Effects of Forest Tree Species Composition on Vernal Pond Water Chemistry, Decomposition, Primary Productivity and Amphibian Larval Fitness Traits

Submitted by

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Abstract

Human activities have dramatically changed the composition of tree species in North American forests. These changes have altered the composition of leaf litter in woodland ponds, which has been linked to the decline of amphibian populations. Leaves from different species of trees that accumulate in ponds each fall differ in their chemical composition and produce species-specific effects on water chemistry, nutrient availability and rates of decomposition that ultimately affect the fitness (growth, development and survival) of amphibian larvae that develop in these ponds. These findings have been based on artificial pond research. This project aimed to show that the composition of trees (species, size, and number of individual trees) surrounding temporary woodland ponds directly affected the chemical characteristics (pH, dissolved oxygen, conductivity, and phenolic concentration) and rates of decomposition of the aquatic pond environment. Documenting this relationship in natural pond communities will provide an impetus for developing forest management practices that benefit amphibians and other organisms that live in these aquatic environments. We found that high levels of red maple had a positive correlation with polyphenol levels, as well as a negative correlation with pH and conductivity levels. Elms showed to have a relationship with the decomposition rates of ponds. These each could be associated with the variation in juvenile tadpole mass at metamorphosis.

Introduction

Human activities, including logging, fire suppression, and the introduction of pest species, have altered the composition of native forest plant communities in North America (Ellison et al., 2005). The introduction of non-native insects and diseases have resulted in the near eradication of green ash and American chestnut tree species and contributed to a dramatic decline in hemlock and oak species. In contrast, fire suppression during the last century is thought to be the single most important factor contributing to the expansion of red maple, which now dominates the mid-canopy of many northern hardwood forests, and which will likely replace historically dominant trees in the overstory during the next century (Abrams, 1998). As Ellison et al. (2005) have reported, the dramatic changes in these ecosystems can impact fluxes of energy and nutrients, food webs, and biodiversity.

The composition of tree and other plant species in an ecosystem, specifically once such as a forest, can greatly impact its structure and function (Bangert et al., 2006). The foundation species of ecosystems are found in great abundance and are available to create the locally stable environments that other biota rely on (Ellison et al., 2005). This study will look into the effects of the decline in these foundation species, and how ecosystems are changed with the changing plant community.

In the Midwest, specifically the Great Lakes region, there has been a large number of changes to its plant communities both local and regional. For example, a 20% loss in conifers in this region was found to have occurred primarily through human activity. Excessive logging, repeated slash first, and attempts at creating farmland caused a reduction in hemlock, pine, and tamarack genera, and an increase in hardwoods (Williams, 1989; Gough, 1997; Schulte et al., 2007).

The hardwoods replacing this decrease in conifers is abundantly found to be red maple. Red maple has been increasing so quickly in the eastern United States that it is now deemed the “Red

maple paradox" (Abrams, 1998). Red maple is known to have a modest leaf physiologic response to environmental conditions, but this cannot explain its extreme expansion in this region. The spread of red maple has caused many foundation species in the Midwest and eastern United States to become eradicated. The red maple requires less light, water, and nutrients when compared to many of these native species, and this can help explain why it has been so successful where natural species were not in recent years.

Recent studies have linked changes in forest tree species to the decline of amphibian populations (Stephens et al., 2013). Autumn shed leaves that accumulate in small woodland ponds each year provide a nutrient source (carbon-C, nitrogen-N and phosphorus-P) for the growth of primary producers (microbial and algal communities) which are the primary food source for amphibian larvae that develop in these ponds. Along with nutrients, decomposing leaves also release secondary compounds such as phenolics (i.e. tannins) that can inhibit the growth of primary producers and affect amphibian larval survival (Cohen et al. 2012, Mozerolle et al. 2012).

Changes in the foundation species of ecosystems can also disrupt fundamental processes that many organisms rely on for survival, such as decomposition of organic matter, nutrient cycling, etc (Ball et al., 2008). Jenkins et al. (1999) found this kind of alteration with respect to the decline of eastern hemlock. Initially, with the presence of hemlock, the litter decomposed slowly and the dark and acidic soil allowed for slow nitrogen cycling and a low amount of nutrients in the soil. As hardwoods began to replace the hemlocks in these areas, the dynamics of decomposition were greatly altered due to the faster rate at which hardwood litter decomposes. This increased the nitrogen cycling rate and produced a more nutrient-rich soil. This is an obvious, and dramatic, change to these stable ecosystems.

These changes in plant community and the ecosystem can lead to changes in other organisms that make up this system. For example, Stephens et al. (2013) found that leaf litter from green ash,

which is a species in decline, allowed frog larvae to grow larger and develop faster, allowing for better survival when compared to larvae in other litter conditions. This reinforces the important role that tree species play in the development of its ecosystem and the other components in it. Stephens et al. concluded that the quality of the leaf litter strongly affected amphibian fitness.

A large body of research (see Stoler et al., 2015), using mesocosms (artificial pond communities), has demonstrated that tree species (leaves) differ in their chemical composition and produce species-specific effects on water chemistry (pH, dissolved oxygen, conductivity and polyphenolics), nutrient availability (N and P) and rates of decomposition. These studies have also demonstrated a direct relationship between the chemical composition of leaves and larval amphibian fitness traits, including growth and development rates, and survival.

While there is an assumption that differences in leaf litter input to ponds has the potential to alter aquatic ecosystems, to date there are no published studies demonstrating a link between the species of trees growing around ponds and the chemical characteristics of those ponds. The current study aims to analyze a natural forest community, surveying the tree species surrounding temporary woodland ponds. These ponds will also be examined, looking at the water chemistry of each throughout a single pond cycle. The levels of nitrogen and phosphorus, as well as the rate of leaf decomposition, will also be documented. These measures will be used to look for a statistical relationship between the forest community (tree species composition, number of individuals, and tree size) growing around these ponds with the chemical characteristics (pH, DO, conductivity, polyphenolics) of the ponds. Additionally, this will attempt to show that the nutrient levels in the ponds are related to both tree species composition surrounding the pond, as well as the rate of decomposition within the pond.

