Invasive Plant Leaf Litter Affects Anuran Embryo Survival Rates, Timing of Hatching, and Hatchling Size

The negative impacts of invasive species on native amphibians have been well studied (Moyle 1973; Bradford 1989; Blossey 1999; Simberloff 2000; Bucciarelli et al. 2014). Although much of this research has focused on the negative impacts of animals, invasive plants are of particular interest because they are disproportionately wetland species (Zelder and Kercher 2004) that pose a substantial risk to aquatic amphibians. Invasive plants can alter aquatic systems by leaching phytochemicals and causing changes to water chemistry (Leonard 2008; Watling et al. 2011a; Montez 2016). Phenolic compounds of invasive plants have been suggested as the proximate causes in the reduction of survival of some native aquatic amphibians (Maerz et al. 2005; Brown et al. 2006). Other plant invasions have been associated with changes in water chemistry that subsequently alter the behavior and metamorphosis of larval anurans (Watling et al. 2011a; b; Saenz et al. 2013; Saenz and Adams 2017).

Although there is a growing amount of literature regarding the impacts of invasive plants on amphibian larvae, less is known about the effects on amphibian embryos. Adams and Saenz (2012) suggested that amphibian eggs may be particularly vulnerable to changes in water chemistry. They demonstrated that changes in water chemistry caused by the addition of Chinese Tallow (Triadica sebifera) leaf litter reduced the survival of Rana sphenoecephala eggs in experimental trials. They concluded that reduced pH and dissolved oxygen associated with the leaf litter were the proximate causes of mortality, two water-quality parameters known to affect amphibian embryo development and survival (Gosner and Black 1957; Adolph 1979; Schlichter 1981; Pierce 1985; Seymour and Bradford 1995).

Chinese Tallow is an exotic plant species of particular interest because of its extensive invasion of the southeastern United States. It was first introduced into North America in the late 1700s (Bell 1966) and many forests throughout the southeast have experienced rapid replacement of native vegetation by Chinese Tallow (Oswalt 2010). The invader has the ability to outcompete native vegetation across many different habitat types, in part due to its rapid growth and effective seed dispersal (Jubinsky and Anderson 1996; Bruce et al. 1997). Chinese Tallow often grows in wet areas (Scheld and Cowles 1981; Siemann and Rogers 2003) and, as a result, co-occurs with many aquatic organisms. Several recent studies suggest that Chinese Tallow can negatively affect the behavior and survival of larval and embryonic anurans (Leonard 2008; Cotten et al. 2012; Adams and Saenz 2012; Saenz et al. 2013, Saenz and Adams 2017).

Leonard (2008) and Cotten et al. (2012) found that leaf litter of invasive Chinese Tallow negatively affected tadpole survival when compared to the leaf litter of native species. Leonard (2008) also found that leaf litter from different tree species affected water quality parameters differently. Specifically, he determined that Chinese Tallow treatments had lower dissolved oxygen than treatments with leaf litter from native tree species. He attributed this decrease in dissolved oxygen to an increase in biological oxygen demand likely from bacterial and fungal blooms associated with Chinese Tallow leaf litter decomposition in water (Weyers and Suberkropp 1996; Wright and Covitch 2005). This association with microbial communities and Chinese Tallow leaf litter was later demonstrated by Montez (2016).

Egg hatching plasticity, in response to various cues, is well documented in anurans (Warkentin 1995; Chivers et al. 2001; Johnson et al. 2003; Saenz et al. 2003; Gomez-Mestre et al. 2006; Touchon et al. 2006). Predator cues are known to cause changes in the timing of hatching of amphibian eggs (Warkentin 1995; Sih and Moore 1993; Johnson et al. 2003; Saenz et al. 2003) and similar results were reported in the presence of a fungal pathogen (Gomez-Mestre et al. 2006). In addition to predators and pathogens, Warkentin (2002) documented that hypoxic conditions induced early hatching in Agalychnis callidryas, resulting in less-developed tadpoles at the time of hatching.

Because Chinese Tallow leaf litter is known to reduce dissolved oxygen, we would expect lower concentrations to affect indirectly the development of anuran eggs and the timing of hatching. In this study, our primary objective was to compare the effects of leaf litter leachate from invasive Chinese Tallow and two native tree species on the hatching success of R. sphenoecephala. We also tested if leaf litter from invasive and native species had effects on the timing of hatching, and post-hatching size and development of hatching anurans. We hypothesized that Chinese Tallow leaf litter will accelerate hatching, decrease size, and result in less developed hatchlings compared to native species leaf litter. We also hypothesized that a lower concentration of Chinese Tallow leaf litter will have less of an effect on water chemistry and anuran eggs hatching and development.

