

# **Impact of Leaf Species on Northeastern Macroinvertebrate Populations**

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## **Abstract:**

### **Impact of Leaf Species on Northeastern Macroinvertebrate Populations**

Macroinvertebrates are small, invertebrate insects that play essential roles in aquatic ecosystems. The primary macroinvertebrate food source is leaves from trees. Therefore it is likely that different leaves impact macroinvertebrates differently, and it is possible that invasive plant species have negative impacts on these macroinvertebrates, considering that the evolution of these invertebrates took place in an environment containing native leaves, not those of invasives. To test whether three invasives in particular, Norway Maple, Tree of Heaven, and Knotweed, are negatively impacting macroinvertebrates, four different types of leaf packs (as designed by the LaMotte Company) were assembled: one of each invasive leaf and the control, composed equally of three native tree leaves: American Beech, American Sweetgum, and Red Oak. Thirteen sets of these packs were placed in the Bronx River for three weeks and then removed. The macroinvertebrates present in the packs at the end of the three weeks were recorded. After data analysis, two conclusions were made: the control pack had statistically significantly greater macroinvertebrate diversity (alpha level 0.1), and had a significantly higher mean of pollution non-tolerant macroinvertebrates (alpha level 0.02). These outcomes strongly suggest that the native trees are more conducive to healthy, diverse populations of macroinvertebrates.

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## **Introduction**

Macroinvertebrates are small, invertebrate insects that live in and play essential roles in freshwater ecosystems, particularly streams and rivers. The size and type of macroinvertebrates vary greatly; all macroinvertebrates must be visible to the naked eye to be considered a macroinvertebrate, but can grow up to thirty centimeters (though that is rare). As for different types of these insects, some invertebrates function as only a stage in the life of an insect that will one day be capable of flight (i.e. the dragonfly) or that will live outside the water (Peckarsky et al., 1990). Others are forever aquatic creatures, and instead of changing form only grow in size.

The populations and presence of certain types of macroinvertebrates signal different things about the habitat in question. For example, there is only a strong presence of form-static macroinvertebrates in an environment, it is probably that this area is significantly polluted. However, if there is an abundance of physically dynamic macroinvertebrates, the ecosystem is usually unpolluted, diverse, and healthy. The macroinvertebrates typically labeled as pollution tolerant are therefore mostly form-static insects, and those categorized as pollution intolerant are mostly physically morphing macroinvertebrates. In this way, the populations of macroinvertebrates are good measures of the health of an ecosystem.

Aside from serving as a meter of the health of an aquatic environment, macroinvertebrates also play essential and irreplaceable roles in their ecosystem. First and foremost these invertebrates act as an energy bridge by break down plant matter (mostly tree leaves), which is the main source of nutrients for macroinvertebrates and consequently much of riparian ecosystems, particularly in ecosystems located under a

canopy of leaves (Sweeney, 1993). Second, some macroinvertebrates act as filters, cleaning out the water and removing things like algae and dead organic matter that could otherwise poison a body of water and the other life forms in the environment. Macroinvertebrates also have an important role in the sense that they are one of the most basic groups of organisms on the food chain. Consequently, much of the upper hierarchy that the ecosystem is depends heavily on the state of the macroinvertebrates in their system. Ultimately, because macroinvertebrates are dependent on the plant life around them, the entire ecosystem that the macroinvertebrates are a part of become reliant on that plant life. For this reason, the state of plant life is very much influential on the state of the macroinvertebrates and their more specific ecosystem.

