Effects of Chinese tallow leaf litter on water chemistry and surfacing behaviour of anuran larvae

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The establishment of exotic invasive species, including plants, has been linked to the decline of some amphibian populations. Of particular concern with invasive plants, from an amphibian conservation perspective, is that they are disproportionately wetland or riparian species. Recent evidence suggests that Chinese tallow (Triadica sebifera), an exotic deciduous tree species, is expanding its range and becoming more abundant where it occurs in the United States. This is particularly relevant to amphibian conservation considering that Chinese tallow tends to invade wetlands, and recent studies have demonstrated that the leaf litter causes mortality of anuran eggs and larvae by reducing the dissolved oxygen and pH of water. The lethal effect of Chinese tallow leaf litter is short lived and concentrated soon after leaf fall, typically December through to February in the south-eastern United States. In this study, we were interested in determining the sub-lethal effects of Chinese tallow leaf litter on the surfacing frequency and air-gulping behaviour of overwintering anuran larvae. Lithobates catesbeianus and L. clamitans clamitans are two frog species that commonly overwinter as aquatic larvae and extensively overlap in range with invasive Chinese tallow, which may expose their tadpoles to the deleterious effects of the leaf litter. We conducted experiments where we exposed tadpoles to four different concentrations of tallow leaf litter and recorded water chemistry and tadpole surfacing frequency. We found that as Chinese tallow concentration increased, oxygen levels decreased. Both anuran species responded similarly to our treatments and dissolved oxygen levels, where tadpoles swam to the water’s surface to air gulp at a significantly higher rate in the treatments with greater tallow concentration. Such changes in behaviour induced by Chinese tallow could have negative consequences on tadpole foraging efficiency and predator avoidance, ultimately reducing fitness. As biological invasions will continue to be an important part of global change, more attention should be given to sub-lethal impacts, as they pertain to fitness.

Key words: Anuran larvae; air gulping; Chinese tallow; leaf litter; invasive plants

INTRODUCTION

The establishment of exotic invasive species has been linked to the decline of some amphibian populations (Doubledee et al., 2003; Ryan et al., 2009; Bucciarelli et al., 2014). Introduced vertebrates, such as fish and exotic amphibians, are known to compete with and induce behavioural changes in native amphibians, and reduce or eliminate amphibian populations through direct predation (Lawler et al., 1999; Crossland, 2000; Knapp & Matthews, 2000; Pyke & White, 2000; Gillespie, 2001; Smith, 2005). Even invasive invertebrates have been known to reduce the breeding success of native amphibians (Gamradt & Kats, 1996) and cause population declines by degrading foraging habitat (Maerz et al., 2009). Invasive plants can affect chemical and physical habitat features, influencing decomposition and nutrient dynamics that can alter the trophic structure of invaded ecosystems, which could ultimately affect native amphibian populations (Brooks et al., 2004; Maerz et al., 2005a; Brown et al., 2006; Maerz et al., 2010; Martin & Murray, 2011; Watling et al., 2011). Amphibian larvae exposed to Amur honeysuckle (Lonicera maackii) in aquatic environments make more trips to the water surface (Watling et al., 2011; Hickman & Watling, 2014) and have shown reduced survival compared to larvae exposed to native plants, likely due to effects of phytochemicals acting on tadpole respiratory surfaces (Brown et al., 2006; Watling et al., 2011). Maerz et al. (2005b) speculated that gill tissue damage, caused by compounds in the extract of L. maackii, might compromise the ability of the anuran larvae to take up oxygen. Invasive plants not only influence amphibian biology directly, they can have strong indirect effects on amphibians through decreasing dissolved oxygen, as suggested in a study on the effects of Chinese tallow (Triadica sebifera) on amphibian egg hatching (Adams & Saenz, 2012).

