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## The effects of the invasive exotic Chinese tallow tree (*Triadica sebifera*) on amphibians and aquatic invertebrates

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The effects of the invasive exotic Chinese tallow tree (*Triadica sebifera*)  
on amphibians and aquatic invertebrates

A Dissertation

Submitted to the Graduate Faculty of the  
University of New Orleans  
in partial fulfillment of the  
requirements for the degree of

Doctor of Philosophy  
in  
Conservation Biology

by

Norman E. Leonard  
B.S. University of Georgia, 1998

May, 2008

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## Dedication

This work is dedicated to...

... my daughter, Jessica Marie Leonard. She is the light of my life and the eye of my storm.

... my father, Kenneth E. Leonard, whose guidance and example have been invaluable. He fostered my love of the natural world, encouraged me to pursue knowledge, and taught me that one man *can* make a difference. He is a man of superior intellect, uncommon courage, and the extraordinary strength to believe in and work for a better world.

... my mother, Anna L. Leonard, whose support, encouragement, and faith have never faltered. She told me when I was young that I could be whatever I wanted to be, and then she saw to it that I had every opportunity. From her I have learned compassion, empathy, generosity, and perseverance.

... my sister and friend, Stephanie Higdon, whose strength of spirit, fierce independence, and stubborn faith in my abilities provided me with both inspiration and the capacity to believe in myself, even when I did not want to.

... my friend and soul mate, Elizabeth Sassler, whose love and affection brought me encouragement and inspiration when I needed it most. Elizabeth's smile has its genesis in the depths of her heart, and it is expressed more with her eyes than with her lips. I love that smile, and I love her. Elizabeth's warmth, support, and genuine interest in my work are gifts that I shall always cherish. Her thoughtfulness and love of learning are a fountain of interesting and new ideas that I find invigorating and challenging. I look forward to a lifetime with this amazing woman.

... my friend, Philip Agcaoili, whose admonition to "follow the road less traveled" provided the map for my "winding road". It has, indeed, been a long, strange trip, but never a lonely one. Men do not encounter friends of Phil's good character and enduring loyalty but once in a lifetime.

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## Abstract

This dissertation addresses the question of how leaf litter from trees affects animals that live in aquatic environments, with an emphasis on the effect of Chinese tallow (*Triadica sebifera*) leaf litter on anuran larvae (i.e., frog tadpoles). This question is important to our understanding of how allochthonous inputs to aquatic habitats drive biodiversity in wetlands. It also addresses a timely conservation concern in southeastern Louisiana where invasion by Chinese tallow trees (*Triadica sebifera*) is displacing native trees. The invasion process is homogenizing forest composition and changing the quantity and quality of litter inputs to ponds from those produced by a mixture of native species to that of a single invasive species. This change in litter quality may have important effects on aquatic animals because leaf litter that falls into ponds is an important source of nutrients and energy in wetland foodwebs. Leaf litter also affects water quality via effects on dissolved oxygen and leaching of defensive compounds, which may subsequently affect the diversity and performance of aquatic animals. Herein I address these issues by presenting a series of studies in which tadpole and aquatic invertebrate responses were tested using leaf litter from Chinese tallow leaves and three native tree species. The major findings of this research are:

- (1) Leaf litter has a direct effect on water quality
- (2) Chinese tallow can cause differential survival and performance of tadpoles
- (3) Differences in water quality due to leaf litter can cause changes in tadpole behavior
- (4) Chinese tallow leaf litter breaks down much faster than litter from native trees
- (5) Difference in litter breakdown rates influence aquatic community composition.

Keywords: Invasive species, amphibian, *Triadica sebifera*, dissolved oxygen, temporary pond

## **Preface**

Each chapter within this dissertation is intended for publication as a research paper in a peer-reviewed journal. The chapters stand alone. The introduction is provided in order that readers unfamiliar with the study system and questions addressed in the body of this dissertation might gain some understanding of the basic concepts and issues addressed herein.

## Introduction

Norman E. Leonard

The effects of invasive plants on invaded communities are poorly understood (Soulé and Orians 2001). Studies of the effects of invasive species across trophic levels or ecosystem boundaries are rare. I propose to research the effects of leaf litter from invasive Chinese tallow trees (*Triadica sebifera* [L.] Small) on amphibian diversity in temporary ponds. This research will encompass three questions that are active areas of ecological and conservation research: 1) How do invasive terrestrial plants affect aquatic detrital food webs? 2) How do they affect the structure of a native faunal community? 3) What role do they play in amphibian declines? These topics will be addressed in St. Tammany Parish, Louisiana where Chinese tallow is invading temporary ponds used by amphibians for reproduction and completion of larval life stages. Many of these ponds are small and shaded, making them dependent on detritus for energy and nutrients. As Chinese tallow displaces native trees, the type and quality of detrital inputs to the ponds will change. Such shifts may alter competitive interactions or cause local extinctions of amphibians due to changes in the rate of nutrient cycling or the disturbance regime (Soulé and Orians 2001).

Many aquatic ecosystems in the Southeast are dependent upon plant detritus for energy and nutrients (Wallace et al. 1999). Leaves, wood, and humic compounds leached from the soil are the primary sources of energy and nutrients for aquatic detrital food chains (Allan 1995, Findlay et al. 2003). The type and abundance of detritus may affect the availability and processing of limiting resources (Horne and Goldman 1994). These factors may influence the

composition of aquatic faunal assemblages (Gessner and Chauvet 2002, Mancinelli and Rossi 2002), which are uniquely adapted to the type, abundance, and timing of detrital inputs from native plants (Barlocher and Kendrick 1975, Allan 1995, Hieber and Gessner 2002). The effects of variation of detritus types and abundance on microbes and invertebrates are well known (e.g., Wallace et al. 1982, Tank et al. 1998), but few have addressed the effect of this variation on vertebrates in temporary ponds.

The establishment of invasive tree species might cause variation in litter type and abundance, thereby affecting animals that depend on detrital pathways. However, the affect of invasive plants on higher trophic levels is relatively unknown (Soulé and Orians 2001). One possible effect of invasive plants is bottom-up control of consumer abundance and diversity. For instance, algal community composition might be affected by nutrients released from invasive plant detritus and thereby alter resource availability for algivorous larval anurans. Alternatively, the primary role of leaves that fall into ponds may be the provision of habitat structure as tadpoles prefer structurally complex habitats (Alford and Richards 1999). The invasion of ephemeral wetlands by *T. sebifera* and the resultant displacement of native trees provide an opportunity to study the community-level effects of shifting litter quality due to the presence of an invasive species.

***I hypothesize that a shift from native to novel floras in temporary pond basins and subsequent shifts in detritus composition will decrease the abundance and richness of aquatic amphibians because of shifts in nutrient pathways or habitat availability.***

## Study system

### *Chinese tallow*

Chinese tallow, *Triadica sebifera* (L.) Small, is a tree in the family Euphorbiaceae that is native to southeastern Asia (Webster 1994, Bruce et al. 1997). It was first introduced to the United States 230 years ago (Franklin et al. 1966) and has since become an invasive species. The tree became popular for its attractive fall foliage and was widely used in landscaping (Brown 1956). It is still sold as an ornamental tree in some parts of Louisiana (personal observation).

The range and invasive ability of *T. sebifera* seems to be limited by wintertime low temperatures (Bruce et al. 1997) and water availability (Barrilleaux and Grace 2000). *T. sebifera* tolerates shade (Jones and McLeod 1989), flooding (Jones and Sharitz 1990), salinity (Conner 1994, Conner et al. 2001), and soil pH ranging from 3.9 to 8.5 (Lin et al. 1958). It is an excellent competitor (Bruce 1993) that seems to be mostly free of grazers in the New World (Siemann and Rogers 2003a, b). It produces copious numbers of seeds that are dispersed by birds (Renne et al. 2000, Conway et al. 2002) or water (Jubinsky and Anderson 1996). Germination rates are high and rapidly growing seedlings may form mono-specific stands (personal observation). *T. sebifera* may pose one of the largest threats to biodiversity in the Southeast (Matlack 2002).

### *Temporary ponds*

Chinese tallow is most commonly found in ephemeral wetlands in St. Tammany Parish (personal observation). These seasonally inundated wetlands comprise systems with readily defined boundaries and discreet aquatic communities. Temporary ponds are the primary habitat of many amphibians because fish populations and predation are kept low by periodic drying. Drying has made temporary ponds an endangered habitat in need of conservation because they



are difficult to identify as wetlands and may seem unimportant when dry (Semlitsch and Bodie 1998).

### *Amphibians*

Amphibians are important components of most ecosystems and may comprise more biomass in a system than any other vertebrate taxon (Burton and Likens 1975, Stebbins and Cohen 1995). They may be the most important consumers of invertebrates where they live (Stebbins and Cohen 1995) as well as an abundant, high-quality food resource for predators (Stebbins and Cohen 1995, Pough 2004). Nevertheless, amphibians are often over-looked in food web and ecosystem studies, probably because many are secretive, live in areas difficult to access, are nocturnal, or are difficult to capture (Dundee et al. 1989). In St. Tammany Parish, 40 amphibian species have been recorded (Dundee et al. 1989), several of which are rare or endangered.

### **Scope**

The biological hypothesis presented above is enormous in scope, so this dissertation addresses the more tractable issue of testing for direct effects of leaf litter on aquatic organisms and key features of their habitat such as dissolved oxygen, light attenuation, and litter breakdown rates. I addressed these questions in a three-pronged approach. The first prong, which was never completed due to complications arising from Hurricane Katrina, consisted of aural surveys and drop box sampling of amphibians in temporary ponds invaded by Chinese tallow. The second prong utilized the mesocosm approach to ecological studies in order to achieve the best possible balance between a highly-replicated experimental set-up and nature-like conditions.

Specifically, I conducted two large mesocosm experiments, one each in 2004 (40 replicates) and 2005 (12 replicates). In 2004 I simply tested for and described the effects of leaf litter from Chinese tallow trees and 3 native tree species on the survival and development of frog tadpoles. Then in 2005, I explored the interaction of leaf litter and leaching on tadpole performance. These two experiments are described and discussed in Chapter 1. In Chapter 2, I describe the effects of leaf litter and dissolved oxygen on tadpole behavior; the observations of behavior were made using mesocosms and tadpoles raised in tupelo and pine tanks in 2004. The final prong of my research is discussed in Chapter 3, where I present the results of several litterbag experiments that were done in naturally occurring ponds and streams in St. Tammany Parish, Louisiana. The purpose of the litterbag experiments was to measure the breakdown of leaves from Chinese tallow and native trees and then use the observations to describe differences in the animal communities associated with each leaf type. Although I had hoped that using litterbags in this way would capture more amphibian larvae, the overwhelming majority of the animals I caught were invertebrates in the subphyla Crustacea and Hexapoda. Thus, Chapter 3 addresses the effect of leaf litter on these organisms rather than amphibians, as I do in chapters 1 and 2.

# Chapter 1: Leaf litter from invasive Chinese tallow trees (*Triadica sebifera*) affects native tadpoles

Norman E. Leonard and Joseph H.K. Pechmann

## Abstract

Many vernal pond food webs rely upon plant litter to provide energy and nutrients; therefore, changes in litter quality or quantity because of succession or nonnative plant invasion might affect the performance of vernal pond fauna. In southeastern Louisiana, *Triadica sebifera* is replacing native tree communities above vernal ponds. We used two mesocosm experiments to examine the effects of native leaf litter replacement by *T. sebifera* litter on three larval amphibian species. In the first experiment, *Pseudacris fouquettei*, *Bufo terrestris*, and *Hyla cinerea* tadpoles were added sequentially to tanks containing litter from *T. sebifera*, *Quercus laurifolia*, *Nyssa aquatica*, or *Pinus elliottii*. We measured the survival to, mass at, and time to metamorphosis of the tadpoles. No *P. fouquettei* and only 10 *B. terrestris* tadpoles survived in mesocosms with *T. sebifera*. *H. cinerea* survival was not significantly different, larval periods were shorter, and mass at metamorphosis was larger in *T. sebifera* treatments than native leaf treatments. Other research suggested that low dissolved oxygen (DO) results from chemical oxygen demand from litter leachates; therefore, we devised a second experiment to compare DO and performance of *P. fouquettei* and *B. terrestris* tadpoles in mesocosms with *Q. laurifolia* or *T. sebifera* leaves that were or were not leached prior to use. We also measured diel DO cycles weekly. DO was lowest in unleached *T. sebifera* treatments and increased through time in all treatments. All *P. fouquettei* died in *T. sebifera* tanks but neither litter type nor leaching had an effect on *B. terrestris* survival. Our results suggest that litter quality shifts from *T. sebifera* invasions may negatively affect tadpoles through effects on abiotic conditions such as DO.

## Introduction

Detritus is an important driver of ecosystem processes and biodiversity (Chapter 3, Moore et al. 2004). Investigations of how detrital amendments affect animal habitats are important to our understanding of how animals will be affected by both natural ecological succession and colonization by invasive exotic plants. Local patterns of amphibian presence and absence (Werner and Glennemeier 1999) and amphibian performance (Skelly et al. 2002) can be influenced by pond canopy closure and its effects on detrital inputs to ponds. Changes in forest canopy composition, e. g., the shift from oak (*Quercus sp.*) to red maple (*Acer rubrum*) dominance in forests of northeastern North America, can decrease amphibian biomass and survival (Rubbo and Kiesecker 2004).

Changes in canopy composition and subsequent changes to the quantity and quality of litter inputs to nearby aquatic habitats may alter nutrient availability, the structure of aquatic food webs, and the physical attributes of pond habitats. Nutrient availability changes with changes in detritus because trees vary in their allocation of resources to the structure and chemical defense of their leaves, and the composition of nutrients and secondary compounds left in the leaves at abscission can also vary greatly (Niinemets and Tamm 2005). Variation in the amount of essential nutrients (N and P), structural fiber, and secondary compounds can affect the rate of detrital decomposition and the productivity of associated fungi, bacteria, and algae (Webster and Benfield 1986a, Ostrofsky 1997). For example, replacing oak litter with red maple litter may cause a shift towards increased heterotrophy, because the replacement favors fungi and bacteria over algae (Rubbo et al. 2006). Detritus with higher C:N ratios, higher percentage lignin, or higher phenolic concentrations decomposes slower, supports less productivity of bacteria and algae, and ultimately reduces the performance of secondary consumers (Tuchman et al. 2003a, Tuchman et al. 2003b).

These changes in nutrient availability and trophic status are confounded by concurrent changes in the concentration of secondary chemicals such as hydrolysable phenolics (e.g. tannins). Tannins and other litter leachates may affect abiotic conditions such as dissolved oxygen (Chergui et al. 1997), which could negatively affect aquatic fauna. The rates at which nutrients and secondary chemicals become available is a function of how quickly detritus breaks down (Webster and Benfield 1986a, Ostrofsky 1997), so the changes described here are inextricably related to the structural role of leaves. Leaves are an important structural component of the habitat for many aquatic fauna (e.g., Tarr and Babbitt 2002), so while rapidly decomposing plants might provide nutrients for high productivity of food resources, they may provide insufficient cover for animals over longer periods.

