Leaf litter of invasive Chinese tallow (*Triadica sebifera*) negatively affects hatching success of an aquatic breeding anuran, the Southern Leopard Frog (*Lithobates sphenocephalus*)

C.K. Adams and D. Saenz

Abstract: Chinese tallow (*Triadica sebifera* (L.) Small) is an aggressive invasive tree species that can be abundant in parts of its non-native range. This tree species has the capability of producing monocultures, by outcompeting native trees, which can be in or near wetlands that are utilized by breeding amphibians. Existing research suggests that leaf litter from invasive Chinese tallow reduces survival in larval anurans. The purpose of this study was to determine the effects of Chinese tallow leaf litter on anuran eggs. We exposed eggs of the Southern Leopard Frog (*Lithobates sphenocephalus* (Cope, 1886)) at various stages of development to different concentrations of Chinese tallow leaf litter to determine survival. Eggs in the earliest stages of development that we exposed to tallow leaf litter died, regardless of concentration; however, some more-developed eggs exposed to tallow leaf litter did hatch. We determined that the greater the concentration of tallow leaf litter, the lower the dissolved oxygen and pH levels we observed. We suggest that changes in these water-quality parameters are the cause of the observed mortality of anuran eggs in our experiments. Eggs exposed to water containing tallow leaf litter with dissolved oxygen <1.59 mg/L and a pH <5.29 did not survive to hatching.

Key words: Southern Leopard Frog, *Lithobates sphenocephalus*, Chinese tallow, *Triadica sebifera*, invasive species, dissolved oxygen, pH, egg hatching.

Résumé : Le suif végétal de Chine (*Triadica sebifera* (L.) Small) est une essence d'arbre envahissante agressive qui peut localement être abondante hors de son aire de répartition naturelle. Cette essence est capable de produire des monocultures en délogeant des essences indigènes pouvant se trouver dans des zones humides ou à proximité et que des amphibiens utilisent pour la reproduction. Les travaux existants suggèrent qu'une couverture de feuilles mortes de suif végétal de Chine réduit la survie de larves d'anoures. Le but de l'étude consiste à déterminer les effets des feuilles mortes de suif végétal sur les œufs d'anoures. Nous avons exposé des œufs de grenouille léopard du Sud (*Lithobates sphenocephalus* (Cope, 1886)) à différents stades de leur développement à différentes concentrations de feuilles de suif végétal dans la couverture de feuilles mortes afin de déterminer leur taux de survie. Les œufs exposés aux stades les plus précoces de leur développement à des feuilles mortes de suif végétal sont morts, peu importe la concentration de ces dernières, alors que certains œufs à des stades plus avancés ont éclos. Nous avons déterminé que plus la concentration de feuilles mortes de suif végétal dans la couverture était grande, plus les teneurs d'oxygène dissous et le pH étaient faibles. Nous suggérons que les modifications de ces paramètres de qualité de l'eau sont à l'origine de la mortalité observée parmi les œufs d'anoures dans nos expériences. Les œufs exposés à de l'eau contenant des feuilles mortes de suif végétal et présentant des teneurs d'oxygène dissous <1,59 mg/L et un pH <5,29 n'ont pas survécu jusqu'à l'éclosion.

Mots-clés : grenouille léopard du Sud, *Lithobates sphenocephalus*, suif végétal de Chine, *Triadica sebifera*, espèce envahissante, oxygène dissous, pH, éclosion d'œufs.

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Introduction

The negative impacts of invasive species range from direct predation on native organisms to habitat alterations that subsequently affect native communities. The impacts of introduced predators can be obvious because predation affects native organisms directly (Moyle 1973; Bradford 1989; Rodda and Fritts 1992; Gamradt and Kats 1996; Wiles et al. 2003). Conversely, the impacts of invasive plants may be less obvious, but their impacts are beginning to receive more consideration.