Because of the strong link between the plant life and the aquatic ecosystem, it is quite probable that an alteration of the plant life would lead to a change in the aquatic life. A likely example would be the presence of invasive trees in what were otherwise native forests. These invasive plant species are plants that are indigenous to another region, and that spread and grow quickly in a new environment and consequently pose a threat to native species of that region. Due to the fact that these plants are frequently aggressive and often outcompete native species, what is often seen where invasives are present is the existence of few other plant species. It is common that the invasive plant will eventually have more or less totally outcompeted all other comparable plant growth and have severely decreased the plant diversity of that area. It is known that invasive plants often harm native plants (and some land animals as a result), but what is possible and has yet to be seen is whether or not leaves from these invasive plants negatively impact aquatic environments surrounding the invasive plant growth. It is very possible

that the invasives are negatively impacting macroinvertebrates for two reasons: one, macroinvertebrates likely have evolved to thrive when feeding off of native plants, and two, the presence of primarily one leaf species may not provide the adequate nutrients the body of water needs to maintain a healthy ecosystem.

Three very prevalent invasive plant species in the Northeastern United States are the Norway Maple, Tree of Heaven, and Japanese Knotweed. The former two species are trees, and the latter is a large herbaceous plant. These three plant species spread rapidly and display vigorous growth that allows them to outcompete comparable native plants. The streams and rivers in the forests of this region could potentially be suffering from the presence of the invasives for the reasons previously stated. It has been shown repeatedly that all three species work to prevent the growth of neighboring species. The Norway Maple is a very hardy tree that releases allelopathic chemicals into the soil to prevent the growth of other plants, and also prevents the growth of a significant understory by having a very thick canopy (Wyckoff et al., 1996). The Tree of Heaven is a very fast growing tree that rebounds violently when killed by sending out underground shoots, and also releases allelopathic chemicals (Canham et al., 2008) and Knotweed characteristically forms monocultures in riparian zones (Sullivan et al., 2007). A monoculture is a region where only one plant grows. Also, like the two invasive trees mentioned above, Knotweed releases allelopathic chemicals (Murrell et al. 2011) that inhibit other plant growth. Although it has been shown that these plants negatively impact other plant species, further research needs to be done to determine whether or not these species are impacting aquatic ecosystems as well. It is therefore important to conduct a study to assess whether this is occurring and determine whether increased emphasis should be

placed on preventing the spread of invasives plants and actively working to clear them out.

A few other studies have attempted to test something similar, whether certain leave species impact macroinvertebrate populations differently. However, these studies have been inconsistent with one another in terms of their specific aims and their outcomes. For example, a study by Barker in 2011 concluded that Knotweed, *Lonicera maackii*, had significant impacts on macroinvertebrate populations, yet a study by Braatne et al. in 2007 determined Knotweed to not have a significantly different effect on macroinvertebrate populations than native species. Furthermore, studies have not incorporated the Tree of Heaven or the Norway Maple in the leaf litter analysis, and these two tree species are very invasive and abundant in the Northeast. For these reasons, a study testing Knotweed, the Tree of Heaven, and the Norway Maple in comparison to common native Northeastern trees and how they respectively impact macroinvertebrate populations is strongly merited.

## **Methodology**

The first step in the procedure was collecting leaves. Leaves were collected from the following trees (or large herbaceous plant in the case of Knotweed): American Beech, American Sweetgum, Red Oak, Knotweed, Norway Maple, and Tree of Heaven. American Beech, American Sweetgum, and Red Oak were chosen as the native trees because they are among the most common trees along the Bronx River where I worked. The leaves were removed from the trees and dried in large plastic containers. After the leaves were dried they were put into leaf packs. Leaf pack were made by putting thirty grams of leaves in a plastic mesh bag (with an identification tag) and then secured the bag

with string. The control pack was made up of 10g of each of the native trees, and the experimental leaf packs were made up of 30g of one of the invasive leaves. After the packs were formed they were placed in the river and fully submerged under water (this was done by tying one end of the leaf pack to a heavy rock). The leaf packs were placed in the river in sets, meaning that four leaf packs (one of each type: control, Norway Maple, Knotweed, and Tree of Heaven) were placed in the same area.