To study the effects of changing detritus quality on animals, and amphibian tadpoles in particular, we turned our attention to temporary ponds experiencing changes in canopy composition because of invasion by Chinese tallow (*Triadica sebifera*) trees. In southeastern Louisiana, USA, invasion by *T. sebifera* is displacing native tree species in many wetlands (Bruce et al. 1995, Conner et al. 2002). Canopy composition around these ephemeral wetlands ranges from a mixture of native species of oak (*Quercus spp.*), pine (*Pinus spp.*), and gums (*Liquidambar spp.* and *Nyssa spp.*), to pure stands of Chinese tallow. Chinese tallow is a tree in the family Euphorbiaceae that is native to southeastern Asia (Bruce et al. 1995, Jubinsky and Anderson 1996, Barrilleaux and Grace 2000). In North America it now ranges in areas near the coast from the Delmarva Peninsula south to Florida and west to Texas (Radford et al. 1968, Correll and Johnston 1970, Bruce et al. 1997) as well as isolated areas in California (Oswald et al. 1994). Where it invades, tallow commonly forms monospecific stands (Bruce et al. 1995, Barrilleaux and Grace 2000). Invaded habitats include terrestrial landscapes as diverse as

bottomland hardwood forests (Harper 1995), Texas prairie (Bruce et al. 1995), and high marsh (Barrilleaux and Grace 2000). Because of its potential to invade and homogenize the canopy of many habitats, some propose that tallow is a threat to biodiversity in the Southeast (Matlack 2002). While tallow can displace native tree species, there is no evidence to date that tallow has a negative effect on aquatic animals. Chinese tallow is toxic to cattle (Russell et al. 1969), a possible consequence of the high concentrations of hydrolysable tannins in their leaves (Neera et al. 1992, Neera and Ishimaru 1992). Cameron and LaPoint (1978) found hydrolysable tannins composed 3.6% of tallow dry leaf mass after abscission.

Here we report on two experiments designed to improve our understanding of how changes in litter quality affect anuran tadpoles. The first study evaluated the performance (survival to, time to, and size at metamorphosis) of three tadpole species in mesocosms containing leaf litter from Chinese tallow or one of three native tree species. In a similar study comparing *B. americanus* tadpole performance in bins and mesocosms treated with invasive *Lythrum salicaria* or native *Typha latifolia* and field enclosures in naturally occurring *Lythrum* and *Typha* marshes, tadpole survival was lower and development was slower in experimental units treated with the invasive plant (Brown et al. 2006). A study by Maerz et al. (2005) in which *B. americanus* and *H. versicolor* tadpoles were raised in extracts of *L. salicaria* or *T. latifolia* litter, *B. americanus* tadpoles were stunted by the invasive plant litter extract although the *H. versicolor* tadpoles showed no effect. These studies of the invasive *L. salicaria* are relevant to our study of *T. sebifera* because both species decompose rapidly (Cameron and Spencer 1989, Ehrenfeld 2003) and have high phenolics concentrations in their leaves (Cameron and LaPoint 1978, Maerz et al. 2005). These similarities between the invasive species thus lead to predict that *B. terrestris* tadpoles reared in mesocosms containing invasive Chinese tallow

leaves would perform poorly relative to tadpoles raised in tanks containing leaves from native trees, but predicted that *P. fouquettei* and *H. cinerea* tadpoles (both species being in the family Hylidae) would not be affected by litter type.

The objective of the second study was to relate tadpole performance to the effects of litter type on water chemistry, specifically dissolved oxygen levels, in mesocosms containing leached or un-leached tallow and native leaf litter. Due to the demonstrated effects of litter type on tadpoles observed in the first part of this paper and the hypothesized link between litter extracts and tadpole performance reported by others (e.g., Maerz et al. 2005, Brown et al. 2006), we chose to explore the possible effects of litter leachates on dissolved oxygen. There is evidence that suggests that litter leachates affect dissolved oxygen concentrations (Chergui et al. 1997). The first experiment was done using unleached leaf litter that was collected in fall, dried, and stored until needed, as is common in this type of investigation (e.g., Brown et al. 2006). Nevertheless, we think that tadpoles rarely encounter un-leached leaves in natural habitats, and Cameron and LaPoint (1978) reported that aquatic isopods performed differently on leached and un-leached Chinese tallow litter. Thus, we crossed two litter treatments (Chinese tallow and laurel oak) with two leaching treatments (leached and unleached) in this study. We predicted that tadpoles exposed to leached leaves would perform better and that tadpole performance across all treatments would be related to leaf litter effects on dissolved oxygen.

## **Methods**

### *Tallow v. native tree species*

We constructed 40 artificial ponds (mesocosms) from 1000-L plastic cattle watering tanks placed in an unshaded lawn. We covered the tanks with fiberglass window screen. Each

tank was equipped with a screened adjustable standpipe to control the water level within the tanks. On 15 and 16 February 2004, we filled clean tanks with 1180 L of New Orleans city water and treated the water with chloramine remover. Water levels were initially set to 45 cm and allowed to fluctuate with rainfall through the duration of the experiment. All mesocosms received plankton amendments collected from at least 3 naturally occurring ponds on 3 occasions. At each pond, 30 tows of a 500- $\mu$ m plankton net were rinsed into an 19-L bucket, then strained through a 1mm mesh net to exclude insect larvae, large crustaceans, and debris. We inoculated tanks with the resulting plankton slurry in randomly assigned 350 ml aliquots.

On 18 February 2004, the mesocosms received 1500 g of one of four possible leaf treatments: Chinese tallow (*Triadica sebifera*, invasive), slash pine (*Pinus elliottii*, native), laurel oak (*Quercus laurifolia*, native), or water tupelo (*Nyssa aquatica*, native). The amount of leaves used in each tank was determined by raking freshly senesced leaves from the bottom of dry ponds, drying the leaves to a constant mass, and calculating the average leaf biomass per m<sup>2</sup> of pond substrate. We found an average litter standing stock of 537 g/m<sup>2</sup> in 24 air-dried samples raked from four un-invaded ponds (i.e., 6 samples per pond), which means that our tanks, which had a bottom surface area of 2.63 m<sup>2</sup>, should have received approximately 1412 g of litter. We rounded to 1500 g of litter per tank, or approximately 1.27 g/L. Leaves were collected from trees in St. Tammany Parish, Louisiana, immediately prior to abscission (Chinese tallow leaves, leaves fell from trees with minimal disturbance) or from the forest floor immediately following abscission (oak, pine, and tupelo leaves) during December 2003 (tallow) and January 2004 (native tree species). The range in collection dates is because of differences in the timing of abscission among tree species. Leaves were air dried to a constant mass in the laboratory and



measured into paper bags for storage. Bags of leaves were randomly assigned to tanks according to a randomized block design (10 replicate blocks of four treatments each).

*Pseudacris fouquettei* (upland chorus frog), *Bufo terrestris* (southern toad), and *Hyla cinerea* (green tree frog) tadpoles were added to the tanks in this sequence, according to the breeding phenology observed in local ponds. Tadpoles were obtained by collecting at least 25 egg masses from naturally occurring ponds (*P. fouquettei*) or a minimum of 4 amplexing pairs of adults that were captured and placed in a container during oviposition (*B. terrestris* and *H. cinerea*). Eggs were transported to the lab where tadpoles hatched and were held until they had absorbed their yolk sacs. For each species, 45 tadpoles were randomly assigned to each mesocosm in groups of 5, stratified by clutch or clump. *P. fouquettei* were added to the tanks on 21 February 2004, hereafter referred to as day 1 of the experiment. *B. terrestris* were added to the tanks on 03 May, which was day 75 of the experiment, and *H. cinerea* were added on 28 May, which was day 100. All chorus frogs had metamorphosed prior to the introduction of toads, but a few toads remained in the tanks when the green tree frogs were added. Upon metamorphosis, defined as the emergence of the forelimbs, tadpoles were collected and transferred to the lab. In the lab, newly metamorphosed frogs were allowed to absorb their tails and then weighed. Metamorphs were returned to their natal ponds.

Water quality was measured on days 25 and 118, and light attenuation was measured on day 118. We measured temperature, percent saturation of dissolved oxygen, mg/L dissolved oxygen, conductance, specific conductance, and salinity using a YSI 556 MPS (Yellow Springs Instruments, Inc.) Measurements were made at 10-15 cm depth beginning at noon. We measured light attenuation in 16 tanks (4 spatial blocks) using a Li-Cor LI-192 underwater quantum sensor (Li-Cor, Inc., Lincoln, Nebraska, USA). For each tank, we measured the

intensity of light at the water surface and at 35 cm depth and calculated percent absorbance from the difference between them.

*Leached v. un-leached tallow and oak litter*

We used a similar mesocosm design to determine whether leaching of Chinese tallow or laurel oak leaves would alter the effects of those leaf types on tadpole performance. Again, we used 1000-L cattle watering tanks for mesocosms. Leaves of Chinese tallow and laurel oak were collected as in the first experiment, dried, and divided into 1500 g samples. The leaves were placed into mesh bags constructed of window screen. Half of the bags were soaked in approximately 100 L rainwater for 14 days prior to placement in tanks on 13 February 2005 to create the leaching treatment. Prior to placing the leaf litter in tanks, water was drained from leached leaves and leachates were discarded. After leaching, the different treatments were randomly assigned to replicate mesocosms. Tanks were arranged in 3 replicate blocks of four tanks each, one for each possible treatment combination.

As in the previous experiment, all tanks received 45 *Pseudacris fouquettei* and 45 *Bufo terrestris* tadpoles. Eggs were collected and tadpoles were assigned to tanks as described for the previous experiment. *P. fouquettei* tadpoles were added to the tanks on 14 February 2005 (day 1) and *B. terrestris* tadpoles were added on 04 June (day 110). We did not use *Hyla cinerea* in this experiment. There was no overlap between *P. fouquettei* and *B. terrestris* presence in mesocosms. Mesocosms were checked daily and metamorphs removed and processed as described previously.

We measured water quality on the 6<sup>th</sup> day of weeks 1, 2, 3, 4, 5, 9, 11, and 17 in every tank once every two hours for a complete 24 hr cycle. Measurements began at 1600 hrs EST and

continued through 1600 hrs the following day, and included parameters measured as described in the previous experiment. We calculated 24-hour minimum and maximum values of dissolved oxygen for each tank and focused our discussion of the results on the minimum values because animals are most stressed when these values occur. We measured light attenuation in all tanks on days 48 and 62 of the experiment following the methods described in the first experiment.

### *Statistical analyses*

We used the mesocosm as the unit of replication for all analyses. For each species in each mesocosm we determined the proportion of tadpoles that metamorphosed, mean mass at, and mean time to metamorphosis. Block terms were not significant in preliminary analyses and were dropped from the final analyses, with the exception of light attenuation in the first experiment. Proportion data were arcsine-square root transformed and mass data were log transformed to homogenize variances. We used Statistica (StatSoft, Tulsa, OK, [www.statsoft.com](http://www.statsoft.com)) to perform one way (first experiment) and two-way (second experiment) analyses of variance (ANOVA) to determine whether the litter treatments had a significant effect on our observations of tadpole performance. Post-hoc comparisons were performed with Tukey's HSD for the first experiment in order to determine which treatments differed. Complete mortality of *P. fouquettei* in the tallow tanks in both experiments necessitated excluding the tallow treatments from statistical analyses for *P. fouquettei*. This reduced the *P. fouquettei* analyses for the second experiment to t-tests comparing the leached and unleached oak treatments.

Water quality data recorded in the first experiment were analyzed using univariate repeated measures ANOVAs for each water quality parameter (e.g., specific conductance). In

the second experiment, we recorded all the same water quality parameters as in the first experiment, but we only analyzed the dissolved oxygen and light attenuation data. We chose to focus on DO percent saturation because this measurement accounts for the effects of temperature and salinity on oxygen solubility in water. We focused on the minimum value observed during each 24-hr observation period because stresses on animals should occur during periods of low dissolved oxygen concentrations. Dissolved oxygen data for the second experiment were analyzed using multivariate repeated measures ANOVA of the 24-hr dissolved oxygen minimum values for each tank (the multivariate analysis is more appropriate than the univariate when multiple measurements are made over time). Percent saturation and light attenuation data were divided by 100 and arcsine-square root transformed to homogenize variances.

## Results

### *Tallow v. native tree species*

In the first experiment, 3323 of 5400 tadpoles metamorphosed. Experiment-wide survival rates were lowest in tallow treatments ( $20.7\% \pm 3.2\%$ ) and highest in native leaf treatments (73.9% to 75.9%, Table 1.1). The fewest frogs produced by a single tank was 7 (a tallow tank, all *Hyla cinerea*), and the most frogs produced was 121 (a pine tank; 45 *Pseudacris fouquettei*, 33 *Bufo terrestris*, and 43 *H. cinerea*). No tallow tank produced more than 45 frogs (all *H. cinerea*) while no native-leaf treatment produced fewer than 77 (a pine tank; 44 *P. fouquettei*, 7 *B. terrestris*, 26 *H. cinerea*). Treatment differences in amphibian biomass produced by each tank were not significant (Table 1.1). The standard error of the mean biomass per tank observed in the Chinese tallow treatment was at least twice that observed for any other treatment (Table 1.1). Tanks treated with Chinese tallow leaves produced both the lowest (5,555 mg) and

highest (43,867 mg) frog biomass per tank over the duration of the experiment despite mean survival rates of 0% for *P. fouquettei* and 4% for *B. terrestris* in these tanks.

Table 1.1. Mean survival and biomass ( $\pm 1$  SE), and ANOVA, for amphibian tadpoles reared in mesocosms treated with different types of leaf litter. Treatments labeled with the same lower case letter could not be distinguished statistically.

Treatment	Survival (%)				Total biomass (mg)			
Oak	75.9	$\pm$	3.1	a	25721.41	$\pm$	1663.42	a
Pine	73.9	$\pm$	3.4	a	26750.94	$\pm$	1999.24	a
Tupelo	75.6	$\pm$	1.8	a	26174.85	$\pm$	1007.83	a
Tallow	20.7	$\pm$	3.2	b	23024.28	$\pm$	4273.03	a
F-ratio $F_{3,36}$	66.25				1.58			
p-value	<0.001				0.2102			

#### *Pseudacris fouquettei*

Mesocosms with tallow had 0% survival of *P. fouquettei* tadpoles while the mean survival rate for native leaf treatments was  $91.5\% \pm 1.5\%$ . Because all 1255 metamorphs of this species came from the native litter treatments, the tallow treatment was removed from the statistical analyses. There was no significant effect of native litter type on mortality, time to metamorphosis, or total metamorph biomass (Table 1.2). *Pseudacris fouquettei* mass at metamorphosis averaged 11% higher in the tupelo litter treatment than in the pine treatment ( $F_{2,27}=5.6$   $p=0.009$ ).

Table 1.2. Mean survival, larval period, mass at metamorphosis and total biomass of tadpoles reared in mesocosms treated with different types of leaf litter, and associated ANOVAS. Tallow treatments not included in ANOVAS due to 0% survival. All data are mean  $\pm 1$  SE, and treatments labeled with the same lower case letter could not be distinguished statistically.