Exotic invasive plants are known to have negative impacts on some animal populations (Blossey 1999; Schmidt and Whelan 1999; Herrera and Dudley 2003; Borgmann and Rodewald 2004). In some cases, these exotic plant invasions can affect native amphibians (Maerz et al. 2005*a*, 2005*b*; Brown et al. 2006; Watling et al. 2011*a*). Watling et al. (2011*a*) found that soil surrounding and leaf litter from Amur honey-

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suckle (*Lonicera maackii* (Rupr.) Herder) decreased the time to metamorphosis of tadpoles of the American Toad (*Anaxyrus americanus* (Holbrook, 1836)) compared with a native soil and leaf litter. Japanese knotweed (*Fallopia japonica* (Houtt.) Ronse Decr.) is known to indirectly reduce terrestrial foraging habitat quality of adult amphibians by reducing arthropod abundance (Maerz et al. 2005*a*), while the toxic tannins found in Eurasian purple loosestrife (*Lythrum salicaria* L.) cause a direct negative effect on the growth and survival of American Toads and alter food availability (Maerz et al. 2005*b*; Brown et al. 2006).

Another invasive plant that has been shown to negatively affect native organisms is the Chinese tallow (*Triadica sebifera* (L.) Small) (Leonard 2008; Cotten et al. 2012). Chinese tallow (henceforth simply tallow) was first introduced in the United States in the late 1700s (Bell 1966). It now occurs mostly in the eastern United States, from Texas to Florida to North Carolina, and also California (Renne et al. 2000; Hrusa et al. 2002). In addition to rapid range expansion, the abundance of tallow has continued to increase in areas where it occurs. Oswalt (2010) reported alarming increases in tallow abundance from the early 1990s to the late 2000s in eastern Texas, Louisiana, and Mississippi. Oswalt (2010) suggests that tallow has increased 174% from 1992 to 2007 in east Texas, more than 500% from 1991 to 2005 in Louisiana, and 445% from 1994 to 2006 in Mississippi; it is now the fifth most common tree species in east Texas and Louisiana.

Tallow exhibits many characteristics that make it a good invader, such as rapid growth, high fecundity, maturity at a young age, the ability to grow in a wide range of soil types, effective seed dispersal mechanisms, and long-term survival in the seed bank (Scheld and Cowles 1981; Cameron and Spencer 1989; Jubinsky and Anderson 1996; Bruce et al. 1997; Cameron et al. 2000; Renne et al. 2000). In the southeastern United States, tallow can aggressively displace native plants and form monoculture stands (Jubinsky and Anderson 1996; Bruce et al. 1997; Siemann and Rogers 2003). Although tallow can grow in virtually every habitat type, it is often found in wetter areas (Siemann and Rogers 2003). As a result, tallow is often found in areas used by breeding amphibians.

Tallow leaves readily leach tannins and phenolics; in addition, leaves decompose much more quickly than other deciduous leaves in terrestrial and aquatic environments (Cameron and Spencer 1989; Leonard 2008). Maerz et al. (2005*b*) hypothesized that tannins from leaves of Eurasian purple loosestrife were the direct cause of differential survival of tadpoles of American Toads when compared with tadpoles of American Toads exposed to leaves of the broadleaf cattail (*Typha latifolia* L.). However, Maerz et al. (2005*b*) did note that dissolved oxygen (DO) may have played a role in the differential survival of tadpoles of American Toads. Chergui et al. (1997) suggested that the toxic effects of tannins could be due to a reduction in DO. The process of leaf decomposition, including leaching and microbial decomposition, can reduce DO (Tremolieres 1988; Weyers and Suberkropp 1996; Chergui et al. 1997; Wright and Covich 2005; Canhoto and Laranjeira 2007).

The impact that tallow has on breeding amphibians is not well understood; however, some recent work by Leonard (2008) and Cotten et al. (2012) has provided reason for concern. Leonard (2008) and Cotten et al. (2012) found that tallow leaf litter negatively affected tadpole survival when compared with leaf litter of native species, although the effect differed among anuran species. Leonard (2008) also found that leaf litter significantly affected many water-quality parameters, especially DO which tended to be lower in tallow treatments compared with other species of leaf litter. Leonard (2008) attributed this phenomenon to a variety of factors including bacterial and fungal production, which could create high biological oxygen demand (Weyers and Suberkropp 1996; Wright and Covich 2005).

