

Spring 5-25-2019

# Effects of Leaf Litter on Amphibian Site Selection

Rebekah Magee  
*Louisiana Tech University*

Follow this and additional works at: <https://digitalcommons.latech.edu/theses>

---

## Recommended Citation

Magee, Rebekah, "" (2019). *Thesis*. 13.  
<https://digitalcommons.latech.edu/theses/13>

This Thesis is brought to you for free and open access by the Graduate School at Louisiana Tech Digital Commons. It has been accepted for inclusion in Master's Theses by an authorized administrator of Louisiana Tech Digital Commons. For more information, please contact [digitalcommons@latech.edu](mailto:digitalcommons@latech.edu).

**EFFECTS OF LEAF LITTER ON AMPHIBIAN  
SITE SELECTION**

by

Rebekah Magee, B.S.

A Thesis Presented in Partial Fulfillment  
of the Requirements of the Degree  
Master of Science

COLLEGE OF APPLIED NATURAL SCIENCES  
LOUISIANA TECH UNIVERSITY

May 2019

LOUISIANA TECH UNIVERSITY  
GRADUATE SCHOOL

**May 28, 2019**

Date of thesis defense

We hereby recommend that the thesis prepared by

**Rebekah Magee**

entitled **Effects of leaf litter on amphibian oviposition site selection**

be accepted in partial fulfillment of the requirements for the degree of

**Master of Science in Biology**

\_\_\_\_\_  
Julia E. Earl, Supervisor of Thesis Research

\_\_\_\_\_  
William J. Campbell,  
Head of Biological Sciences

**Members of the Thesis Committee:**

Natalie Clay  
Julia E. Earl  
Terri Maness

**Approved:**

\_\_\_\_\_  
Gary A. Kennedy  
Dean of Applied & Natural Sciences

**Approved:**

\_\_\_\_\_  
Ramu Ramachandran  
Dean of the Graduate School

## ABSTRACT

Leaf litter plays an important role in the forest ecosystem, impacting various processes and hindering erosion. While there is variability in the chemical and nutritional properties of leaf litter, the effects these variables have on organisms within the environment are not well known. In this study, we examined the effects of leaf litter chemistry on amphibian oviposition site selection. Artificial ponds were created using small, plastic pools, and leaf litter of 15 different tree species (including two invasive species) was added. During the 60 day experiment, water quality measurements (including temperature, pH, dissolved oxygen, conductivity, and water depth) were taken weekly from each individual pool, and the amount of eggs deposited by Cope's gray treefrog (*Hyla chrysoscelis*) was recorded. Using zero inflated models, results show that tree species was the most accurate predictor of the amount of eggs deposited into each pool. Frogs had a strong preference for post oak leaves, while they completely avoided southern red oak leaves. Tree species also had an effect on the amount of nitrogen, phosphorus, and tannins (a type of secondary compound in tree leaves). These results indicate that cues from tree species have a strong impact on habitat selection for amphibians, which may impact ecosystems in broader ways through changes in amphibian abundance and diversity.

## APPROVAL FOR SCHOLARLY DISSEMINATION

The author grants to the Prescott Memorial Library of Louisiana Tech University the right to reproduce, by appropriate methods, upon request, any or all portions of this Thesis. It is understood that “proper request” consists of the agreement, on the part of the requesting party, that said reproduction is for his personal use and that subsequent reproduction will not occur without written approval of the author of this Thesis. Further, any portions of the Thesis used in books, papers, and other works must be appropriately referenced to this Thesis.

Finally, the author of this Thesis reserves the right to publish freely, in the literature, at any time, any or all portions of this Thesis.

Author \_\_\_\_\_

Date April 11, 2019

## **DEDICATION**

This thesis is dedicated to my fiancé for his constant support and encouragement through the last year of my project, and to my mom for her loving help throughout all of the most stressful points of this process.

## TABLE OF CONTENTS

ABSTRACT .....	iii
APPROVAL FOR SCHOLARLY DISSEMINATION.....	iv
DEDICATION .....	v
LIST OF FIGURES .....	vii
LIST OF TABLES .....	viii
ACKNOWLEDGMENTS .....	ix
CHAPTER 1 INTRODUCTION.....	1
CHAPTER 2 AIMS AND OBJECTIVES.....	5
CHAPTER 3 MATERIALS AND METHODS .....	6
CHAPTER 4 RESULTS.....	13
CHAPTER 5 DISCUSSION .....	21
BIBLIOGRAPHY .....	26

## LIST OF FIGURES

<b>Figure 3-1:</b> Field site, located on LA Tech’s South Campus.....	9
<b>Figure 3-2:</b> Pool blocking schematic.....	9
<b>Figure 4-1:</b> Effects of tree species on tannin concentration of water.....	17
<b>Figure 4-2:</b> Percentage of phosphorous in collected tree leaves.....	18
<b>Figure 4-3:</b> Percentage of nitrogen in collected tree leaves.....	19
<b>Figure 4-4:</b> Average number of eggs laid in each species’ pools.....	19
<b>Figure 4-5:</b> Relationship between leaf tannin content and average pool water tannin concentration.....	20



## LIST OF TABLES

<b>Table 3-1:</b> Tree species used in the experiment and their leaf chemistry.....	7
<b>Table 3-2:</b> T Models for AICc comparisons of models representing the competing hypotheses. ....	12
<b>Table 4-1:</b> Pearson-correlation matrix for all water quality variables and leaf nutrients.....	14
<b>Table 4-2:</b> ANOVA results for effect of tree species on amount of leaf nutrients and water quality variables.....	15
<b>Table 4-3:</b> Binomial process of model ranking predicting the initial egg laying choice made by female treefrogs.....	16
<b>Table 4-4:</b> Poisson process of model ranking predicting the amount of eggs laid in each pool.....	16

## **ACKNOWLEDGMENTS**

I would like to thank my advisor, Dr. Julia E. Earl, for all of her guidance and support during the design, execution, and writing of this experiment and thesis. Thank you to Ian Lovemore for helping to set up this experiment, and Joseph Phillips for continuing with the examination of this experiment's data. Also, thank you to my committee for their guidance in the design of this experiment, and interpretation of the data.

# **CHAPTER 1**

## **INTRODUCTION**

Across the globe, amphibian populations are declining (Stuart et al., 2004). As many as 41% of amphibian species are threatened, and many species are on the brink of extinction (Hoffmann et al., 2010). Research has been done to assess the factors driving these declines, showing many anthropogenically related causes such as climate change and habitat loss to be contributing factors in these population declines (Alford & Richards, 1999). However, not much research has been done to assess smaller population level changes in amphibian populations due to variation in natural environmental cues, which could interact with anthropogenic changes across the globe. Understanding these interactions could help further the understanding of what is driving global amphibian declines and aid amphibian conservation efforts.