Recent studies have shown that exposure to Chinese tallow leaf litter has negative effects on tadpole survival compared to leaf litter of native tree species (Leonard, 2008; Cotten et al., 2012). Of concern are the changes in water chemistry, particularly decreases in dissolved oxygen, caused by Chinese tallow leaf litter (Adams & Saenz, 2012; Saenz et al., 2013). Moderate concentrations of Chinese tallow leaf litter will decrease...
In addition to affecting survival, low dissolved oxygen has been linked to marked changes in behaviour in amphibian larvae (West & Burggren, 1982; Feder, 1983a; Wassersug & Feder, 1983; Crowder et al., 1998). Chinese tallow leaf litter has been observed to decrease dissolved oxygen levels in water and cause mortality in anuran larvae (Adams & Saenz, 2012; Saenz et al., 2013) and lower oxygen levels are known to affect rates of air breathing in larval amphibians, we suggest the potential for induced behavioural responses by native amphibian species in the presence of sub-lethal concentrations of tallow leaf litter.

We suggest that tallow will affect activity by lowering dissolved oxygen to levels where cutaneous and gill respiration by larval anurans would need augmentation with aerial oxygen uptake. In addition, aerial uptake of oxygen could affect the larva’s relative position in the water column, as air-breathing affects buoyancy (Wassersug & Feder, 1983). The movement required for air-breathing is of particular concern because activity levels in anuran larvae have been associated with alerting predators to the prey’s location, resulting in increased predation and reduction in foraging time (Feder, 1983b; Moore & Townsend, 1998; Watling et al., 2011; Hickman & Watling, 2014).

Our primary objective was to determine if low concentrations of Chinese tallow leaf litter can cause behavioural changes in aquatic larval anurans. First, we explored the effects of different concentrations of Chinese tallow leaf litter on water chemistry. We then determined if increasing concentrations of tallow leaf litter increase air gulping behaviour of anuran larvae and affect their resting position in the water column. We then explored the relationship between dissolved oxygen and observed tadpole behaviour (see Moore & Townsend, 1998; McIntyre & McCollum, 2000), in the context of invasive plant/amphibian interactions.

**MATERIALS AND METHODS**

**Study Species: Invasive Tree** — The invasive Chinese tallow is a fast-growing deciduous tree native to China and Japan (Bruce et al., 1997). Currently, the invasive distribution of Chinese tallow includes much of the South Atlantic and Gulf Coastal Plain of the United States (Conner et al., 2002) and it is now the fifth most numerous tree species in the entire state of Louisiana and the fifth most common species found in east Texas (Oswalt, 2010). The Chinese tallow has high reproductive potential and is known to replace native vegetation and produce monocultures, particularly in wetland habitats (Cameron & Spencer, 1989; Jubinsky & Anderson, 1996; Bruce et al., 1997).

All Chinese tallow leaves used in the study were collected from trees in the Stephen F. Austin Experimental Forest in Nacogdoches County, Texas, in the autumn of 2009. We collected newly senesced Chinese tallow leaves, those that had changed colour from green to red or orange, by stripping them directly from tallow trees or by raking freshly fallen leaves from the ground. Leaves were immediately air-dried in a dark climate-controlled room and stored in black plastic bags until used in experiments. Although leaves used in this study had been stored for over eight months, earlier work suggests that the leaves would not lose their “potency” in this amount of time and had similar effects on water chemistry as leaves that had been stored for shorter periods of time (Saenz et al., 2013).

**Study Species: Native Anurans** — American bullfrog (Lithobates catesbeianus) and bronze frog (L. clamitans) larvae were the focal animal subjects of this study. Both species breed in the summer months and regularly overwinter as larvae (Saenz et al., 2006). The ranges of both anuran species overlap significantly where Chinese tallow occurs (Conant & Collins, 1998; Conner et al., 2002), making them potentially vulnerable to negative effects of this invasive plant. All anuran larvae were collected in August 2010 by dipnet from ponds in the Davy Crockett National Forest in Houston County, and the Stephen F. Austin Experimental Forest in Nacogdoches County, Texas. Upon capture, we examined all tadpoles for signs of tail damage and kept only individuals that appeared undamaged. Tadpoles were housed in plastic bins filled with aged tap water at a density of approximately one tadpole per litre and provisioned with ground tropical fish flakes, ad libitum. Tadpoles were kept in the lab no more than one week before being used in behaviour trials.