Methods and Materials

Study Site

The study was conducted in Bald Mountain State Recreation Area in SE Michigan. The temporary ponds surveyed were identified based on their location near Heart Lake (HL 1-6, 11, 13) and Graham Lake (GL 1, 3-6). The forested area around the surveyed ponds consisted of a variety of hills, marshes, grassy plains, and rugged terrains. The surveys were conducted May-June 2017.

Tree Survey

The tree communities around each pond were surveyed by constructing four 10x10m quadrats along the NSEW margins of the ponds. Within each quadrat, each tree was identified and its diameter at breast height (dbh) measured using a meter stick. All dead trees, excluding Ash, and trees with a dbh < 4 cm were not included in the surveys. Trees that were not within the quadrat, but had branches overhanging into the quadrat, were included. Nomenclature followed Barnes and Wagner (1981).

The total basal area of each tree species around each pond was calculated as the sum of the cross-sectional surface area (m^2 using the dhb of each tree) of each individual tree. For analyses, the total basal area of each tree family was used.

Water Chemistry

Dissolved oxygen (DO), pH, and conductivity were measured for each pond on three dates (May 10, May 23, June 8) at three different locations within each pond using a Hana Instruments HI 9828 multisensor probe.

Dissolved polyphenolic substances were assayed twice (May 1 and June 12). One 250ml water sample from each pond was stored at -20ºC for polyphenolic determination. Dissolved polyphenolic compounds were analyzed using the Folin-Ciocalteu method (Clesceri & Eaton 1998).

Decomposition, periphyton and microbial biomass

To measure rates of decomposition in each pond, 5g (dry mass) of cottonwood leaves were added to fine-mesh (1 mm pore size) and coarse-mesh (10 mm pore size) litter bags. The coarse-mesh bags allowed tadpoles access to the leaves. Five bags of each mesh size were placed on the bottom of each pond in about 30 cm of water. To measure periphyton and microbial biomass each pond received 5 unglazed ceramic tiles (121 cm^2). Each tile was grouped with a fine-mesh and coarse-mesh bag and secured with wire stake.

On the last day of the experiment (July 10), the bags were returned to the lab and the leaves removed and washed of any debris. They were put into labelled containers dried in an oven at 40ºC for 48 hours. The leaves were then reweighed. Decomposition was determined as the difference between the initial and final leaf mass.

Temperature

The temperature of each pond was taken every hour via a Hobo Pendant Light/Temperature recorder. The Hobo was placed in the middle of each pond, submerged about 30 cm below the surface, and retrieved at the end of the experiment (approximately 60 days). The Hobos were attached to a brick in order to maintain this depth throughout the experiment. Temperature was recorded in degrees Fahrenheit.

Light Quantity

The amount of light supplied to each pond was measured using two different methods. The first was using the Hobo that was placed in the middle of each pond. This recorded the amount of light exposure (lumens/ft^2). Additionally, an analysis using GAP (Gap Light Analyzer) determined percent canopy openness and total transmitted light (moles of light per square meter per day, mols/m^2/d). Pictures were taken of the canopy. The camera was placed at water level (facing north) and pictures were taken at three locations across the long axis of each pond.

Periphyton Abundance

Periphyton from each pond was analyzed on July 10. To assess periphyton abundance, one-quarter of the tile (5 per pond) was scraped (using a razor blade) into a 15ml tube containing 3ml of water and 9ml of methanol. The slurry was then filtered using a GF/F glass filters (Whatman Inc, Kent, U.K.) wrapped in aluminum foil, and stored at -20℃ until fluorometric analysis. Fluorometric analysis (Trilogy Model, Turner Instruments) was used to determine chlorophyll *a* (chl *a*) following a modified version of the EPA method 445.0 (Arar and Collins 1997).

Bacterial Count

To determine microbe abundance, one-quarter of the tile (5 per pond) was scraped (using a razor blade) into a 15ml tube containing 3ml of water and 9ml of methanol. These samples were homogenized and stored in the dark at 3℃ until used. Three 1 ml samples of each of these water samples were collected and transferred into microcentrifuge tubes containing formaldehyde. These were spun down at 1000x for 4 minutes, and 900 µL of the formaldehyde supernatant was removed. 1 mL of McDowell Trump's fixative was then added to each tube, and these samples were refrigerated when not in use. Slides were created of these samples, using acridine orange dye (10 µ/mL). 150 µL of the AO dye was mixed with each sample, and this mixture was filtered using GF/F glass filters (Whatman Inc., USA). The filter was sealed onto a slide with glycerol.

These slides were analyzed using a fluorescent microscope. Five areas from each slide were photographed (5 slides/pond) to obtain 5 different bacterial counts per slide. Each photo was captured using a green light and then a blue light, resulting in 10 photos per slide. Using NIS-Elements Advance Research software, the microbes on each of these photos were counted. We only counted microbes between .20 µm and 10 µm. Each microbe counted was also analyzed for length, diameter, width, area, and perimeter. This information was used to find an average bacterial count per pond.

Juvenile Mass at Metamorphosis

To determine mass at metamorphosis approximately 20 recently metamorphosed juveniles (tails completely resorbed (Gosner stage 46, Gosner, 1960) were collected from 12/13 ponds, returned to the lab and weighed on an electronic balance (to the nearest 0.001g.)

Statistical Analysis

For all variables measured, the raw data were $\log_{10}$ transformed when distributions deviated from normal. All percentage data were arcsine-transformed. To assess whether water chemistry variables varied during the summer a repeated-measures analysis of variance was used to test for differences among sampling dates. ANOVA's were used to compare differences in response variables among ponds using sampling dates as random variables.