Leaf litter plays an important role within forest ecosystems. It impacts many processes, such as deterring erosion (Li, Niu, & Xie, 2014; Sayer, 2006) and providing nutrients to the ecosystem (Vitousek, 1982; Vitousek & Sanford, 1986). These processes can be impacted by the tree species within a region, as tree leaf characteristics vary by species. Characteristics such as leaf structure (Abrams & Kubiske, 1990) and nutrients vary greatly among species (Hättenschwiler et al., 2008), which alter ecosystem level processes including decomposition (Nykqvist, 1963). Leaf litter provides nutrients to the ecosystem in a few different ways, one of which is leaching. As leaves decompose, their

soluble nutrients are leached into the soil or water, which may affect other organisms in the environment by possibly limiting photosynthesis rates and therefore reducing primary productivity levels (Chapin, Matson, & Mooney, 2002).

Leaf litter is a substantial source of resources and nutrients for many organisms within both terrestrial and aquatic environments. In forests, leaves provide elements to the aquatic ecosystem, such as nitrogen and phosphorus, which fuel algal photosynthesis - an important food source for aquatic organisms (Loman, 2001). The nutrient leaching varies by type of leaf (conifer vs. broadleaf), as well as tree species. Depending on the environment, some of the leached components may be decomposed or utilized quickly, causing no increase in the nutrient concentration (Nykqvist, 1963). However, in some environments, leached components can increase nutrient concentrations, and increases in elements such as nitrogen are seen to influence species diversity in ponds (Knutson et al., 2004), as well as an increased mortality in some organisms (e.g., amphibians) (Rouse, Bishop, & Struger, 1999).

In addition to nutrients, leaves also leach secondary compounds that can affect animals (Bernays & Cornelius, 1992; Janzen, Juster, & Beyll, 1977). These compounds are commonly grouped into three major categories: terpenes/steroids, alkaloids, and tannins. Some of these compounds are commonly found in many plant species (tannins), and some are more species specific (alkaloids) (Bourgaud, Gravot, Milesi, & Gontier, 2001). Tannins are a class of secondary compounds that many plants use as a defense mechanism against herbivory and plant pathogens. Toxicity to tannins has been observed in a variety of organisms such as earthworms (Gonzalez & Zou, 1999) and tadpoles, as they are indigestible and slow down overall digestion rates (Dodd & Buchholz, 2018;

Earl, Cohagen, & Semlitsch, 2012). This makes habitats containing high amounts of tannins potentially detrimental to many aquatic organisms that may ingest them and may be a habitat selection cue if they are recognized as a compound to be avoided.

Organisms often use different habitat characteristics to select the best habitat to live and raise offspring in to maximize fitness (Laurila & Aho, 1997). Many animals lay eggs and provide no parental care following egg deposition. In these species, oviposition site selection is crucial, because these eggs' survival is dependent on the environmental characteristics of the habitat where they were deposited (Refsnider & Janzen, 2010). Since tannins can decrease survival, secondary compounds in tree leaves may also impact the habitat selection of species that are negatively affected by them. On the other hand, some studies of frogs have found a preference for water with high tannin and acidic levels when compared to a control, although the same conditions cause a high mortality rate in their tadpoles (Dodd & Buchholz, 2018). Habitat selection is important for the individual organisms, as well as the ecosystem, as any changes in habitat selection may alter community composition. This is found in organisms such as aquatic beetles that avoid colonization of ponds due to differences in water quality, potentially altering ecosystem function (Pintar & Resetarits 2017b). These selection preferences occur in other organisms that have a life stage involving an aquatic ecosystem, because variables such as compound or nutrients leached into pond can impact their survival (Martin, Rainford, & Blossey, 2015).

Female frogs use a variety of factors to select an oviposition site, such as rain event frequency (Cayuela et al., 2014), canopy cover (Binckley & Resetarits, 2002), and pond depth (Klesecker, Blaustein, & Belden, 2001). Female frogs typically select

oviposition sites that will increase their tadpoles' survival and growth (Pintar & Resetarits, 2017a), so they avoid habitats containing predators (Blaustein et al., 2004; Touchon & Worley, 2015) and pesticides (Vonesh & Buck, 2007). The variability of factors affecting larval survival could lead to differences in oviposition site selection and thus potentially impact species diversity if different species are selecting oviposition sites based on different cues. Some of this variability can be attributed to leaf litter contained within the water, as different leaf species contain differing nutrient and secondary compound levels (Sayer, 2006).

Amphibians play many roles in ecosystems, because throughout their complex life cycles they live in both the aquatic and terrestrial environments. This makes them important members of both ecosystems through contributions such as energy and nutrient cycling, as well as increasing species diversity (Hocking & Babbitt, 2014). When frogs are in their tadpole stage, they increase the primary productivity (primarily algae) in aquatic environments (Altig, Whiles, & Taylor, 2007; Hocking & Babbitt, 2014), through excretion metabolization, and consumption of eatable algae allowing non-eatable algae to thrive (Kupferberg, 1997). Along with this, tadpoles are a nitrogen sink, decreasing the amounts of available nitrogen in the pond water (Seale, 1980). In the terrestrial stage, they affect decomposition of plant and leaf litter via excretory processes as well as decomposition of their carcasses (Beard, Vogt, & Kulmatiski, 2002). This makes them extremely important to various ecosystem processes and makes understanding what cues drive their oviposition site selection choices crucial to understanding changes in those processes, as their presence/absence could alter ecosystem functions.

## **CHAPTER 2**

### **AIMS AND OBJECTIVES**

Since female treefrogs tend to select oviposition sites to maximize their offsprings' survival (Laurila & Aho, 1997), I examined treefrog oviposition site selection in relation to leaf litter species and water quality, which are known to affect tadpole survival. I examined different tree species' leaf nutrients and tannins, as both can alter tadpole survival (Earl et al., 2012). I predicted that frogs will select pools with tree leaves containing higher nutrient and lower tannin concentrations. I also predicted that frogs will prefer pools with high dissolved oxygen and neutral pH. Measuring these variables, I created models containing different chemical variables as well as different water quality variables to ultimately use in a model ranking analysis to determine which variables best predict oviposition site selection.

## CHAPTER 3

### MATERIALS AND METHODS

To assess the oviposition habitat selection of *Hyla chrysoscelis*, I provided the frogs with potential oviposition sites that varied in leaf litter tree species. I used 15 tree species (Table 3-1) that varied in leaf nutrients (nitrogen and phosphorous) and secondary compounds (tannins). I collected 100g of each of the 15 species from three different locations (Ruston public parks and Louisiana Tech University campus) in Lincoln Parish, Louisiana for each species. Within the 15 species, two species (*Pyrus calleryana* and *Triadica sebifera*) are invasive in Northern Louisiana, and the remaining 13 species are native. All leaves were collected from the ground (Fall and Winter 2017-2018) after they had senesced and fallen from the trees except for *Triadica sebifera* (Chinese tallow). The Chinese tallow leaves were too decomposed when leaves were collected, so fresh leaves were stripped from the branches. All leaves were rinsed briefly in tap water to remove dirt and pollen and dried in a drying oven at ~40°C.