**Behaviour Trials** — All behaviour trials were conducted in commercially available 5-gallon (18.93 L) glass aquaria in a 22 °C climate-controlled room. Ten litres of dechlorinated aged tap water, maintained at a constant temperature of 21.5 °C, was added to each aquarium resulting in approximately 12 cm of water depth. Mesh bags, resembling teabags, were added to each aquarium and provisioned with Chinese tallow leaves according to concentration levels for each treatment. The bags all sank to the bottom of each aquarium. Our study included four treatments: control (0.0 g tallow/L water, included an empty mesh bag), low concentration (0.15 g tallow/L water), medium concentration (0.25 g tallow/L water) and high concentration (1.0 g tallow/L water). For reference, a typical dry averaged-sized Chinese tallow leaf weighs approximately 0.25 g (Saenz, unpublished data). Bags with tallow leaves were allowed to soak for 48 hours prior to adding the anuran larvae. No aeration was added to the experimental aquaria at any time and the tadpoles were not fed during the trials. We measured dissolved oxygen (DO; mg/L), pH, and salinity of water
in each trial immediately prior to behaviour trials using a Hach Hydrolab Quanta®. The Hach Hydrolab Quanta® uses the Practical Salinity Scale (PSS) to measure salinity. One tadpole was placed in each aquarium containing the Chinese tallow treatments, and was allowed to acclimate for 30 minutes prior to activity trials. Each tadpole was used only once in this study. All larvae were measured (total length) and developmental stage was determined after each trial (Gosner 1960). Developmental stage and total length did not differ among treatments for bullfrog (Gosner stage, $F = 0.07, P = 0.9756$, $df = 3, 117$; total length, $F = 0.04, P = 0.9899$, $df = 3, 117$) or for bronze frog larvae (Gosner stage, $F = 0.17, P = 0.9175$, $df = 3, 111$; total length, $F = 0.09, P = 0.9674$, $df = 3, 111$) (Table 1). Overall, bullfrog larvae averaged 65.04 mm ($SE = 1.38$) in total length with a mean developmental stage of 33.3 ($SE = 0.40$) while bronze frogs averaged 35.18 mm ($SE = 1.06$) in total length with a mean developmental stage of 28.8 ($SE = 0.26$).

Each aquarium setup was used for five trials before replacing with fresh water and tallow treatments. The five trials were considered a block. Trials consisted of a 10-minute observation period where the total number of air gulps taken by the tadpole and the relative resting position (when not actively swimming to the surface) in the water column were recorded. Air gulps were recorded only when a gas bubble was expelled from the mouth of the tadpole following surfacing behaviour of the animal. Trials were replicated 30 times per treatment for bronze frogs and 32 times per treatment for bullfrogs, resulting in 248 total trials.

In addition to laboratory trials, we sampled the water chemistry of 51 wetlands in the Davy Crockett National Forest in eastern Texas during 27-30 October 2009 as a reference to compare to our laboratory treatments. It is important to note that the samples were taken preceding typical annual leaf-fall and all sites sampled were devoid of the invasive Chinese tallow tree. We used a Hach Hydrolab Quanta® to measure dissolved oxygen, pH, and salinity from naturally occurring wetlands to use as a reference to compare to our laboratory treatments.

**Analyses** — For each anuran species, we compared water chemistry variables and tadpole size and developmental stage in the different treatments with a mixed effect, complete block ANOVA (block considered a random effect). The Tukey-Kramer adjustment was used to control the error rate of pairwise comparisons of least-squares means. Air gulping rates across treatments were compared with a generalised linear mixed model (block considered a random effect) with a Poisson distribution. We modelled the relationship between gulp rate and dissolved oxygen with Poisson regression, including block as a random effect. A Cochran-Armitage test was used to determine if a trend was present in the relationship between buoyant behaviour and tallow leaf concentration. All results were considered statistically significant at the alpha < 0.05 level (version 9.1, SAS institute, 2003).

**RESULTS**

Dissolved oxygen and pH values differed significantly among treatments in the bullfrog (DO, $F = 792.05, P < 0.0001$, $df = 3, 117$; pH, $F = 1157.13, P < 0.0001$, $df = 3, 117$;) and bronze frog (DO, $F = 955.08, P < 0.0001$, $df = 3, 111$; pH, $F = 213.25, P < 0.0001$, $df = 3, 111$;) experiments with no block effect. Post hoc tests revealed that DO and pH levels were lower in treatments with greater Chinese tallow leaf litter concentrations. Salinity values had very little variation within treatments and were consistent across treatments (Table 2).