Because many of the variables measured were related, principle component analysis (PCA) was used to reduce the number of predictor variables. The mean of the sampling dates was used for variables measured more than once. The PCA reduced the initial predictor variables to six components, which were not collinear. Components with Eigen values that were >1 were retained. Varimax rotation of component loadings was used to help interpret the components resulting from the PCA. The significance of factor loadings on each component was assigned if factors had loadings >0.560 at $a<0.05$. The factor scores were then used in multiple linear regression (backward selection) to explain variation in size at metamorphic climax in each pond. All statistical analyses were carried out in SPSS (version 24.0 IBM Corporation, Armonk, NY, U.S.A.) and reported means include ± SE.

Results

Tree Survey

We identified 19 species (including dead ash), representing 13 families surrounding the thirteen ponds (Table 1). The ponds had on average 6.23 ± 0.61 species (range: 4 to 13 species). Red maple and elm were the most common tree species (12/13 ponds) followed by white oaks

(9/13), black cherry (9/13), Shagbark and pignut hickory (6/13 each), and red oak (6/13). The total basal area of all tree species around each pond varied among ponds ($\bar{X}$ = 4.08 ± 0.47; range: 1.39-6.86 m^2). Red maple accounted for 43.6% of the total basal area of all species, followed by white oak (21.3%), red oak and hickory (8%). All other tree species accounted for less than 5% of the total basal area around each pond.

Water Chemistry

The pH varied significantly among ponds ($F_{(12,26)}$ = 54.46, P < 0.001; range 5.81-7.44; Fig 1a), and among sampling dates ($F_{(1,25)}$ = 5.44, P =0.028; Fig 1a). Dissolved oxygen levels were generally low (<2.0 mg/L) and did not differ among ponds ($F_{(12,26)}$ = 0.988, P = 0.485; range 0.048-1.56 mg/L; Fig 1b), but did decrease significantly between the first sampling date and the last two sampling dates ($F_{(1, 25)}$ = 13.96, P < 0.001; Fig 1b). Conductivity levels also varied significantly among ponds ($F_{(1,26)}$ = 74.29, P < 0.001; range 29.44-534.22 µS/cm; Fig 1c) and among sampling dates ($F_{(1,25)}$ = 6.79, P = 0.015; Fig. 1c). Polyphenolic levels varied significantly among ponds ($F_{(1,26)}$ = 16.09, P < 0.001; range: from 3.70- 25.79 mg/L; Fig 1d) but did not differ between sampling dates ($F_{(1,12)}$ = 1.39, P < 0.26; Fig. 1d).

Decomposition

The percent mass loss of fine mesh leaf bags did not differ among ponds ($F_{(12,50)}$ = 1.46, P = 0.17; range: 20.6-46.9 %; Fig 2a). However, the percent mass loss from coarse mesh leaf bags did differ significantly among ponds ($F_{(12,52)}$ = 2.33, P = 0.18; range: 35.8-46.3 %; Fig. 2b). The difference in mass loss between coarse- and fine-mesh bags averaged 12.2± (range: -8.7-21.4%) and differed significantly among ponds ($F_{(12,37)}$ = 1.46, P = 0.004).

Canopy cover, light and temperature

Percent canopy openness differed significantly among ponds ($F_{(12,32)}$ = 13.25, P < 0.001; range: 16.4-98.4%; Fig. 3a). In addition, both light intensity (as measured by submerged Hoboware)

and total transmitted light (GAP analysis) differed significantly among ponds ($F_{(12,16606)} = 78.630$, $P < 0.001$; range: 55.17 -704.50 lumens/ft^2; $F_{(12,32)} = 11.34$, $P < 0.001$; range: 4.54-29.23 moles m^{-2}d^{-1} respectively; Fig 3b, c). Average pond temperature also differed significantly among ponds ($F_{(12,16606)} = 67.297$, $P < 0.001$; range: 59.1-62.6°F; Fig. 3d).

Periphyton

Periphyton biomass differed significantly among ponds ($F_{(12,51)} = 13.17$, $P < 0.001$; range: 0.88-2.0 chl *a* µg tile^{-1}; Fig 4).

Microbial biomass

Microbial counts based on both DNA ($F_{(12,312)} = 9.07$, $P < 0.001$; range: 177.8-484.6; Fig 5) and RNA ($F_{(12,312)} = 13.75$, $P < 0.001$; range: 111.2-713.0; Fig 5) assays.

Juvenile metamorphic mass

Juvenile wood frogs metamorphosed from 12/13 ponds. Mass at metamorphosis differed significantly among ponds ($F_{(11,63)} = 55.11$, $P < 0.001$; range: 203-1039 mg, Fig. 6).

PCA analysis

Six components were extracted from the 19 variables measured in each pond. The six components accounted for 89.3% of the total variation in the data set (Table 2). Average pond temperature, percent canopy openness, light (measured by light exposure to hobo's, and total transmitted light from GAP analysis) dissolved oxygen and Chl *a* all loaded positively on Component 1 and accounted for 31.5% of the variance in the original data set. Because each of the variables producing the highest loadings on this component can be related to light, we labeled this component 'canopy cover'. Polyphenolics, ph, conductivity and total basal area of maple trees loaded significantly on Component 2, accounting for 21.6% of the variation in the original data set. The correlation coefficients of polyphenolics and total basal area of maple trees for Component 2 were negative indicating that they were inversely related to ph and conductivity. We refer to this

component as ‘polyphenolics/water chemistry’. Both measures of microbial abundance loaded positively on Component 3 and accounted for 11.2% of the variation in the original data set. We labeled this component ‘microbial biomass’. Mass loss from fine mesh and coarse mesh bags and total basal area of Elm trees loaded positively on Component 4 and explained 11.2% of the variation. We labeled this component ‘decomposition’. The total basal area of oak and hickory species and total basal area of all trees around the pond loaded positively on Component 5 and accounted for 7.6% of the total variation. The total basal area cherry trees loaded positively on Component 6 accounting for 6.2% of the total variation. Total basal area of all tree species loaded negatively on Component 6.