Although previous studies have documented the potential negative impacts of invasive species on larval amphibians, to our knowledge, no work has been conducted on the effects of invasive species on amphibian eggs. Amphibian eggs are immobile and could be considered one of the most vulnerable life stages of amphibian development. Amphibian egg hatching success can be affected by pH and available oxygen (Gosner and Black 1957; Adolph 1979; Schlichter 1981; Pierce 1985; Seymour and Bradford 1995). This raises a profound concern that tallow leaves, which are known to reduce DO, might adversely affect anuran development in the egg or larval stage.

In this study, our objective was to determine the impacts of tallow leaf litter on amphibian egg hatching. Specifically, we wanted to reveal the effects of tallow leaf-litter concentration on water-quality, hatching success of amphibian eggs exposed to tallow leaf litter, and the effects of tallow leaf litter on timing of amphibian egg hatching. Also, we wanted to investigate the mechanisms by which tallow leaf litter may affect hatching success.

Materials and methods

We used the Southern Leopard Frog (*Lithobates sphenocephalus* (Cope, 1886)) as our study organism to determine the potential effects of tallow leaf litter on amphibian egg hatching. The Southern Leopard Frog is a common species in eastern Texas and breeds in a variety of aquatic habitats ranging from ephemeral to permanent. They lay large egg masses ranging from a few hundred to several thousand per clump. The rate of egg development can vary depending on temperature but can take as long as 10 days (Saenz et al. 2003). This species is capable of breeding any month of the year in eastern Texas (Saenz et al. 2006). Also, the Southern Leopard Frog and tallow co-occur over much of their respective ranges (Conant and Collins 1998; Oswalt 2010).

Tallow leaves were collected from 4 November to 4 December 2009 from multiple trees located on the campus of Stephen F. Austin State University in Nacogdoches, Texas. Leaves were collected by stripping loose leaves from low hanging branches just prior to abscission. Leaves were air dried in large plastic containers (1.2 m diameter × 0.3 m deep) indoors and then stored in black plastic bags in a dark room.

We investigated the effects of different concentrations of tallow leaf litter (0, 1, 2, and 4 g/L) on the hatching success of eggs of the Southern Leopard Frog in two different experiments. We chose our leaf-litter concentrations based on Leonard (2008), who found a mean standing stock of 537 g/m^2 of leaf litter in natural ponds. This translates to approximately 1.27 g of leaf litter/L of water. We suggest that tallow leaf litter can create lower or much higher concentrations in nature because it can produce dense monocultures in and around wetlands; therefore, we included a range of concentrations below and above Leonard's (2008) estimate for this study.

Tallow leaf-litter concentration experiment

We compared the effects of four concentrations of tallow leaf litter (0, 1, 2, and 4 g/L) on the hatching success of eggs of the Southern Leopard Frog (15 egg masses of Gosner stages 1–9) (Gosner 1960) and water chemistry. Tallow leaf litter was placed in 13 L white plastic buckets (30 cm diameter × 28 cm deep; $n = 60$) with 4 L of aged tap water. A plastic container containing 20 eggs of the Southern Leopard Frog was placed in each bucket. Containers (14 cm × 14 cm × 9 cm) had mesh-covered holes to allow water flow between the container and bucket. Each treatment was replicated 15 times using the 15 egg masses collected on 20 January 2010; therefore, each replicate was blocked by egg mass. Within each block, eggs were randomly assigned a treatment.

To determine hatching success, the eggs were observed every 12 h until hatching had initiated. Once hatching had begun, eggs were observed every 1–8 h and the number of hatchlings recorded. We considered an egg mass hatched once half (10 eggs) of the eggs hatched (Laurila et al. 2001). Once an egg mass hatched, the hatchlings and remaining eggs were euthanized using MS222 and then preserved in formalin. If an egg mass appeared to stop developing, the egg mass was removed from the bucket and Gosner (1960) stage determined, then returned to the bucket. Twelve hours later, the same egg mass was removed and Gosner (1960) stage was again determined to ensure that the egg mass was no longer developing. At this point, the eggs were considered dead and preserved in formalin.