Tree	Species Name	Family	Nitrogen %	Phosphorus %	Average [Tannin]
<b>Sweetgum</b>	<i>Liquidambar styraciflua</i>	Altingiaceae	0.80	0.11	5.09
<b>Bald Cypress</b>	<i>Taxodium distichum</i>	Cupressaceae	1.14	0.07	3.88
<b>Chinese Tallow</b>	<i>Triadica sebifera</i>	Euphorbiaceae	0.69	0.053	19.71
<b>American Beech</b>	<i>Fagus grandifolia</i>	Fagaceae	1.46	0.069	11.66
<b>Post Oak</b>	<i>Quercus stellata</i>	Fagaceae	0.76	0.081	4.62
<b>Southern Red Oak</b>	<i>Quercus falcata</i>	Fagaceae	0.90	0.071	8.18
<b>Water Oak</b>	<i>Quercus nigra</i>	Fagaceae	1.04	0.052	4.98
<b>White Oak</b>	<i>Quercus alba</i>	Fagaceae	1.64	0.131	6.86
<b>Mockernut Hickory</b>	<i>Carya tomentosa</i>	Juglandaceae	0.77	0.068	6.47
<b>Southern Magnolia</b>	<i>Magnolia grandiflora</i>	Magnoliaceae	0.51	0.025	5.47
<b>Loblolly Pine</b>	<i>Pinus taeda</i>	Pinaceae	0.46	0.038	2.04
<b>Shortleaf Pine</b>	<i>Pinus echinata</i>	Pinaceae	0.59	0.043	2.67
<b>Sycamore</b>	<i>Platanus occidentalis</i>	Platanaceae	0.87	0.101	3.48
<b>Bradford Pear</b>	<i>Pyrus calleryana</i>	Rosaceae	0.65	0.053	37.87
<b>Black Willow</b>	<i>Salix nigra</i>	Salicaceae	1.37	0.08	4.62

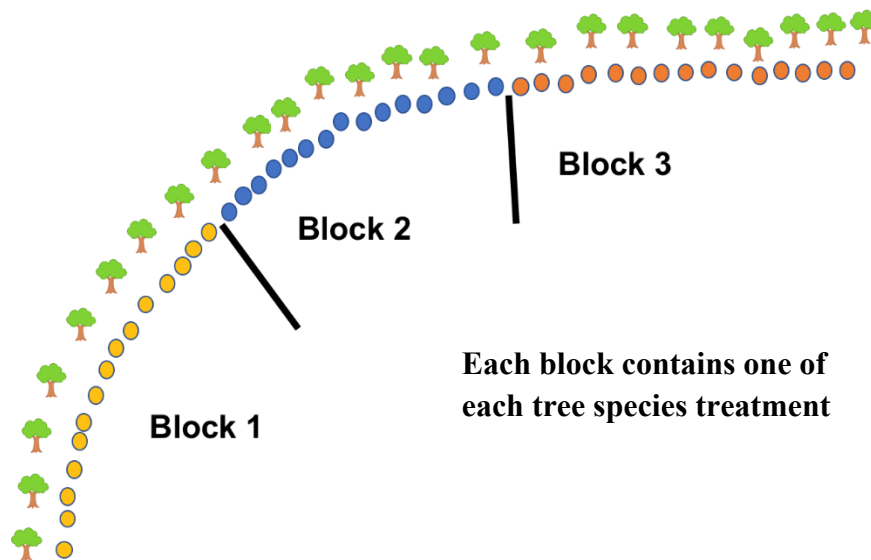
**Table 3-1:** Tree species used in the experiment and their leaf chemistry

The 15 species of leaf litter were added to 45 wading pools (1m in diameter, 30cm deep) with three replicates per leaf species treatment. Pools were placed approximately 1m apart from each other in a field within 1-6m of the forest edge at Louisiana Tech University's South Campus farm property and were filled with tap water to 10cm deep (approximately 80L) on 6-7 May, 2018 (Figure 3-1). Pools were covered with window screen covers (1-2mm mesh diameter) secured with bungee cord immediately after filling

and allowed to sit for 24 hours for the water to dechlorinate. Pools were separated into three blocks of 15 pools. Blocks were used to account for any variability within geographical placement of the pools along the tree line. Each of the 15 tree species treatments was put into three blocks of 15 pools each. The pools were randomly assigned a leaf species, and the layout was checked to make sure treatments were well-interspersed (Figure 3-2). At the beginning of the experiment (10 May, 2018), 80g of leaf litter of the appropriate treatment was added to each pool, resulting in a concentration of ~1g leaf litter/L of water. This concentration is equivalent to leaf input in dense uncut forest and has been used in previous studies (e.g., Earl et al. 2014). Once the leaf litter was added, window screen covers were pushed down below the water surface (10 May 2018) in order to easily separate any deposited eggs from the water. Pools were checked daily at the beginning of the experiment, but it was discovered that a rain event was needed for egg-laying to occur. Following this, pools were checked for three days following each rain event for the remainder of the 60 days. Any eggs deposited by treefrogs were removed and counted. Eggs were counted manually, using images taken of eggs right after oviposition. After photos were taken, the eggs were released into a nearby natural pond. The experiment ran for 60 days (May 10-July 10, 2018) and was conducted under Louisiana Department of Wildlife and Fisheries Scientific Research and Collecting Permit LNHP-18-075.



**Figure 3-1:** Field site, located on LA Tech's South Campus



**Figure 3-2:** Pool blocking schematic

Water quality measurements were taken beginning at 10am once a week over the course of the experiment, including pH (EcoSense® pH10A), water temperature (to 0.1°C; YSI® ODO200), dissolved oxygen (to 0.1 mg/L; YSI® ODO200), conductivity (to 0.1  $\mu$ S/cm; Hach Pocket Pro Cond<sub>LR</sub> Tester), and the depth of water in the center of the

pools (to 0.1 cm). Water samples were taken from each pool four times over the course of the experiment. For each sample, 60mL of water was filtered through 0.7 $\mu$ m pore size (AP40 filters, Millipore) glass fiber filters into plastic sample bottles. Samples were immediately put on ice, and following collection of all samples, were frozen until analysis. Samples were analyzed using a spectrophotometer (Hach DR 3900) to estimate the tannin concentration (mg/L) using the Tyrosine method (Clesceri et al., 1989).

Leaves from each species and site were also analyzed for their nutrient and tannin concentrations. Nutrient analysis was performed at Louisiana State University's Agriculture Center's Soil Testing and Plant Analysis Lab, to which I sent 5g of each leaf sample. Samples were analyzed for nitrogen and carbon using a LECO CN Analyzer following the Dumas Dry-Combustion procedure. Phosphorous was also analyzed using an inductively coupled plasma procedure (<https://www.lsu.edu/agriculture/>). At Louisiana Tech University, leaf tannin concentration was measured by soaking 0.3g of ground leaves in 600mL of water for 72 hours. Following this, water samples were taken and filtered as above, and tannins were measured in water using a spectrophotometer (Hach DR 3900) using the Tyrosine method (Clesceri et al., 1989) to estimate tannin content per gram of dried leaf.