Species	Leaf	Survival (%)			Larval period (days)			Mass at metamorphosis (mg)			Tank biomass (mg)		
		Mean	SE		Mean	SE		Mean	SE		Mean	SE	
<i>P. fouquettei</i>	Oak	90.0	3.0	a	39.1	0.5	a	242	5	ab	9760	506	a
	Pine	95.3	1.4	a	39.9	0.6	a	235	7	a	9512	400	a
	Tupelo	89.1	2.7	a	39.6	0.4	a	260	2	b	10420	473	a
	F <sub>2,27</sub>	1.229			0.56			5.6			1.00		
	p	0.309			0.578			0.009			0.393		
<i>B. terrestris</i>	Oak	51.3	6.6	a	27.7	1.5	a	97	4	a	2535	354	a
	Pine	55.1	7.6	a	23.8	1.3	ab	93	9	a	2023	377	a
	Tupelo	61.1	6.7	a	20.0	0.5	b	103	6	a	2289	312	a
	Tallow	2.2	1.1	b	52.9	8.8	c	77	12	b	89	55	b
	F <sub>3,36</sub>	28.81			23.45			10.86			13.56		
p	<0.001			<0.001			<0.001			<0.001			
<i>H. cinerea</i>	Oak	74.7	5.6	a	41.1	1.9	a	400	26	ab	13426	1415	a
	Pine	70.9	6.0	a	42.1	2.2	a	473	35	a	15215	1863	a
	Tupelo	84.0	4.1	a	39.8	1.8	a	360	7	b	13466	833	a
	Tallow	58.7	9.1	a	31.7	1.1	b	931	34	c	22935	4279	a
	F <sub>3,36</sub>	2.18			7.49			66.85			1.17		
p	0.107			<0.001			<0.001			<0.001			

### Bufo terrestris

Overall survival of *B. terrestris* was relatively low with only 764 of 1800 (42.4%) reaching metamorphosis. Only 10 individuals emerged from tallow mesocosms, and survival in native litter treatments was low (average 56%; Table 1.2) relative to *P. fouquettei*. ANOVA results were highly significant for all response variables (Table 1.2). Mean larval period was shortest in tupelo tanks followed by pine and oak. Larval period of toads exposed to native litter treatments was approximately half the larval period of toads exposed to Chinese tallow litter. We observed no effect of native litter type on mass at metamorphosis or tank biomass, but both were higher for native litter treatments than for tallow (Table 1.2).

## Hyla cinerea

*Hyla cinerea* had the highest overall survival of the three frog species. 1304 of 1800 green tree frogs survived to metamorphosis, with a mean survival rate of  $72.1\% \pm 3.4\%$ . No significant effect of leaf species on survival or total frog biomass per tank were detected (Table 1.2). The remaining two variables – time to metamorphosis, mass at metamorphosis – showed significant effects of litter type (Table 1.2). Whereas tadpoles of *P. fouquettei* and *B. terrestris* did not survive or grow in tallow treatments, the *H. cinerea* metamorphs in tallow treatments were more than twice as large and transformed an average of 8 days earlier than frogs in native leaf treatments. Post-hoc analyses revealed significant differences between native litter effects on mass at metamorphosis (Table 1.2). *H. cinerea* metamorphs were smallest in the tupelo treatments, whereas *P. fouquettei* metamorphs from tupelo tanks were the largest. While *H. cinerea* tadpoles in pine tanks were 31% larger than tadpoles in tupelo tanks (Table 1.2), the effects of native litter were small relative to the effects of Chinese tallow.

## *Water Quality*

Water quality was affected by both leaf species and day (Table 1.3). We did not measure light attenuation on day 25, but there was a significant effect of leaf type on light absorbance on day 118 (Table 1.3). Averaged across treatments, salinity decreased between days 25 and 118, pH increased, dissolved oxygen increased, and turbidity decreased (Table 1.3). All parameters except temperature had a significant Leaf\*Day interaction effect (Table 1.3), indicating that the effect of leaf changed over the course of the experiment. Specifically, DO increased over time except in all treatments except tallow tanks, specific conductance and salinity differed among treatments more on day 118 than day 25, and pH and turbidity differed among treatments much

more on day 25 than on day 118. The amount of incident light varied by block ( $F_{3,12}=27.3$   $p<0.001$ ) because of variation in cloud cover on the day observations were made, so block was included as a factor in our ANOVA for light attenuation. In pine tanks, only one quarter of the light incident at the surface was absorbed by the water column at 35 cm depth, roughly one half of incident light was absorbed by 35 cm depth in oak tanks, and almost all light was absorbed in tupelo and tallow tanks (Table 1.3). Observations of light attenuation are in agreement with the differences in other water quality measurements. In general, water that transmitted more light had higher dissolved oxygen, higher pH, lower salinity and lower specific conductance.

#### *Leached v. un-leached tallow litter*

##### *Pseudacris fouquettei*

All *Pseudacris fouquettei* tadpoles in tallow treatments died, regardless of leaching treatment (Table 1.4). The oak treatments produced 256 *P. fouquettei* metamorphs, 126 from the un-leached oak leaves and 130 from tanks with leached oak leaves. Across all oak tanks, time to metamorphosis for *Pseudacris fouquettei* tadpoles ranged from 47 to 64 days and mass at metamorphosis ranged from 1448 to 3211 mg. The 100% mortality rate in the tallow treatments precluded their inclusion in analyses of chorus frog survival, mass at, and time to metamorphosis. Analyses for these three variables were performed for the oak treatments only, using t-tests. There was no significant effect of leaching on survival of chorus frog in oak tanks (Table 1.4;  $t=-0.392$ ,  $df=4$ ,  $p=0.715$ ). Mass at metamorphosis for *P. fouquettei* was marginally higher in tanks containing unleached oak leaves than leached oak leaves (Table 1.4;  $t=2.657$ ,  $df=4$ ;  $p=0.057$ ). There was no significant effect of leaching treatment on time to metamorphosis of the chorus frog tadpoles (Table 1.4;  $t=-0.065$ ,  $df=4$ ,  $p=0.951$ ).



### Bufo terrestris

*Bufo terrestris* mortality was high with only 167, or 30.9%, surviving to metamorphosis. Tadpoles of this species survived in all treatments. Neither the leaf treatment nor the leaching treatment had a significant effect on survival ( $F_{1,8}=1.22$ ,  $p=0.301$  and  $F_{1,8}=0.202$ ,  $p=0.665$ , respectively), but the leaf\*leaching interaction had a marginally significant effect ( $F_{1,8}=5.15$ ,  $p=0.053$ ). Leaching greatly increased toad survival in oak tanks but decreased it in tallow tanks (Table 1.4). The larval period of toads in tallow tanks was shorter than that of toads in oak tanks (Table 1.4;  $F_{1,8}=5.58$ ,  $p=0.046$ ), but there was no effect of leaching or the leaf\*leaching interaction on larval period ( $F_{1,8}=0.02$ ,  $p=0.897$  and  $F_{1,8}=1.13$ ,  $p=0.319$ , respectively). Toads raised in tallow tanks had a larger mass at metamorphosis than those in oak tanks (Table 1.4; leaf effect  $F_{1,8}=13.54$ ,  $p=0.006$ ). The leaching treatments did not have a significant effect on mass at metamorphosis ( $F_{1,8}=0.03$ ,  $p=0.871$ ), but the leaf\*leaching interaction term was significant ( $F_{1,8}=8.44$ ,  $p=0.020$ ). Leaching increased toad metamorph mass in oak leaves but decreased toad metamorph mass in tallow leaves (Table 1.4).

Table 1.3. Means  $\pm$  1 SE for water quality measurements made in experimental mesocosms on days 25 and 118 of the first experiment. Values presented under the headings “Effect of Leaf,” “Effect of Day,” and “Effect of Leaf\*Day” are p-values for the repeated measures ANOVA with 3 df for Leaf, 1 df for Day, and 12 error df. Light attenuation was not measured on day 25; p-value presented is for a main effects ANOVA of the effect of leaf on light attenuation with 3 df for Leaf and 3 df for Block.

Variable	Leaf	Mean (day 25)	SE	Mean (day 118)	SE	Effect Leaf	Effect Day	Effect Leaf*Day
Temp. °C	Oak	21.7	$\pm$ 0.3	33.7	$\pm$ 0.7			
	Pine	23.2	$\pm$ 0.5	34.7	$\pm$ 0.5			
	Tupelo	21.4	$\pm$ 0.7	32.8	$\pm$ 0.6			
	Tallow	20.0	$\pm$ 0.3	32.4	$\pm$ 0.4	<0.001	<0.001	0.742
Specific Cond. $\mu$ S/cm	Oak	0.277	$\pm$ 0.004	0.126	$\pm$ 0.007			
	Pine	0.296	$\pm$ 0.002	0.120	$\pm$ 0.005			
	Tupelo	0.274	$\pm$ 0.003	0.142	$\pm$ 0.006			
	Tallow	0.333	$\pm$ 0.003	0.196	$\pm$ 0.004	<0.001	<0.001	<0.001
Salinity ppt	Oak	0.135	$\pm$ 0.003	0.068	$\pm$ 0.003			
	Pine	0.140	$\pm$ 0.000	0.063	$\pm$ 0.003			
	Tupelo	0.130	$\pm$ 0.000	0.073	$\pm$ 0.003			
	Tallow	0.160	$\pm$ 0.000	0.100	$\pm$ 0.000	<0.001	<0.001	<0.001
pH	Oak	6.7	$\pm$ 0.1	6.9	$\pm$ 0.1			
	Pine	7.0	$\pm$ 0.1	7.2	$\pm$ 0.3			
	Tupelo	6.5	$\pm$ 0.0	6.6	$\pm$ 0.0			
	Tallow	5.7	$\pm$ 0.1	6.9	$\pm$ 0.0	<0.001	<0.001	<0.001
Dissolved oxygen %	Oak	31.2	$\pm$ 8.3	66.1	$\pm$ 7.9			
	Pine	63.1	$\pm$ 5.1	74.6	$\pm$ 7.8			
	Tupelo	11.6	$\pm$ 1.9	34.9	$\pm$ 2.3			
	Tallow	9.4	$\pm$ 4.4	8.5	$\pm$ 3.7	<0.001	<0.001	0.031
Dissolved oxygen ppm	Oak	2.80	$\pm$ 0.71	4.72	$\pm$ 0.56			
	Pine	5.51	$\pm$ 0.42	5.20	$\pm$ 0.49			
	Tupelo	1.14	$\pm$ 0.19	2.53	$\pm$ 0.18			
	Tallow	0.91	$\pm$ 0.38	0.62	$\pm$ 0.27	<0.001	<0.001	<0.001
Light Attenuation % abs.	Oak	NA	NA	47.5	$\pm$ 3.4			
	Pine	NA	NA	27.1	$\pm$ 2.6			
	Tupelo	NA	NA	83.5	$\pm$ 3.9			
	Tallow	NA	NA	94.1	$\pm$ 1.6	<0.001	NA	NA
Turbidity NTU	Oak	42.5	$\pm$ 36.9	0.7	$\pm$ 0.3			
	Pine	7.1	$\pm$ 1.5	7.7	$\pm$ 4.6			
	Tupelo	13.4	$\pm$ 3.0	0.6	$\pm$ 0.4			
	Tallow	636.0	$\pm$ 249.1	3.4	$\pm$ 0.4	0.009	0.018	0.010

Table 1.4. Tadpole performance data for *Pseudacris fouquettei* and *Bufo terrestris* stocked in mesocosms treated with Chinese tallow or oak leaves crossed with two levels of leaching, leached and un-leached. Letter designations are T=un-leached tallow, W=leached tallow, O=un-leached oak, and K=leached oak. Values are means  $\pm$  1 SE, minimum, and maximum for mortality, larval period, and mass at metamorphosis.

Species	Treatment	Survival (%)		Larval period (days)		Mass (mg)	
		Mean	SE	Mean	SE	Mean	SE
<i>P. fouquettei</i>	Tallow, unleached	0	0	NA		NA	
	Tallow, leached	0	0	NA		NA	
	Oak, unleached	94.8	2.7	51.6	0.4	235.7	9.2
	Oak, leached	97.0	1.5	51.7	0.2	209.2	3.9
<i>B. terrestris</i>	Tallow, unleached	47.4	14.4	21.1	0.5	126.7	14.2
	Tallow, leached	27.4	13.5	22.9	2.7	93.6	12.2
	Oak, unleached	9.6	0.7	26.1	0.6	55.8	3.5
	Oak, leached	39.3	11.6	24.7	0.6	85.3	10.1

#### Water Quality

Dissolved oxygen minimum levels were hypoxic (i.e.,  $< 2.0\%$  saturation) in all treatments at the beginning of the experiment (Table 1.5). DO minima increased rapidly with time (weeks) in the oak treatments, but increased little in the tallow treatments until after week 5 (Table 1.5; Figure 1.1). By week 3, oak tanks with leached or unleached litter had DO minima in the range of 20% saturation (Table 1.5;  $20.97\% \pm 10.11\%$  and  $19.27\% \pm 2.82\%$ , respectively), but leached and unleached tallow tanks remained hypoxic (Table 1.5;  $0.97\% \pm 0.17\%$  and  $0.93\% \pm 0.09\%$ , respectively). The condition of hypoxia was not alleviated in tallow tanks until the 5<sup>th</sup> week (Table 1.5). By the 17<sup>th</sup> week, dissolved oxygen levels had increased to  $59.57\% \pm 3.52\%$  in leached oak tanks and  $66.90\% \pm 5.08\%$  in unleached oak tanks, but levels in leached and unleached tallow tanks were half of these levels (Table 1.5,  $30.53\% \pm 1.24\%$  and  $27.20\% \pm 2.06\%$ , respectively). DO minima were about twice as high in the oak treatments than in the tallow treatments from weeks 9 to 17 (Figure 1.1). Multivariate repeated measures ANOVA of

the minimum dissolved oxygen concentrations showed a significant effect of leaf and week (Table 1.6). The effects of leaching and the interaction terms were not significant (Table 1.6).

Table 1.5. Percent saturation of dissolved oxygen (mean  $\pm$  1 SE) for weeks 1, 3, 5, 9, and 17 of the leaching experiment.

Treatment	Percent Saturation of Dissolved Oxygen									
	Wk 1		Wk 3		Wk 5		Wk 9		Wk 17	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Leached Tallow	1.13	0.34	0.97	0.17	3.07	0.15	28.23	1.22	30.53	1.24
Leached Oak	1.20	0.26	20.97	10.11	42.10	3.09	48.40	2.06	59.57	3.52
Un-leached Tallow	0.73	0.07	0.93	0.09	3.00	0.25	15.97	0.44	27.20	2.06
Un-leached Oak	1.20	0.26	19.27	2.82	40.03	3.68	49.63	3.06	66.90	5.08

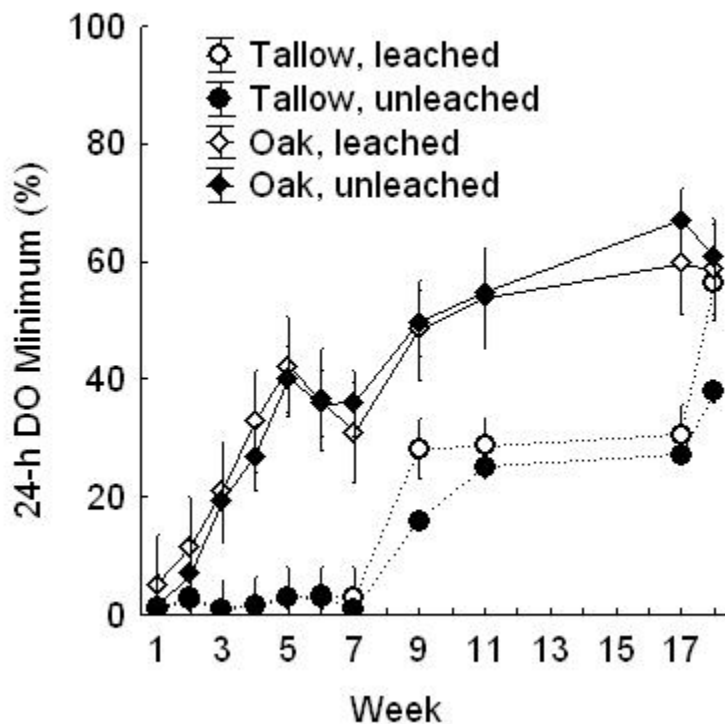


Figure 1.1. 24-hour minimum dissolved oxygen percent saturation plotted as a function of time (week). Shaded points represent unleached treatments and open points represent leached treatments; circles represent tallow treatments and diamonds represent oak treatments. Error bars represent mean  $\pm$  1 SE.