**MATERIALS AND METHODS**

We used the Southern Leopard Frog (Rana sphenoecephala) as our study organism to compare the physiological effects of invasive Chinese Tallow and two common native tree species, Red Maple (Acer rubrum) and Swamp Chestnut Oak (Quercus michauxii). Rana sphenoecephala is an ideal species to investigate potential negative impacts of invasive Chinese Tallow because these two species overlap substantially in their ranges and the frog's timing of oviposition coincides with Chinese Tallow leaf fall (Saenz et al. 2006; Adams and Saenz 2012).

We collected leaves of Chinese Tallow, Red Maple, and Swamp Chestnut Oak from 4 November to 4 December 2009 from multiple trees located on the campus of Stephen F. Austin State University in Nacogdoches, Texas. Leaf collection was done by stripping loose leaves from low hanging branches just prior to abscission or by collecting freshly fallen leaves. 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 until used.

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We collected 10 *R. sphenocephala* egg masses from the Stephen F. Austin Experimental Forest, Nacogdoches County, Texas, on 20 January 2010. All egg masses were in developmental Gosner (1960) stages 10–14, with all eggs highly synchronous within each egg mass. We developed five leaf-litter treatments to compare the effects of invasive and native plants on the embryos. Our treatments consisted of aged tap water with no leaf litter (control) and aged tap water with 0.5 g/L of Chinese Tallow leaf litter, 1.0 g/L of Red Maple leaf litter, 1.0 g/L of Swamp Chestnut Oak leaf litter, or 1.0 g/L of Swamp Chestnut Oak leaf litter. These leaf-litter concentrations are well within what could be expected in nature (Leonard 2008). Each replicate was conducted in 13-L white plastic buckets (30 cm diameter × 28 cm deep; N = 50) which contained 20 *R. sphenocephala* eggs and 4 L aged tap water. Within 45 minutes of setting up the treatments, eggs were placed in a square plastic containers (14 cm × 14 cm × 9 cm) with mesh-covered holes to allow water flow between the container and the bucket (Fig. 1). Each treatment was replicated 10 times and blocked by egg mass. As a result, each replicate within a block contained full siblings of the exact same developmental stage.

To determine hatching success, we checked the eggs every 12 h to observe embryo development. If eggs failed to develop from one check to the next, they remained in the container for an additional 24 h before making a determination on mortality. After 24 h of no development, mortality was apparent because embryos that died would begin to become discolored and show signs of decomposition.

Once hatching began, we checked eggs hourly and recorded the number of hatchlings. Because some eggs will fail to hatch, we considered an egg mass hatched and terminated the trial once half of the eggs hatched in each container (Laurila et al. 2001). We defined hatching as the point at which the hatching completely leaves the inside of the egg jelly envelope (Laurila et al. 2001). Once an egg mass hatched, the hatchlings and remaining eggs were euthanized with MS222 and preserved in formalin. Developmental stage (Gosner 1960) was determined for all remaining eggs and total length was determined for hatchlings. We used a randomized, incomplete block ANOVA.

Fig. 1. Image of *Rana sphenocephala* eggs in a plastic container with mesh openings, within a 13-L bucket containing 4 L of water and 4 g of leaf litter. The image shown was taken of a trial in the 1.0 g/L Chinese Tallow treatment.

Fig. 2. Hatching results for Swamp Chestnut Oak, Red Maple and Chinese Tallow leaf-litter treatments. A) Hatching success out of 10 possible clutches and least square means reported for B) time to hatch, C) developmental stage, and D) total length for *Rana sphenocephala* hatchlings in the different leaf-litter treatments. Different letters in a column indicate significant differences among five leaf-litter treatments (P < 0.05). Error bars represent standard error of the means.
where egg mass was considered a random block, and a Kenward-Rogers denominator degrees of freedom approximation, followed by Tukey-adjusted pairwise t-tests to compare Gosner stage, total length, and hours to hatching among treatments.