Bullfrog air gulping rates differed among treatments ($F = 52.02, P < 0.0001$, $df = 3, 117$) with post hoc tests indicating that treatments with higher concentration of Chinese tallow leaf litter induced higher gulping rates with no block effect (Fig. 1), although tadpole response did not differ in the low concentration treatment in comparison to the control. Results for bronze frog air gulping trials ($F = 50.44, P < 0.0001$, $df = 3, 111$) were very similar to the results from the bullfrog experiments (Fig. 1). When we considered only trials containing Chinese tallow leaf litter, Poisson regression indicated a significant relationship between air gulping and dissolved oxygen for bullfrogs and bronze frogs (Table 3; Fig. 2).

The typical resting position of the tadpoles of both species was at the bottom of the aquarium. A trend of floating behaviour, with increasing Chinese tallow concentration, was observed in bronze frogs with no block effect ($Z = -5.73; P < 0.0001$). Bronze frogs were never observed floating in the control and the low concentration treatments while 36% and 64% of the individuals were observed floating at the water’s surface.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total length</th>
<th>Gosner</th>
<th>Total length</th>
<th>Gosner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>64.81 A</td>
<td>2.77</td>
<td>33.56 A</td>
<td>0.82</td>
</tr>
<tr>
<td>Low</td>
<td>65.28 A</td>
<td>2.90</td>
<td>33.16 A</td>
<td>0.85</td>
</tr>
<tr>
<td>Medium</td>
<td>65.52 A</td>
<td>2.79</td>
<td>33.41 A</td>
<td>0.81</td>
</tr>
<tr>
<td>High</td>
<td>64.53 A</td>
<td>2.74</td>
<td>33.25 A</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Table 1. Total length and Gosner stage of tadpoles exposed to four concentrations of Chinese tallow leaf litter (control = 0.0 g/L, low = 0.15 g/L, medium = 0.25 g/L, and high = 1.0 g/L). Within anuran experiments, different letters in a column represent significant differences across treatments.
In the medium and high concentration treatments, respectively. Floating behaviour was observed on only one occasion in the bullfrog experiments, in a high concentration trial; therefore, no analyses were performed.

**DISCUSSION**

Chinese tallow leaf litter concentration significantly influenced air gulping activity in the two species of anuran larvae in our study. Even at relatively low concentrations, submerged Chinese tallow leaf litter had significant effects on the behaviour of bullfrog and bronze frog larvae, suggesting that the results of interactions between the invasive plant and native anurans may be more complex than simple mortality versus survival outcomes demonstrated in other recent studies (Watling et al., 2012; Adams & Saenz, 2012; Cotten et al., 2012; Saenz et al., 2013).

We suggest that changes in dissolved oxygen levels, caused by the introduction of Chinese tallow leaf litter, was the driver that regulated gulping behaviour in our experiments. Results from many previous studies have demonstrated the link between dissolved oxygen and air gulping behaviour by amphibian larvae (West & Burggren, 1982; Feder, 1983a; Wassersug & Feder, 1983; Crowder et al., 1998). Even under normoxic aquatic conditions, some amphibian larvae species still regularly take air breaths (West & Burggren 1982). Our results were consistent with their findings, as we regularly

**Table 2.** Water chemistry results from 51 wetlands from the Davy Crockett National Forest and from bullfrog and bronze frog experiments. Tadpoles were exposed to four concentrations of Chinese tallow leaf litter (control = 0.0 g/L, low = 0.15 g/L, medium = 0.25 g/L, and high = 1.0 g/L). Within anuran experiments, different letters in a column represent significant differences across treatments.