We used a Hach Hydrolab Quanta® to measure DO (mg/L), pH, and turbidity (NTU, nephelometric turbidity units) on 4 of 15 replicates every 12 h until all egg masses had hatched or stopped developing. We continued to collect water chemistry every 24 h for an additional 3 days and then once more 7 days later. We were not able to collect water chemistry measurements on all 15 replicates because of time constraints involved with taking water chemistry measurements. As a result, we randomly selected the four replicates used. We calculated a mean value for each water chemistry variable.

Tallow leaf-litter concentration and aeration experiment

We compared the effects of four concentrations of tallow leaf litter (0, 1, 2, and 4 g/L) and two levels of aeration (aerated or nonaerated) on water chemistry and hatching success of eggs of the Southern Leopard Frog. We used a standard aquaculture air compressor, air hose, and air stones to supply air to each aeration treatment. One air stone was placed in each bucket in aeration treatments and air flow was set to saturate the water with oxygen.

For this experiment, we used the 6 egg masses (Gosner stages 10–15) (Gosner 1960) collected on 22 January 2010. Eggs (10 per bucket) and replicates were maintained in 13 L white plastic buckets (30 cm diameter × 28 cm deep; $n = 48$) maintained and blocked as previously described. Hatching success and water-quality variables were measured as in the previous experiment. DO, pH, and turbidity were collected on each replicate at the beginning of the experiment when eggs were placed in treatments and again on each replicate once that replicate had hatched or the eggs were considered dead. We calculated a mean value for each water-quality variable for each replicate.

All research followed 2004 *Guidelines for Use of Live Amphibians and Reptiles in Field and Laboratory Research* (American Society of Ichthyologist and herpetologists, The Herpetologists' League, and the Society for the Study of Amphibians and Reptiles).

Data analysis

Because egg masses did not hatch in all leaf-litter concentration and aeration combinations, we considered each combination a separate treatment level and did not test for interaction between leaf-litter concentration and aeration. We used a randomized incomplete block analysis of variance (egg masses random blocks and tallow leaf-litter concentration and aeration as fixed treatment) to determine if treatment affected hours to hatching, followed by a Tukey–Kramer's test on the least squares means (lsmeans) ($P < 0.05$) to separate treatments. We used a one-way analysis of variance (ANOVA) to determine if water quality differed among treatments ($P < 0.05$), followed by a Tukey–Kramer's test to separate treatments.

Results

Tallow leaf-litter concentration

DO (mg/L) and pH were significantly different among tallow leaf-litter concentration treatments (ANOVA; $F_{[3,12]} = 3080$, $P < 0.0001$; $F_{[3,12]} = 107$, $P < 0.0001$, respectively). Treatments with higher concentrations of tallow leaf litter had significantly lower DO and pH (Table 1). Turbidity was also significantly different among tallow leaf-litter concentration treatments ($F_{[3,12]} = 13.43$, $P = 0.0004$). Treatments with higher concentrations of tallow leaf litter had significantly higher turbidity (Table 1). The effect of tallow leaf litter on DO, pH, and turbidity changed depending on the length of time the leaf litter had been in the water (Figs. 1*a*–1*c*). DO and pH both decreased initially and then increased towards the end of the study. The concentration did not seem to affect how low the DO went; however, the amount of time the DO remained low appeared dependent on concentration (Fig. 1*a*). The higher the tallow leaf-litter concentration, the lower the pH reached and the greater amount of time the pH remained low (Fig. 1*b*). The higher the tallow leaf concentration, the higher the turbidity was reached and the greater amount of time the turbidity remained high (Fig. 1*c*).

No egg masses exposed to any concentration of tallow leaf litter survived. Only eggs without leaf litter survived and the mean time to hatching was 104 h.

