Effects of Invasive Plant Leaf Litter on a Lake Ecosystem

by Amar A. Bhardwaj

Abstract – Invasive plants are introduced into foreign ecosystems and proliferate easily. These plants often have no natural consumers in their new environment, allowing them to outcompete neighboring native plants, causing a plethora of detriments. This research aimed to help solve a rarely investigated aspect of this problem: the effect of invasive plant leaf litter on lake ecosystem dynamics. It was predicted that invasive leaf litter would have more adverse effects on a lake than native litter, because overwhelming evidence in the literature indicates the harm of invasive plants. This was tested with four groups of mesocosms corresponding to four leaf litter treatments: a native mix of red maple and American beech litter, a monoculture of invasive common reed leaf litter, an invasive monoculture of Japanese knotweed litter, and a mixture of litter from all four species. Using repeated measures analysis of variance statistical tests, each treatment was compared in its effects on the lake ecosystem’s phosphate concentration, nitrate concentration, algae growth, mosquito development, pH, dissolved oxygen concentration, and water conductivity. The common reed leaf litter treatment resulted in the highest nitrate, phosphate, and oxygen levels, leading to the conclusion that this leaf litter can potentially cause eutrophication in lakes. Additionally, Japanese knotweed resulted in the highest conductivity and lowest oxygen levels, showing its leaf litter has the fastest breakdown. The rapidity of this breakdown poses a harm to a lake as adjustment to rapid changes places various stresses on ecosystems. These discoveries can be implemented in lake conservation to more effectively maintain a healthy ecosystem.

I. INTRODUCTION

Invasive species are organisms that have been introduced into and become well established in a foreign ecosystem. These species pervade and disrupt ecosystems of every variety across the globe through various mechanisms, causing an estimated $1.4 trillion in damages per year [1]. Invasives are the second largest cause of biodiversity loss worldwide [2]. Once invasive species are integrated into their new environment, they are virtually impossible to eradicate, a trait that exacerbates their detriment. Naturally, extensive research has been conducted into the impact of invasive species and methods of combating this effect. However, the majority of research has been directed towards animals, and has largely neglected the threat of invasive plants. This research aimed to address the lack of inquiry on the topic by providing additional knowledge of the influence of invasive plants. By learning more about the impact of the leaf litter of invasive plants in a lake context, we will be able to better understand and subsequently remediate the harm it causes lake ecosystems.

A. Invasive Plants

An invasive plant is one that has become established in a foreign area, and is able to flourish and beget reproductive offspring continuously in its new environment [3]. Invasive plants reach the ecosystems in which they are invasive through a variety of pathways. These include natural factors, such as transportation of seeds by wide-ranging animals through endozoochory or epizoochory: seed dispersal through animal ingestion or on the outside of animals, respectively [4]. However, capable of transporting plants vastly greater distances and considerably more common are pathways due to human activity, either intentional or unintentional, such as trade, shipping, travel, or tourism. Once introduced, plants have the potential to reach the aforementioned level of integration into the ecosystem, earning them the title of invasive. Invasive plants perpetuate their damage by decreasing the species richness, diversity, and evenness in the new areas they occupy [5].

B. The Success and Harm of Invasive Plants

A widely accepted explanation of introduced plants’ success is the natural enemy release hypothesis, which maintains that invasive plants are able to thrive because they are untouched by their natural enemies in their new ecosystem [6]. For example, an herbivore that grazes on a specific plant in its endemic region and limits its proliferation will not be able to do so when the plant species is transported a significant distance away, allowing the plant to flourish uninhibited.

In addition to this mechanism of success, invasive plants have also been found to gain an advantage through their harmful effects on native species. Firstly, invasive plants cause a greater decrease in biomass of neighboring plants in their invasive region compared to their native area through more aggressive competition in their new ecosystems [7]. They grow rapidly and crowd out native plants, outcompeting them for vital resources such as water, sunlight, and nutrients [8].

Moreover, invasive plants are under altered natural selection pressures (such as aforementioned predation) in their new environment, providing the possibility of rapid adaptive evolution. Plants may evolve novel characteristics that also cause damage to surrounding life [10]. In one example, an invasive plant can experience selection on biochemical composition in its nonnative environment. As a result, the plant’s leaf litter, dead leaves from the plant that fall to the ground, can release more volatile chemicals, for example Peroxycetyl nitrate, than it did in the plant’s native ecosystem, harming the surrounding species richness [11] [12].