Zero inflated models were used to examine differences in the number of eggs laid per pool (using R-studio Version 3.4.1). These models use a two-step process, first examining variables predicting whether eggs were present or not present (binomial choice). Following this, a Poisson process is applied to detect which model is best at predicting the number of eggs deposited, while using the binomial process as a baseline. An informational theoretical approach (Burnham & Anderson, 2004) was used to

compare models based on hypotheses for the impact of water quality and leaf traits on oviposition site selection. I created seven models (including the intercept-only model and the block model), each representing a hypothesis explaining which variables were most influential in oviposition site selection (Table 3-2). Before models were created, correlations between model parameters were analyzed (using Pearson-correlations) and parameters were removed if the correlations were above 0.70, as was the case with the percentages of nitrogen and phosphorous in the tree leaves. Models were ranked using Akaike's Information Criterion for small sample size (AICc), with the top model being the model with the lowest AICc value and consideration was taken for any competing hypotheses (models within two AICc units of the top model). Model selection occurred in two steps with model ranking for the binomial process first. Then the best model for the binomial process was included in all models to determine the best model for the Poisson process. This helped me evaluate potential cues that treefrog females use during oviposition site selection. ANOVAs were also used to examine differences among treatments in each water quality variable and leaf nutrient content, and a Tukey post hoc test was used to analyze differences between species (using SPSS Version 24).

Model	Model Name	K	Parameters
1	Tannin	3	Water Tannin Concentration
2	Tree Leaf Nutrients (continuous)	3	Nitrogen %
3	Tree Species Treatment	17	Tree species (15)
4	Water Depth (continuous)	4	Average Water Depth Average Water Temperature
5	Water Quality (continuous)	5	Average pH Average Conductivity Average Dissolved Oxygen
6	Null	2	Intercept-Only
7	Block	4	

**Table 3-2:** Models for AICc Comparisons of Models Representing the Competing Hypotheses. In all models, I used the average for that parameter per pool across the entire experiment.

## CHAPTER 4

### RESULTS

Over the course of the experiment, a total of 11,333 *Hyla chrysoscelis* eggs were deposited into the pools over six nights (June 4, 6, 11, 19, 20, 21). The average number of eggs deposited per night that ovipositing occurred was 500. Of the 44 pools, 23 received eggs at least once during the experiment (52.3%); the mean for each pool was 493 total eggs with number of eggs/pool ranging from 25-2,075.

A significant correlation was found between average conductivity and the following: Average water temperature ( $r = 0.45$ ,  $p = 0.002$ ), average water depth ( $r = -0.43$ ,  $p = 0.004$ ), and average pH ( $r = 0.32$ ,  $p = 0.018$ ), indicating that conductivity levels may be influenced by a many other variables (Table 4-5). The average concentration of tannins in pool water samples were negatively correlated with average pool water depth ( $r = -0.32$ ,  $p = 0.035$ ) (Table 4-1). The measured leaf tannins were positively correlated ( $r = 0.53$ ,  $p < 0.01$ ) with the mean tannin concentrations measured in samples taken from the tanks (Figure 4-5).

The zero inflated model ranking indicated that water depth alone was the best predictor of the initial choice whether or not to lay eggs in a pool (i.e., the binomial process, Table 4-3), as the treefrogs strongly chose deeper water when compared to shallow water. This model was competing with a water quality model containing dissolved oxygen, pH, and conductivity, since it was within two AICc units of the top

model. As such, water depth was included as the sole predictor of the binomial process in all subsequent models predicting the number of eggs deposited into each pool (i.e., the Poisson process). The tree species treatment (plus block, to ensure there were no block effects) model was clearly the best supported model (Table 4-4) as frogs laid eggs most in the post oak treatments, and none in the southern red oak treatment (Figure 4-4).

Tree leaf species differed in phosphorus ( $p \leq 0.01$ ) and nitrogen ( $p < 0.01$ ) content and the tannin ( $p < 0.01$ ) concentration in the pool water, but tree leaf species had no significant effect on any of the other measured variables (Table 4-2). Chinese tallow had significantly higher phosphorus percentage than all other tree species (Figure 4-2). It was also significantly higher than all other tree species in nitrogen percentage, and black willow was significantly different than loblolly pine, although Chinese tallow was the highest of all species. (Figure 4-3). The average tannin concentration in water was significantly higher in Bradford pear leaves compared to the other species, followed by the other invasive tree species, Chinese tallow (Figure 4-1).

		Dissolved Oxygen	Conductivity	Water Temperature	Water Depth	pH	Leaf % P	Leaf % N
Water Tannin Concentration	r	-.080	.126	.139	-.315	-.050	.117	.185
	P	0.600	.411	.361	.035	.744	.444	.224
Dissolved Oxygen	r	1	.164	.155	-.060	.084	.037	.015
	P		.282	.310	.695	.584	.807	.920
Conductivity	r		1	.450	-.426	.351	.000	.124
	P			.002	.004	.018	.999	.416
Water Temperature	r			1	.201	.861	.111	.118
	P				.185	.000	.466	.441



Water Depth	r				1	.430 <sup>*</sup>	.072	-.128
	P					.003	.639	.404
pH	r					1	.230	.208
	P						.128	.170
Leaf % Phosphorous	r						1	.806 <sup>*</sup>
	P							.000

**Table 4-1:** Pearson-correlation matrix for all water quality variables and leaf nutrients.

The sample size is 44 for each correlation.

<b>Factor</b>	<b>d.f.</b>	<b>F</b>	<b>P value</b>
<b>Leaf Phosphorus %</b>	14, 29	28.66	<0.01
<b>Leaf Nitrogen %</b>	14, 29	13.08	<0.01
<b>pH</b>	14, 29	1.19	0.33
<b>Conductivity</b>	14, 29	<0.01	0.49
<b>Water Tannin Concentration</b>	14, 29	5.86	<0.01
<b>Dissolved Oxygen</b>	14, 29	0.94	0.53
<b>Water Depth</b>	14, 29	0.79	0.67
<b>Water Temperature</b>	14, 29	0.90	0.57