Multiple regressions of the six components with mass at metamorphosis using backward selection indicated that the polyphenolic/water chemistry (Component 2), microbial biomass (Component 3) and decomposition (Component 4) best explained wood frog metamorphic mass (whole model $R^2 = 0.911$, $F_{(4,7)} = 29.1$, $P<0.001$). Increasing values of all three components were associated with larger metamorphic body size, however because polyphenolics and total basal area of maples loaded negatively on Component 2, lower values for Component 2 were associated with higher total basal area of maples and polyphenolics and smaller mass at metamorphosis.

Discussion

Water chemistry (ph, DO, conductivity and dissolved polyphenolic substances), rates of decomposition and primary productivity (periphyton and microbial biomass) all differed dramatically among ponds. The total basal area of maple trees around the ponds explained the variation in water chemistry, while the total basal area of elm was associated with rates higher rates of decomposition. Primary productivity appeared to be unrelated to specific tree species surrounding the ponds. In particular, periphyton abundance appeared to be more closely related to canopy cover and not to specific tree species and microbial biomass was not associated with any of

the measured variables. Metamorphic body size was best explained by conductivity, ph, polyphenolics, microbial biomass and rates of decomposition.

A study done by Stephens et. al (2013) examined many of the same factors as the current study, although they manipulated leaf litter in artificial mesocosms as opposed to natural ponds. By comparing my results to the results of the Stephens study, we can see if any of these previous findings can be applied to the natural environment. pH, for example, was similar between the natural and artificial studies ($\bar{X}$ = 6.93±0.05 vs. 6.42 ± 0.01 respectively). Conductivity comparisons were similar, with the natural and artificial studies having similar means ($\bar{X}$ = 235.22±15.62 µS/cm vs. 246.63 ± 2.45 µS/cm).

On the other hand, dissolved oxygen significantly differed between the current study and the Stephens et al. study. In the mesocosms of the Stephens study, DO was much higher than in the natural ponds of the current study, and even increased over the 30-day span of the experiment. The current study showed a drastic decrease in DO over time (Fig 1b). This could be due to the artificial component of experiment by Stephens et al. By using small pools that were relatively uncovered and exposed to sunlight, the amount of dissolved oxygen would have been much higher than in natural ponds in forests, which were more covered by canopies. Additionally, the natural ponds had a lot more organisms that could be utilizing the oxygen when compared to the mesocosms that had a less diverse biome.

Polyphenolics also differed between the current study and the Stephens et al. (2013) study. The current study's average polyphenolic levels were only about half of the previous study's ($\bar{X}$ = 11.36±1.21 mg/L, vs. 22.83±3.54 mg/L). The range also differed significantly, with the natural study's range from approximately 4-26 mg/L, and the mesocosm study's range from approximately 10-90 mg/L. Stephens et al. looked at individual species of leaves in their study, finding the specific polyphenolic levels due to an individual type of leaf in a mesocosm. The current study alternatively

looked at a mixture of leaves in each pond, which could have caused lower polyphenolic levels, but also produced a more realistic result. By only looking at individual species of leaves in each polyphenolic measurement, the previous study may have obtained less realistic polyphenolic levels.

Another similar measure between the current study and the Stephens et al. (2013) study was the rate of decomposition. Both studies analyzed percent mass lost of fine and course mesh leaf litter bags. The previous study analyzed 10 different types of leaf litter, while we only looked at Cottonwood leaves in the bags. When comparing both studies' the percent mass loss of Cottonwood leaves was similar in the two studies (~35% mass loss in both fine and coarse mesh bags).

Periphyton biomass between these two studies differed dramatically. In the Stephens et al. (2013) study, periphyton levels ranged from 20 to 55 chl *a* µg tile^{-1}, representing an approximate 3-fold range. The current study periphyton levels measured in the ponds ranged from 40 to 6600 chl *a* µg tile^{-1}: a 165x increase. The amount of periphyton in the natural ponds were clearly must greater than in the mesocosms. A study by Holgerson et al. (2016) also analyzed periphyton biomass in natural ponds, and also found a larger range of measurements per pond. Their range approximately 30 to 325 chl *a* µg cm^{-1}, a 10-fold increase. While their range did not quite reach the numbers of the current study, one must also consider the difference in units. If Holgerson et al.'s findings were converted to the chl *a* µg tile^{-1} unit of the current study, the values would be much larger, and even more comparable to our findings. This reinforces a natural environment's better conditions for periphyton biomass when compared to an artificial setting. It allowed for the flourishing of periphyton that the mesocosms could not achieve.

Additionally, Stephens et al. (2013) concluded that periphyton was an important food source for juvenile tadpoles. This is not found in the current study, where the amount of periphyton did not relate to the juvenile tadpole mass. Those ponds with the highest amount of periphyton actually had the smallest massed tadpoles in this study (Fig. 4, 6).

The factors responsible for variation in water chemistry among ponds In both studies, mesocosms/ponds with an abundance of red maple were shown to have higher polyphenolic levels, and smaller tadpoles. This could be considered a predictor variable for the masses of these juvenile tadpoles.

The PCA analysis performed in the current study revealed six components, four of which explained variation in metamorphic mass. Dissolved oxygen, temperature, light intensity, total transmitted light, percent canopy openness, and periphyton biomass all were positively loaded on Component 1. These factors were not, however, associated with any particular species of tree, but rather seems to be influenced by the overall forest structure. A more open canopy leads to an increase in light (light intensity, total transmitted light), which provides more chlorophyll a to the ponds and the periphyton. More sunlight also allows the flourishing of algae, which increase the dissolved oxygen available in these ponds.

Component 2 related conductivity, pH, polyphenolics, and maples. Conductivity and pH had a positive association with component 2, while polyphenolics and maple had a negative association. This reinforces previous studies findings about maple and its relationship with polyphenolics (Stephens et al., 2013). Ponds that had a negative component 2 value were associated with an abundance of maple, an increase in polyphenolic levels, and a decrease in conductivity and smaller metamorphic mass, while positive values were associated with an increase in pH and conductivity, and larger metamorphic mass. Conductivity is generally associated with nutrient availability (Stephens et al., 2013), so a lower level of conductivity indicates a low level of nutrients. Less nutrients will cause less developed tadpoles. Therefore, component 2 confirms maple and polyphenolics' ability to predict juvenile tadpole mass.