C. Motivation for Project

The current and potential future impact of invasive plants must be addressed to minimize their prominent detriments. Almost 60% of plant species in the United States are nonnative, and cause
the loss of billions of dollars every year, through causes such as damage to crop production and the cost of controlling invasive plants' excessive growth [1]. Their ability to succeed in new ecosystems allows them to outcompete natives, decrease biodiversity, and even transform the environment and its structure [13]. Fortunately, investigation has been undertaken to gather more information on this issue, as previously referenced. However, this has remained focused on terrestrial plants, and literature on the influence of invasive plants in an aquatic environment is limited, but points to detrimental effects. For example, rapidly multiplying invasive aquatic weeds can choke waterways, depriving other aquatic life of space, sunlight, and oxygen [14].

Despite this information, there is still more to be investigated on the topic of invasive plants. This is the problem this project seeks to address. As mentioned, research has explored the detrimental effects of invasive leaf litter on land, and the harmful effects of invasive plants in an aquatic setting are evident, but there is a gap in the literature regarding research on the synthesis of these two concepts: the effect of invasive plant leaf litter on aquatic ecosystems, which could uncover unprecedented mechanisms by which invasive plants influence the water bodies with which they interact.

D. Goals

Therefore, the goal of this project was to contribute to the range of knowledge on invasive plants by investigating the effect of multiple plants' leaf litter on a lake ecosystem. More specifically, the goal was to research the impact of red maple (Acer rubrum), American beech (Fagus grandifolia), Japanese knotweed (Fallopia japonica), and common reed (Phragmites australis) leaf litter. This goal was divided into three parts, by focusing on three different lake factors:

1) To determine the effect of invasive and native plant leaf litter on vital nutrients in a lake ecosystem.

2) To determine the effect of invasive and native plant leaf litter on prevalent food web components in a lake ecosystem.

3) To determine the effect of invasive and native plant leaf litter on various 'healthy ecosystem metrics' indicative of a lake ecosystem's well-being.

Leaf Litter Species Chosen

Because the research was conducted in a Northeastern United States forest and lake setting, the project focuses on organisms and aspects particular to this region and ecosystem type. Red maple and American beech were chosen for use as a gauge of comparison for the effect of the invasive plants because they are plants native to the area and also because their leaf litter can be easily acquired from the forest floor during the fall. Japanese knotweed and common reed were selected, as they are prevalent and harmful invasive plants in the region and because their leaf litter is not difficult to obtain.

Japanese knotweed is an herbaceous perennial plant native to eastern Asia that was introduced to North America, and has since escaped control and become invasive in ecosystems across the continent, including lakes [15]. The plant is considered highly invasive in both the US and Canada. Common reed is a perennial grass that was introduced in North America from Europe and has gained invasive status in the new region [16]. It is abundant along the borders of lakes throughout the mainland United States and southern Canada, and is considered an indicator of wetland disturbance [17].

Goal 1

The goal to monitor nutrient levels was set because nutrients are an integral part of a functioning ecosystem, nourishing plants and other producers to support all trophic levels. As such, a change in nutrient levels in a lake has the potential for drastic repercussions throughout the ecosystem. The nutrients selected to focus on were nitrate and phosphate, as these are two of the most prominent and important nutrients in a lake ecosystem.

Goal 2

In keeping with the importance of analyzing nutrients because of their effect on trophic dynamics, a goal was set to determine effects of leaf litter on multiple organisms within this food web itself. Through this, a more comprehensive analysis of the trophic structure in a lake was achieved, incorporating levels beyond the nutrient base. The two organisms analyzed were algae and mosquitoes, which represented data from a producer and consumer in the ecosystem.

Goal 3

A healthy lake ecosystem can only exist within specific ranges of a variety of factors, and the third part of this project's goal was to measure these factors and determine whether the leaf litter treatments resulted in a thriving ecosystem as defined by these healthy ecosystem metrics. The three metrics tested for this goal were water pH, dissolved oxygen concentration, and conductivity. pH was measured because balanced water chemistry is vital to support a successful lake ecosystem, and harmful modifications in this chemistry by leaf litter would be revealed by extreme pH levels. Similarly, conductivity is dependent on the presence of ions in the water, and is therefore another useful measure of water chemistry. As oxygen is necessary to sustain heterotrophic life and also an indicator of photosynthesis, the concentration of dissolved oxygen was recorded to monitor the ecosystem's support of this aspect of life.

E. Hypothesis

Corresponding with the three components of this research's goal, three hypotheses were established.

1) The leaf litter of all treatments, both invasive and native, would increase nitrate and phosphate levels.

This hypothesis was made because it was expected that leaf litter would begin to break down in the water, and the nutrients existing in the leaves would be released, leading to heightened nutrient levels. No distinction for hypothesis could be found between invasive and native plants in this regard.