Tallow leaf-litter concentration and aeration

As in the previous experiment, we found a significant difference in mean DO, mean pH, and mean turbidity among treatments (ANOVA; $F_{[7,40]} = 2664.71$, $P < 0.0001$; $F_{[7,40]} = 91.39$, $P < 0.0001$; and $F_{[7,40]} = 30.73$, $P < 0.0001$, respec-

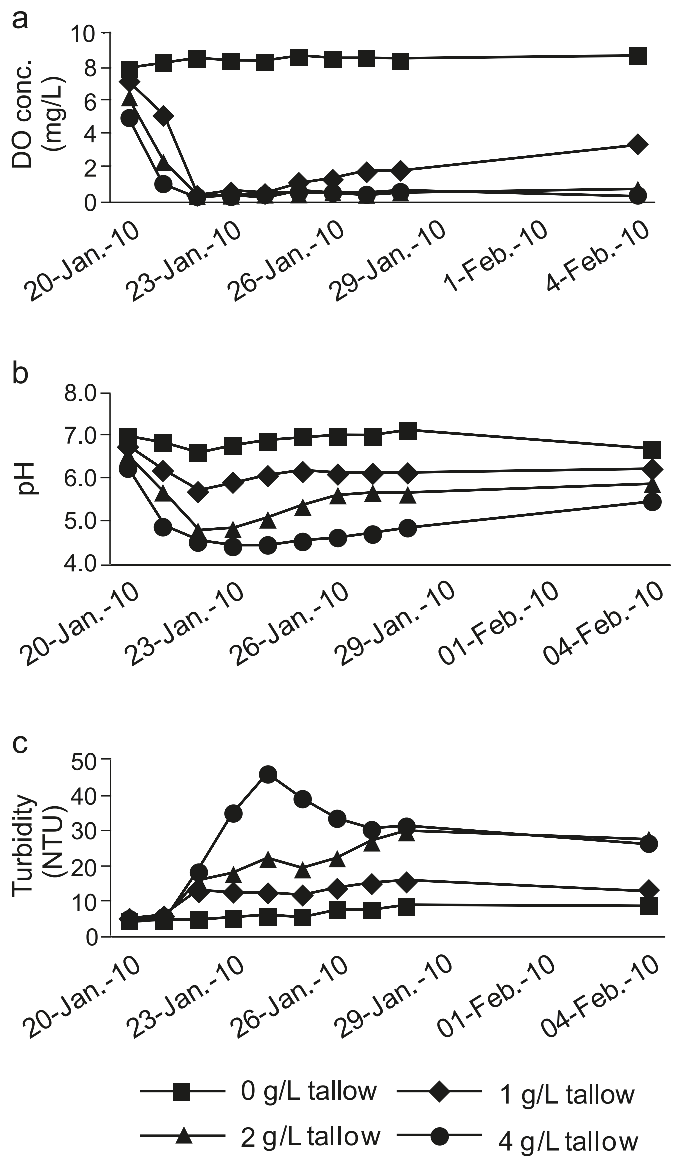
Table 1. Dissolved oxygen (DO; mg/L), pH, and turbidity (NTU) across four concentrations (0, 1, 2, and 4 g/L) of leaf litter of the Chinese tallow (*Triadica sebifera*) (n = 4 per treatment).

	DO		pH		Turbidity	
Treatment	Mean	SE	Mean	SE	Mean	SE
0	8.38 A	0.11	6.84 A	0.15	5.8 A	0.9
1	2.04 B	0.04	6.07 B	0.03	11.4 AB	0.5
2	1.11 C	0.04	5.36 C	0.17	20.9 BC	4.5
4	0.79 D	0.04	4.77 D	0.06	26.8 C	2.3

Note: Different letters indicate significant differences among the four leaf-litter treatments (P < 0.05).

tively). Treatments with higher concentrations of tallow had significantly lower DO, significantly more acidic pH values, and significantly higher turbidity (Table 2). Treatments that contained leaf litter and did not have aeration had significantly lower DO than other treatments (Table 2). All egg masses without tallow leaf litter hatched regardless of aeration (Table 3). Four egg masses (66%) hatched in buckets that contained 1 g/L of tallow leaf litter with no aeration, while all six egg masses (100%) hatched in buckets containing 1 g/L of tallow leaf litter with aeration. No egg masses survived to hatching in nonaerated treatments containing 2 or 4 g/L of tallow leaf litter. Five egg masses (83%) hatched in aerated treatments containing 2 g/L of tallow leaf litter and no egg masses survived to hatching in aerated treatments containing 4 g/L of tallow leaf litter. Differences in DO and pH may have contributed to differential hatching success. Eggs exposed to water with DO <1.59 mg/L or a pH <5.29 did not survive (Figs. 2*a*, 2*b*). We found significant differences in time to hatching among treatments ($F_{[9,17]}$ = 16.09, P < 0.0001). Both tallow leaf-litter concentration and aeration affected the time to hatching (Table 3). Aeration delayed hatching, while an increase in tallow leaf-litter concentration accelerated hatching.

