of means, IQRS, and standard error of means are displayed for each group. The difference between the means for initial and final growth were not significantly different ($p = 0.087$).

Fig. 6. Average leaf strength per species. Boxplot corresponds to the mean weight required to tear dried leaves of each species. The means, IQRs, and standard error of means are displayed for each leaf species. Overlapping standard errors between groups represent non-significant differences. Outliers are represented by black dots. The average force required to tear each leaf did not vary significantly between species ($t = 0.258$).

Fig. 7. Average leaf strength based on number of days soaked. Boxplots correspond to the mean weight required to tear leaves soaked for different time intervals. The means, IQRs, and standard error of means are displayed for each soaking period. Overlapping standard errors between

groups represent non-significant differences. The number of days soaked increased the mean weight required to tear leaves but was not significantly different.

Figure 1

Figure 2

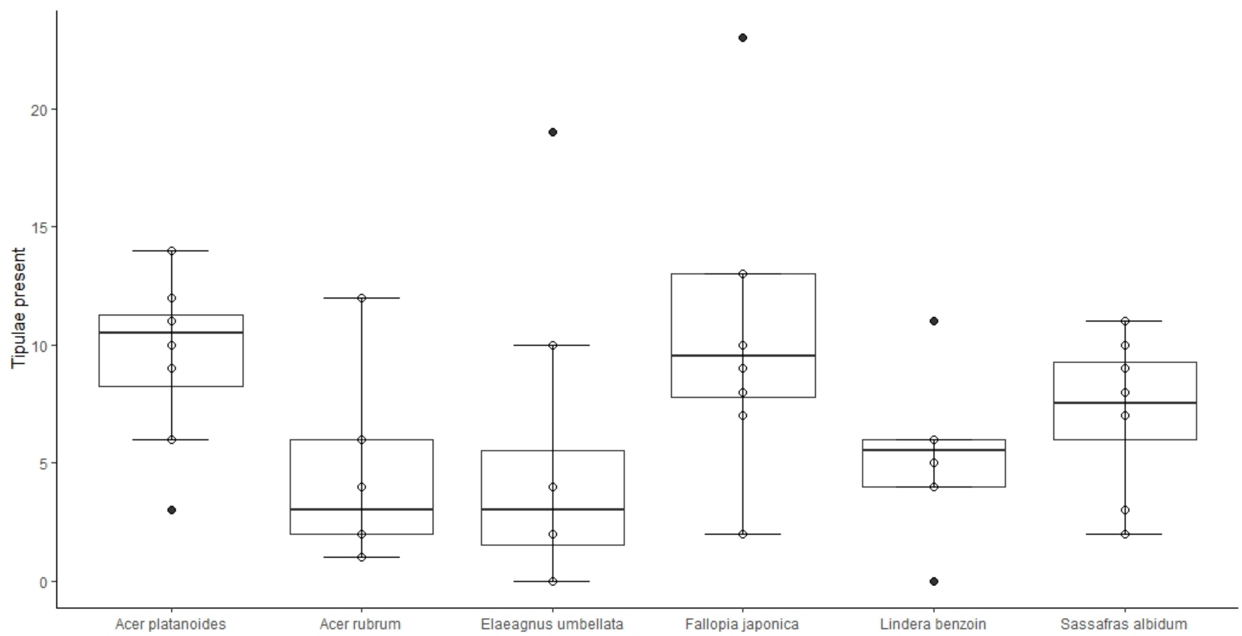

Figure 3

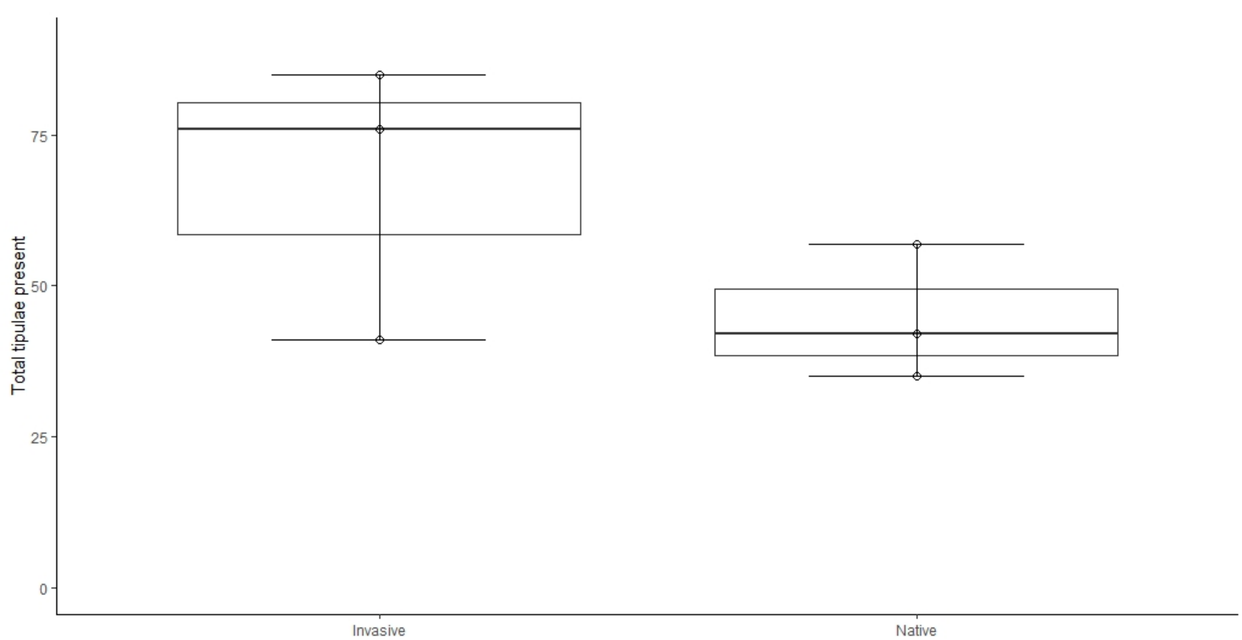

Figure 4

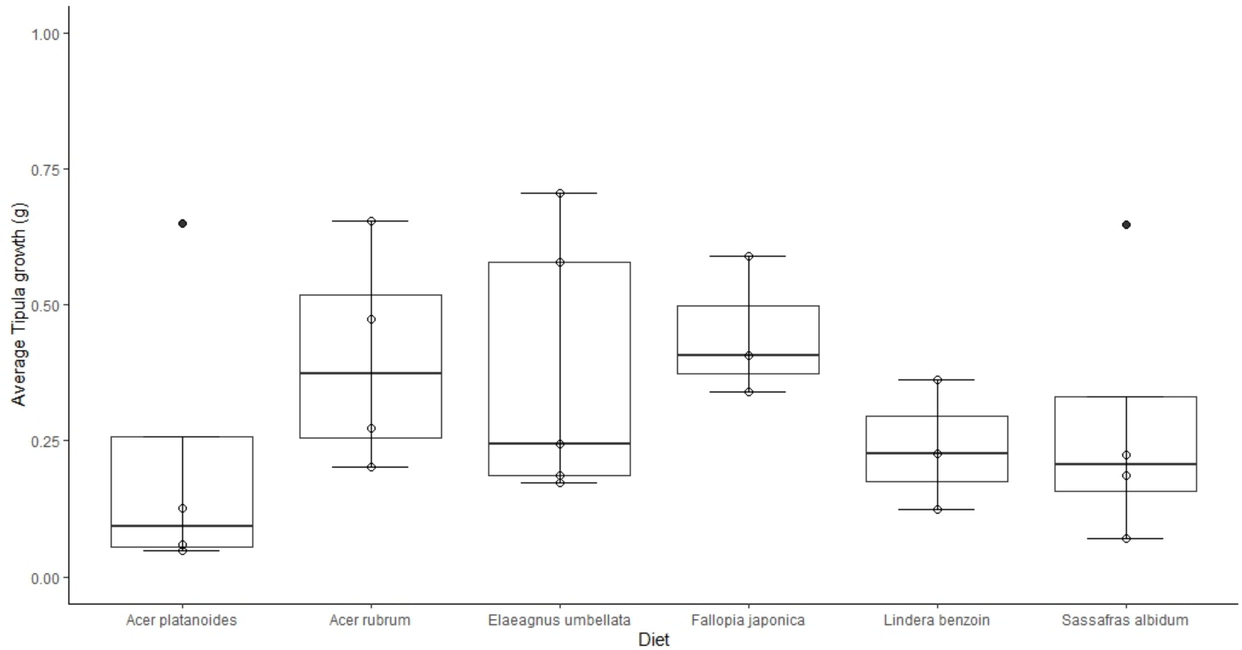

Figure 5

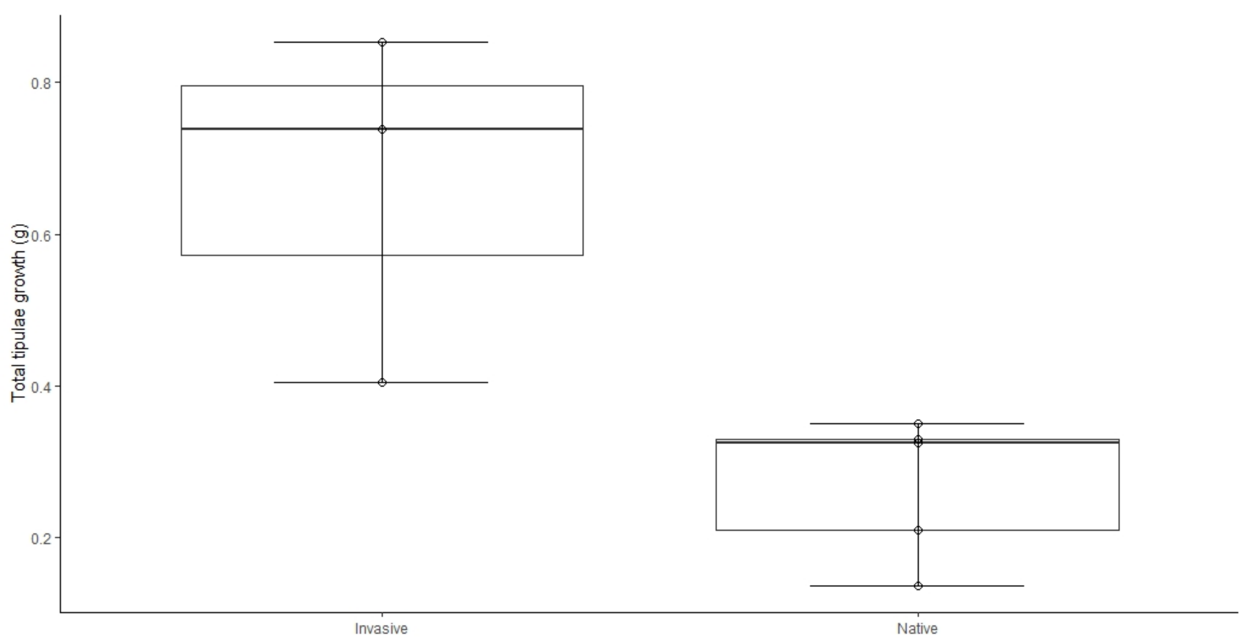

Figure 6

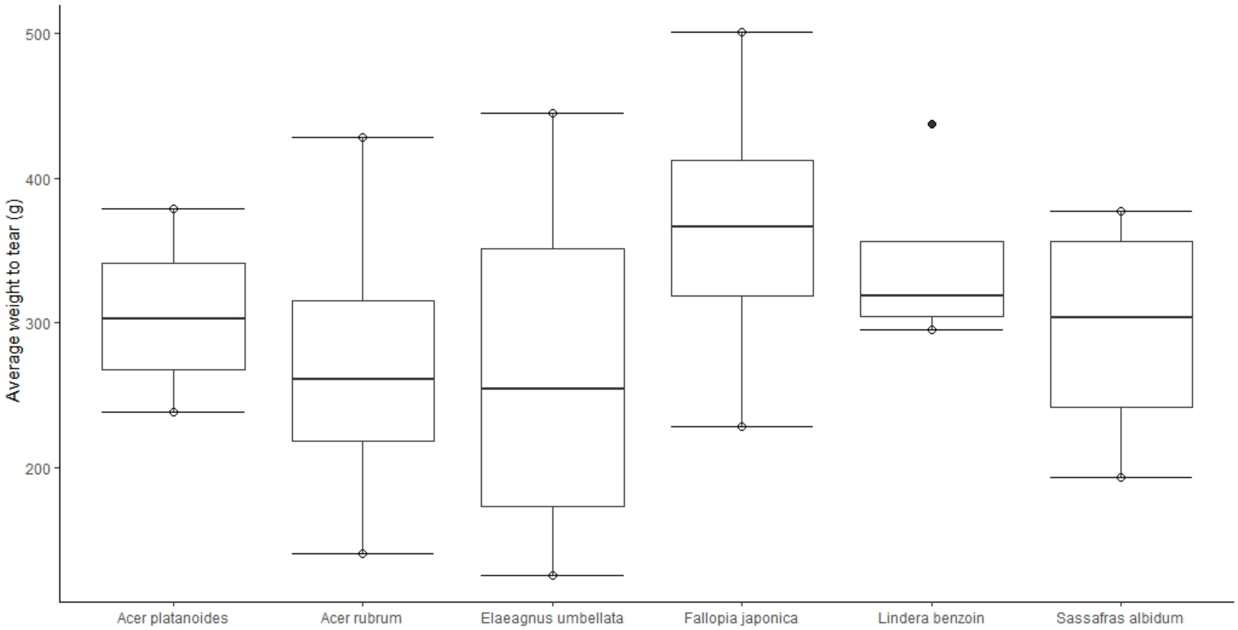

Figure 7