Component 3 related the two measures of bacterial count, using the red and green fluorescent lights. Microbial counts did not group with any of the other variables measured but was positively

related to tadpole mass at metamorphosis. As mentioned previously, our study contradicted studies such as Stephens et al. (2013) by not having periphyton as an influencer or primary food source of the developing tadpoles. This could be explained by the bacterial count measures, suggesting that the tadpoles may be consuming the microbes in the ponds.

Component 4 related oak and hickory trees. While they were not associated with any variables measures. These two species have similar components when comparing amounts of lignin and nitrogen present, as well as the carbon-nitrogen ratio of the leaves. These three quantities were relatively higher than the other species present around the ponds researched (Ostrofsky, 1997), which could explain why they (oak, hickory) are related in the PCA analysis.

Component 5 related decomposition to the presence of elms. Decomposition was measured using the percent mass loss in both the fine and coarse mesh leaf litter bags. There is a positive correlation with the presence of elms around a pond and their rate of decomposition. This could be explained by the relatively small amount of lignin present in elm leaves (24.5%), along with the higher amounts of nitrogen (0.59) and carbon-nitrogen ratio (74.3). All of these factors play a role in increasing the decomposition rate of the ponds they surround (Ostrofsky, 1997).

Component 6 was only composed of cherry trees. This could be due to their relatively low levels of lignin (22.4%), high levels of nitrogen (1.67%), and low carbon-nitrogen ratio (29.8). This indicates that these leave decompose extremely quickly, and could have its own separate relation to the components of the ponds cherry trees surround (Ostrofsky, 1997).

Conclusions

When comparing the current study to previous, artificial studies of similar objectives, it can be said that there is a difference depending on the naturality of the environment used. It is more difficult to be able to replicate the true dissolved oxygen and polyphenolic levels of a natural pond in comparison to pH and conductivity, as seen when comparing the findings between the current

study and the study done by Stephens et al. (2013).

Decomposition was found to be comparable between the two studies, but periphyton biomass was drastically different. It has been concluded in various studies that periphyton is an essential food source for developing tadpoles (Holgerson et al., 2016; Stephens et al., 2013), but the current study did not support these findings. By examining the periphyton biomass between ponds and comparing these to the variation of juvenile mass in ponds, there does not seem to be the positive relationship that these previous studies have suggested. This could indicate another food source present in the ponds that the tadpoles feed on, such as microbes present in the ponds.

This study helps reinforce the assumptions about water chemistry, leaf litter, and tadpole growth made by previous studies. It also allows the application of these assumptions to the natural environment, where until now they were mostly confirmed by artificial experiments (Stephens et al., 2013). Additionally, it helps connect various factors that make up a forest ecosystem in order to explain the development of tadpoles, which has become a major topic in the recent years with the declining population of amphibians.

Certain limitations to this study involve some techniques used. This experiment was only done for about two months due to the lack to precipitation in the early summer of 2017, when the study was occurring. Certain factors could be different had we been able to measure the water chemistry more than three times. Additionally, we only focused on a handful of tree species, and there could be another unstudied species that could play a part in the structure of the forest and the amphibians living there.

Future studies could focus on strengthening the limitations of this study. Additionally, more research could be done looking into the primary food source of tadpoles in these natural temporary ponds, looking at a preference of bacteria over periphyton. Further research could be done with other aspects of tadpole metamorphosis, such as survival and length of larval period.

This study has confirmed the influence of leaf litter on the water chemistry of natural temporary ponds. The red maple present in large quantities in these midwestern forests have increased the levels of polyphenolics in the water, which in turn can also explain the decrease in development of the tadpoles that inhabit these areas. Due to the eradication of natural species such as ash, and the invasion of species such as red maple in these areas, there is an effect on the rest of the forest ecosystem, including the other organisms that live there.

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Table 1 *Families and Tree Species identified around the ponds*

Family	**Tree Species**
Oleaceae	(Dead) Ash
Salicaceae	Cottonwood
Tileaceae	Basswood
Betlaceae	Birch
	Hop-Hornbeam
Rosaceae	Black Cherry
Ulmaceae	Elm
Juglandaceae	Pignut Hickory
Aceraceae	Shagbark Hickory
	Box Elder
	Red Maple
	Silver Maple
	Sugar Maple
Fagaceae	Black Oak
	Red Oak
	White Oak
Pinaceae	White Pine
Lauraceae	Sassafrass
Hamamelidaceae	Witch-Hazel

Table 2 *PCA Analysis of Dependent Variables*

	Component					
	1	2	3	4	5	6
pH	0.033	**0.966**	-0.142	-0.049	0.132	0.007
Dissolved Oxygen	**0.74**	0.277	-0.149	0.432	-0.221	-0.006
Conductivity	0.1	**0.964**	-0.153	-0.024	0.095	0.079
Polyphenolics	-0.088	**-0.96**	0.178	-0.031	-0.088	-0.068
Fine Mesh Loss	0.087	0.004	0.384	-0.056	**0.555**	0.407
Coarse Mesh Loss	-0.154	0.068	-0.197	0.129	**0.858**	0.004
Temperature	**0.958**	0.02	0.045	0.146	0.079	0.009
Light Intensity	**0.865**	-0.034	0.116	-0.273	0.118	-0.197
Total Transmitted Light	**0.972**	0.082	0.009	-0.093	-0.054	0.054
% Canopy Openness	**0.959**	0.084	0.07	-0.029	-0.187	0.037
Maple	-0.351	**-0.581**	0.373	-0.236	-0.156	0.419
Oak	0.021	-0.249	0.19	0.842	-0.084	-0.171
Elm	-0.019	0.405	0.015	-0.162	**0.75**	0.081
Hickory	0.013	0.45	-0.101	0.773	0.095	-0.005
Cherry	0.017	0.11	-0.168	-0.083	0.137	**0.871**
Total Basal Area	-0.318	-0.42	0.425	0.57	-0.024	0.436
Periphyton Biomass	**0.564**	0.444	-0.35	0.137	-0.443	0.328
Microbe (green)	0.121	-0.291	**0.895**	0.17	0.065	-0.016
Microbe (red)	0.026	-0.175	**0.948**	-0.005	-0.116	-0.134