Table 1.6. Repeated measures ANOVA table for the effect of leaf, leaching, and time on the 24-hr minimum dissolved oxygen concentrations observed on weeks 1, 2, 3, 4, 5, 7, 9, 11, and 17 of the second experiment. Results for univariate analysis are presented on the left side of the table, and the results for the multivariate analyses are presented on the right. Note that main effects are only listed once and are the same for both analyses. Although the assumption of sphericity was not rejected ( $W=0.0002$ ,  $\text{Chi-square}=43.37$ ,  $\text{df}=35$ ,  $p=0.157$ ), we consider the multivariate analysis more appropriate for the within-treatment comparisons.

	SS	df	F	P	Wilks $\lambda$	F	Effect df	Error df	p
Leaching	122.24	1	2.67	0.141					
Leaf	17118.4	1	373.59	<0.001					
Leaching*leaf	1.18	1	0.03	0.876					
Error	366.57	8							
Wk	26831.5	8	291.59	<0.001	0.000	1372.27	8	1	0.021
Wk*leaching	176.38	8	1.92	0.073	0.018	6.77	8	1	0.289
Wk*leaf	3369.8	8	36.62	<0.001	0.003	40.24	8	1	0.121
Wk*leaching*leaf	491.28	8	5.34	<0.001	0.002	53.76	8	1	0.105
Error	736.14	64							

The assumption that incident light did not differ among spatial blocks was not rejected ( $F_{2,30}=1.99$   $p=0.155$ ). Light attenuation was affected by leaf type, leaching treatment, and day, and there was strong litter\*day interaction (Table 1.7; other interactions were not significant). The intensity of incident light was lower on day 48 than it was on day 62 (Figure 1.2). On both days, the order of treatments from least opaque to most opaque was leached oak < un-leached oak < leached tallow < un-leached tallow (Figure 1.2). The tallow treatments consistently absorbed between 97.9% and 99.8% of all incident light. The oak treatments became less opaque between days 48 and 62.

Table 1.7. Repeated measures ANOVA table for the effect of leaf, leaching, and day on light attenuation (% absorbance) at 35 cm depth in tanks on days 48 and 62 of the second experiment.

	SS	df	F	P
Intercept	32.9135	1	69881.73	<0.001
Leaf	2.1277	1	4517.49	<0.001
Leaching	0.0154	1	32.61	<0.001
Leaf*leaching	0.0000	1	0.00	0.977
Error	0.0038	8		
Day	0.0212	1	34.74	<0.001
Day*leaf	0.0120	1	19.59	0.002
Day*leaching	0.0005	1	0.77	0.406
Day*leaf*leaching	0.0002	1	0.30	0.600
Error	0.0049	8		

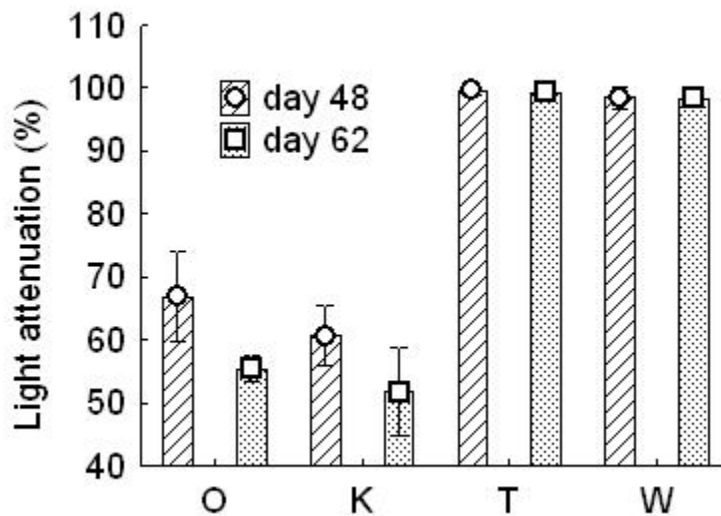


Figure 1.2. Light attenuation (% absorbance) in cattle tanks. Treatments were unleached oak (O), leached oak (K), unleached tallow (T), and leached tallow (W). Error bars represent 95% confidence intervals.

## Discussion

In the first experiment, the results for *B. terrestris* and *H. cinerea* reared in mesocosms with Chinese tallow are consistent with our predictions. However, we were surprised by the

strong effect of tallow litter on *P. fouquettei*. In addition to the effects of Chinese tallow leaf litter, we also observed that the native types of leaf litter affected tadpole performance. Tadpoles of *P. fouquettei* did better in tupelo tanks than *H. cinerea*, which generally did better in oak or pine tanks. These species-specific responses to litter type may be partly explained by limnological differences between litter treatments.

Leaf litter treatments produced significant variation in dissolved oxygen, specific conductance, pH, and turbidity. Although our observations of amphibian performance cannot be linked to our observations of water quality by deductive inferences, we provide strong evidence for an effect of litter type on both. Our results are best interpreted in light of factors as diverse as the timing of tadpole introductions to tanks, the chemical properties of the leaf litter, light attenuation and internal shading effects, and subsequent variation in dissolved oxygen levels between treatments could all affect our observations.

The difference in *B. terrestris* tadpole performance between 2004 and 2005 seems to be related to the timing of their introduction to tanks. Although we could not test this hypothesis, we note the fact that the *B. terrestris* tadpoles were added later in the second experiment than they were in the first (day 110, 04 June 2005, and day 76, 03 May 2004, respectively). The effect of this difference in the timing of tadpole introductions to tanks may be increased performance (i.e., larger mass at metamorphosis, shorter larval period, and increased survival) resulting from the effect of time on the metabolism or neutralization of chemicals leached from leaves, increased opportunity for algal production and subsequent increases in foodstuffs for tadpoles or production of oxygen via photosynthesis. Dissolved oxygen data collected for the second experiment seem to support the last of these possibilities although it is unlikely that DO completely explains differences in tadpole performance. Thus, it is plausible that the tadpole

performance in our experimental tanks can be attributed to the role of leaves as a resource, direct toxicity effects of tallow leachates on tadpoles, or leachate effects on algae – and subsequently lower algal production and photosynthesis.

The addition of leaf litter to cattle tanks to provide experimental systems with nutrients is a common practice in mesocosm studies. The idea is that the leaves provide nutrients to producers as they decompose. Thus, leaves with different breakdown rates should release their constituent nutrients to tanks at different rates. We measured litter breakdown of tallow, oak, and red maple leaves in ponds and streams (Chapter 3), and found that tallow leaves breakdown at rates as much as 11 times faster than litter from any native tree species (Chapter 3). In this experiment, we did not measure the chemical properties of the leaf litter added to tanks. However, Chinese tallow litter left over from this experiment was analyzed in the lab of Dr. Richard Durtsche at Northern Kentucky University (Thermo (Carlo Erba) Flash EA 1112 Series NC Soil Analyzer). On average, tallow leaves placed in the mesocosms in 2004 were 1.06% nitrogen, 43.3% carbon, and had a C:N ratio of 40.8. This C:N ratio is much lower than published values for *Nyssa sylvatica* (C:N=58.3; Ostrofsky 1997) and 5 species of *Quercus* (C:N=64.7 to 69.3; Ostrofsky 1997), suggesting that Chinese tallow leaves are a relatively higher quality substrate than either tupelo or oak leaves. Furthermore, data from our own work on litter breakdown rates (Chapter 3) and published values suggest that relatively more abundant nitrogen in tallow leaves is released faster than that of the other species of litter because the tallow leaves are more labile than the other types of litter by at least an order of magnitude (Chapter 3, Webster and Benfield 1986b, Ostrofsky 1997). From these data, we would have expected Chinese tallow leaves to provide ample nutrients to tanks early in both experiments, stimulating



algae growth, and supporting tadpoles, except in cases where the leaves had already been leached.

In our second experiment, litter type was more important to amphibian performance and dissolved oxygen measurements than the leaching treatments (Table 1.4 and Figure 1.1). These results run against our predictions, which were based on other work showing that litter and wood leachates have a significant effect on gastropods (Chergui et al. 1997), fishes (Temmink et al. 1989), and amphibians (Maerz et al. 2005). The fact that we did not observe a detectable effect of leaching on dissolved oxygen concentrations may be explained by the loss of nutrients from leached leaf material. Leachates from wood pulp contain utilizable forms of several nutrients and micronutrients including Mg and Zn, in addition to tannins (Catricala et al. 1996, McArthur and Richardson 2002). In natural ponds, material that leaches from leaf litter remains in the system (Schofield et al. 1998) where it can be broken apart by microbial and fungal activity (Vasudevan and Mahadevan 1990), which makes nutrients available to algae and other organisms throughout the course of the season. By leaching leaves before adding them to tanks, we decreased the tannin concentrations in tanks, but we also deprived tanks of labile nutrients. By retaining nutrients in the system, there are more resources available to primary producers, which in turn produce more food for tadpoles to graze as well as more oxygen from photosynthesis. The benefit of these nutrients may be ameliorated by decreased light attenuation, but even in dark tanks some light manages to permeate the surface of the water and increased algal growth would be expected relative to similarly dark but relatively nutrient-poor tanks. Thus, the usual practice of using un-leached leaves in mesocosm experiments seems preferable to using leached leaves. Leaching leaves prior to an experiment increases the possible sources of variation as leaching processes are difficult to control. Future work on the effect of leaf litter leachates should employ

methods that decouple the effects of leaf extracts and leaching from the concurrent loss of nutrients from the system.

An alternative consequence of rapid decomposition that we did not anticipate was the possibility that the tallow leaves would support a robust fungal or bacterial community and deplete dissolved oxygen in the tanks biologically. Litter breakdown subsequent to leaching of tannins and other phenolics from leaves may promote bacterial and fungal production (Weyers and Suberkropp 1996, Wright and Covich 2005) that create high biological oxygen demand (Tao et al. 2005). Although microbes can be adversely affected by tannins (Scalbert 1991, Nelson et al. 1997), they can also break down tannins (Vasudevan and Mahadevan 1990). The fungal and bacterial communities were not characterized or measured in these experiments, but tallow tanks had a putrid odor and formed a thick, oily biofilm on their surface early in the experiment. Both the odor and biofilm eventually dissipated but could be interpreted as signs of bacterial or fungal blooms early in the experiment.

Direct toxicity effects of leachates on tadpoles or their food resources are unlikely because leachates are ubiquitous in natural amphibian habitats, and the majority of naturally occurring amphibian habitats in southeastern Louisiana are darkly stained with leachates. The blackwater ecosystems of the southeast, which get their name from the high concentrations of tannins in the waters, support diverse communities of invertebrates (Spieles and Mitsch 2000, Battle and Golladay 2001), amphibians (Werner and McCune 1979), and fishes (Leyse et al. 2004, Pazin et al. 2006). However, the rapid leaching of tannins from leaves may create high chemical oxygen demand (Chergui et al. 1997, Tao et al. 2005). This would partially explain why some organisms in experimental enclosures seem to be directly harmed by tannins that occur naturally in their native habitats (e.g., Temmink et al. 1989, Maerz et al. 2005). Biological

suppression of oxygen via allelopathic effects on algae may be a factor, given that many of the chemicals in leaf litter are part of a plant's defensive strategy. Nevertheless, the plankton that we added to tanks in both experiments was drawn from naturally occurring wetlands, including areas invaded by Chinese tallow. Thus, the plankton introduced to the tanks should have been resistant to potential allelopathy by litter leachates, and toxicity to algae (and subsequent suppression of oxygen production) is unlikely.

A more likely explanation of how litter leachates might have affected amphibians in our tanks is the indirect effect of staining on algal production. In the end, the outcome is nearly indistinguishable from a toxicity effect, but the mechanism is important because one, toxicity, can be peculiar to a particular tree species or litter type while the other, staining or shading, is a function of complex biochemical interactions that may not be peculiar to any given litter type. Staining of the water column and subsequent light attenuation can be treated as an "internal" shading effect, meaning that light availability to producers was limited by low light penetration of the water rather than mechanical shading due to trees or other foliage. Water in the pine tanks was not heavily stained whereas water in the tupelo tanks was, with oak tanks being intermediate between the two (personal observation). The high light attenuation we observed in tupelo tanks supports this idea (Table 1.3). Such high light attenuation may limit algal production, thus explaining our observations of low dissolved oxygen levels in treatments with the lowest light availability.

In the field, increased canopy cover has been linked to reduced dissolved oxygen concentrations (Schiesari 2006), but no study that we are aware of has controlled for the effect of shading while measuring the effect of leaf litter on dissolved oxygen. All mesocosms used in this study were placed in full sun and covered with identical screen lids. Incident light striking

the water surface within tanks did not differ among tanks. If light was limiting photosynthesis and the production of oxygen by algae, the effect occurred within the tank rather than resulting from exogenous shading. Nevertheless, even the tanks with low light produced algae, and the dissolved oxygen levels in all treatments in 2005 eventually converged, thereby indicating that algal production may have been sufficient to provide food and oxygen to tadpoles.

We linked low DO levels to tadpole behavior in a separate study that used a subset of the tanks from 2004 (Chapter 2). Our findings from that study indicate that tadpoles move less in low oxygen environments. As movement is associated with foraging and predator avoidance in tadpoles (McIntyre and McCollum 2000), the study provides support for our assertion that litter-induced differences in tadpole performance are linked to litter-induced variation in water quality. However, such observations are correlative at best and prevent us from drawing deductive inferences linking water quality to behavior and, by extension, performance.

Identifying mechanisms that link litter-induced amphibian responses to litter-induced water quality requires independent, controlled manipulation of the various factors described above. For instance, the possibility that leachates suppressed algal production and tadpoles were food-limited would require manipulation of food availability in the tanks. Likewise, the effects of leachates on DO might be addressed by aeration into some tanks, but this and similar types of manipulation of DO would likely affect other components of the study system as well. Aeration might stimulate bacterial or fungal growth in tanks, thereby confounding observations of amphibian responses. In another manipulation, researchers might choose to use extracted leachates while discarding the leaves to test for toxicity effects or minimize the effect of heterotrophy on decomposing litter. Each of the options would be informative and represent potentially profitable avenues for future research. In this study, our use of mesocosms as a black

box – leaves and tadpoles in and metamorphs out – was chosen because it is an approach that allows for compromise between rigorously controlled laboratory conditions and “real life” natural conditions encountered in field studies (Wilbur 1989).

Our interpretation of this work is further limited by the fact that we did not measure the concentration of leaf litter leachates in mesocosm water. So while there is a clear effect of litter type on tadpole performance and dissolved oxygen levels, we lack the ability to attribute these effects to any particular differences between litter or leaching treatments. We assume that the leaching treatments changed the tannin concentrations in tanks based on information in the literature (e.g., Chergui et al. 1997), but the magnitude of these changes remains an unknown. Similarly, differences in the tannin content of leaves may be an important factor explaining why some animals seem sensitive to changes in detrital inputs to aquatic habitats. A recent study of another invasive plant, purple loosestrife, found the leaves of that plant contain ~3% hydrolysable tannins, and that hydrolysable tannins were contributing to the negative effect that plant species has on the survival of American toad tadpoles (Maerz et al. 2005, Brown et al. 2006). In addition to potentially damaging the gills of some aquatic fauna (Temmink et al. 1989), tannins increase the chemical oxygen demand in aquatic systems (e.g., Grismer et al. 2003, Tao et al. 2005). Chergui et al. (1997) hypothesized that the toxic effects of tannin-rich leachates on gastropods was mostly due to oxygen suppression, and another study with tiger shrimp reported reductions in dissolved oxygen levels as a consequence of high leaf-loading rates (Hai and Yakupitiyage 2005). While Maerz et al. (2005) found negative effects of tannin rich loosestrife extracts on American toad tadpoles, which are obligate gills breathers, they found no negative effects on Gray treefrog tadpoles (*Hyla versicolor*), which have functioning lungs and can gulp air from the surface in low oxygen environments.