After the leaves had been in the river for three weeks they were removed and analyzed. The leaves were sorted through on metal tins, and any macroinvertebrates found were placed in petri dishes for counting. All four leaf packs per set were analyzed in the same day. The macroinvertebrates were organized by order, and after the leaves were finished being searched the number of macroinvertebrates in each order was recorded. Six sets were placed in together in the span of a week, and all six sets were analyzed three weeks after they were placed in the river. After all the original sets had been analyzed seven more sets were placed in the river (six of which went in the locations used in the first round). Again, after three weeks the packs were analyzed.

After the thirteen leaf pack sets had been sorted and the macroinvertebrates recorded the data was processed. The means of the types of macroinvertebrates were calculated, as was the average diversity per leaf pack. After this had been completed statistical analysis was performed to determine if there was a significant different in the populations of macroinvertebrates in the different types of leaf packs.



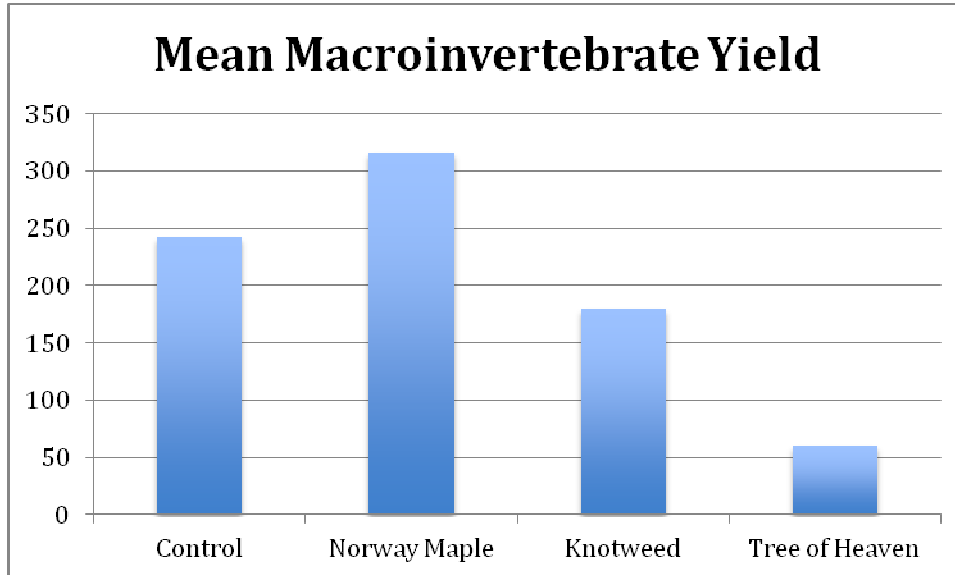
## Results

**Table 1:** Important Statistics

	<b>Control Pack</b>	<b>Norway M.</b>	<b>Knotweed</b>	<b>T. of Heaven</b>
<b>Av. Orders Obs.</b>	6.5	5.31	5.31	3.75
<b>St. Dev. Of Above</b>	1.8	1.68	1.59	1.16
<b>Mean Orders Obs.</b>	7	5	5	4
<b>Av. Total Yield</b>	242.33	316	180.23	59.83
<b>St. Dev. Of Above</b>	124.58	221.97	172.88	67.31
<b>Av. Pol. No.-Tol Yld.</b>	11.28	3.69	2.53	2.17
<b>St. Dev. Of Above</b>	8.77	4.33	3.47	4.78
<b>Av. Pol. Tol Yld.</b>	231.05	312.31	177.7	57.66
<b>Av. Pol. N.T. : Total</b>	1 to 20.48	1 to 84.64	1 to 70.24	1 to 26.57