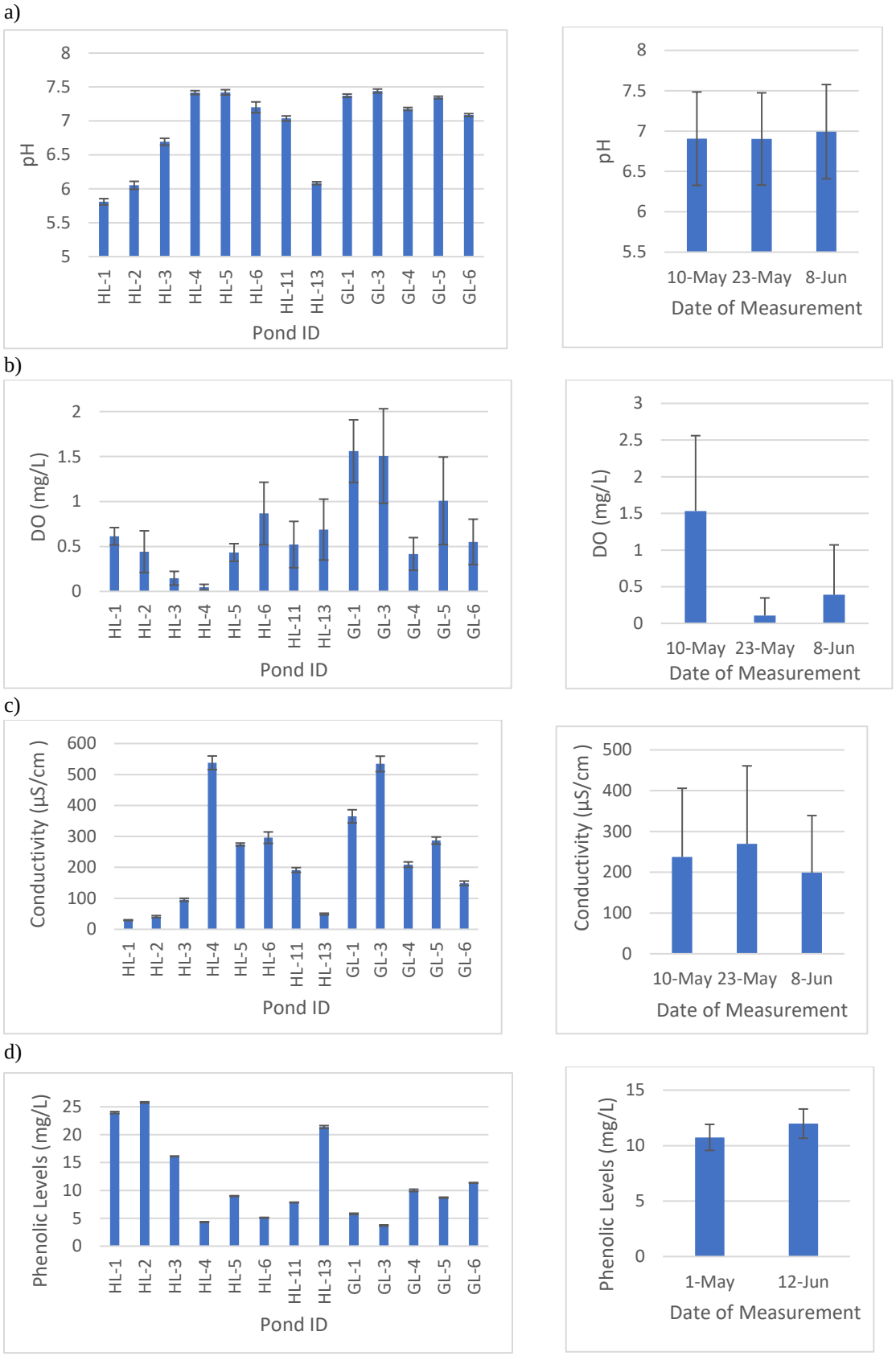
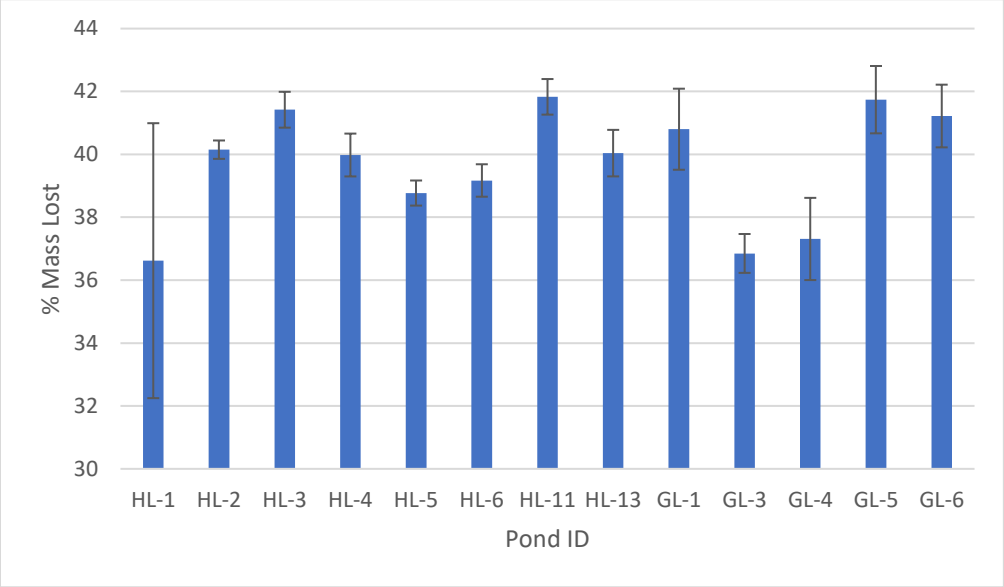