2) The growth and survival of algae and mosquitoes would remain at normal levels in the exclusive presence of native leaf litter, but would decrease with invasive plant leaf litter.
When being affected solely by native leaf litter, the food web was predicted to be typical as this situation was a natural ecosystem with no abnormal influences included. However, it was predicted that invasive leaf litter would be detrimental to the food web components, as aforementioned research has shown that invasive plants, and specifically their leaf litter, can harm native species in order for the invasives to outcompete them.

3) Native plant leaf litter would yield favorable pH, dissolved oxygen, and conductivity metrics, but that of invasive plants would result in extreme and deleterious values.

As in hypothesis 2, native leaf litter was not hypothesized to affect these healthy ecosystem metrics because a lake ecosystem tends to maintain the proper ranges of these factors in a native species setting. Invasive plant leaf litter was predicted to lead to unhealthy ranges because invasives are an ecosystem disruptor, and through damage to other organisms and abnormal effects on water quality factors will most likely create more inhospitable conditions. This means abnormally acidic or basic pH, an oxygen concentration so low that it cannot support life, and extremely high or low conductivity.

II. METHODS

As invasive plants have largely unexplored effects on lake ecosystems, the objective of this research was to study the response of a lake ecosystem to the leaf litter of invasive plants. As per standard procedure in research of this nature, aquatic mesocosms were used to investigate this effect [18]. An aquatic mesocosm is a small enclosure of water under controlled and close to natural conditions. This tool allowed for an environmental factor to be manipulated in a realistic simulation of a lake ecosystem, avoiding the unfeasibly large scale and potentially hazardous nature of experimenting on a lake itself. Four different combinations of leaf litter were implemented as treatments, and the impact of each on water quality factors as well as mosquito larvae development recorded and compared.

A. Creation of Mesocosms

Water

Eleven mesocosms were created in eleven separate plastic rectangular containers, each 50 centimeters long and 20 centimeters wide. The mesocosms were composed of water, leaf litter, and mosquitoes. First, four liters of water were added to each container to fill it close to the top. Natural lake water from a lake in a northeastern United States forest region, containing phytoplankton and zooplankton, was used to accurately replicate the conditions of a lake ecosystem.

Leaf Litter

Next, varied treatments of leaf litter were added because this was the experimental variable to be tested. Leaf litter was collected by hand during the fall from the surface layer of litter on the ground in a northeastern United States forest ecosystem. It was sorted by species and stored in a dry environment until use. Leaf litter from native red maple (Acer rubrum) and American beech (Fagus grandifolia) trees were used, along with Japanese knotweed (Fallopia japonica) and common reed (Phragmites australis) as the two invasive plants. Four different treatments of leaf litter were used in the mesocosms to observe and compare their various effects. Treatment A consisted of 5 grams of red maple and 5 grams of American beech leaf litter, and was added to two of the mesocosms (Fig. 1). For treatment B, 10 grams of Japanese knotweed leaf litter were added to three mesocosms. Treatment C, the second invasive plant treatment, used 10 grams of common reed leaf litter in three mesocosms. Lastly, treatment D comprised 2.5 grams each of red maple, American beech, Japanese knotweed, and common reed leaf litter added to the remaining three mesocosms.

Treatment A was used as a control treatment to provide insight into the water quality and mosquito development data that occurs in the absence of invasive plants. As the two natives chosen are natural inhabitants of the northeastern United States, where the research was conducted, this provided a frame of reference with which to determine how the invasive treatment changed the selected environment compared to an ecosystem that hasn’t been invaded. Treatments B and C contained leaf litter of two individual invasive plants as the experimental treatments, to be compared to the native treatment as explained. Two separate invasive plants were used to learn if and how different invasive plants can produce different effects in the same area. Specifically, common reed and Japanese knotweed were chosen because these two species are invasive to and prominent in the region, and often have influence on the area’s lakes. Treatment D was the closest approximation to the true composition of leaf litter in a lake ecosystem, and was implemented to observe the interactions between all leaf litter components in nature. While treatments B and C were used to the effect of isolating the specific effects of each invasive, treatment D contained a mix of every plant species analyzed, as a typical ecosystem will contain both native and invasive plants. In this way, it could be determined whether the presence of native leaf litter affected the impact of invasives, and any other unforeseen differences compared to the other treatments could be observed with regard to interactions between native and invasive plant leaf litter.