<table>
<thead>
<tr>
<th>Source treatment</th>
<th>Dissolved Oxygen SE</th>
<th>pH SE</th>
<th>Salinity SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wetlands</td>
<td>6.63 0.16</td>
<td>6.06</td>
<td>0.07 0.03</td>
</tr>
<tr>
<td>Range</td>
<td>4.90-8.69</td>
<td>4.11-6.64</td>
<td>0.01-0.05</td>
</tr>
<tr>
<td>Bullfrog experiments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.24 A 0.03</td>
<td>7.59 A 0.02</td>
<td>0.11 0.00</td>
</tr>
<tr>
<td>Low</td>
<td>3.23 B 0.25</td>
<td>6.72 B 0.04</td>
<td>0.11 0.00</td>
</tr>
<tr>
<td>Medium</td>
<td>0.64 C 0.09</td>
<td>6.51 C 0.03</td>
<td>0.11 0.00</td>
</tr>
<tr>
<td>High</td>
<td>0.12 D 0.01</td>
<td>5.46 D 0.06</td>
<td>0.12 0.00</td>
</tr>
<tr>
<td>Bronze frog experiments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8.20 A 0.06</td>
<td>6.89 A 0.05</td>
<td>0.11 0.00</td>
</tr>
<tr>
<td>Low</td>
<td>3.15 B 0.23</td>
<td>6.49 B 0.04</td>
<td>0.11 0.00</td>
</tr>
<tr>
<td>Medium</td>
<td>0.64 C 0.07</td>
<td>6.28 C 0.03</td>
<td>0.11 0.00</td>
</tr>
<tr>
<td>High</td>
<td>0.17 D 0.01</td>
<td>5.49 D 0.05</td>
<td>0.12 0.00</td>
</tr>
</tbody>
</table>

**Figure 1.** Bar graphs depicting the mean rate of air gulps taken by bullfrog and bronze frog tadpoles in each of the Chinese tallow treatments over a ten minute time-period. Different letters over the bars indicate statistical differences among treatments at the P < 0.05 level. Error bars represent Standard Error.

**Figure 2.** Predicted number of air gulps by bullfrog and bronze frog tadpoles over a ten minute time-period (as calculated with a Poisson regression model) in relation to dissolved oxygen. Points represent data for each trial from all four treatments.
observed air-gulping behaviour in our control trials that averaged more than 8 mg DO per litre of water. The primary difference we found between treatments was in activity, not with position in the water column. Although the consistent trend was higher gulping frequency associated with higher concentration of leaf litter and lower oxygen (Figs. 1 & 2), statistically significant differences from the control were not apparent until compared with the medium concentration treatment of 0.25 g of leaf litter per litre of water. We suggest that a threshold for inducing a significant increase in the rate of gulping behaviour in amphibian larvae exists somewhere between 0.15 g and 0.25 g of Chinese tallow leaf litter per litre of water. Interestingly, our data showed a marked decrease in oxygen levels between the low and medium treatments, despite only a small difference in the amount of leaf litter (Table 2).

In addition to increased gulping rates, bronze frog tadpoles exposed to higher levels of Chinese tallow leaf litter had a significantly higher tendency to float at the surface of the water. This behaviour could be an indication of extreme stress induced by hypoxic conditions mediated by a positional change in the water column by the tadpoles in an attempt to seek more dissolved oxygen at the air/water interface. However, the position in the water column could simply be a result of changes in buoyancy caused by intake of air at the water’s surface (Wassersug & Feder, 1983). The floating behaviour was never observed in the control or low concentration treatments, suggesting that the extreme conditions found in the other treatments were required to induce this particular behaviour. Lithobates pipsiens (formerly Rana pipsiens) tadpoles at earlier developmental stages were more apt to float near the surface than individuals at later stages (Wassersug & Siebert, 1975). Interestingly, the buoyant/float ing behaviour in our study was primarily observed in bronze frogs and only once in 128 bullfrog trials. Animal size and developmental stage could be factors affecting our observations, as bullfrog larvae were much larger and more developed than bronze frog tadpoles (Table 1). Early stage tadpoles may lack the lung development and vascularisation to exchange oxygen via air gulping behaviour as efficiently as later stage tadpoles (Strawinski, 1956; Wassersug & Siebert, 1975), thus taking position near the water’s surface may aid in obtaining oxygen subcutaneously if oxygen levels are higher there than deeper water.