Fig. 1. Effects of concentration of leaf litter of the Chinese tallow (*Triadica sebifera*) and time on water quality. Dissolved oxygen (DO) (*a*), pH (*b*), and turbidity (*c*) represent means (n = 4 per treatment per date).



Discussion

Tallow affected the hatching success of the Southern Leopard Frog in both of our laboratory experiments. Our results indicate that even at low concentrations, tallow leaf litter can be lethal to eggs of the Southern Leopard Frog. We observed reduced survival in treatments containing only 1 g/L of tallow leaf litter. Tallow leaf litter caused significant changes in water quality. Treatments with higher concentrations of tallow leaf litter had lower DO and pH and higher turbidity. The reduced hatching success that we observed could be due to these changes in water quality caused by tallow leaf litter. Amphibian eggs require oxygen from the environment to survive and can be sensitive to changes in pH (Gosner and Black 1957; Adolph 1979; Pierce 1985; Seymour and Bradford 1995). We observed that both DO and tallow leaf-litter concentration affected time to hatching. Changes in the timing of the transition from the egg to larval stage are common among anurans. These changes can occur in the presence of predator cues (Warkentin 1995, 2000; Schalk et al. 2002; Johnson et al. 2003; Saenz et al. 2003) and can also be a result of hypoxia (Bradford and Seymour 1988; Mills and Barnhart 1999; Seymour et al. 2000; Warkentin 2002). The impacts of tallow leaf litter on DO likely contributed to differential hatching times. Warkentin (1995) found that early hatching led to higher vulnerability of less-developed tadpoles to aquatic predators. More research is needed to understand the potential cost of early hatching caused by tallow.

No egg masses exposed to 1 g/L of tallow leaf litter hatched in the initial experiment, but 67% of similarly exposed egg masses hatched in the second experiment. This is likely due to the different developmental stages of the egg masses. Egg masses used in the first experiment were less developed (Gosner stage 1–9) than those in the second experiment (Gosner stage 10–15), initially. As a result, eggs in the first experiment were exposed to tallow leaf litter for a longer period of time than those in the second experiment. Another potential cause of differential survival across experiments is that eggs may be more sensitive to changes in oxygen early in development.

Table 2. Dissolved oxygen (DO; mg/L), pH, and turbidity (NTU) across four concentrations (0, 1, 2, and 4 g/L) of leaf litter of the Chinese tallow (*Triadica sebifera*) and aeration treatments (n = 6 per treatment).

	DO		pH		Turbidity	
Treatment	Mean	SE	Mean	SE	Mean	SE
Nonaerated						
0	8.52 A	0.02	7.00 A	0.07	7.0 A	0.5
1	1.50 D	0.15	6.15 BC	0.03	14.9 AB	1.9
2	0.47 E	0.04	5.34 D	0.07	23.0 B	2.1
4	0.43 E	0.04	4.62 E	0.05	37.9 C	3.0
Aerated						
0	8.68 A	0.03	6.96 A	0.10	7.0 A	0.3
1	8.43 AB	0.04	6.58 AB	0.08	14.2 AB	1.0
2	8.16 BC	0.06	5.72 CD	0.18	20.8 B	1.6
4	7.86 C	0.12	4.80 E	0.12	34.7 C	3.8

Note: Different letters indicate significant differences among the eight treatments ($P < 0.05$).