Case studies such as this one are also limited by their scope of inference. We cannot predict the direction or magnitude of the effect for any litter types other than those explicitly tested herein. Not all species of trees invading temporary ponds have litter with similar qualities. For species like Chinese tallow, in which the lignin concentration in leaves is low compared to other trees (Cameron and Spencer 1989) and litter break down is relatively rapid (Chapter 3, Cameron and LaPoint 1978, Cameron and Spencer 1989), the effects are likely to be similar to those we report. However, for pond invaders that produce more recalcitrant litter, the effects of invasion may be quite different.

Despite these shortfalls, this study provides support for the idea that shifts in canopy composition, and hence litter quality, will change the limnologic characteristics of ponds. It also shows that dissolved oxygen concentrations in the water will change and that amphibian performance will subsequently be affected. We used leaves from invasive exotic Chinese tallow trees (*T. sebifera*) to provide a contrast to the litter from native species such as laurel oaks (*Q. laurifolia*), but changes in canopy composition need not include invasive species to have an effect on amphibians (e.g., Werner and Glennemeier 1999). We simply used the invasive species because it provided a convenient, stark permutation of the system in which we work. Its invasion of temporary ponds thus qualifies it as a novel permutation of the habitat. By taking advantage of this fact, we successfully demonstrated that changes in detrital resources can affect amphibian performance and survival.

Issues related to amphibian conservation arise from this work. For example, the strong negative effect of Chinese tallow on *P. fouquettei* and *B. terrestris* survival suggests that efforts to protect these species may require efforts at controlling invasions. Conversely, the survival rate of *H. cinerea* in tallow tanks was not significantly different from the survival rates in tanks

treated with native leaves, and managers faced with the responsibility of conserving this species may reasonably conclude that Chinese tallow does not pose any greater risk than native trees. Similarly, the differences in tadpole performance between litter treatments might help conservation biologists predict the effects of changes in canopy cover because of fire suppression or conversion from one forest type to another. For instance, a shift in forest composition from pine to oak or tupelo may produce larger *P. fouquettei* and smaller *H. cinerea* while a shift in the other direction might favor the chorus frogs. Thus, future efforts at amphibian conservation need to account for the effects of canopy composition in and around breeding ponds. Other studies have also found links between canopy cover and amphibian performance (e.g., Skelly et al. 2002, Thurgate 2006). The production of larger individuals of late-breeding species, as seen in *Hyla* in tallow treatments, will change species interactions among those species that are able to cope with Chinese tallow invasion. If these species metamorphose at larger sizes in natural ponds as they did on our mesocosms, then they may experience greater survival in terrestrial habitats (Chelgren et al. 2006) and increased fitness (Altwegg and Reyer 2003). Ultimately, these changes could cause changes in community composition.

Another conservation concern that arises from this work is the possibility that continued, unchecked Chinese tallow invasion will result increase the risk of local extinction (e.g., *P. fouquettei*) or reduced abundance and fitness (e.g., *B. terrestris*). Alternatively, populations in invaded wetlands may experience selection for later breeding as time between pond flooding and breeding events seems to partially explain the difference in *B. terrestris* performance between years.

The results of this experiment support the idea that changes in canopy composition and subsequent changes in detrital quality can affect dissolved oxygen levels in ponds and thereby

affect amphibian performance. Our data demonstrate that it matters what kind of plant produces the litter falling into a pond because even the native tree species used in this study elicited differential performance in the tadpoles we tested. As similar invasions of wetlands by non-native trees and plants are becoming widespread (e.g., Sher et al. 2000, Harrod 2001, Johnson and Rothfels 2001), important effects on amphibians are likely to occur.

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## Chapter 2: Leaf litter effects on dissolved oxygen influence time spent moving by *Hyla cinerea* tadpoles

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### Abstract

In June 2004, we observed *Hyla cinerea* tadpoles in 10 mesocosms containing one of two different types of leaf litter, slash pine (*Pinus elliottii*) or water tupelo (*Nyssa aquatica*), to determine whether litter type and subsequent effects on water chemistry led to differences in tadpole activity. Water chemistry (N=9) and tadpole behavior (N=10) were observed in each mesocosm in the morning and afternoon. For each set of observations, three haphazardly selected tadpoles from each mesocosm were transferred to a screen enclosure within the mesocosm and duration of movement and air gulping behavior were observed for 2 minutes after a 30-minute acclimation period. Litter type had an effect on dissolved oxygen and other water quality parameters. Dissolved oxygen was 13.4% higher in pine mesocosms in the mornings and 21.8% higher in the afternoons. Morning tadpole movements were greater in pine (mean  $\pm$  1SE:  $12.04 \pm 2.72$  s) than tupelo mesocosms ( $2.76 \pm 0.96$  s). In the afternoons, the amount of activity in pine tanks ( $18.40 \pm 7.88$  s) remained high relative to tupelo tanks ( $2.16 \pm 1.01$  s). This research suggests that litter effects on dissolved oxygen may affect tadpole activity. As activity is often positively associated with food intake, litter effects on activity via decreased dissolved oxygen may lead to effects on growth and development not directly related to effects on algal resource production.

## Introduction

Ephemeral ponds in many landscapes are often shaded or partially covered by trees whose leaf litter provides an important source of energy and nutrients. The quality and quantity of these detrital inputs vary among ponds and through time due to differences in canopy composition (Skelly et al. 1999, Werner and Glennemeier 1999). Humans may change canopy composition, and consequently detritus quantity and quality by the propagation of economically important species, thinning, harvesting, fire suppression, and other activities. Understanding the consequences of such changes on adjacent aquatic habitats is necessary for predicting the subsequent effects on aquatic animals.

Ephemeral ponds in southeastern Louisiana are often shaded by water tupelo (*Nyssa aquatica*) or slash pine (*Pinus elliottii*) trees. Typically, ponds dominated by water tupelo are completely shaded while those dominated by slash pine are only partially shaded, because slash pines rarely grow in the pond center and their canopies are sparser than those of tupelo. These ponds differ in kind and quantity of litter inputs, and presumably also in temperature, algal growth, and dissolved oxygen concentrations (Chapter 1).

Ephemeral ponds support many aquatic animals, including the early life history stages of some amphibians. Amphibian larvae obtain oxygen from the water through their skin, across gill membranes, or, in some cases, through lungs (West and Burggren 1982, Burggren and Vitalis 2005). The amount of oxygen in the water is thought to affect amphibian performance measures such as growth rates and survival to metamorphosis (Werner and Glennemeier 1999, McIntyre and McCollum 2000). There has also been much discussion of the effects of dissolved oxygen on activity levels (McIntyre and McCollum 2000) and air gulping (e.g., Wassersug and Murphy 1987), a behavior exhibited by some species in oxygen-poor habitats (Wassersug and Seibert 1975, Feder and Wassersug 1984).

The green tree frog, *Hyla cinerea*, is common in a wide range of aquatic habitats in the southeastern U. S. (Gunzburger and Travis 2005), from ephemeral ponds with a 60-90 day hydroperiod to permanent aquatic habitats such as bayous. *H. cinerea* tadpoles are not frequently found in closed-canopy ponds (Leonard, personal observation), but many habitats used by *H. cinerea* receive litter from both tupelo and pine trees. We used *H. cinerea* as a model to examine differences between pine and tupelo-dominated systems.

This study used *H. cinerea* tadpoles raised in experimental mesocosms to address two questions: 1) Does the dissolved oxygen level in ponds vary in response to leaf litter? 2) Does dissolved oxygen level affect tadpole activity level and air gulping behavior?

## **Methods**

### *Setup*

The mesocosms used in this study were part of a larger study (Chapter 1). We filled tanks with 1180 L of New Orleans city water on 15 and 16 February 2004 and treated them with chloramine remover the next day. We then treated each tank with 1500 g of litter from 1 of 4 tree species on 18 February and added plankton on 19 February and 14 March. Plankton were collected with a 500- $\mu$ m plankton net from at least 3 naturally occurring ponds. The resulting zooplankton slurry was divided into 350-ml aliquots and randomly assigned to tanks, stratified by pond of origin. This experiment utilized 10 tanks treated with either slash pine (*Pinus elliotii*) or water tupelo (*Nyssa aquatica*) leaves arranged in five spatial blocks. Leaf treatments within the blocks were randomly assigned to tanks. Details of tank set-up followed well-established standard methods, including screen lids and adjustable standpipes (e.g., Morin 1983, Fauth 1990).

All tanks received 45 *Pseudacris fouquettei* tadpoles on 21 February, 45 *Bufo terrestris* on 03 May, and 45 *H. cinerea* tadpoles on 28 May. This sequence of species followed the natural breeding phenologies observed at ponds in southeastern Louisiana. All individuals of *P. fouquettei* metamorphosed prior to the introduction of *B. terrestris*, but toads were still present in several tanks when we added *H. cinerea*. This paper deals only with *H. cinerea* tadpoles, which we collected from seven amplexing pairs of *H. cinerea* at a single temporary pond near Florenville, LA. The fertilized eggs were hatched in the lab and tadpoles absorbed their yolk sack before we randomly assigned them to tanks in groups of 5, stratified by clutch or clump (Chapter 1).

#### *Water quality*

We measured water temperature, dissolved oxygen, pH, turbidity, and specific conductance in the artificial ponds with a Hydrolab Quanta at 10-15 cm depth at the same time of day and in the same order as we made behavior observations. Morning observations started at 06:30 h and evening observations started at 16:00 h in order to detect diurnal dissolved oxygen minima and maxima, respectively. We measured water quality in each tank on 9 different occasions between 21 and 29 June 2004 comprising 5 mornings and 4 afternoons.

Measurements were taken immediately prior to behavioral trials on 6 of the 9 periods.

#### *Behavior*

Activity levels and air gulping behavior were observed on 5 mornings and 5 afternoons between 21 and 29 June 2004. Activity was defined as movement of any type, and no effort was made to discern the purpose of movements (e.g., feeding, fleeing, thermoregulation, etc.). Three

tadpoles were selected haphazardly from the population of each tank, placed in a screen enclosure, and allowed 30 minutes to acclimate prior to the start of observations. Enclosures were constructed of fiberglass window screen and plastic-coated aluminum clothesline wire. The enclosures were 40 cm deep, 30 cm in diameter, and had a volume of 32 L. The tops of the enclosures were open to the air, and the screened bottoms were set on top of the tank's leaf litter substrate. Each enclosure was placed in the center of the tank for observations. After the acclimation period, the tadpoles were observed for a period of 2 minutes. The amount of time tadpoles spent moving and the number of air gulps observed were recorded. We pooled data for the three tadpoles.

### *Statistics*

Data were analyzed using Statistica 7.1 (StatSoft, Inc., Tulsa, OK, USA). Our experimental design crossed leaf litter treatment (pine or tupelo) with time of day (morning or afternoon). Although repeated-measures ANOVA would have been the most appropriate choice of methods, the unbalanced design of our water quality measurements (i.e., 5 mornings and 4 afternoons, with several observations made on different days) precluded this approach. We instead calculated tank averages for each water quality variable across days and used two separate ANOVAs to analyze morning and afternoon observations separately. Averaging was appropriate because our observations spanned only 9 days (21-29 June 2004), and conditions within tanks underwent little directional change during this period. We tested the effect of leaf treatment on dissolved oxygen percent saturation, turbidity, pH, salinity, specific conductance, and temperature. For these analyses, we transformed dissolved oxygen percent saturation data by dividing by 100 and then applying an arcsine-square root transformation. All other water

quality variables were included without transformation. There was not a significant block effect (all  $p > 0.23$ ) for any water quality variable or the movement data, so this term was dropped from our models.

We treated the behavior data in a manner consistent with the water quality data, meaning that we averaged our observations across days (movement observations) or pooled the data for all days (air gulping observations). We analyzed the movement data using ANOVAs, but we did not analyze the air-gulping data because 50% of our observations had a value of zero even after pooling all days (we report only the mean number of air gulps  $\pm$  one standard error across all tanks for each litter treatment and time of day). With the ANOVAs for the movement data, we tested the effect of leaf treatment on the time tadpoles spent moving in the top 10 cm of the water column within experimental enclosures. The movement data were log transformed ( $\ln(x+1)$ ). Our observations of tadpole movement at the bottoms of tanks were not analyzed because data from the darkly stained tupelo tanks were not reliable (Chapter 1; see Discussion). All means are reported as the mean  $\pm$  one standard error.

## **Results**

In the mornings, pine tanks had higher dissolved oxygen) and turbidity than tupelo tanks, but marginally lower salinity and specific conductance (Tables 2.1 and 2.2). In the afternoons, pine tanks had higher dissolved oxygen, but differences in turbidity could no longer be attributed to the effect of litter type (Tables 2.1 and 2.2). Salinity and specific conductance remained lower in pine tanks in the afternoons, with observations of these variables moving closer to significance (Tables 2.1 and 2.2). Variation in pH tracked changes in dissolved oxygen concentration as

expected, but the difference in pH between litter treatments was not significant in either the morning or afternoon observations (Tables 2.1 and 2.2).

Table 2.1. Results of ANOVAs of the effects of leaf treatment on dissolved oxygen, turbidity, pH, salinity, specific conductance, and water temperature.

		Morning (AM)				Afternoon (PM)		
		df	MS	F	P	MS	F	p
Dissolved oxygen	Treatment	1	0.05	7.03	0.029	0.12	10.11	0.013
	Error	8	0.01			0.01		
Turbidity	Treatment	1	7.53	12.01	0.008	1.56	2.64	0.143
	Error	8	0.63			0.59		
pH	Treatment	1	0.01	0.84	0.386	0.00	0.15	0.704
	Error	8	0.02			0.02		
Salinity	Treatment	1	0.00	5.09	0.054	0.00	6.90	0.030
	Error	8	0.00			0.00		
Specific conductance	Treatment	1	0.00	5.02	0.055	0.00	5.27	0.051
	Error	8	0.00			0.00		
Water temperature	Treatment	1	0.07	0.36	0.563	0.23	1.30	0.287
	Error	8	0.20			0.17		

In the mornings, tadpole activity was significantly greater in pine litter mesocosms ( $12.04 \pm 2.72$  s) than tupelo litter mesocosms ( $2.76 \pm 0.96$  s;  $F_{1,8}=10.58$ ,  $p=0.012$ ). In the afternoons, the amount of activity in pine tanks ( $18.40 \pm 7.88$  s) remained high relative to tupelo tanks ( $2.16 \pm 1.01$  s;  $F_{1,8}=5.60$ ,  $p=0.045$ ).

Table 2.2. Table showing the mean  $\pm$  1 SE for dissolved oxygen, turbidity, pH, salinity, specific conductance, and water temperature in experimental mesocosms. AM measurements were taken between 06:30 and 08:00 h; PM measurements were taken between 16:00 and 17:30 h.