Figure 1 *Water chemistry (a) average pH level variation by pond and by measurement dates (b) average dissolved oxygen level variation by pond and by measurement date (c) average conductivity level variation by pond and by measurement date (d) average polyphenol level variation by pond and by measurement dates*

a)



b)

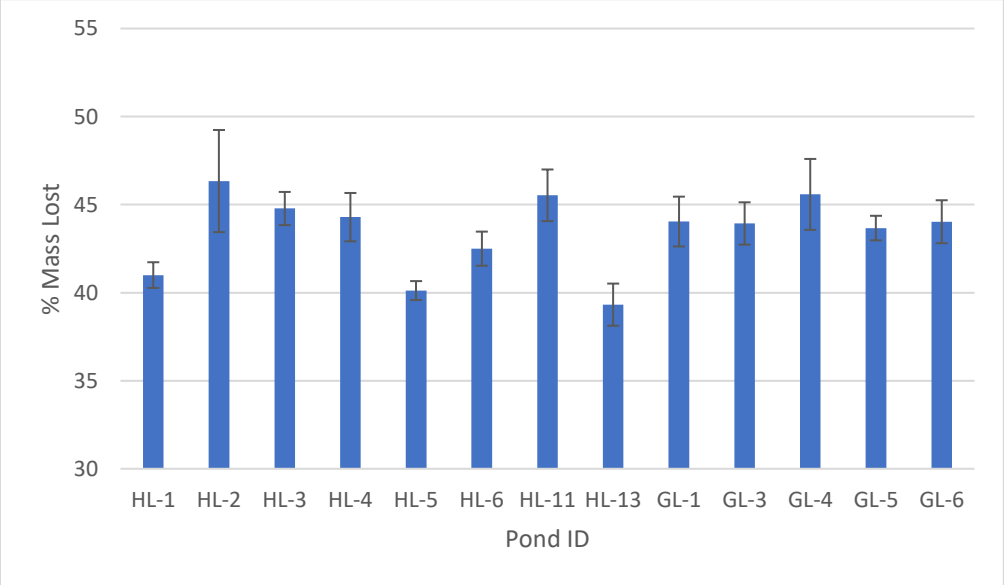
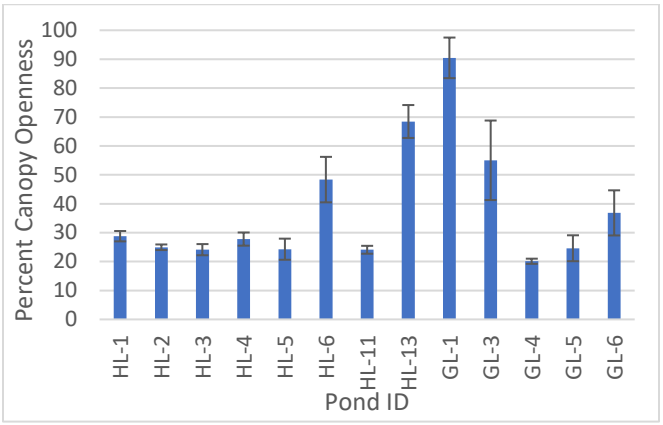
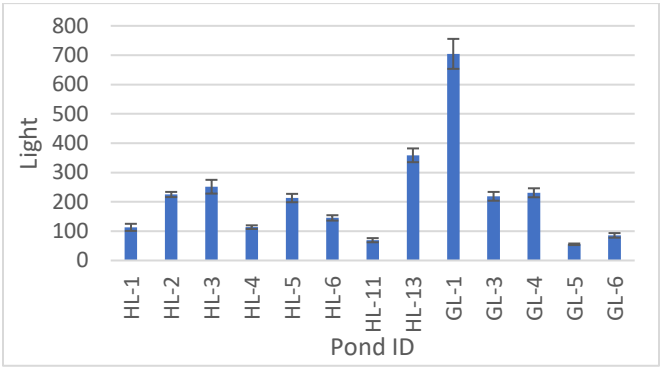


Figure 2 *Decomposition rates (a) percent mass lost in fine mesh leaf litter bags (b) percent mass lost in coarse mesh leaf litter bags*

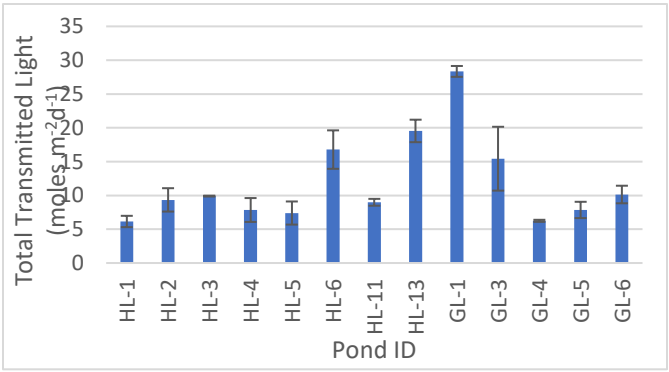
a)



b)



c)



d)

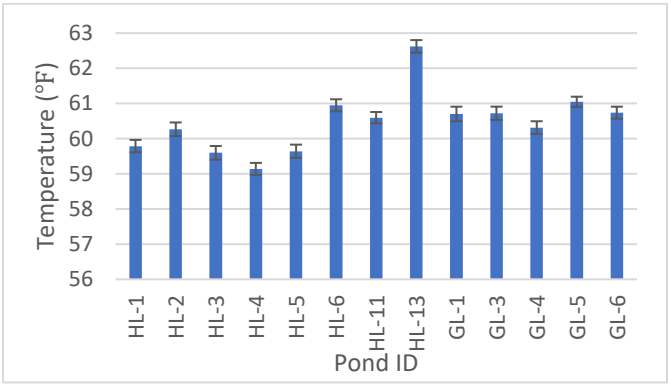


Figure 3 *Canopy, light, and temperature (a) percent canopy openness per pond (b) light intensity variation per pond (c) total transmitted light per pond (d) average temperature per pond*