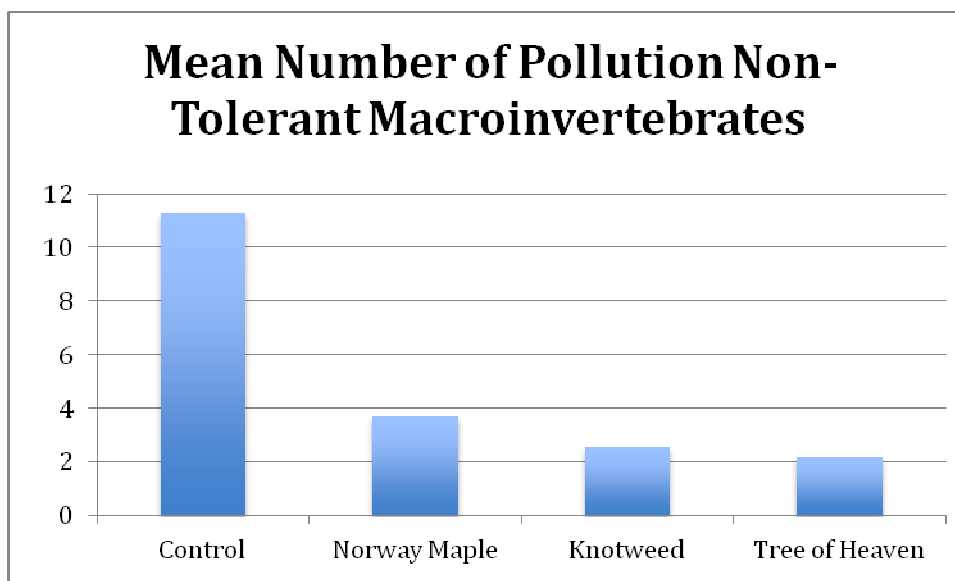
**Table 1:** Shows the average orders of macroinvertebrates observed, the median number of orders observed, the average total invertebrate yield, the average pollution non-tolerant and average pollution tolerant yield, and finally the ratio of average pollution non-tolerant invertebrates to average pollution tolerant. These statistics highlight a few things. The average orders and median orders observed were higher for the control pack than for the other. This was statistically significant at alpha level 0.1 or lower. The average total yield was the highest for Norway Maple, and the Tree of Heaven was the smallest. This was not statistically significant, except for the difference between the Tree of Heaven and the other three. The Tree of Heaven had statistically significantly fewer total macroinvertebrates than the other three did. The table also displays the abundance of more sensitive invertebrates in each of the packs. The ratio of pollution non-tolerant to tolerant was highest in the control pack, and the control pack also had a statistically significantly higher mean of pollution non-tolerant invertebrates than the other packs did at alpha level 0.02 or lower.

**Figure 1:** Graph of Mean Macroinvertebrate Yields



**Figure 1:** Expresses graphically the mean (or average) macroinvertebrate yield expressed numerically in Table 1. The mean macroinvertebrate yield was highest for the Norway Maple, followed by control, then Knotweed, and finally Tree of Heaven. This data was not statistically significant, with one exception. The Tree of Heaven mean was statistically significantly lower, at alpha level 0.05.

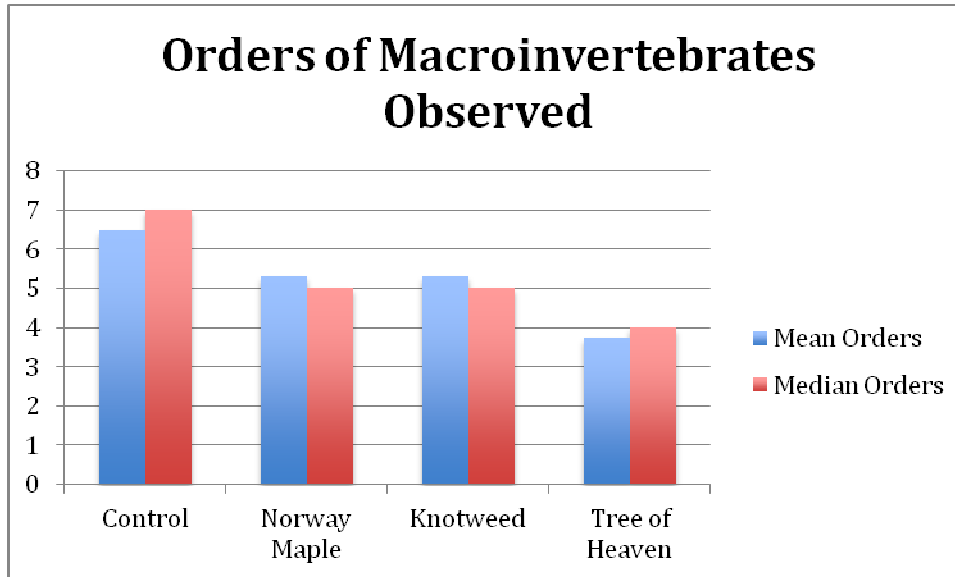
**Figure 2:** Graph of Mean Number of Pollution Non-Tolerant (Sensitive) Macroinvertebrates