Variable	Treatment	Time	Mean	SE
Dissolved oxygen (%)	Pine	AM	39.9	2.3
		PM	56.6	3.2
	Tupelo	AM	26.5	1.4
		PM	34.8	1.7
Turbidity (NTU)	Pine	AM	2.33	0.65
		PM	1.49	0.25
	Tupelo	AM	0.59	0.13
		PM	0.70	0.18
pH	Pine	AM	6.49	0.03
		PM	6.64	0.04
	Tupelo	AM	6.57	0.02
		PM	6.60	0.02
Salinity (ppt)	Pine	AM	0.057	0.001
		PM	0.054	0.002
	Tupelo	AM	0.066	0.002
		PM	0.064	0.002
Specific Conductance ( $\mu\text{S}/\text{cm}$ )	Pine	AM	108	3
		PM	104	3
	Tupelo	AM	129	3
		PM	125	3
Water Temperature ( $^{\circ}\text{C}$ )	Pine	AM	26.6	0.3
		PM	28.7	0.3
	Tupelo	AM	26.4	0.2
		PM	28.4	0.2

We observed very little air gulping by tadpoles. In the mornings, the frequency of air gulping was  $0.80 \pm 0.37$  (mean  $\pm$  1 SE) in pine tanks and  $1.00 \pm 0.45$  in tupelo tanks, and in the afternoons the frequency was  $0.80 \pm 0.37$  in pine tanks and  $0.20 \pm 0.20$  in tupelo tanks.

## Discussion

This research suggests that low concentrations of dissolved oxygen resulting from changes in detrital inputs to ponds can affect tadpole activity. Our results confirm those of previous studies suggesting that dissolved oxygen can vary in response to leaf litter (Hai and



Yakupitiyage 2005, Magnusson and Williams 2006) and that dissolved oxygen is an important factor regulating tadpole behavior (Wassersug and Seibert 1975, McIntyre and McCollum 2000). This is the first study we are aware of to link tadpole behavior to type of leaf litter.

The oxygen results reported here are important because they reflect the effect of litter type on dissolved oxygen independent of canopy cover, shading, and temperature. In natural ponds other factors that affect dissolved oxygen are autocorrelated with canopy composition and confound differences resulting from detrital subsidies to ponds. For instance, the tupelo species used in this study (*Nyssa aquatica*) is more tolerant of flooding than the pine species we used (*Pinus elliottii*), so that ponds containing tupelo have more trees growing within the pond and denser canopy cover. Increased canopy cover would further decrease dissolved oxygen concentrations in the water by limiting photosynthesis and algal productivity. The change in canopy cover would thus add to litter-mediated effects on dissolved oxygen by limiting both photosynthetic production of oxygen and food availability for algivorous *H. cinerea* tadpoles.

We observed that *H. cinerea* tadpoles in mesocosms were less active when dissolved oxygen concentrations were relatively low and more active when dissolved oxygen concentrations were high. Activity is often positively associated with food intake, as shown in studies that examined the interaction between activity, tadpole performance, and the effect of a predator (e.g., Eklov and Halvarsson 2000, McIntyre and McCollum 2000), a pollutant (e.g., Gurushankara et al. 2007), or parasite (e.g., Goater et al. 1993). Thus, we conclude that the effect of oxygen on activity reported herein, together with likely reductions in overall food availability in tupelo-dominated ponds, may reduce tadpole foraging success. This is corroborated by the larger study done in these mesocosms, in which mean size at metamorphosis for *H. cinerea* was smaller in tupelo than in pine (Chapter 1). We speculate that these effects

may ultimately lead to declines, shifts in distribution, or local extinctions of frogs like *H. cinerea*. Most hylid frogs are not commonly found in ponds with a dense canopy (Halverson et al. 2003, Horn et al. 2005), and the behavioral observations reported here may be an important factor in explaining local observations of frog presence or absence.

We had expected to observe an effect of leaf litter and dissolved oxygen concentration on the frequency of tadpole air gulping behavior because the limited literature on this topic suggests a strong negative relationship between these variables (e.g., Wassersug and Seibert 1975, Moore and Townsend 1998, McIntyre and McCollum 2000). Our observations ran contrary to our predictions. In fact, we observed very little air gulping behavior whatsoever. Only 14 occurrences of air-gulping behavior were observed in 100 2-minute observation periods. In these tanks the dissolved oxygen concentration was hypoxic (<2 ppm) and similar to the concentration that induced increased bobbing behavior in the Wassersug and Seibert (1975) study. Nevertheless, we only recorded 0.006 bobs/tadpole/minute during our observations of these tanks. This rate is 4 orders of magnitude smaller than the rates of 1.1 to 2.5 bobs/tadpole/minute reported by Wassersug and Seibert.

We did not directly manipulate dissolved oxygen, so we cannot rule out alternative explanations for the differences in activity that we observed. For example, differences between the pine and tupelo litter treatment ponds in algal productivity, light penetration, water color, or water clarity (see Chapter 1) could also influence tadpole activity. Had we bubbled oxygen or carbon dioxide into the tanks, we might have produced stronger evidence for the relationship between dissolved oxygen and tadpole activity levels. However, additions of either gas might also have affected algal productivity within our tanks, thereby changing food availability, pH of the water, or providing an unnatural disturbance (natural ponds are usually still, quiet bodies of

water with few bubbles). The cleanest investigations of the relationship between tadpole behavior and dissolved oxygen are most likely to occur in lab settings, but these settings sacrifice a great deal of “reality” for control over experimental conditions.

A more fundamental problem with manipulating oxygen in our tanks directly is that it would have decoupled the link between leaf litter type and dissolved oxygen. The point of this study was to examine potential links between leaf litter, dissolved oxygen, and tadpole behavior. This study accomplished that goal by showing that leaf litter can affect the dissolved oxygen concentration of small limnetic systems and that the activity of tadpoles within these systems may decrease with decreasing oxygen. This finding lends mechanistic support to papers describing patterns of amphibian species richness (Skelly et al. 1999, Werner and Glennemeier 1999) and performance (Skelly et al. 2002) in ponds with varying degrees of canopy closure. Our results suggest that the presence or absence of anurans from various habitat types may be mediated by the effects of oxygen on activity.

### **Acknowledgements**

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### **Chapter 3: Differential processing and invertebrate utilization of leaf litter from invasive Chinese tallow trees (*Triadica sebifera*) in southeastern Louisiana**

Norman E. Leonard and Joseph H.K. Pechmann

#### **Abstract**

Chinese tallow (*Triadica sebifera*) is an invasive exotic tree that grows well in wetlands along the Gulf Coastal Plain of North America. It is rapidly invading ephemeral ponds, displacing native vegetation, and causing shifts in the quantity and quality of allochthonous inputs to aquatic habitats. We examined the processing rates ( $k$ -values) of leaf litter from invasive *T. sebifera* trees and native trees in St. Tammany Parish, Louisiana to better understand the consequences of *T. sebifera* invasion in three separate field experiments using litterbags. Two experiments were in a small blackwater creek, and one experiment was done in three naturally occurring temporary ponds. We found that *T. sebifera* litter mass loss rates ( $k$ ) average  $0.080 \text{ d}^{-1}$ , a rate 8.4 times faster than the pooled rates of native tree litters tested herein. Changes in mass loss rate were not additive for litterbags containing a 50% mixture of tallow and oak leaves. Litterbags containing mixed litter lost mass 5.0 times faster than litterbags of *Q. laurifolia* and 3.3 times slower than *T. sebifera*. Animal colonization of litterbags was driven by small crustaceans in both the stream and pond habitats. In 2002, the pooled abundance of the six most common taxa was higher in *Acer rubrum* than *T. sebifera* litterbags ( $F_{1,22}=7.74$ ,  $p=0.011$ ). In 2005, litter type affected animal abundances in one pond ( $F_{2,24}=4.89$ ,  $p=0.017$ ), but not the other two ( $F_{2,24}=2.24$ ,  $p=0.128$  and  $F_{2,24}=1.38$ ,  $p=0.270$ ). The majority of animals encountered were shredders or detritivores that depend heavily on seasonal litter inputs for their survival. Our observation that *T. sebifera* litter disappeared entirely by the end of all three experiments

suggests that the rapid breakdown and non-additive effects of *T. sebifera* litter may force drastic shifts in the faunal composition of invaded wetlands.

## **Introduction**

Leaf litter detritus is important to many aquatic ecosystems because it may provide nutrients and energy that drive food webs or provide animals with refugia from predators (Walls 1995, Wehr et al. 1998, Moore et al. 2004). Energy and nutrients from leaf litter are made available to aquatic organisms by mechanical and chemical breakdown processes that may be mediated by the physical (Webster and Benfield 1986b) and chemical (Ostrofsky 1993, 1997) properties of the leaves. Thus, leaf litter breakdown rates can mediate the availability of nutrients as well as the persistence of physical habitat structure. Since this important property of leaf litter may vary between tree species, the types and amounts of leaf litter falling into aquatic habitats can affect the ability of native animals to persist where forest composition is undergoing transition (Rubbo and Kiesecker 2004).

The potential for transitions in forest composition to affect litter dynamics has thus caused conservation biologist to pay particular attention to invasive tree species. One such species is Chinese tallow (*Triadica sebifera*), a tree from southeastern Asia that is invasive along the Gulf Coast of the North America (Bruce et al. 1995, Barrilleaux and Grace 2000). It invades wetland as well as terrestrial habitats, and may out-compete native trees due to its shade tolerance of shade (Jones and McLeod 1989), flooding (Jones and Sharitz 1990), and salinity (Conner 1994, Conner et al. 2001). In some cases, Chinese tallow forms novel forests where grassland once existed (Bruce et al. 1995) or homogenizes forests by establishing monoculture stands in the place of native trees (Smith et al. 1997, Conner et al. 2002). Invertebrate terrestrial

herbivores of Chinese tallow are few within novel habitats (Siemann and Rogers 2003b), and there is evidence that leaf litter from Chinese tallow affects the survival of terrestrial isopods in ponds in Texas, which died of starvation when fed Chinese tallow litter in the laboratory (Cameron and LaPoint 1978, Cameron and Spencer 1989). In our own work, we found that chorus frog and toad tadpoles reared in mesocosms with Chinese tallow leaves were less likely to survive and metamorphosed smaller than their counterparts reared in mesocosms with native types of leaf litter (Chapter 1). Thus, there is evidence that shifts in detrital composition and quality can affect the survival and performance of aquatic animals.

Here we used a series of litterbag experiments in a stream and temporary ponds to address the question of whether there is the potential for Chinese tallow invasion to affect aquatic animals in naturally occurring streams and temporary ponds via changes in litter processing dynamics. We test the hypothesis that Chinese tallow litter in water breaks down more rapidly than native species. Chinese tallow litter in terrestrial habitats breaks down rapidly (Cameron and Spencer 1989); we reasoned that similarly rapid breakdown of *T. sebifera* litter in aquatic habitats will occur in aquatic habitats. Based on evidence from the literature that leaves can provide animals with food resources (e.g., Moline and Poff 2008) and refugia (e.g., Walls 1995, Glos et al. 2007), we also predicted that the abundance of animals colonizing experimental litterbags would show an effect of litter type. Specifically, we predicted that animals assemblages recovered from Chinese tallow litterbags would be less abundant than assemblages in native type litterbags, given our observations of low amphibian survival in mesocosms treated with Chinese tallow litter (Chapter 1).

## Methods

### *Litter processing*

In 2002 we used green leaves from Chinese tallow and red maple (*Acer rubrum*) trees because senesced leaves were not available when we constructed the litterbags. We collected the leaves by hand from trees growing near the study site and returned them to the lab where we air-dried them to a constant mass and then used them to construct litterbags. We used commercially available mesh produce bags (21 in. plain red vexar bags, item #A-100140 from Packer's Supply Company, Ft. Pierce, Florida, USA). Each bag contained  $20 \pm 1$  g of leaves and was anchored in the thalweg of Abita Creek near the west gate of The Nature Conservancy's Abita Preserve near Abita Springs, St. Tammany Parish, Louisiana, USA. Abita Creek is a small blackwater stream with little to no tallow invasion along its banks except for points along its channel where there are road crossings or pasture adjacent to the stream. The most common trees in the riparian zone of this stream are bald cypress (*Taxodium distichum*), tupelo (*Nyssas spp.*), and sweetgum (*Liquidambar styraciflua*). On 25 July 2002 (day 0), we set 20 litterbags of each litter type (40 bags total) in the thalweg of the stream. Litterbags were placed in the stream in four blocks by stringing 10 litterbags of the same litter type together on nylon line (i.e., 4 strings). On each collection date, we haphazardly selected 2 litterbags from each string for collection. We planned to collect 4 litterbags of each treatment according to a geometric time scale on days 3, 7, 14, 28, and 56. However, a flood on days 13 and 14 of the experiment forced us to modify our sampling regime such that litterbags were removed from the stream on days 3, 7, 28, 35, and 56. After collection, litterbags were returned to the lab, rinsed, and dried to constant mass. In 2002 we did not correct for handling loss or ash litter samples. Invertebrates from each litterbag were retained using a 500- $\mu$ m sieve and placed in ethanol until they could be sorted and identified. We identified invertebrates to the family level, and all vertebrates were identified to species.

In 2003, we replicated the 2002 experiment using green Chinese tallow and red maple leaves as well as senescent (brown) Chinese tallow leaves. Green leaves were collected and dried in a manner consistent with the 2002 experiment; senescent leaves were collected from trees immediately prior to abscission (see Chapter 1 methods) in December 2002, air-dried to constant mass, and stored in paper bags in the laboratory. Initial mass of litterbags was  $35 \pm 2$  g (the large variation here is due to our use of whole leaves). On day 0, we retained 3 bags of each litter type in order to adjust our observations for handling loss. Bags were deployed into Abita Creek on 31 August 2003 and we collected 3 replicate bags of each treatment from the stream on days 1, 3, 8, 16, and 32. Thus, our analysis comprised a total of 54 litterbags, including the 9 held aside on day 0 and the 45 collected through the course of the experiment. Leaf litter was not ashed, and no animals were retained.

On 11 March 2005, we placed 45 litterbags ( $35 \pm 2$  g) containing laurel oak (*Quercus laurifolia*), Chinese tallow, or a 50% mix of oak and tallow leaves in each of 3 ephemeral ponds that had similar levels of tallow invasion (135 litterbags total). All leaves used in 2005 were senescent. The three ponds were within 0.5 km of each other on commercial pine plantations near Florenville, LA. Surrounding uplands were planted with slash pine (*Pinus elliottii*), but the pond basins were relatively undisturbed and supported tupelo (*Nyssa spp.*), laurel oak (*Q. laurifolia*), sweet gum (*Liquidambar styraciflua*), and Chinese tallow (*T. sebifera*). In addition to the 135 litterbags placed in ponds, we constructed 8 additional litterbags (3 tallow, 3 oak, and 2 mix) that we did not place in ponds but returned to the lab and weighed to estimate mass loss due to handling. We then collected the bags according to a geometric time scale on days 4, 8, 16, 32, and 64. Samples from oak and tallow litterbags were retained for ash-free dry mass (AFDM) determination; litterbags with mixed leaves were not ashed. In both years, we



identified and counted animals in litterbags still containing leaves when they were pulled from the Abita Creek. We retained animals from litterbags by rinsing the leaves in a 500- $\mu$ m sieve and preserving the animals in ethanol until they could be sorted and identified. In the stream ecology literature on litter breakdown, results of litterbag experiments are most often reported in terms of AFDM due to the confounding effects of sediment accumulation on samples as well as the nutrient- and energy-cycling emphasis of most studies (e.g., Benfield et al. 2001, Webster et al. 2001). Thus, we analyzed both ashed and un-ashed samples in 2005. The ashed samples provided an estimate of carbon content through time while the un-ashed samples provided an indication of habitat structure and persistence. Control bags were not ashed and are not included in our analysis of the effects of ashing.