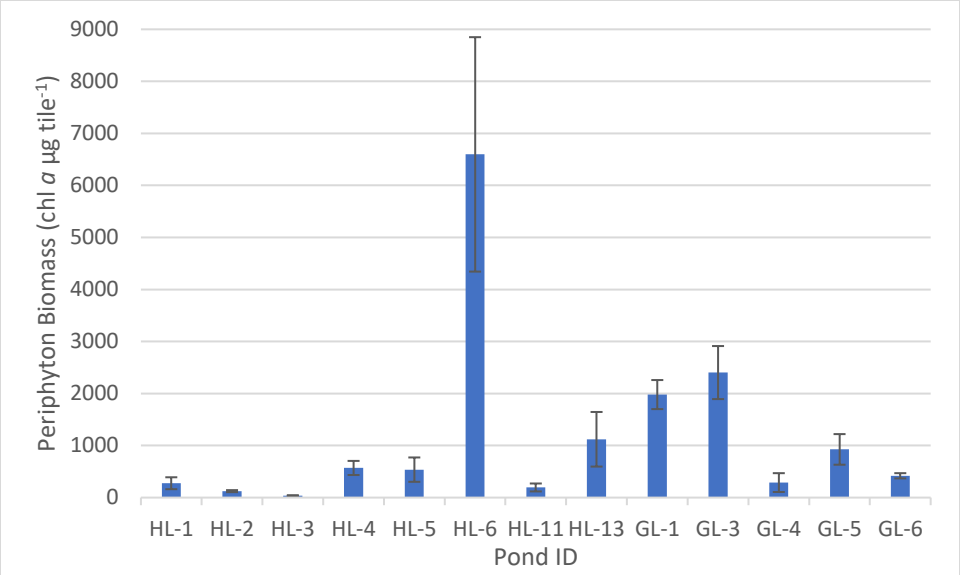


Figure 4 *Periphyton biomass measures per pond*

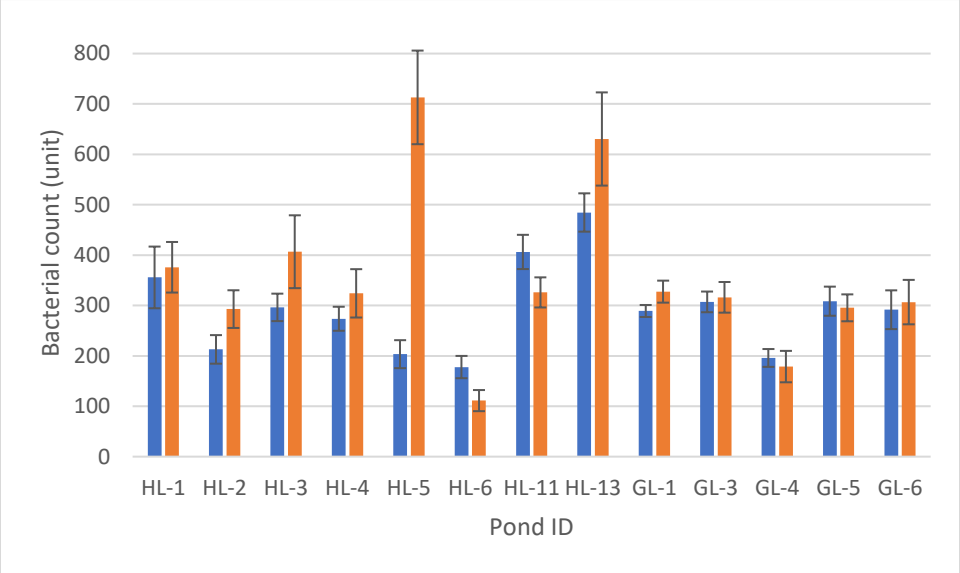


Figure 5 *Average bacterial count per pond, using green (left) and red (right) lights on a fluorescent microscope*

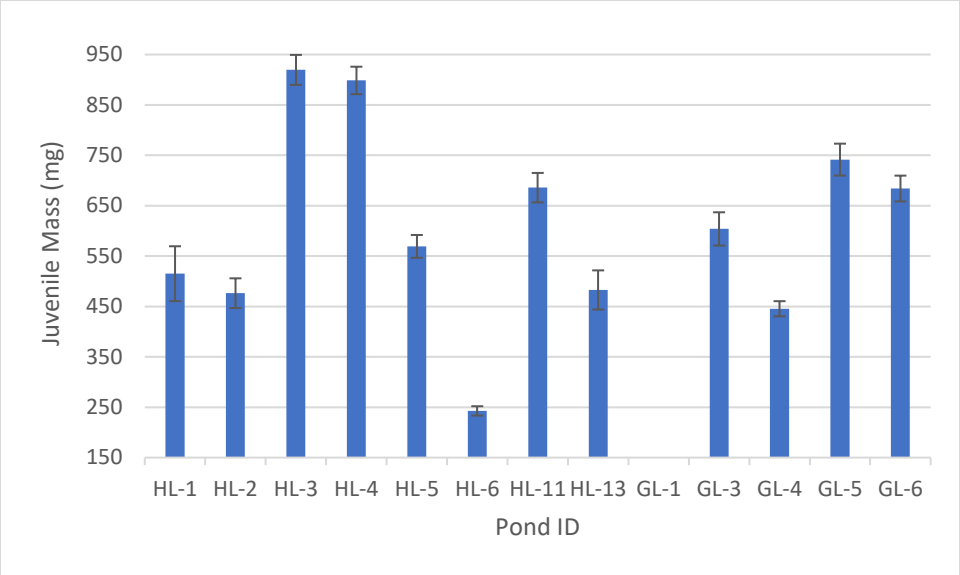


Figure 6 *Average juvenile tadpole mass at metamorphosis per pond (GL-1: no tadpoles)*