**Figure 2:** Expresses graphically the mean pollution non-tolerant yield expressed in Table 1. This graph shows that the control had the highest mean number of pollution non-tolerant

macroinvertebrates. This was statistically significant at alpha level 0.05, as well as lower level for Tree of Heaven as well.

**Figure 3:** Graph of Median and Mean of Orders of Macroinvertebrates Observed



**Figure 3:** Shows graphically the mean and median number of orders observed per leaf pack. The mean and median number of orders observed was highest in the control. The median was not tested for statistical significance, but the mean of the control was statistically significantly higher than the other three. The mean of the control was significantly higher at alpha level 0.1 for the Norway Maple and Knotweed and at 0.001 for Tree of Heaven.

## Discussion

### Overview and Explanation of Criteria in Analysis

The testing of leaf packs composed of native and invasive plant leaves yielded a number of different results. Before delving into the results, however, it is important to explain the connotations of conducting this research in the Bronx River. The Bronx River is the only freshwater stream in New York City. The river begins in Valhalla and lets out on the water connecting the East River with the Long Island Sound. The river travels over twenty miles through some of the most densely populated metropolitan area in the world. As such, the river is quite polluted, increasingly so further South. Because of the high

level of pollution in the river, the leaf packs are expected to have a very high percentage of pollution tolerant macroinvertebrates and a low percentage of pollution non-tolerant invertebrates. Studies in the past, including one by Kratzer et al. in 2006 demonstrate that water systems in and around southern New York, particularly those east of the Hudson River, have a high density of macroinvertebrates, yet a low overall diversity. Therefore, what will be considered in the results is the comparative number of pollution non-tolerant and relative diversity among the leaf packs. Overall the results did not show a diverse macroinvertebrate community. However, there were significant differences in the biodiversity in the different kinds of leaf packs.

#### **Total Average Macroinvertebrate Yields**

The leaf pack type that had the overall greatest average yield of macroinvertebrates was the Norway Maple, followed second by the control pack, then by Knotweed, and finally by Tree of Heaven (Figure 1). However, the difference in means between the Norway Maple pack and the control pack was not statistically significant, and would only have been so if the alpha level was greater than 0.3, which is very high. Additionally, the standard deviation of the Norway Maple invertebrate yields was close to twice as high as the control pack, almost three times as high as the Tree of Heaven standard deviation, and considerably larger than the Knotweed standard deviation (Table 1). For this reason, the statistics of the greatest average yield should be considered cautiously. In fact, none of the average yields are statistically significantly higher than any of the others, with the exception of the Tree of Heaven. All of the leaf pack means were significantly higher than Tree of Heaven pack mean. What the average yield of the leaf packs show is that there is no mathematically significant difference between the control, Norway Maple, and Knotweed mean yields. At the same time however, those

three leaf pack type yields had significantly higher yields than Tree of Heaven at the 0.05 alpha level, which strongly suggests that overall the Tree of Heaven seems to have a negative impact on macroinvertebrate populations.