### *Statistical Methods*

We analyzed each experiment separately using Statistica 7.1 (StatSoft, Tulsa, OK, [www.statsoft.com](http://www.statsoft.com)). The mass loss rates ( $k$ -values) of leaves were calculated by regressing the natural log of the proportion of mass remaining against time (Benfield 1996). The  $k$ -values reported herein are the negative slope of the regression line (Benfield 1996) calculated using the model parameters provided by the statistical software. We used ANCOVA and tested for heterogeneity of slopes (significant interaction of categorical and continuous predictor variables) to determine whether the slopes of the regression lines ( $k$ -values) for different leaf types were statistically distinguishable (Benfield 1996). In 2003 and 2005, when we used control bags to measure mass loss due to handling, we included the control bags in our analyses as Day 0 samples. In 2002, when control bags were not used, bags collected on Day 3 provided the first data points in the regressions, and any breakdown that occurred between Day 0 and Day 3 had no

influence on the calculated  $k$ -value. In 2005 we also used the heterogeneity of slopes model to test whether ashing the samples changed the slope of the mass loss regressions. This analysis was done both across all ponds and litter treatments and separately for each litter type in each pond. The effect of ashing was not significant (see Results section), so we report data for un-ashed samples only. This decision allowed us to report data consistently, as samples were not ashed in all years, and analysis of ashed samples would have required the omission of mixed-leaf litterbags since we could not have distinguished between the relative contributions of oak and tallow litters to the amount of carbon remaining in litterbags.

We examined the animal colonization of litterbags in 2002 and 2005. In both years, we identified and counted animals in litterbags still containing leaves when they were pulled from the Abita Creek. Litterbags not containing leaves were omitted from this portion of our analysis, and newly-hatched young still clinging to their parent (small crustaceans) were not counted in tallies of animal abundance. We described trends in the abundances of common groups and made note of rare groups. The large number of zeros in the data sets precluded most statistical analyses. However, by pooling the most common taxa (6 groups in 2002 and 4 groups in 2005) into as single variable, we were able to do two-way ANOVA for each year to examine the effects litter type and day on the abundance of the most common animals. We treated the litterbag as the unit of replication because litterbags were spaced about a meter from each other in the stream, far enough apart that we felt each pack could be treated independently. In 2002, two bags were missing (one from day 28 and one from day 32), and the day 56 samples were discarded because the tallow leaves were gone. Thus, 30 litterbags from 4 days were included in our analysis. In 2005, no bags were missing, and we discarded the day 56 samples. Thus, the 2005 analysis comprised 36 litterbags per pond (3 per treatment per sampling day over 4 dates). For these

analyses, count data were square root transformed to satisfy the assumptions of the statistical tests.

## Results

### *Litter processing rates*

All day 56 samples were discarded because the Chinese tallow leaf litter had completely broken down by then. The green Chinese tallow leaves had  $k=0.116$  and green (non-senescent) red maple leaves had  $k=0.008$ . Thus, the rate of breakdown was approximately 14.5 times, or 1 order of magnitude, greater for Chinese tallow than red maple (Fig. 3.1). The regression was significant for both maple ( $r^2=0.3440$ ,  $p=0.007$ ;  $y=-0.2421-0.008*x$ ) and tallow ( $r^2=0.9698$ ,  $p<0.001$ ;  $y=0.0090-0.116*x$ ). The day\*leaf type interaction term in the heterogeneity of slopes analysis was significant ( $F_{1,30}=337.55$ ,  $p<0.001$ , Figure 3.1).

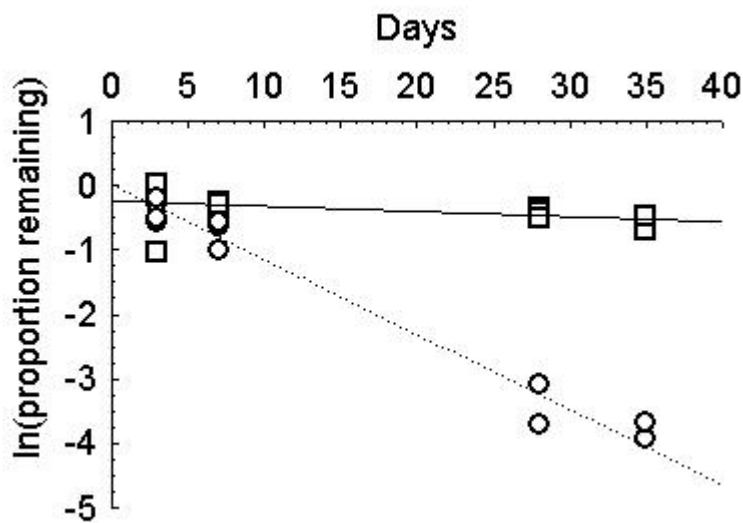


Figure 3.1. Mass loss plot for litterbags placed in Abita Creek in 2002. Open circles and small dashes represent fresh *Triadica sebifera* litter, and open squares and solid line represent fresh *Acer rubrum*. Slopes of the regression lines were significantly different (see Results section).

In 2003, the green tallow leaves broke down fastest ( $k=0.097$ ;  $r^2=0.58$ ,  $p<0.001$ ), the breakdown rate in senescent tallow leaves was intermediate ( $k=0.044$ ;  $r^2=0.84$ ,  $p<0.001$ ), and the green maple leaves were slowest ( $k=0.017$ ;  $r^2=0.78$ ,  $p<0.001$ ). Thus, green tallow leaves broke down about 5.7 times faster than green maple leaves and about 2.2 times faster than senescent tallow leaves. The slopes of the regression lines were significantly different (interaction of day\*litter type  $F_{2,47}= 11.77$ ,  $p<0.001$ , Figure 3.2).

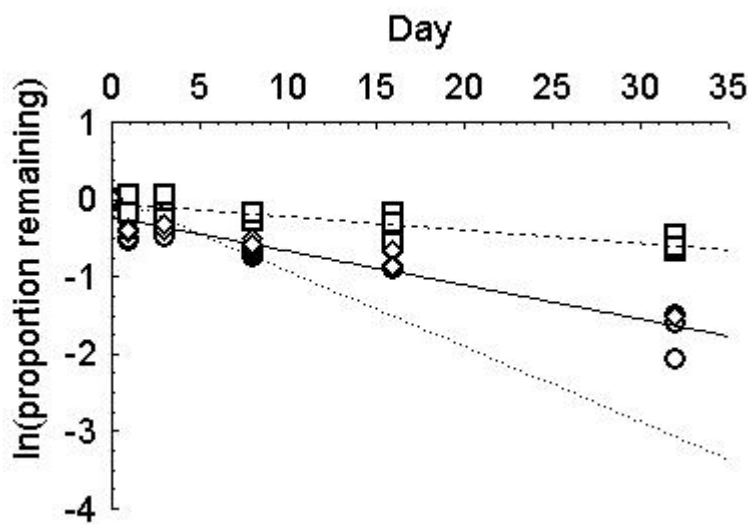


Figure 3.2. Mass loss plot for litterbags placed in Abita Creek in 2003. Open diamonds and small dashes represent fresh *Triadica sebifera* litter, open circles and solid line represent senescent *T. sebifera*, and open squares and large dashes represent fresh *Acer rubrum*. Slopes of the regression lines were significantly different (see Results section).

In 2005 we found that ashing our samples did not affect our estimates of breakdown rates (interaction of ashing\*day  $F_{1,137}=0.58$ ,  $p=0.448$ ). The  $k$ -value for all ashed samples was  $k=0.030$  and all un-ashed samples was  $k=0.040$ . Ashed and un-ashed  $k$ -values for oak were identical ( $k=0.002$ ), but the estimated breakdown rate  $k$  was 0.068 for ashed tallow samples and 0.083 in un-ashed tallow samples. Thus, we report only the data for un-ashed samples below.

We analyzed the litter breakdown rates in each pond separately. In all ponds, Chinese tallow litter broke down faster than mixed-leaf litter, which was faster than oak litter (Table 3.1).

Litter type had as significant effect on breakdown rates in each pond (Table 3.1). The most appropriate mean of a set of rates is the harmonic mean of the set (Chou 1975). Taking the harmonic mean of the  $k$ -values across ponds, Chinese tallow ( $k=0.066$ ) broke down 3.3 times faster than mixed-leaf litter ( $k=0.020$ ) and 16.5 times faster than oak litter ( $k=0.004$ ). Mixed-leaf litter broke down 5.0 times faster than oak litter. We calculated the harmonic mean of the  $k$ -values for the tallow and oak leaves in each pond for comparison to the observed mixed-leaf rates. The calculated mean was 0.008 for Amy's pond, 0.010 for Hyla Pond, and 0.006 for Sevosa Pond. These means of the oak and tallow  $k$ -values are slower than the observed values we report in Table 3.1. When we pooled our mass loss data across years and habitats, we found that mean mass loss rates for all treatments containing tallow litter (includes mixed-leaf treatments;  $k=0.080$ ;  $r^2=0.63$ ,  $p<0.001$ ,  $y=-0.0211-0.0802*x$ ) were 8.4 times faster than the mean mass loss rate all other leaf types ( $k=0.010$ ;  $r^2=0.26$ ,  $p<0.001$ ,  $y=-0.1243-0.0096*x$ ). Thus, our data indicate that Chinese tallow leaves have the effect of increasing mass loss rates of oak leaves.

Table 3.1. ANCOVA results showing the effect of litter type, day, and litter\*day interaction on amount of leaf litter remaining in litterbags from 3 ponds. All  $k$ -values were calculated from the model parameters.

Pond	Effect	SS	df	F	p	$k_{\text{tallow}}$	$k_{\text{oak}}$	$k_{\text{mix}}$
Amy's	Litter	0.85	2	2.54	0.092	0.139	0.004	0.024
	Day	15.89	1	95.05	0.000			
	Litter*Day	17.11	2	51.18	0.000			
	Error	6.19	37					
Hyla	Litter	0.11	2	2.74	0.078	0.038	0.006	0.016
	Day	2.26	1	115.36	0.000			
	Litter*Day	1.06	2	26.93	0.000			
	Error	0.75	38					
Sevosa	Litter	0.13	2	0.73	0.489	0.085	0.003	0.022
	Day	7.53	1	84.30	0.000			
	Litter*Day	7.13	2	39.92	0.000			
	Error	3.39	38					

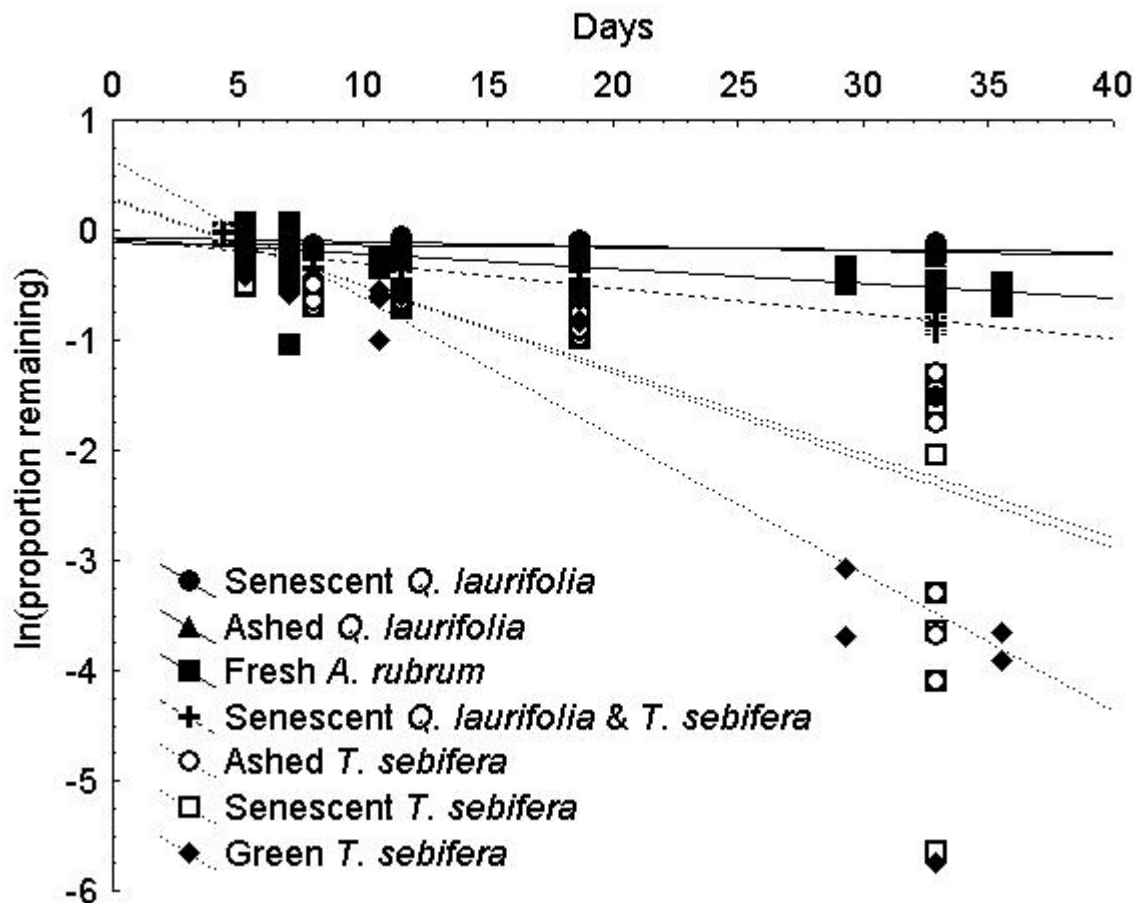


Figure 3.3. Plot of mass loss regressions for litterbags placed in ponds and streams between 2002 and 2005. Each point represents the natural log of the proportion of leaf material remaining in one litterbag on the day it was collected. Each line represents the mass loss rate for each treatment pooled across years (if a treatment was repeated) and across ponds in 2005. Slopes were not homogenous and all regressions were significant at  $\alpha < 0.01$  (see text).

#### *Animal Colonization of Litter Bags*

We identified 4,785 animals from 26 taxonomic groups in our 2002 samples from Abita Creek, with chironomids being the only animals found in every sample. Organisms representing 8 taxa (Table 3.2) were found only in maple litterbags; no organism was found only in tallow litterbags. Invertebrates from 6 families were represented in at least half of the litterbags for

each treatment. These groups, in order from most common to least common, were the Chironomidae (chironomid larvae), Hydropsychidae (caddisfly larvae), *Hyallela azteca* (amphipods), *Caecidotea spp.* (isopods), Heptageniidae (mayfly larvae), and Gyrinidae (gyrinid beetle larvae). The pooled abundance of the six most common taxa found in litterbags was higher in maple litterbags than tallow litterbags (ANOVA  $F_{1,22}=7.74$ ,  $p=0.011$ ; Fig. 3.3). In both litter types, the pooled abundances of the six most common taxa increased with time (effect of day;  $F_{3,22}=18.14$ ,  $p<0.001$ ; Fig. 3.3) although it appears that the abundance of these taxa in tallow litterbags began to decline by day 32 (Figure 3.3). This observation is weakly supported by the leaf\*day interaction, which was nearly significant ( $F_{3,22}=2.85$ ,  $p=0.061$ ).

Table 3.2. List of taxonomic groups observed only in *Acer rubrum* litterbags in 2002.