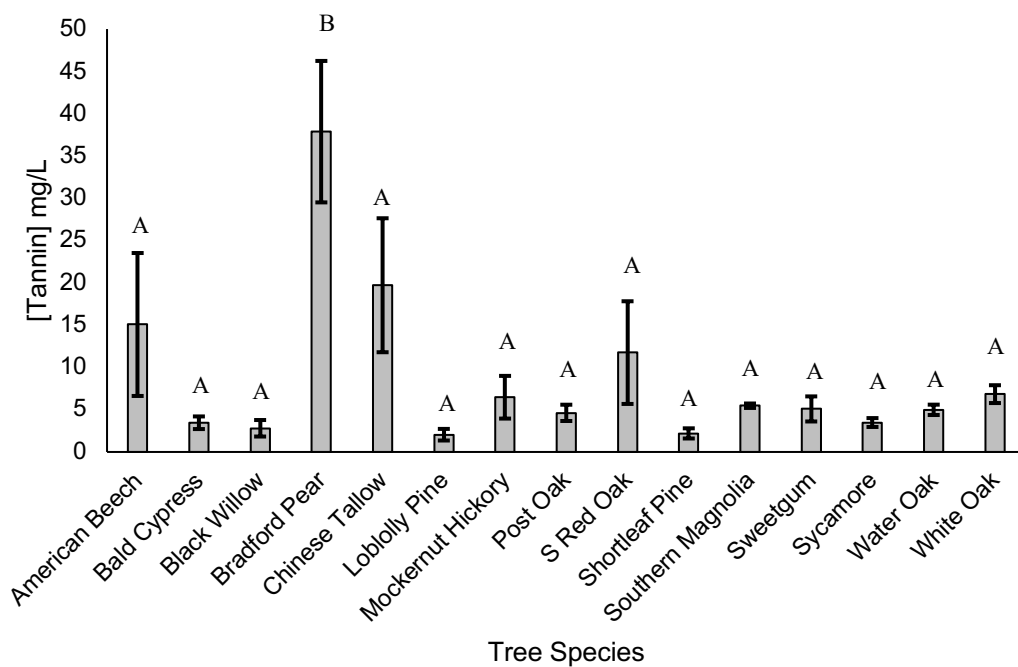
**Table 4-2:** ANOVA results for effect of tree species on amount of leaf nutrients and water quality variables.

Model	K	Log-Likelihood	AICc	Delta AICc	Weight
Water Depth (Alone)	3	-7090.65	14187.89	0.00	0.51
Water Depth	4	-7090.64	14190.31	2.42	0.15
Null	2	-7093.16	14190.61	2.72	0.13
Water Quality	5	-7089.67	14190.91	3.02	0.11
Tannins	3	-7093.02	14192.63	4.74	0.05
Tree Leaf Nutrients	3	-7093.16	14192.91	5.02	0.04
Block	4	-7093.14	14195.30	7.41	0.01
Tree Species Treatment	16	-7087.01	14226.16	38.27	2.48E-09
Tree Species Treatment + Block	18	-7086.97	14237.31	49.42	9.40E-12

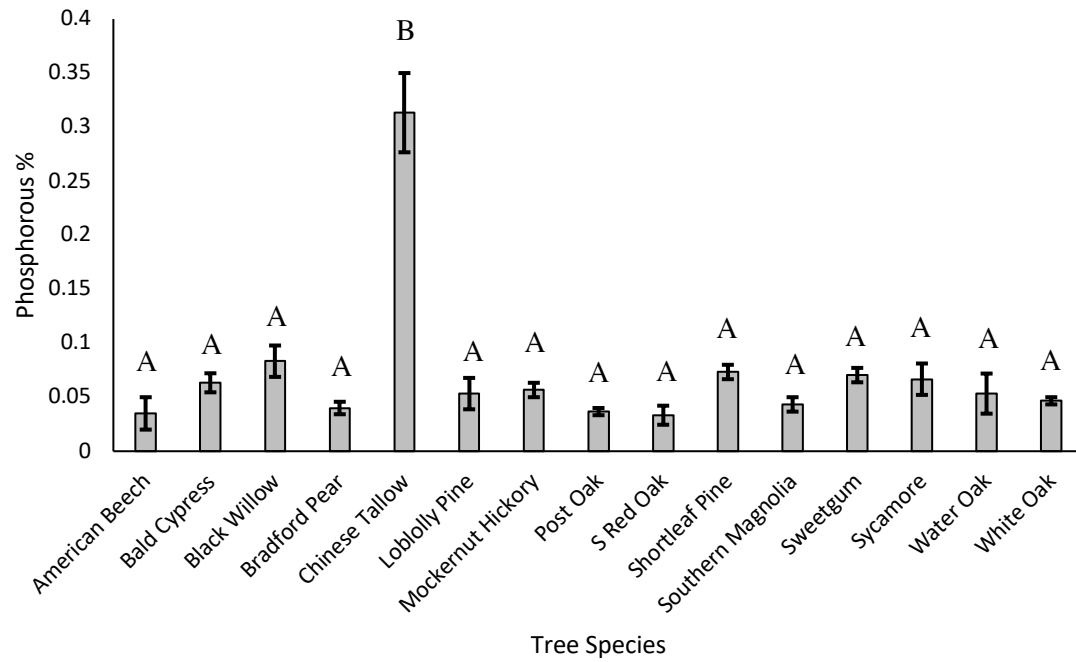
**Table 4-3:** Binomial process of model ranking predicting the initial egg laying choice made by female treefrogs

Model	K	Log-Likelihood	AICc	Delta AICc	Weight
Tree Species Treatment + Block	18	-757.45	1584.57	0.00	1
Tree Species Treatment	16	-1362.04	2781.63	1197.05	1.16E-260
Water Depth	3	-2958.17	5927.92	4343.34	0.00
Water Quality	5	-3273.88	6562.03	4977.45	0.00
Block	4	-4544.38	9100.33	7515.76	0.00
Tannins	3	-6960.85	13930.72	12346.148	0.00
Tree Leaf Nutrients	3	-7087.51	14184.05	12599.48	0.00
Null	2	-7093.16	14190.61	12606.04	0.00

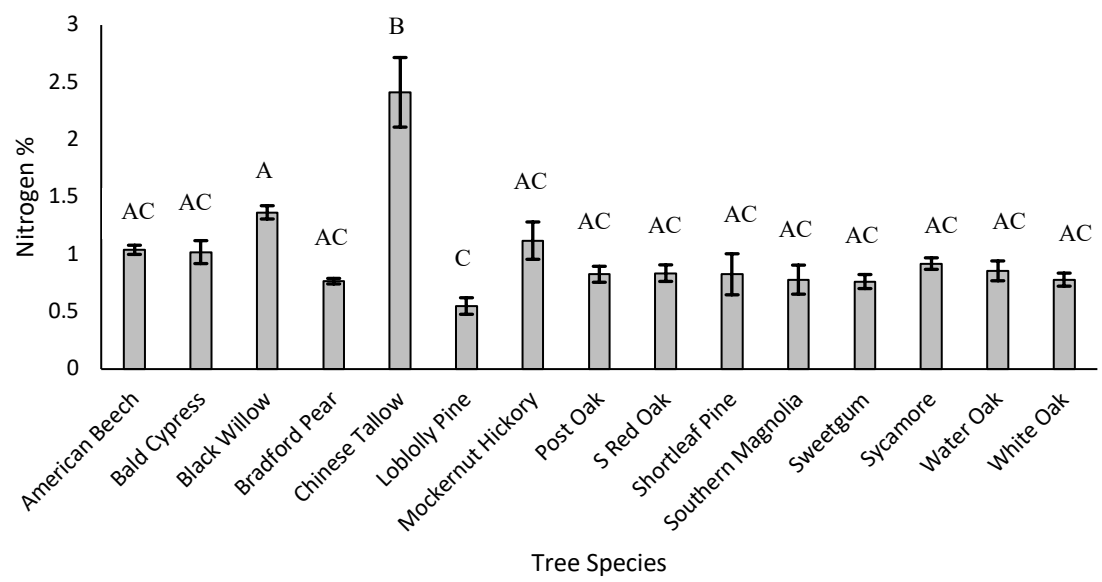
**Table 4-4:** Poisson process of model ranking predicting the amount of eggs laid in each pool