Mosquitoes

Once these ingredients were added, ten 2nd instar Culex sp. mosquitoes were added to each of the mesocosms using a pipette in order to monitor effect on mosquito larvae development. Culex sp. were used because these are a common species to the region, and a natural part of the ecosystem being modeled. 2nd instar larvae were chosen because they were close to the adult stage and would become developed during the data collection period. Ten were placed in each mesocosm as a standardized quantity at a

Figure 1: An unfinished mesocosm (50 cm by 20 cm) containing Treatment A in lake water (Photo by A. Bhardwaj)
comfortable population density. These mosquitoes were obtained from a larger container of stagnant water in which they were grown. Each mesocosm was covered with window screen mesh material to prevent the escape of any newly emerged adult mosquitoes, and a rubber band placed around the container to secure the mesh in place. A 9-cm diameter hole was cut out of the center of the mesh and the top of a 0.5-liter cylindrical mosquito breeder attached over the hole to catch the adult mosquitoes. These completed mesocosms (Fig. 2) were arranged in an array of random distribution by treatment type in a greenhouse, and remained in place for the duration of the project.

B. Collection of Data

After creation, the mesocosms were left untouched for a period of five days, to let the leaves sink in the container and to allow for algae and microorganisms such as zooplankton to become established. This time period was derived from convention and common practice in this area of research [18] [19]. Following this, the mesocosms were sampled and data collected every two days for one week. Admittedly, a sampling period of one week did pose a limitation in this research, as the measured factors were still changing at the conclusion of the week and results and trends may have varied over a longer period of time. Sampling was carried out every two days because changes in ecosystem factors from day to day would have been marginal and rendered daily sampling unnecessary. In sampling, water samples were taken from the mesocosms and water quality factors analyzed. Measurements of pH, dissolved oxygen concentration, conductivity, algae content, nitrate concentration, and phosphate concentration were recorded.

Water Quality Data

A multipurpose instrument, the YSI ProPlus meter was used to measure the dissolved oxygen, pH, and conductivity of the water samples by placing the meter's probe in the sample. For the dissolved oxygen reading, the probe was stirred gently in the water. To measure algae content, a pipette was used to fill a plastic cuvette with a small amount of sample water. The cuvette was then placed in a Turner Designs Aquafluor for reading.

Both the nitrate and phosphate concentrations of the water were taken using a Hach DR/890 colorimeter. The nitrate mid range test used the colorimeter's program 54. Two sample cells were filled with 10 mL each of sample water, one to be used as the blank and the other to be treated. One NitraVer 5 Nitrate Reagent Powder Pillow was added to one cell, and then the cell was capped and shaken vigorously for one minute. Following this, a 5-minute reaction period took place in which the sample was left untouched. Next, the outsides of the cells were wiped clean with Kimwipes, the blank was placed in the colorimeter for calibration, and then the prepared sample was placed in the colorimeter for reading of nitrate concentration.

Figure 2: Array of completed mesocosms for each treatment type, fitted with mesh and breeder to contain mosquitoes (Photo by A. Bhardwaj)

Figure 3: Photos of the YSI ProPlus, Turner Designs Aquafluor, and Hach DR/890 colorimeter used in data collection, respectively (Photos by EcoEnvironmental, Hoskin Scientific, and Pine Environmental)

The phosphate concentration test used the same colorimeter with a varied procedure. To start, two cells were filled with 25 mL of sample each, and 1 mL of Molybdate Reagent was added to one using a calibrated dropper. Next, 1 mL of Amino Acid Reagent Solution was added to the same cell, and the cell was capped and inverted several times to mix. It was then left to sit for the duration of a 10-minute reaction period. Following this, the cell containing only sample water, the blank, was used to calibrate the colorimeter, and the prepared cell was then placed in the colorimeter to obtain a phosphate concentration reading.

Mosquito Development Data

In addition to water quality factors, data were also collected regarding mosquito development, in order to further simulate an authentic lake ecosystem and gain insight into the effects of the treatments on animal life in addition to simply the water quality. Every two days, on the same days that water quality factors were measured, each mesocosm was inspected for the presence of adult mosquitoes that had developed from the initial 2nd instar larvae. First, the cylindrical mosquito catcher was examined and the number of adult mosquitoes inside was recorded. However, not all adult mosquitoes that developed found their way into the cylinder, so the space inside the covered mesocosm was inspected as well. The mesh covering the containers was pulled back slowly and carefully, and the adult mosquitoes were counted as they became visible.

Every adult mosquito observed in the mesocosm was recorded, whether it was dead or alive, as the object was to determine how many mosquito larvae under each treatment were able to develop into adults, and their subsequent survival or death as adults was irrelevant to these data. All mosquitoes counted on a given day were released or removed from the mesocosms so as not to impact the count recorded on the next day, and to ensure the data collected each day reflected the number of mosquitoes that developed into adults within the past two days only.
III. RESULTS

Data collection was designed with the purpose of achieving the project’s three goals of investigating nutrients, food web components, and healthy ecosystem metrics, in addition to testing the three hypotheses formulated that correspond to these goals. As such, data were collected for nitrate and phosphate concentrations, algae growth, mosquito development, pH, dissolved oxygen concentration, and water conductivity.