Order/ Family	Genus	Common name	Total individuals
Spongillidae		Sponge	4
Erpobgellidae	<i>Erpodella</i>	Leech	2
Ostracoda		Ostracod	4
Coenagrionidae	<i>Argia</i>	Dragonfly	1
Libellulidae	<i>Libellula</i>	Dragonfly	1
Cuculionidae		Beetle	3
Siluriformes	<i>Noturus</i>	Madtom catfish	2
Ranidae	<i>Rana</i>	Frog tadpole	3

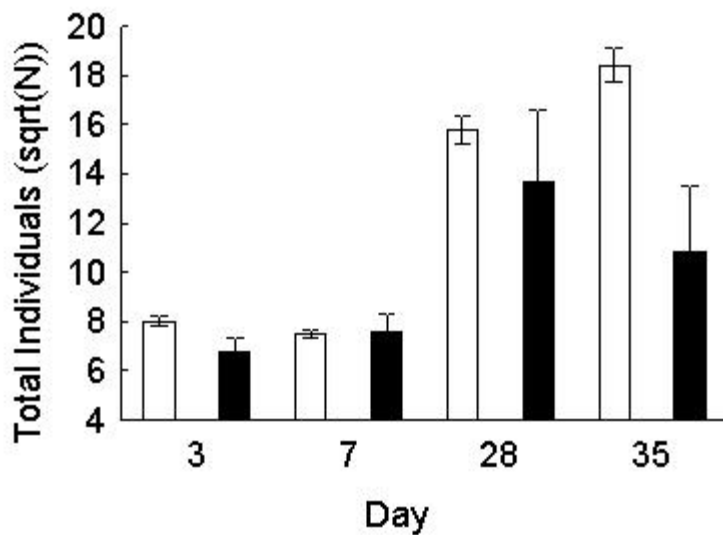


Figure 3.4. Bar plot showing the total number of individual animals from the 6 most common taxa in litter bags from Abita Creek in 2002. Light bars represent counts from *Acer rubrum* litterbags and dark bars represent counts from *Triadica sebifera* litterbags. Bars represent mean  $\pm$  1 SE.

In 2005, the litterbags in ponds hosted a less diverse assemblage that was characterized by four common animals: amphipods (*H. azteca*), isopods (*Caecidotea sp.*), crayfish (*Carambellus schmittii*), and midges (Chironomidae). Of 11,242 animals recovered from 144 litterbags, 9181 were isopods, 1004 were chironomids, 702 were amphipods, and 299 were crayfish. The remaining 56 animals were distributed among 20 taxonomic groups. To these 20 groups, Hyla Pond contributed 29 specimens from 13 taxa, Sevosa Pond contributed 18 invertebrates from 11 families, and Amy's Pond contributed 9 organisms from 4 groups. Over all ponds and days, 3168 animals from 14 taxa were in oak litterbags, 4137 animals from 13 taxa were in mixed-leaf treatments, and 3937 animals from 16 taxa were in tallow litterbags.



Table 3.3. Two-way ANOVA table for the effect of litter type and day on the pooled abundances of the four most common taxa collected from litterbags in ponds in 2005.

Pond	Effect	SS	df	F	p
Amy's Pond	Litter	28.34	2	2.24	0.128
	Day	385.26	3	20.33	<0.001
	Day*Litter	66.13	6	1.74	0.154
	Error	151.63	24		
Hyla Pond	Litter	136.57	2	4.89	0.017
	Day	259.52	3	6.20	0.003
	Day*Litter	39.52	6	0.47	0.822
	Error	334.98	24		
Sevosa Pond	Litter	14.70	2	1.38	0.270
	Day	120.85	3	7.58	0.001
	Day*Litter	70.19	6	2.20	0.078
	Error	127.46	24		

Among the less common organisms were the 7 *Ambystoma talpoideum* larvae found in litterbags from Amy's Pond (N=3) and Hyla Pond (N=4). We also found 2 *Bufo terrestris* tadpoles in litterbags from Hyla Pond. Despite the occasional vertebrate, the majority of animals collected in the litterbags in ponds were small crustaceans, many of which were gravid or carrying newly hatched young (not included in counts). Two-way ANOVA showed that there was no significant effect of litter type on the pooled abundance of the four most common taxa in Amy's or Sevosa ponds. However, litter did have a significant effect in Hyla Pond (Table 3.3), where abundances were largest in mixed litter treatments and lowest in oak treatments, with tallow having intermediate abundances. As expected, the effect of time was significant in all ponds, but there was no effect significant of the litter\*day interaction in any pond (Table 3.3). Through time, there was a general pattern of increasing abundance followed by decreasing abundance sometime after day 16 (Figure 3.4). Although the abundance of animals was lowest in litterbags from Sevosa Pond (2923 animals, Figure 3.4), 915 of the 1004 (91%) Chironomidae larvae collected in 2005 came from this pond. However, only 1161 (13%) of the isopods were

from Sevosa Pond. Of the remaining isopods, 5152 (56%) were from Hyla Pond and 2868 (31%) were from Amy's Pond (31%). Only one *H. azteca* was collected in Hyla Pond, but 208 were collected in Sevosa Pond and 493 in Amy's Pond. Crayfish were more evenly distributed among ponds, with 120 (40%) from Amy's Pond, 98 (33%) from Hyla Pond, and 81 (27%) from Sevosa Pond.

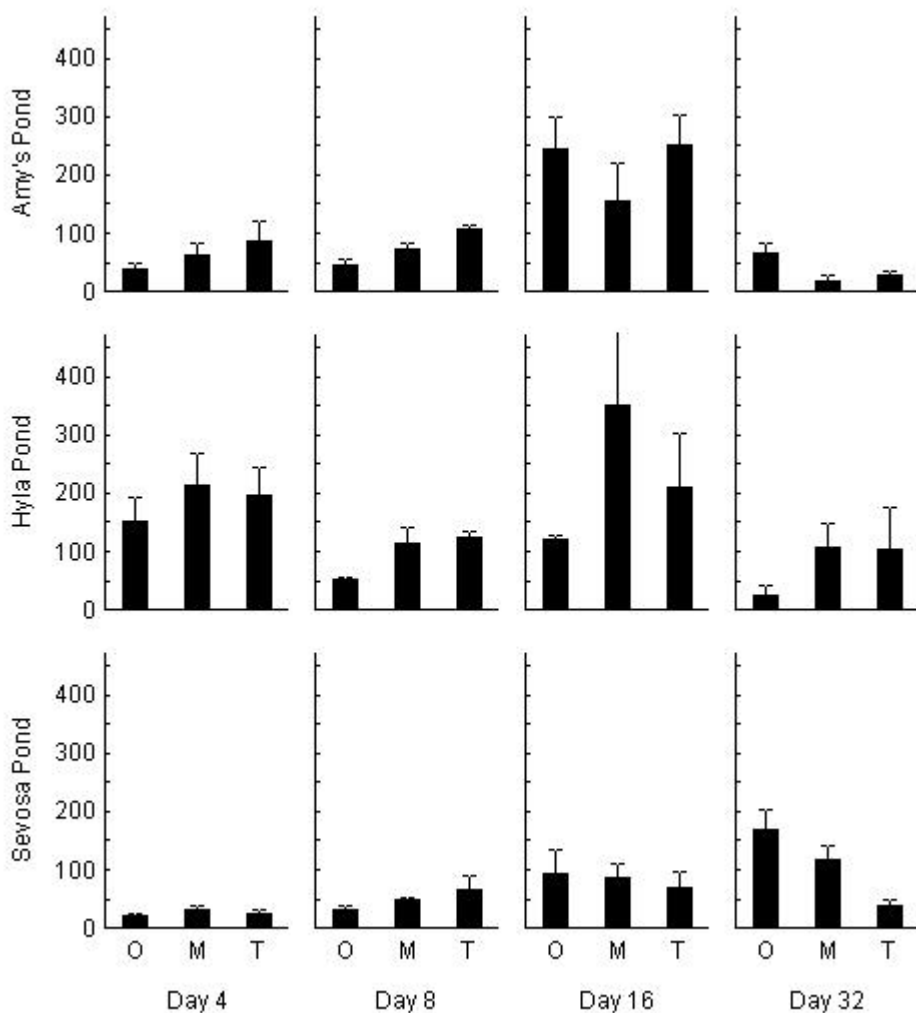


Figure 3.5. Plots of the total number of individuals in the four most common taxa in litterbags from ponds on 4 dates in 2005. Bars represent means of three litterbags  $\pm$  1 SE. The letters "O", "M", and "T" denote the oak, mixed, and tallow treatments, respectively.

## Discussion

Our data confirm our prediction that, in water, Chinese tallow litter breaks down more rapidly than the two native species tested. This observation corroborates observations of rapid mass loss by Chinese tallow litter in terrestrial habitats (Cameron and LaPoint 1978, Cameron and Spencer 1989). Rapid breakdown by Chinese tallow litter relative to native types of leaf litter in aquatic habitats may explain the differences in animal colonization of litterbags we report from 2002. Our observations of litterbags from streams confirm our prediction that animal assemblages in Chinese tallow litterbags would be less diverse and less abundant than assemblages in bags of native litter. We were surprised that abundances in the 2005 experiment in ponds did not show a stronger, more consistent effect of litter type. There are several possible explanations for this result, but the one we think is most likely is that we simply under-sampled the ponds, meaning that we lacked statistical power due to low samples sizes. The lack of a clear pattern in the 2005 animal data for two of the ponds may also be explained by the ubiquity of small crustaceans that are generalist shredders, which we would not expect to exhibit strong preferences for one litter type over another. Hyla pond, which is the only pond where the litter effect was significant (see Results, Table 3.3), was the only pond without large numbers of *H. azteca* (only 1 individual of this species was caught across all days in all bags).

Our data also indicate that there is considerable variation in the  $k$ -values for Chinese tallow. This variation was affected by whether the leaves were green or senescent and whether they were placed in a stream or a pond. The most rapid rate we report was measured for fresh (green) tallow leaves in a stream in the summer of 2002 while the slowest rate we reported for tallow leaves is from litterbags of senescent material placed in ponds during spring 2005. It makes sense that the most rapid break down was observed in a stream because the continual rinsing by the current and the abrasive action of moving sediment are likely to accelerate

mechanical decomposition of the litter. Although ponds lack current, the presence of small vertebrates and high density of shredding crustaceans may possibly result in mechanical agitation of the litterbags. Breakdown of leaf material in aquatic ecosystems can be influenced by numerous factors including but not limited to mechanical action of currents, mechanical actions of grazing and scraping animals, water temperature, dissolved oxygen, and enzymes from microbes and fungi (Webster and Benfield 1986a, Webster et al. 1999). Consequently,  $k$ -values such as those reported here may vary considerably for litter from a given species of tree, and the environmental context of studies reporting such values must be considered. For instance, the  $k$ -values we report are faster than those reported by Cameron and LaPoint (1978) who found that tallow leaves in ponds in Texas broke down at rates between 0.89% and 1.1% per day. The discrepancy between previously reported decay rates and those we report is probably explained by the time of year when the studies were done. Seasonal temperature differences (Garden and Davies 1988) or seasonal differences in species assemblages (Inkley et al. 2008) between the studies could explain the differences in reported decay rates.

Our observations of mixed-leaf litterbags support the notion that the dynamics of litter processing change in non-additive ways as leaf litter diversity changes (Swan and Palmer 2004, Taylor et al. 2007). The mass loss rate of oak litter was accelerated when mixed with Chinese tallow litter. The implications of non-additive effects of tallow leaf litter on system-level litter processing rates are far-reaching because they imply more rapid litter breakdown in addition to changes in litter quantity and quality. For some species, the increased cycling of nutrients resulting from increased decay rates will translate into increased food availability. For others, however, the rapid disappearance of litter from systems has the potential to reduce the amount of

substrate available for colonization. This is demonstrated by the absence of some organisms such as sponges and from tallow litterbags in 2002.

The data we present on the colonization of litterbags provide further insight into the consequences of increased litter processing rates subsequent to Chinese tallow invasion. The assemblages we described from our litterbags in both 2002 and 2005 were dominated by shredder organisms that live, feed, and reproduce in allochthonous plant detritus. We described crustacean-rich systems with abundant isopods, amphipods, and crayfish accompanied by a diverse but less abundant suite of insect larvae, many of which also live in or on detritus. Some of the chironomids we found were leaf-boring species that, judging from their large size, found Chinese tallow leaves palatable, but were also exposed to the open water before they could complete their larval period. The tallow leaves simply disappeared too quickly.

The disappearance of the tallow by day 32 highlights the impact of increased litter processing rates on litter-dwelling fauna. When the tallow was gone, only the more recalcitrant types of leaf litter were available for refugia or as a nutrient source. This sort of change in the timing of resource availability constitutes a profound change in habitat quality for animals in affected habitats.

Common criticisms of the litterbag technique, primarily that the methods suppresses the abundance of epiphytic algae (Schnitzer and Neely 2000), are less of a concern in our study system than they might be in some other systems. We did not measure algal growth on the litterbags, but it grew abundantly in all ponds included in this study, as evidenced by the presence of algae growing on collected bags. Instead of being concerned that algae were suppressed, we had the opposite concern. We were concerned that the litterbags might provide substrate for the growth of algae and, along with the leaf material and animals already in the

bags, might have the effect of attracting animals that do not normally utilize litter substrata. The abundance of shredder organisms in our litterbags suggests, however, that attraction of non-detritivores was not a problem.

Our data provide show that invasion by Chinese tallow will influence detrital dynamics in aquatic habitats. Litter from tallow trees breaks down rapidly relative to native tree litter, and we provide some evidence suggesting that differences in litter processing rates influence the structure of aquatic faunal communities. Although some generalist shredders (e.g., *Caecidotea* and *Hyaella*) that are already abundant in the systems we studied did not exhibit strong responses to Chinese tallow litter, the absence of some organisms from litterbags deployed in Abita Creek suggests that some organisms are indeed sensitive to litter type.

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## Appendix A – Institutional Animal Care and Use Committee

### University of New Orleans

Institutional Animal Care and Use Committee (IACUC)

**DATE:** April 26, 2001  
**TO:** Joseph H.K. Pechmann, Ph.D.  
**FROM:** Gerald J. LaHoste, Ph.D.   
Chairman  
**RE:** *IACUC Protocol No. 027*  
*Entitled: Complex Life Cycles and Amphibian Conservation*

Your revised application for the use of animals in research (referenced above) has been received and found to be acceptable. The approval date is May 2, 2001 and the expiration date is May 2, 2004. Annual renewal forms (which do not require re-submission or re-review) will be sent to you at yearly intervals until the expiration date.

## Appendix B – Coauthor release letter from Andr  a Forsyth

5341 Carlisle Ct  
New Orleans, LA 70131

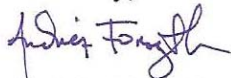
April 2, 2007

Dr. Robert Cashner  
**Department of Biological Sciences**  
Computer Center, Room 200  
2000 Lakeshore Drive  
University of New Orleans  
New Orleans, LA 70148

Dear Dr. Cashner,

I am writing to inform you of my consent for my undergraduate research to be included in Norman Leonard's dissertation. This research took place in 2004 and focuses on the effects of dissolved oxygen on tadpole behavior.

Sincerely,



Andr  a Forsyth

CC: Norman Leonard

## Vita

Norman Eugene Leonard was born in Cleveland, Ohio on 08 May 1973 to Anna L. and Kenneth E. Leonard. He had one sister, Stephanie Marie, born on 02 July 1975 at Mt. Kisco, New York.

Norman graduated from Twin Valley High School in Elverson, Pennsylvania in June of 1991, and attended several undergraduate institutions before earning his Bachelor's of Science degree in Ecology from the University of Georgia in December 1998. His mentors at UGA included Drs. Eugene Odum, Frank Golley, and Judy Meyer.

Norman was married to Heidi Renée Shoup on 19 May 2001 in Lawrenceville, Georgia and divorced 30 May 2007. While married, they lived together in New Orleans, and had one daughter, Jessica Marie, born 25 November 2001 at University Hospital in New Orleans.

On 29 August 2005, Hurricane Katrina made landfall at New Orleans, 2 days after Norman evacuated the city. Following his departure from New Orleans, he re-established residence in Athens, Georgia among his friends at his *alma mater*. There he completed the research he had begun in New Orleans and wrote this dissertation.