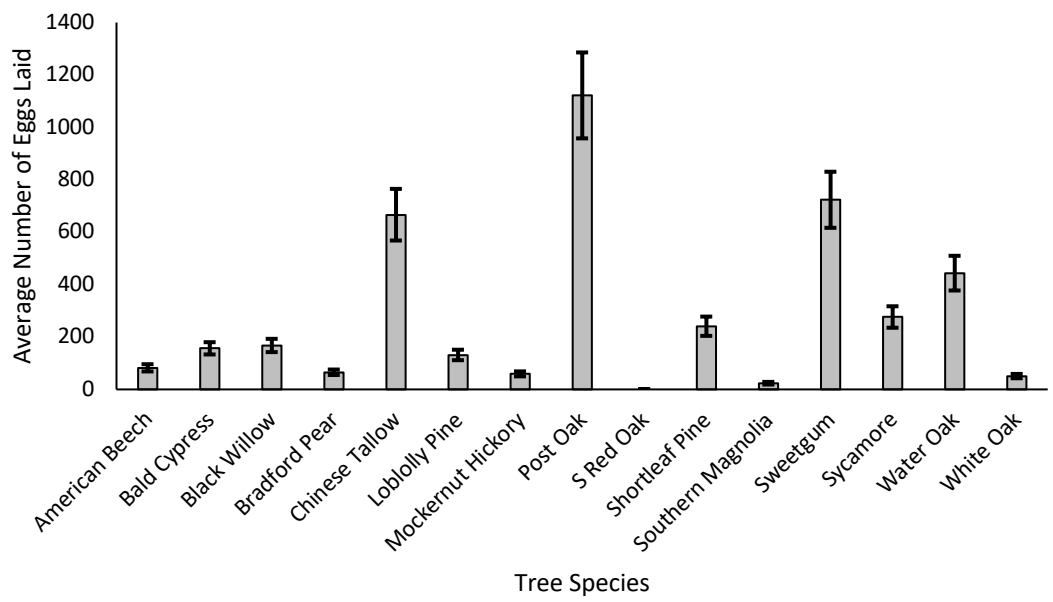
**Figure 4-1:** Effects of tree species on tannin concentration of water, significance from Tukey's post-hoc test indicated by lettering (Error bars are standard error)



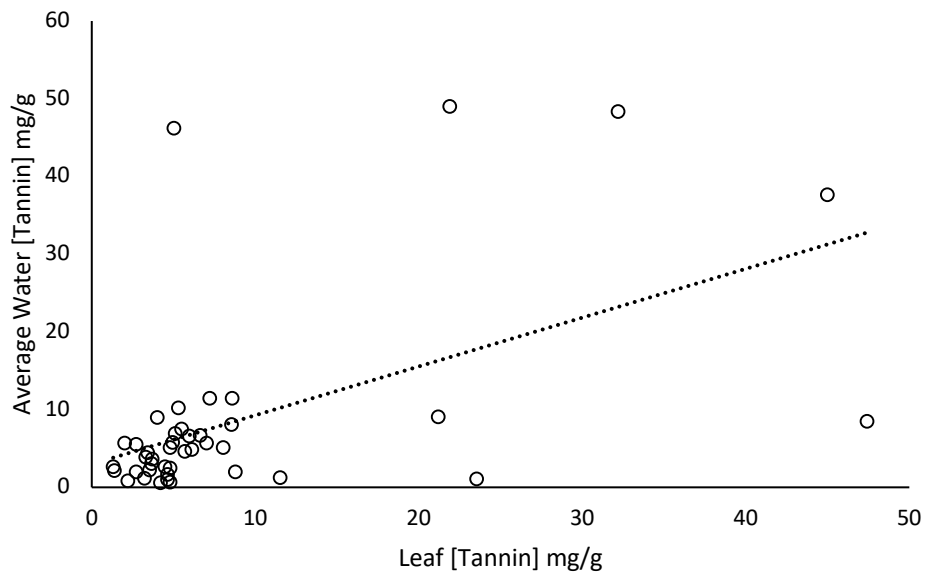
**Figure 4-2:** Percentage of Phosphorous in collected tree leaves, significance from Tukey's post-hoc test indicated by lettering (Error bars are standard error)



**Figure 4-3:** Percentage of Nitrogen in collected tree leaves, significance from Tukey’s post-hoc test indicated by lettering (Error bars are standard error)



**Figure 4-4:** Average number of eggs laid in each species’ pools (Error bars are standard error)



**Figure 4-5:** Relationship between leaf tannin content and average pool water tannin concentration ( $r = 0.531$ ,  $p < 0.01$ )

## CHAPTER 5

### DISCUSSION

This study was designed to investigate the natural cues that may have an effect on amphibian oviposition site selection, specifically in regard to the effects of chemical characteristics unique to different tree species. Overall, the most important factor of treefrog oviposition site selection was species of tree leaves in the pool. Tree species more accurately predicted the amount of eggs deposited in the pools when compared to leaf characteristics or water quality variables. Tree species also affected the percentage of nitrogen and phosphorous in the tree leaves, as well as the concentration of tannins in the pool water (Figures 4-1, 4-2, 4-3). Trees often surround natural ponds, allowing leaf input to the pond water, and this research suggests that tree species will affect the amphibian community composition through frog oviposition site selection. Specifically, *Hyla chrysoscelis* females deposited more eggs into pools containing post oak leaf litter to any of the other species and they laid no eggs in pools containing southern red oak litter (Figure 4-4). This preference and avoidance of specific tree species can lead to further examination of what natural cues that drive amphibian habitat selection. This could be used to examine changes in amphibian species diversity, since plant litter diversity and traits can influence amphibian growth and development (Martin et al., 2015). My results emphasize the impact that tree species can have on oviposition site selection.

The factor that was the most accurate at predicting the oviposition site was the different tree species leaf treatments. Because this was a better model than water quality or tree leaf nutrients, it is likely that either a combination of these variables or an unmeasured variable drives this egg laying pattern. One of these other variables may be the color of the water in ponds. Frogs may select their habitat using color, and tree leaves can impact water color which may impact oviposition site selection. Water color was not quantified in this study, but different species tree leaves altered water color, which made the pool water varying shades of light to dark. For example, both species of pine needles (loblolly and shortleaf) leached almost no pigment into the water, while Bradford pear and Chinese tallow leached darker compounds turning the water almost black. Beetles have a preference for colonizing ponds with a darker color (Williams, Heeg, & Magnusson, 2007), and adult treefrogs select terrestrial refugia locations painted blue instead of white or brown (Cohen et al., 2016), showing a preference for color in habitat selection. Thus, treefrogs may also use color as a cue for oviposition sites. Differences in water color may also alter water temperature, as darker colors absorb more heat.