### **Average Pollution Non-Tolerant Macroinvertebrate Yields**

In contrast to the total macroinvertebrate mean, a measure that was quite statistically significant and was consistent throughout testing was the average yield of pollution non-tolerant, or more sensitive macroinvertebrates. The control leaf pack type had a statistically significantly higher average yield of pollution non-tolerant macroinvertebrates at an alpha level of 0.02 than the comparative yields of Norway Maple, Knotweed, and Tree of Heaven leaf packs. On average the yield of these macroinvertebrates in the control packs was more than three times higher than the yield for Norway Maple and over four times as high as the Knotweed and Tree of Heaven yields (Figure 2). These results are not inconclusive the way the total average yield was. With over ninety eight percent confidence it can be asserted that the native tree leaves generated an on average higher yield of non-pollution tolerant macroinvertebrates. This is important and very noteworthy, as it strongly suggests that the native leaves are more conducive to healthier and more diverse populations of macroinvertebrates. As mentioned in the introduction, a healthy population of pollution non-tolerant macroinvertebrates indicates a healthy aquatic ecosystem. Therefore this data would suggest that the leaves in the control pack would foster a healthier ecosystem than the invasive leaves would.

### **Average Diversity of Macroinvertebrates**

Not only was the average yield of sensitive macroinvertebrates higher for the control pack, but so was the percentage of these invertebrates types when compared to the total population; the average percentage of pollution non-tolerant invertebrates was three times higher in the control pack than in Norway Maple, two times higher than in Knotweed, and larger than in Tree of Heaven. Once again, the control seems most beneficial toward pollution non-tolerant macroinvertebrates. Although the Tree of Heaven average pollution non-tolerant macroinvertebrate population as a percentage of the total was close to the control pack's, because the Tree of Heaven had far fewer macroinvertebrates on average this statistic was much more easily influenced, and consequently less certain. Regardless, the Tree of Heaven is still overall less beneficial to all types of macroinvertebrates because overall it had far fewer totals of all types of macroinvertebrates. All in all, the data describing the average diversity in the packs is parallel with the data of the average yield of more sensitive macroinvertebrates, suggesting that the native leaves are most beneficial to a healthy macroinvertebrate population.

Another statistic that was computed and is statistically significant is the average number of macroinvertebrate orders present per leaf pack, another way of quantifying diversity. The control leaf pack had a statistically significantly higher average number of orders than the other three leaf packs (alpha level was 0.1 for Norway Maple and Knotweed and 0.001 for Tree of Heaven). The Norway Maple and Knotweed were tied for second with the average number of orders per pack, and Tree of Heaven consistently had the fewest present (Figure 3). This is yet more data that suggests that the native trees

are the most beneficial toward biodiversity and the more sensitive macroinvertebrates. This is further evidence that the native trees foster a healthier environment.

### **Discussion Summary**

In summary, there are a few things that the results suggest. First of all, the data consistently and statistically significantly demonstrates that the Tree of Heaven leaves don't allow macroinvertebrate populations to thrive as well as other leaf types allow them to. Second, the mean yield of pollution non-tolerant macroinvertebrates in the control leaf pack was statistically significantly higher than all the experimental leaf packs (at an alpha level of 0.02 or lower). The diversity computed of the leaf packs also points to the native trees as being the more advantageous to a healthy macroinvertebrate distribution, as the control packs had a statistically significantly higher mean number of species present per pack (at alpha level 0.1 and lower).

Past research has looked into whether the invasives tested in this study negatively impacted the plant biodiversity in the areas that these invasives are present, and findings consistently show that these invasive plants tend to cause lower biodiversity and make the surrounding native plants less competitive. However, research hadn't been performed that looked into whether the invasives tested in this study also effect macroinvertebrates in the riparian zones that the trees were occupying. The studies that were performed and did test whether trees had an impact on macroinvertebrates did not test these invasives, were tested in a different region, were often inconclusive, and mostly not conducted at the level of studies that would be published in journals. In addition, the two studies that were performed at the professional level and tested Knotweed contradicted one another. A study by Barker in 2011 (in Ohio) showed that Knotweed significantly impacted the

macroinvertebrate density and invertebrate groups present, while a study by Braatne in 2007 (in Idaho) suggested that Knotweed did not impact macroinvertebrates differently than native leaf litter. The data produced by this study would agree more with the results of study in 2011, as the data obtained also suggests that Knotweed significantly impacts macroinvertebrates. No study, however, had ever tested the Norway Maple or Tree of Heaven leaves, so this study may start to fill a gap in the knowledge of how invasive species, in this case how they impact macroinvertebrates. Also, no study had been performed in the Northeast.