In analysis of these data, similar methods were followed for each of these metrics. The data for the individual mesocosms in each treatment group was averaged to obtain a mean value for each treatment type. These were then plotted in a line graph of a specific metric vs. date of data collection with a separate line for each of the four treatments. Error bars of one standard deviation from the mean were calculated and included at each individual data point.

For further interpretation, repeated measures analysis of variance (rANOVA) tests were performed on all data. rANOVA was used for these data because repeated measures of the same mesocosms were taken for the same variables at different points in time. This test analyzed the effect of both time and treatment on any single variable by considering the variance of each data set. It yielded results for each metric regarding relationships between subjects (effect of treatment on data) and within subjects (effect of time on data in the frame of one treatment type). In this analysis, a result of $P<0.05$ indicated a statistically significant effect.

A. Nutrients

Data regarding nutrient levels were important to collect, as they shed light on the conditions at the most basic level in the ecosystem, which nourishes the entire food web and is vital to ecosystem functioning. Every two days for the duration of one week, data for phosphate and nitrate concentrations, measured in parts per million (ppm), in each mesocosm were taken using a Hach DR/890 colorimeter with two different chemical tests.

Phosphate

As Fig. 4 below showed, the error bar range for the common reed treatment remained at a higher phosphate concentration level than that of all other treatments throughout the experiment, meaning that phosphate levels due to common reed leaf litter were statistically significantly higher than all others, as determined by the standard deviation of each data point.

Nitrate

The results for the four treatments were much more varied for nitrate levels than for phosphate, from zero nitrate concentration in the native/invasive mix to the common reed treatment at the highest comparative concentrations once again (Fig. 5). However, unlike in the data for phosphate, the error bars of the common reed data in this nitrate graph overlapped with others for the first two dates, making the data not completely conclusive. This may have been due to sizeable variation in measurements between mesocosms of the same treatment type at the onset of data collection.

As previously, rANOVA tests were carried out using the data for nitrate concentration (Table I). The test between subjects resulted in a $P$ value of $P<0.001$, showing with statistical significance that the leaf litter treatments had an effect on nitrate levels. However, the outcome of the test within subjects was a value of $P=0.485$. Because this value was greater than 0.05, it led to the conclusion that the nitrate concentrations in each mesocosm didn’t change significantly with time.

B. Food Web Components

In order to gain insight into dynamics at levels throughout the ecosystem’s trophic structure, attention was given to algae growth and mosquito development. These data were collected every two days for one week and analyzed using various methods.

Algae

Data of algae content in each mesocosm were first plotted in a line graph (Fig. 6). These data were measured in relative fluorescence units (RFU), as the instrument used to collect the data (the Turner Designs Aquafluor) employed fluorescence detection in its measurements. The error bars in the graph below showed no significant distinction in algae levels of any particular treatment type. Over the first three data collections, all lines stayed within a restricted range and had largely overlapping error bars. On the fourth data collection, however, all lines varied more, but error bars were also comparatively larger and still overlapped heavily, showing that there was no discernable difference due to treatments in algae levels as determined by standard deviations.

Figure 4: Line graph of average phosphate concentration vs. date of data collection in four separate treatments

Figure 5: Line graph of average nitrate concentration vs. date of data collection in four separate treatments
Next, the rANOVA analysis was used for algae levels (Table I). The test between subjects gave a result of $P=0.367$, which was greater than 0.05 and therefore indicated there was no effect of leaf litter on algae growth. However, analysis within subjects resulted in $P=0.003$, which was low enough to conclude that time affected algae levels.

**Mosquitoes**

The number of new adult mosquitoes in the mesocosm was counted every two days while the mosquito larvae were all still developing, then daily when larvae started becoming adult mosquitoes. The line graph (Fig. 7) representing the data below showed largely inconclusive results. For the first three dates, the number of new mosquitoes remained low and consistent for all treatments because no larva had yet reached the point in development at which it grows into an adult. Following these first three instances, larvae continuously became adults and both the data and error bars ranged widely, giving no comparative distinction between treatment types.

![Figure 7: Line graph of average number of mosquitoes vs. date of data collection in four separate treatments](image)

Given the inconsistency of the data, simply a normal Analysis of Variance test was run for the number of mosquitoes by treatment type. It resulted in $P=0.742$, a value far higher than 0.05 that led to the conclusion that the leaf litter treatments had no effect on mosquito larvae development into adults.