Previous work indicated that leaf-litter leachate affected water chemistry variables after leaf immersion (Adams and Saenz 2012). As a check in the current work, we used a Hach Hydrolab Quanta® to measure pH, dissolved oxygen, and turbidity on two randomly selected replicates in each treatment every 12 h until the termination of the study.

**Results**

All egg masses successfully hatched in the control, 0.5 g/L Chinese Tallow, and oak leaf-litter treatments. Four egg masses (40%) hatched in treatments containing 1.0 g/L Chinese Tallow leaf litter and five egg masses (50%) hatched in maple leaf-litter treatment (Fig. 2A). All leaf-litter treatments hatched significantly faster than the control but were similar to one another ($F_{4,27.2} = 42.06, P < 0.0001$; Fig. 2B). Tadpole developmental stage ($F_{4,34} = 46.28, P < 0.0001$; Fig. 2C) and total length at hatching ($F_{4,33.9} = 45.62, P < 0.0001$; Fig. 2D) were significantly different among treatments. Eggs reared in water containing 1.0 g/L of Chinese Tallow leaf litter were the least developed and smallest in total length but were not significantly different from eggs reared in the 0.5 g/L tallow or maple treatment (Fig. 3).

Adding leaf litter of any of the three tree species to water decreased dissolved oxygen concentrations and pH while increasing turbidity (Table 1). Water chemistry in the 1.0 g/L Chinese Tallow leaf treatment tended to differ the most from the control, with low pH and dissolved oxygen levels and high turbidity, while the oak leaf-litter treatment tended to be the most similar to the control in all the water chemistry values. The water chemistry values in the maple leaf-litter treatments were notable because they were most similar to the 1.0 g/L Chinese Tallow treatment.

**Discussion**

Hatching success was 100% in all treatments except for egg masses reared in the 1.0 g/L Chinese Tallow and 1.0 g/L Red Maple leaf-litter treatments. In these two treatments, dissolved oxygen values approached levels lethal to anuran eggs observed in previous research (Adams and Saenz 2012), leading us to conclude that the differences in mortality between the treatments were likely a result of hypoxia. Other water chemistry values, pH and turbidity, were within ranges that did not negatively affect hatching success in previous studies (Clark and LaZerte 1985; Freda and Dunson 1985; Beattie and Tyler-Jones 1992; Adams and Saenz 2012).

The addition of leaf litter of any tree species in this study caused changes in the water chemistry variables that we measured, with the 1.0 g/L Chinese Tallow leaf-litter treatment causing the greatest changes compared to the control. It is important to note that despite the trend of the 1.0 g/L Chinese Tallow causing a greater change in mean water chemistry values, we were not able to apply statistical analyses to the data because of our small number of samples. Despite the relative low sample size, we determined that dissolved oxygen, pH, and turbidity values were very consistent with other research on Chinese Tallow leaf litter (Adams and Saenz 2012; Saenz et al. 2013; Saenz and Adams 2017).

All leaf-litter treatments reduced the time to hatching and resulted in less-developed and smaller hatchlings (total length) of the Southern Leopard Frog when compared to the control hatchings. We expected time to hatching to vary depending on leaf-litter treatment based on previous research on the effects of Chinese Tallow and native tree leaf litter on dissolved oxygen (Leonard 2008; Adams and Saenz 2012; Montez 2016) and research that demonstrated that dissolved oxygen levels can affect the timing of hatching in anurans (Warkentin 2002).

Eggs exposed to 1.0 g/L Chinese Tallow leaf litter tended to produce the least-developed and smallest (total length) hatchlings; however, the hatchlings were not significantly smaller than hatchlings in the 0.5 g/L Chinese Tallow and Red Maple...
treatments. We were limited by sample size, partly due to egg mortality in several of the 1.0 g/L Chinese Tallow and Red Maple trials. Perhaps a larger sample size may have given us greater statistical power to elucidate the relationships between water chemistry and tadpole size and development. We suggest that the accelerated timing of hatching is a primary driver in the reduced size and development of hatchlings; however, we cannot rule out the role played by tannins or other phenolics, as reduced survival in some larval anurans has been attributed to these compounds (Maerz et al. 2005; Brown et al. 2006; Earl and Semlitsch 2015).