## **Conclusion**

### **Summary of Significance**

As mentioned, little had been done before to determine whether invasive plants are impacting macroinvertebrate populations. Therefore it was seen as valuable to conduct a study looking at whether three very common and aggressive invasives, the Norway Maple, Tree of Heaven, and Knotweed, were negatively impacting macroinvertebrate populations in comparison to how three native tree leaf types, from the American Beech, American Sweetgum, and Red Oak, were impacting the invertebrates. With the conclusion of data collection and analysis it became clear that these invasives were negatively impacting macroinvertebrate populations. The leaf packs composed of invasive leaves had statistically significantly lower means of pollution non-tolerant macroinvertebrates and average number of macroinvertebrate orders per pack. Therefore, what this data suggests is that the native trees tested allow for a healthier and more diverse population of macroinvertebrates than do the invasive plants. Consequently, these native trees will foster an overall healthier ecosystem.



The conclusion that the Norway Maple, Knotweed, and the Tree of Heaven negatively impact macroinvertebrate populations is strongly supported by the data collected. The leaf packs made up of invasive leaves had lower pollution non-tolerant means and diversity means, and all at the statistically significant level. The alpha level for the diversity testing was never higher than 0.1, and was never higher than 0.02 for the tests of pollution non-tolerant yields.

### **Future Work**

There are a number of different experiments that could be performed in the future that would enhance the results of this study. First of all, more invasives should be tested. This study concluded that the three invasives tested negatively impacted macroinvertebrates, but it is important to determine whether invasive plants as a whole tend to hurt macroinvertebrate populations. If they don't, that would suggest that there is something specific about the three invasives already tested that is damaging the macroinvertebrates. Second, the study should be replicated in an aquatic environment that is less polluted. Doing this would allow this experiment to be conducted in an ecosystem with more pollution non-tolerant macroinvertebrates, which would more conclusively show to what degree the more sensitive macroinvertebrates are affected by the invasives in this study. Third, the study should be conducted over a longer period of time. Had time not been an issue, more than two rounds of leaf packs should have been analyzed. If it were possible to have collected more data it could have more conclusively determined, for example, which type of leaf had the greatest mean number of macroinvertebrates per leaf pack. Also, more trials would bring the results closer to the true mean difference between the different types of leaves, like the mean difference between pollution non-

tolerant invertebrates in the control pack and the invasive packs. If more time had been available to continue this study the above are the three things that I would focus on in the project going forward.

### **Remaining Questions**

One aspect that this study did not examine was whether or not the volume of each leaf pack impacts results. In fact, if I were to start the project over and I could change anything about my study, I would look to measuring the average volume of each of the leaf pack. Visually the Norway Maple pack seemed distinguishably larger than the other packs, and the Tree of Heaven smaller than the others. Therefore I wonder whether the volume of the leaf pack had an impact on the number of macroinvertebrates present three weeks later. If the volume does have an impact, it is likely that the Norway Maple has an even more negative impact on macroinvertebrates than this study already suggests.

Another question that has not been answered is whether or not invasives in general are impacting macroinvertebrates. The three invasive plant species tested are three of the most aggressive, commonly occurring, and most difficult to get rid of, and it cannot be said that the results of this study speak to how invasive plants in general affect macroinvertebrates. Because these three invasive species statistically significantly impacted macroinvertebrates it is not unlikely that other invasive species would also have negative effects, but the only way that this can be determined is by future experimentation. This project has been an important step in research of the effects of invasive trees on aquatic environments and macroinvertebrates, but there remains much left to be study and many path left to explore.

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