**C. Healthy Ecosystem Metrics**

**pH**

The pH of the water in each mesocosm was also measured every two days. As pH exists within a small range of values, and seemingly miniscule discrepancies in pH can have magnified effects, the vertical axis of the graph below doesn't include 0, and instead focuses on the relevant range of pH in this experiment. As Fig. 8 showed, the pH in the water of mesocosms treated with maple and beech leaf litter remained consistently and significantly lower than that of the three other treatments.

![Figure 8: Line graph of average pH level vs. date of data collection in four separate treatments](image)

Secondly, the rANOVA analysis for pH resulted in $P<0.001$ for both the between subjects and within subjects test—it can be concluded that the pH varied significantly with regard to leaf litter type, and that pH changed significantly over time (Table I).

**Dissolved Oxygen**

It can be seen from Fig. 9 that the dissolved oxygen levels in the mesocosms with maple and beech, common reed, and the native/invasive mix increased and decreased together throughout the research, and ended with the common reed mesocosms having the highest dissolved oxygen concentration. The water in Japanese knotweed mesocosms, however, remained at significantly lower dissolved oxygen levels consistently.

![Figure 9: Line graph of average dissolved oxygen concentration vs. date of data collection in four separate treatments](image)

In addition, rANOVA tests both between subjects and within subjects yielded $P<0.001$, so it was concluded that the leaf litter treatments as well as time both affected dissolved oxygen concentration (Table I).

**Conductivity**

Conductivity measures a solution’s ability to conduct electricity. Data for conductivity in the water of each mesocosm were taken every two days, and produced the line graph below (Fig. 10). The conductivity in mesocosms treated with maple and beech, common reed, and the native/invasive mix didn’t vary much as the experiment progressed, and all three remained in close proximity to each other. However, the mesocosms containing Japanese knotweed consistently had a much greater conductivity, and the conductivity also increased significantly as time went on.
As indicated by the P value of P<0.001 for the rANOVA test for conductivity (Table I), the results were significant both between subjects and within subjects. Water treated by different leaf litter had statistically significant variation in conductivity, and conductivity also changed significantly as time progressed.

**TABLE I. rANOVA Test Results for Each Metric**

<table>
<thead>
<tr>
<th>Metric Analyzed</th>
<th>Between subjects</th>
<th>Within subjects</th>
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<td>0.000</td>
</tr>
<tr>
<td>Nitrate</td>
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<td>0.485</td>
</tr>
<tr>
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<tr>
<td>Conductivity</td>
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</table>

*a. Bolded numbers indicate statistical significance.*

**IV. DISCUSSION**

The dynamics of a lake ecosystem under the influence of invasive plant leaf litter has gone largely unexplored by research. This project aimed to deepen understanding of the effects of leaf litter from the invasive plants Japanese knotweed and common reed, within the reference frame of American beech and red maple leaf litter. Three hypotheses were formulated at the outset of the project aimed to deepen understanding of the effects of leaf litter treatments. In terms of dissolved oxygen concentrations, the data were evidenced to be statistically significant by the rANOVA test. In addition, the within subjects rANOVA test gave a significant result, demonstrating in conjunction with the line graph that the algae grew over time, an indication that the algae experienced no deleterious effect from any leaf litter. Additionally, the mosquito development data resulted in no statistical significance through an ANOVA test, and the line graph of the data showed no discernable trends, thereby failing to confirm the original hypothesis.

**Hypothesis 2**

Secondly, the hypothesis that algae and mosquito growth would be adversely affected by invasive leaf litter and not by native leaf litter was also inconsistent with the results. The data collected for algae growth was deemed insignificant between subjects by the rANOVA test, showing no difference in the effect of different leaf litter treatments on algae. In addition, the within subjects rANOVA test revealed that both treatment and time had an effect on results, but time did not. Considering the line graph, the mesocosms treated with common reed once again had the highest concentrations, and those containing the mix of native and invasive leaf litter had extremely low levels of nitrate. These results also proved the first hypothesis incorrect, as there was no indication of an increase in nitrate for all mesocosms, and the large range of results between treatment types showed that not all treatments had the same effect, in disagreement with the prediction of the hypothesis.

**Hypothesis 3**

Finally, the hypothesis that pH, dissolved oxygen, and conductivity would be at harmful levels only in the invasive leaf litter mesocosms could not be proven correct by the results. In fact, the most extreme and acidic water was found in the mesocosms containing the mix of native and invasive leaf litter, that of common reed, as confirmed by the rANOVA statistical analysis in concert with the related line graph. This inconsistency once again refutes the hypothesis. Finally, the conductivity data were found to be significant between subjects, demonstrating through analysis of the conductivity line graph that the Japanese knotweed leaf litter yielded significantly greater conductivity levels, while the other invasive leaf litter, common reed, resulted in conductivities intermediate to the other two treatments, a discrepancy that also proves the effect of invasive plant leaf litter in this set of healthy ecosystem metrics was not uniform, a conclusion in disagreement with the hypothesis.