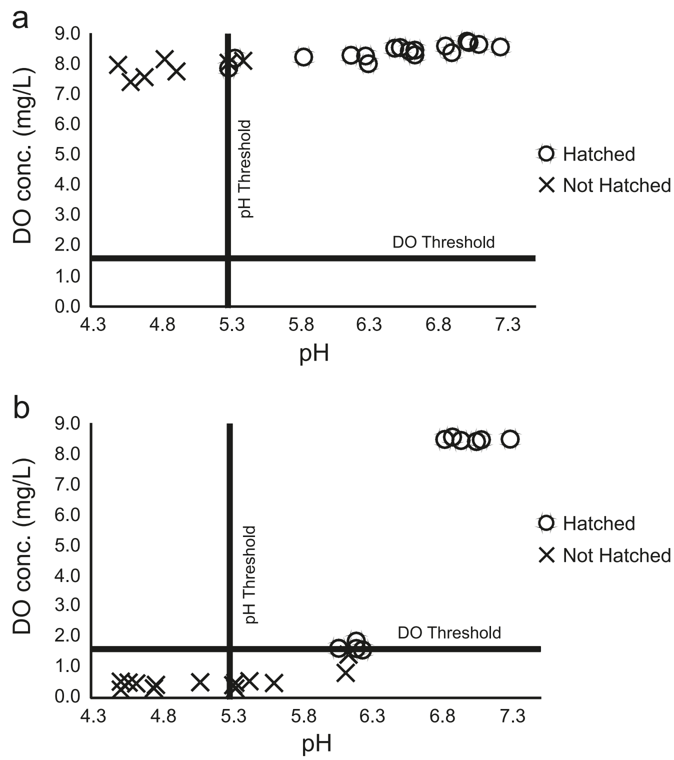
Table 3. Mean number of hours required for half of the eggs of the Southern Leopard Frog (*Lithobates sphenocephalus*) (n = 6 per treatment) to hatch while exposed to four concentrations (0, 1, 2, and 4 g/L) of leaf litter of the Chinese tallow (*Triadica sebifera*) and aeration treatments.

Treatment	Number hatched	Hours to hatching	SE
Nonaerated			
0	6	63.8 B	4.4
1	4	51.2 C	3.2
2	0	—	—
4	0	—	—
Aerated			
0	6	75.5 A	3.5
1	6	61.5 B	2.9
2	5	57.6 BC	4.8
4	0	—	—

Note: Dashes indicate no data because eggs within that treatment did not hatch. Different letters indicate significant differences among the eight treatments ($P < 0.05$).

DO, pH, and turbidity were significantly different among treatments and thus these water-quality variables may be the cause of differential survival that we observed. Both DO and pH were lower in treatments with higher concentrations of tallow leaf litter. By looking at the relationship between DO and pH in aerated and nonaerated treatments, we were able to determine the DO and pH thresholds for hatching (Figs. 2*a*, 2*b*). Egg masses exposed to water with DO <1.59 mg/L or a pH <5.29 did not survive. As a result, we suggest that reduced DO and pH, caused by tallow leaf litter, could be the cause of reduced hatching success. Hypoxia has been linked to reduced survival in some amphibians (Adolph 1979; Bradford and Seymour 1988; Seymour and Roberts 1991; Seymour et al. 1995). Also, the acid tolerance of anuran embryos can vary among species (Pierce 1985). Gosner and Black (1957) reported that Pine Barrens Treefrog (*Hyla andersonii* Baird, 1854) and Carpenter Frog (*Lithobates virgatipes* (Cope, 1891)) can tolerate pH as low as 3.8, while

Fig. 2. Survival of egg masses of the Southern Leopard Frog (*Lithobates sphenocephalus*) exposed to leaf litter of the Chinese tallow (*Triadica sebifera*) under aerated (*a*) and nonaerated (*b*) conditions. Each symbol represents one replicate (n = 48). Egg masses exposed to water with dissolved oxygen (DO) >1.59 mg/L or a pH >5.29 successfully hatched.