Tree species impact water nutrients, which can impact pond productivity. Another unmeasured variable in my study that could influence the effect of tree species on oviposition site selection is the density of algae growing in the pool water. The differences in tree leaf nutrients could provide habitats of varying suitability to algal communities, as nitrogen and phosphorous and necessary components of photosynthesis, since algae is important in tadpole growth and survival (Brönmark & Rundle, 1991). The differing amounts of nutrients leached into the water could produce competition among algae species, as nitrogen and phosphorous fuel photosynthesis (Tilman, 1981). This



could have been an unmeasured cue for *Hyla chrysoscelis* oviposition site selection and ultimately tadpole survival, as decreased algal growth would not be suitable for tadpole growth and development. Future studies would benefit from measuring algal density in assessing oviposition site selection.

In the first process of the zero inflated model (binomial), the water depth (alone) was the highest ranked model in predicting whether or not frogs laid eggs in each pool, competing with the water quality model (Table 4-3). Ponds with deeper water have longer hydroperiods, which is highly selected for among amphibian species to reduce risk of desiccation (Cohen, Maerz, & Blossey 2012). My study could have better examined effects of water quality variables if the water levels were constant, as differences in water depth may have masked effects of water quality. Water depth may also have been a better predictor because of its association with other water quality conditions in the ponds with deeper water, as the conductivity levels were related to shallower water (Table 4-1). Treefrogs prefer water that is higher in dissolved oxygen and lower in conductivity (Pintar & Resetarits 2017a), which is typical of deeper water versus shallower water, and is what was measured in this study.

Tree species also had a significant effect on the nutrient levels and the tannin concentrations (Figures 4-1, 4-2, 4-3). While there were significant differences among the tree species' nutrient levels, the Chinese tallow leaves contained a much larger percentage of nitrogen and phosphorous than the other tree species (Figure 4-2 and 4-3). The high amount of nitrogen and phosphorus seen in the Chinese tallow leaves was most likely due to using fresh rather than senesced leaves. We may have seen different patterns of nutrient levels if we had access to senesced Chinese tallow leaves, which also could

have led to different algal densities in the Chinese tallow ponds. The Chinese tallow and Bradford pear (the two invasive tree species used in this study) had the highest concentration of tannins, highlighting some of the chemical differences that invasive plants have compared to plants native to an area (Kleunen, Weber, & Fischer, 2010). These types of differences in chemical characteristics between invasive and non-invasive species can lead to greater environmental changes, since many invasive species are generalists (Llusià et al., 2010).

Contrary to my predictions, the presence of tannins was not a good predictor of *Hyla chrysoscelis* oviposition preferences. Tadpole survival tends to decrease with increases in water tannin concentration (Earl et al., 2012), as they bind with proteins, which reduces the tadpoles' food quality (Britson & Kissell 1996). However, previous research indicates that frog oviposition may actually be positively related to tannin concentrations despite tannin-caused increases in tadpole mortality (Dodd & Buchholz, 2018). My results are consistently showed no preference for high tannins, but also no avoidance. This could be due to the *Hyla chrysoscelis* females not perceiving tannins as a threat or not being able to recognize them in the water, so they do not actively avoid this compound known to have detrimental effects on their offspring. This perceptive ability could differ across treefrog species and should be investigated further, as this recognition could change species diversity due to variation in tadpole mortality.

Since tannins are seemingly not recognized by the treefrogs, an investigation into what other secondary compounds could produce a preference or avoidance of ponds is warranted. This could be done specifically with invasive plant species in Northern Louisiana, as some invasive species are becoming more abundant and the effect they

have on ecosystems is not well known (Oswalt, 2010). This type of research could also provide data to help conservationists better understand environmental systems, such as the types of cues that drive habitat selection that could in turn alter species diversity. This information could also be applied to forest management as a tool to monitor the conditions that are most favorable for amphibian populations and to create man-made ponds with characteristics that would be most attractive to amphibians. At a broader level, knowledge surrounding the interactions between amphibians and their environments could help inform conservation actions and mitigate amphibian declines (Collins, 2010).

## BIBLIOGRAPHY

- Abrams, M. D., & Kubiske, M. E. (1990). Leaf structural characteristics of 31 hardwood and conifer tree species in central wisconsin: Influence of light regime and shade-tolerance rank. *Forest Ecology and Management*, *31*, 245–253.
- Alford, R. A., & Richards, S. J. (1999). Global amphibian declines: A problem in applied ecology. *Annual Review of Ecological Systems*, *30*, 133–165.
- Altig, R., Whiles, M. R., & Taylor, C. L. (2007). What do tadpoles really eat? Assessing the trophic status of an understudied and imperiled group of consumers in freshwater habitats. *Freshwater Biology*, *52*, 386–395.
- Beard, K. H., Vogt, K. A., & Kulmatiski, A. (2002). Top-down effects of a terrestrial frog on forest nutrient dynamics. *Oecologia*, *133*, 583–593.
- Bernays, E. A., & Cornelius, M. (1992). Relationship between deterrence and toxicity of plant secondary compounds for the alfalfa weevil *Hypera brunneipennis*. *Entomologia Experimentalis et Applicata*, *64*(9), 289–292.
- Binckley, C. A., & Resetarits, W. J. (2002). Reproductive decisions under threat of predation: Squirrel treefrog (*Hyla squirella*) responses to banded sunfish (*Enneacanthus obesus*). *Oecologia*, *130*(1), 157–161.
- Blaustein, L., Kiflawi, M., Eitam, A., Mangel, M., & Cohen, J. E. (2004). Oviposition habitat selection in response to risk of predation in temporary pools: Mode of detection and consistency across experimental venue. *Oecologia*, *138*(2), 300–305.

- Bourgaud, F., Gravot, A., Milesi, S., & Gontier, E. (2001). Production of plant secondary metabolites: A historical perspective. *Plant Science*, *161*(5), 839–851.
- Britson, C. A., & Kissell Jr, R. E. (1996). "Effects of food type on developmental characteristics of an ephemeral pond-breeding anuran, *Pseudacris triseriata feriarum*." *Herpetologica*, 374-382.
- Brönmark, C., & Rundle, S. D. (1991). Interactions between fresh-water snails and tadpoles-Competition and Facilitation Visibility conditions and predator-prey interactions View project Biological invasions in freshwater areas: evaluating native and non-native community interactions. *Oecologia*, *87*(1), 8-18.
- Burnham, K. P., & Anderson, D. R. (2004). *Multimodel Inference: Understanding AIC and BIC in Model Selection Multimodel Inference Understanding AIC and BIC in Model Selection*. *33*(261), 261-304.
- Cayuela, H., Besnard, A., Bonnaire, E., Perret, H., Rivoalen, J., Miaud, C., & Joly, P. (2014). To breed or not to breed: Past reproductive status and environmental cues drive current breeding decisions in a long-lived amphibian. *Oecologia*, *176*(1), 107–116.
- Chapin, S. F., Matson, P. A., & Mooney, H. A. (2002). *Principles of Terrestrial Ecosystem Ecology Library of Congress Cataloging-in-Publication Data*.
- Clesceri, L. S., Greenberg, A. E., & Eaton, A. D. (1998). Standard Methods for the Examination of Water and Wastewater APHA. AWWA, WEF, Monrovia, USA.