**B. Additional Findings**

Although every initial hypothesis made was discovered to be incorrect, the results of this research do point to new conclusions that can be drawn regarding the effect of the leaf litter treatments.
Nutrients

The result of increased levels of phosphate in the mesocosms treated with common reed leaf litter showed that common reed leaf litter released disproportionate amounts of phosphate as it decomposed in the water, thus creating more phosphate-rich water than the other treatments. The statistically significant decrease in phosphate over time for all mesocosms was likely due to algae, zooplankton, and phytoplankton in every mesocosm consuming the nutrient continually.

Similarly, nitrate concentrations were also statistically significantly higher in the mesocosms treated with common reed leaf litter, indicating that the break down of this leaf litter also released heightened amounts of nitrate into the water relative to the other treatments tested. Interestingly, the mesocosms treated with the mixture of both native and invasive plant leaf litter yielded water with the lowest nitrate levels by a significant margin, despite the fact that this mixture of leaf litter included both leaf litter from common reed, which caused extremely high nitrate levels when isolated. Additionally, the native and invasive leaf litter, which were all present in this mixture, each produced noticeable nitrate levels alone, but combining the invasive and native leaf litter resulted in low, undetectable nitrate levels. Evidently, this result showed that the nitrate levels produced by a composition of various invasive and native plant leaf litter was not additive, as intuition would suggest, but instead was likely determined by complex interactions of factors not examined within the scope of this experiment.

Healthy Ecosystem Metrics

Another unexpected result of this research was that the pH of water affected by the native leaf litter was significantly lower and more harmful than that of the other treatments. This result was in agreement with a previous study by Stoler and Relyea that found red maple leaf litter to yield the lowest water pH among mesocosms containing 12 different leaf litter monocultures [19].

In addition, the leaf litter of Japanese knotweed resulted in the outlying lowest dissolved oxygen concentrations compared to the other leaf litter treatments. From this, it can be concluded that the colonization and decomposition of the Japanese knotweed leaf litter occurs at the fastest rate and therefore consumes the greatest amount of oxygen, decreasing the water’s dissolved oxygen concentration. On the same topic, common reed leaf litter-treated mesocosms had the highest dissolved oxygen concentrations on the last three of four data collection dates. The cause of this trend was related to the aforementioned nutrient data in that the heightened nitrate and phosphate levels in the common reed mesocosms can be inferred to have nourished increased phytoplankton growth. As phytoplankton is a photosynthesizer, this proliferation contributed a net increase of oxygen to the water, making dissolved oxygen greater than that of the other three treatments. Although it may seem that algae should also have experienced increased growth in this situation and produced higher levels of dissolved oxygen, algae has in fact been found to merely have an indirect link to oxygen concentrations through influencing the ecosystem’s net primary production and plankton respiration [20]. Therefore, this experiment’s result in which the dissolved oxygen concentration of common reed mesocosms increased in the absence of increased algae growth was indeed consistent with the literature on the subject.

Finally, several conclusions can be drawn regarding the conductivity data. The rANOVA test between subjects was significant, showing that the treatments affected conductivity, and providing additional insight when interpreted using the line graph as well. The mesocosms treated with Japanese knotweed leaf litter resulted in the outlying highest conductivity values, while the Maple/Beech native leaf litter was slightly lower than the others. The heightened conductivity for Japanese knotweed can once again be attributed to the conclusion that the colonization and decomposition of this invasive leaf litter was the fastest of all treatments, and therefore released the greatest number of ions into the water, increasing conductivity by the greatest margin. This trend demonstrated consistency across results, as the dissolved oxygen data also pointed to Japanese knotweed breaking down at an increased rate.

Conversely, the Maple/Beech mesocosm conductivity values were the lowest, indicating the opposite conclusion to that drawn from the Japanese knotweed data: that the Maple/Beech native leaf litter broke down at the slowest rate, releasing the least amount of ions and resulting in the lowest conductivity data. This was consistent with previous findings that water of lower pH slows the rate of leaf litter decomposition, as the pH in the Maple/Beech mesocosms was found to be the most acidic [21].

However, although conductivity varied greatly across treatments, one similarity of all treatments in this respect was that the conductivity values increased over time. The rANOVA test within subjects establishes that the conductivity in each mesocosm changed significantly over the course of the experiment, and the corresponding line graph indicates that this change was a trend upward. This finding confirms the notion on which the previous two conclusions were based: that the conductivity increases over time as a result of ions being released from all leaf litter as the leaves break down in the water.