Schlichter (1981) found that a pH of 5.0 reduced the survival of embryos of the Northern Leopard Frog (*Lithobates pipiens* (Schreber, 1782)) by 50%. Gosner and Black (1957) also reported that embryos of the Southern Leopard Frog were somewhat acid tolerant; with embryos tolerable of pH 4.1. They found that embryos displayed 85% or more mortality at pH 3.7 and they observed high mortality between pH 3.9 and 4.1 but were unable to attribute this mortality to pH alone. We observed that embryos of the Southern Leopard Frog appeared to be affected by a pH as high as 5.4, which differs from the findings of Gosner and Black (1957). This difference could be attributed to a variety of factors including geographical variation in pH tolerance.

Other compounds found in the leaves of tallow may have contributed to reduced survival. As we did not investigate all of the potential water chemistry variables, we cannot rule out the effects that these variables may have on egg hatching. For example, tannins are known to damage the gills of fish (Temmink et al. 1989). Studies have demonstrated that plant extract can reduce larval amphibian survival (Maerz et al. 2005*b*; Watling et al. 2011*b*). Therefore, the tannins found in tallow leaf litter may contribute to differential hatching success. Unfortunately, our experimental design did not allow us to determine if turbidity played a role in the reduced survival of eggs; however, turbidity measurements fell well within the range of those found in natural ponds (C.K. Adams, personal observation). Treatments that contained higher concentrations of tallow leaf litter likely had higher turbidity because more leaves were available for breakdown, thus producing more particulate matter.

Based on our results, we conclude that as little as 1 g/L of tallow leaf litter can cause water to become hypoxic and unsuitable for hatching of eggs of the Southern Leopard Frog in the laboratory. Also 2 g/L of tallow leaf litter can reduce the pH of water to a point that appears to be detrimental to eggs of the Southern Leopard Frog. The amount of time that water is hypoxic and unsuitable for eggs is determined by the amount of leaves in the water. As little as 1 g/L of tallow leaf litter can cause water to reach the threshold for survival and water can stay below the threshold for 5 days. At concentrations of 2 and 4 g/L of tallow leaf litter, water can stay below the DO threshold for >10 days. We observed a similar relationship between leaf-litter concentration and pH. A concentration of 2 g/L or more was required to reach the pH threshold, and the higher the concentration of tallow leaf litter, the longer the water stayed below this threshold. At 2 g/L of tallow leaf litter, water stayed below the pH threshold for 3 days; however, at 4 g/L of tallow leaf litter, water was below the threshold for more than 7 days.

These findings are particularly important when considering which amphibian species may be affected by tallow leaf litter. In the West Gulf Coastal Plain, several amphibian species breed during winter months, including the Southern Leopard Frog (Conant and Collins 1998; Saenz et al. 2006). The timing of leaf fall of tallow trees is variable within and among years and trees can take up to a month to completely shed all of their leaves. In eastern Texas, tallow trees can begin to shed their leaves as early as October, but in some years leaf fall can continue through December (C.K. Adams, personal observation). Another important factor that influences the overlap of anuran breeding and larval stages with tallow leaf fall is rainfall. Rainfall in eastern Texas can be variable; however, rainfall can be more frequent from December through March (Saenz et al. 2006). In Nacogdoches, Texas, November and December are relatively wet months (Chang et al. 1996). Rainfall creates habitat for anurans to breed; however, the amount and timing of rainfall can influence when tallow leaf litter enters the aquatic system. The influence these variables may have on the effect that tallow leaf litter has on anuran hatching is unknown. Still, the potential exists for overlap between tallow leaf fall and amphibian breeding events.

This is one of the first studies to investigate the impacts of Chinese tallow on amphibian eggs. We documented reduced survival of eggs of the Southern Leopard Frog exposed to water with invasive tallow leaf litter. We identified changes in water chemistry that we relate to tallow leaf litter, which probably caused reduced egg survival and altered time to hatching. However, we cannot rule out other impacts of tallow leaf litter such as the potential impacts of tannins and other compounds found in tallow leaves. These results are based on laboratory experiments and the impacts that tallow may have on amphibians in the field may vary; however, we present threshold data that will provide a foundation for future field-based experiments to investigate the impacts of tallow. Future research should focus on the potential impacts of the tannins and other compounds found in the leaves of tallow on amphibians.

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