- Cohen, B. S., MacKenzie, M. L., Maerz, J. C., Farrell, C. B., & Castleberry, S. B. (2016). Color perception influences microhabitat selection of refugia and affects monitoring success for a cryptic anuran species. *Physiology and Behavior*, *164*(October 2017), 54–57.
- Cohen, J. S., Maerz, J. C., & Blossey, B. (2012). Traits, not origin, explain impacts of plants on larval amphibians. *Ecological Applications*, *22*(1), 218–228.
- Collins, J. P. (2010). Amphibian decline and extinction: What we know and what we need to learn. *Diseases of Aquatic Organisms*, *92*(2–3), 93–99.
- Dodd, C. E., & Buchholz, R. (2018). Apparent maladaptive oviposition site choice of Cope's Gray Treefrogs (*Hyla chrysoscelis*) when offered an array of pond conditions. *Copeia*, *106*(3), 492–500.
- Earl, J. E., Castello, P. O., Cohagen, K. E., & Semlitsch, R. D. (2014). Effects of subsidy quality on reciprocal subsidies: how leaf litter species changes frog biomass export. *Oecologia*, *175*(1), 209–218.
- Earl, J. E., Cohagen, K. E., & Semlitsch, R. D. (2012). Effects of leachate from tree leaves and grass litter on tadpoles. *Environmental Toxicology and Chemistry*, *31*(7), 1511–1517.
- Gonzalez, G., & Zou, X. (1999). Plant and litter influences on earthworm abundance and community structure in a tropical wet forest. *Biotropica*, *31*(3), 486–493.
- Hättenschwiler, S., Aeschlimann, B., Coûteaux, M. M., Roy, J., & Bonal, D. (2008). High variation in foliage and leaf litter chemistry among 45 tree species of a neotropical rainforest community. *New Phytologist*, *179*(1), 165–175.

- Hocking, D. J., & Babbitt, K. J. (2014). Amphibian contributions to ecosystem services. *Herpetological Conservation and Biology*, 9(December 2013), 1–17.
- Hoffmann, M., Hilton-Taylor, C., Angulo, A., Bohm, M., & Brooks, T. M. (2010). The impact of conservation on the status of the world's vertebrates repository. *Science*, 330, 1503–1509.
- Janzen, D. H., Juster, H. B., & Beyll, E. A. (1977). Toxicity of secondary compounds to the seed-eating larvae of the bruchid beetle *Callosobruchus maculatus*. 16, 22–27.
- Klesecker, J., Blaustein, A., & Belden, L. (2001). Complex causes of amphibian population declines. *Nature*, 410, 681–684.
- Kleunen, M. van, Weber, E., & Fischer, M. (2010). A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters*, 13, 235–245.
- Knutson, M. G., Richardson, W. B., Reineke, D. M., Gray, B. R., Parmelee, J. R., & Weick, S. E. (2004). Agricultural ponds support amphibian populations. *Ecological Applications*, 14(3), 669–684.
- Kupferberg, S. (1997). Facilitation of periphyton production by tadpole grazing: functional differences between species. *Freshwater Biology*, 37(2), 427–439.
- Laurila, A., & Aho, T. (1997). Do female common frogs choose their breeding habitat to avoid predation on tadpoles? *Oikos*, 78(3), 585–591.
- Li, X., Niu, J., & Xie, B. (2014). The effect of leaf litter cover on surface runoff and soil erosion in Northern China. *PLoS ONE*, 9(9), e107789.
- Llusià, J., Peñuelas, J., Sardans, J., Owen, S. M., & Niinemets, Ü. (2010). Measurement of volatile terpene emissions in 70 dominant vascular plant species in Hawaii: Aliens emit more than natives. *Global Ecology and Biogeography*, 19, 863–874.

- Loman, J. (2001). Effects of tadpole grazing on periphytic algae in ponds. *Wetlands Ecology and Management*, 9, 135–139.
- Martin, L. J., Rainford, S. Kay, & Blossey, B. (2015). Effects of plant litter diversity, species, origin and traits on larval toad performance. *Oikos*, 124(7), 871–879.
- Nykvist, N. (1963). Leaching and decomposition of water-soluble organic substances from different types of leaf and needle litter. *Studia Forestalia Suecica*, (No. 3).
- Oswalt, S. N. (2010). Chinese tallow (*Triadica sebifera* (L.) Small) population expansion in Louisiana, East Texas, and Mississippi. *United States Department of Agriculture (Forest Service)*, (Research Note SRS-20), 1–5.
- Pintar, M. R., & Resetarits, W. J. (2017a). Out with the old, in with the new: Oviposition preference matches larval success in cope's gray treefrog, *Hyla chrysoscelis*. *Journal of Herpetology*, 51(2), 186–189.
- Pintar, M. R., & Resetarits, W. J. (2017b). Tree leaf litter composition drives temporal variation in aquatic beetle colonization and assemblage structure in lentic systems. *Oecologia*, 183(3), 797–807.
- Refsnider, J. M., & Janzen, F. J. (2010). Putting eggs in one basket: Ecological and evolutionary hypotheses for variation in oviposition-site choice. *Annual Review of Ecology, Evolution, and Systematics*, 41(1), 39–57.
- Rouse, J. D., Bishop, C. A., & Struger, J. (1999). Nitrogen pollution: An assessment of its threat to amphibian survival. *Environmental Health Perspectives*, 107, 709–803.
- Sayer, E. J. (2006). Using experimental manipulation to assess the roles of leaf litter in the functioning of forest ecosystems. *Biological Reviews of the Cambridge Philosophical Society*, 81(1), 1–31.



- Seale, D. B. (1980). Influence of amphibian larvae on primary production, nutrient flux, and competition in a pond ecosystem. *Ecology*, 61(6), 1531-1550.
- Stuart, S. N., Chanson, J. S., Cox, N. A., Young, B. E., Rodrigues, A. S. L., Fischman, D. L., & Waller, R. W. (2004). Status and trends of amphibian declines and extinctions worldwide. *Scienceexpress*, 1–5.
- Tilman, D. (1981). Tests of resource competition theory using four species of Lake Michigan algae. *Ecology*, 62(3), 802–815.
- Touchon, J. C., & Worley, J. L. (2015). Oviposition site choice under conflicting risks demonstrates that aquatic predators drive terrestrial egg-laying. *Proceedings of the Royal Society B*, 282(1808), 20150376–20150376.
- Vitousek, P. (1982). Nutrient Cycling and Nutrient Use Efficiency. *The American Naturalist*, 119(4), 553–572.
- Vitousek, P. M., & Sanford, R. L. (1986). Nutrient cycling in moist tropical forest. *Ann. Rev. Ecol. Syst*, 17, 137–167.
- Vonesh, J. R., & Buck, J. C. (2007). Pesticide alters oviposition site selection in gray treefrogs. *Oecologia*, 154(1), 219–226.
- Williams, D. D., Heeg, N., & Magnusson, A. K. (2007). Habitat background selection by colonizing intermittent pond invertebrates. *Hydrobiologia*, 592(1), 487–498.