C. Principal Findings

In the analysis of this project’s results and consideration of the mechanisms driving these observations, several discoveries were made that were corroborated by data from multiple variables. These were the principal findings and outcomes of this research.

Firstly, common reed leaf litter was found to increase the concentrations of both nitrate and phosphate significantly higher than any other leaf litter, because the leaf litter’s breakdown and decomposition released the highest amounts of nutrients into the water. This points to the conclusion that common reed leaf litter has the potential to cause eutrophication in lakes. Eutrophication begins with heightened nutrient levels in a lake, which can nourish explosive algae growth, followed by the die-off and decomposition of this large algal biomass, a process that depletes the lake of dissolved oxygen and causes grave harm to the ecosystem [22]. In addition, the increased levels of dissolved oxygen in the common reed mesocosms were concluded to demonstrate increased
phytoplankton growth, which is an indicator of eutrophication as phytoplankton reacts sensitively to the beginning stages of the process [23]. All considered, the results of this project demonstrate the beginning stages of eutrophication as caused by common reed leaf litter through increased nutrient levels, the latter stages potentially occurring subsequently over a longer period of time.

In addition, Japanese knotweed leaf litter was shown to have the fastest decomposition and breakdown rate. This was evinced by the statistically significant comparatively low values of dissolved oxygen and comparatively high values of conductivity caused by the Japanese knotweed treatment, as previously explained. This high speed of leaf litter breakdown indicated the potential for Japanese knotweed leaf litter to cause abrupt changes in ecosystem balance and dynamics, a harmful effect as rapid shifts place stress on an ecosystem [24]. As a result of this stress, various ecosystem dynamics are disrupted, including nutrient cycling and the balance of species dominance [25].

Before beginning this research, it was predicted that invasive plant leaf litter would have detrimental effects, however all three hypotheses on this assumption were proven wrong. Despite this, the two principal conclusions found new mechanisms by which invasive plant leaf litter caused harm to a lake ecosystem that were not previously anticipated.

V. CONCLUSION

This research set the defining goals of exploring the effect of invasive and native plant leaf litter on 1) lake nutrients, 2) food web components, and 3) healthy ecosystem metrics in order to gain deeper insight into the influences of invasive plant leaf litter on a lake ecosystem. In accordance with these goals, three hypotheses were made with the prevailing prediction that invasive plant leaf litter would be more detrimental to a lake than native litter. In order to test these hypotheses, four groups of mesocosms were created using lake water, mosquitoes, and a different treatment of leaf litter for each of the four groups.

Data were collected regularly over one week for variables tailored to each of the three goals. Phosphate and nitrate data were collected towards the first goal, data for algae growth and mosquito development for the second, and data for pH, dissolved oxygen, and conductivity satisfied the third goal. These collected data, some conclusive of a specific trend and some conclusive of no trend, created a reasonably comprehensive understanding of the various influences of the leaf litter treatments used. In analysis of these data, line graphs were created for each variable with respect to time, and rANOVA significance tests were performed for each metric. This interpretation of the data led to the formulation of many conclusions of varying magnitude.

Of these conclusions, two discoveries held the most significance for this research. It was concluded that the leaf litter of common reed, an invasive plant, had the potential to cause eutrophication in lakes. Secondly, it was found that the leaf litter of invasive Japanese knotweed could create significant change in a lake ecosystem that caused harm because of its rapid nature.

The two major discoveries of this research carry various implications for lake conservation. In lakes experiencing or at risk of eutrophication, conservationists can now use this information to adopt a more targeted approach to combating the eutrophication by specifically earmarking invasive common reed for removal from lakeside areas. Additionally, lake caretakers can focus more efforts on eradicating the invasive Japanese knotweed from beside lakes, as it has now been proven to have adverse effects on a lake ecosystem.

A. Future Research

In extension of this research, a more long term project over multiple months may be taken on, specifically to reinforce the conclusion that common reed may cause eutrophication, as a longer time frame will reveal the later stages of eutrophication, namely the increased growth of algae due to higher nutrient levels and the subsequent decrease in dissolved oxygen concentration as a result of algae death and decomposition. This longer study may also reveal additional influences of the leaf litter treatments that did not present themselves within the one week time span of this project. Future research may also include more food web components, such as tadpoles, in the mesocosms and measure the dynamics of a wider range of variables, such as tannins, to accomplish a more comprehensive analysis of the interactions taking place in the experimental setup.

REFERENCES

[11] Inderjit, Heather Evans, Christoph Crocoll, Devika Bajpai, Rajwant Kaur, Yu-Long Feng, Carlos Silva, Jacinto Treviño Carreón, Alfonso Valiente-Banuet, Jonathan Gershenson,