Regardless of the proximate cause, an implication of reduced size and reduced development at hatching is greater predation risk (Petranka et al. 1987; Warkentin 1995; Moore et al. 1996). Size in particular is negatively associated with predation risk (Petranka et al. 1987). Larger size might help larvae escape predation or move to avoid detection (Moore et al. 1996). The tadpoles in our study in the 1.0 g/L Chinese Tallow and Red Maple treatments were extremely underdeveloped and unable to swim to escape or exhibit any other defensive behavior.

It is clear that under laboratory conditions, Chinese Tallow and Red Maple leaf litter induce premature hatching of anuran eggs, likely as a result of hypoxia driven by leaf decomposition in the aquatic trials (Montez 2016). Cameron and Spencer (1989) reported that Chinese Tallow leaves readily leached tannins and total phenolics and that leaves break down much more quickly than other deciduous leaves in the terrestrial environment. We report that Chinese Tallow leaves readily leached tannins and total phenolics, and that leaves break down much more quickly in the aquatic trials (Montez 2016). Cameron and Spencer (1989) reported that Chinese Tallow leaves readily leached tannins and total phenolics, and that leaves break down much more quickly than other deciduous leaves in the terrestrial environment. We suggest that Chinese Tallow likely poses a risk to hatching success and larval survival in the natural environment due to its ability to colonize wetlands and outcompete native vegetation creating monoculture stands (Jubinsky and Anderson 1996; Bruce et al. 1997; Siemann and Rogers 2003). More research in natural settings should be conducted to determine the effects of Chinese Tallow and native leaf litter on amphibian eggs and larvae.

<table>
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<th>Treatment</th>
<th>N</th>
<th>pH mean</th>
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</table>

**Table 1.** Means and standard errors for water chemistry parameters of egg hatching trials of different leaf litter treatments. Reported values are from samples taken 48 hours after leaf litter was submerged in the water in all treatments.

**Literature Cited**


Bell, M. III. 1966. Some notes and reflections upon a letter from Benjamin Franklin to Noble Wimberly Jones, October 7, 1772. Ashantiilly Press, Darien, Georgia.


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Several studies have documented the effects of predation in aquatic communities wherein the predator-prey interactions are influenced by the type of predators, the composition of prey communities (e.g., size, density), and prey behavior (Morin 1983; Brodie and Formanowicz 1983; Wellborn et al. 1996; Skelly 1997; Jara 2008; Mogali et al. 2012, 2020). Further, predation efficiency of a predator depends upon the gape size limitation of the coexisting predator. However, most aquatic insect predators are not considered gape-limited, because they usually do not swallow prey whole, instead breaking prey into smaller pieces or ingesting body fluids (Jara 2016).

Predation of anuran tadpoles by aquatic insects and their larvae is well known, but the relative vulnerability of tadpoles in relation to their body sizes during development is less well studied (Semlitsch 1990; Richards and Bull 1990; Crump and Vaira 1991; Wellborn et al. 1996). As far as Indian Bronze Frogs (Hylarana temporalis) are concerned, their breeding period extends from October–January (our unpublished observations) and the tadpoles are routinely found in gently flowing streams and in isolated pockets of water along streams in the Western Ghats (Mogali et al. 2012, 2016). During the extended breeding period, bronze frog tadpoles of different sizes and developmental stages are found simultaneously. Along with the tadpoles, certain invertebrate predators may have to eat smaller prey but in greater numbers (Baber and Babbitt 2003). Therefore, the vulnerability of tadpoles greatly depends upon the gape size limitation of the coexisting predator. However, most aquatic insect predators are not considered gape-limited, because they usually do not swallow prey whole, instead breaking prey into smaller pieces or ingesting body fluids (Jara 2016).

Vulnerability of Hylarana temporalis Tadpoles to Predation by Water Scorpions (Laccotrephes, Nepidae)

Several studies have documented the effects of predation in aquatic communities wherein the predator-prey interactions are influenced by the type of predators, the composition of prey communities (e.g., size, density), and prey behavior (Morin 1983; Brodie and Formanowicz 1983; Wellborn et al. 1996; Skelly 1997; Jara 2008; Mogali et al. 2012, 2020). Further, predation efficiency of a predator depends upon the gape size limitation of its mouth (Kishida and Nishimura 2004; Urban 2007; Ramamonjisoa et al. 2018). A large gape size enables a predator to ingest larger prey which provides more nourishment than smaller prey (Scott and Murdoch 1983; Stephens and Krebs 1986). However, predators with small gapes...