Lung development also differs among species and could be an important factor affecting behaviour and survival of species that develop lungs later in the larval stage (Wassersug & Siebert, 1975). The differences in survival between the toad (Anaxyrus americanus) and the plains leopard frog (L. blairi) observed in the Watling et al. (2011) study could be related to ontogenetic differences in lung development between the taxonomic groups. Wassersug & Siebert (1975) dissected toads (Bufo woodhousii) and found that their lungs developed much later in the larval stage than in northern leopard frogs (R. pipsiens). Both species in our study are in the Family Ranidae, as are the leopard frogs (Hillis & Davis, 1986), and are known to develop lungs early in the larval stage. Thus, ranids should be better suited to behaviourally compensate for low dissolved oxygen levels induced by Chinese tallow than some other native species of anuran such as toads. Fortunately, most of the anuran species that are likely to occur in the larval stage during the winter months, when Chinese tallow leaf litter is most likely to affect water chemistry (Saenz et al., 2013), are ranid species. The species most susceptible to low oxygen levels, the toads, are restricted to breeding in the summer months (Saenz et al., 2006) and will likely not be affected, as dissolved oxygen has been shown to rebound over time (Saenz et al., 2013). Time and energy costs are associated with air breathing, which suggests that changes in tadpole behaviour could have effects on fitness (Kramer, 1983). Based on extrapolations from our results, bullfrog larvae would average approximately 32 more forays to the water’s surface per hour for aerial consumption of oxygen in the high concentration treatment (1.0 g tallow/L water) compared to the control, while bronze frog larvae in the high concentration treatments would surface, on average, 35 more times per hour than in the control aquaria. Obviously, natural wetlands would vary in depth, as would the energy expenditure to secure oxygen at the water’s surface.

We argue that predation risk should be considered as an effect of Chinese tallow exposure, given the amount of time that larvae would be exposed to predators while engaging in surfacing behaviour, as it is well known that activity levels are positively correlated with predation in aquatic ecosystems (Woodward, 1983; Wolf & Kramer, 1987; Skelly, 1996; Bridges, 2002). Moore & Townsend (1998) found that lower dissolved oxygen increased surfacing by L. clamitans and increased predation by fishing spiders (Dolomedes triton) and Feder (1983b) showed that air breathing in R. berlandieri affected predation by the painted turtle (Chrysemys picta), concluding that air breathing increased visual recognition by a visual predator. Despite the risk of predation, larval amphibians will continue engage in risk-prone surfacing behaviour when exposed to the leachate of some exotic plants (Hickman & Watling, 2014). The two

### Table 3. Results of regression analyses exploring the relationship between dissolved oxygen and gulp rate for tadpoles exposed to four concentrations of Chinese tallow leaf litter (control = 0.0 g/L, low = 0.15 g/L, medium = 0.25 g/L, and high = 1.0 g/L).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Bullfrog (n=128 tadpoles)</th>
<th>Bronze frog (n=120 tadpoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.83 ± 0.08, t = 23.15, P &lt;0.0001</td>
<td>0.09 ± 0.06, t = 21.35, P &lt;0.0001</td>
</tr>
<tr>
<td>DO</td>
<td>-0.54 ± 0.06, t = -8.85, P &lt;0.0001</td>
<td>-0.48 ± 0.06, t = -8.60, P 0.0004</td>
</tr>
</tbody>
</table>
anuran species in our study might be less susceptible to fish predators due to their unpalatable skin (Adams et al., 2011); however, they are still at risk of predation by invertebrate predators that are likely more abundant in ephemeral wetlands that could be at greater risk of oxygen depletion due to Chinese tallow leaf litter.

A closer look at water chemistry results revealed that dissolved oxygen levels in natural wetlands were approximately twice as high as in the lowest Chinese tallow treatment in our experiments; however, pH in our treatments was well within the normal range of naturally occurring wetlands in the study sites. This suggests that even modest invasion of Chinese tallow could significantly impact some water chemistry variables that could, in turn, affect the behaviour and survival of native amphibians. We do know, based on our results, that much variation occurs in the concentration of oxygen in natural wetlands, but we are not certain of the effects that Chinese tallow will have on water chemistry in nature.

Biological invasions are an important part of global change, as suggested by Dukes & Mooney (1999), and it is a complex problem that is presenting challenges to native biota that we are only beginning to understand. Given that there are over 2,000 species of alien plants established in the United States alone (Vitousek et al., 1997) and invasive plant species are disproportionately wetland or riparian species (Zedler & Kercher, 2004), it is logical to conclude that Chinese tallow is only one of many invasive plants that pose a threat to native aquatic ecosystems. Our results revealed that extremely low levels of Chinese tallow could have profound impacts on species and potentially ecosystems. Small changes in the proportion of invasive trees and shrubs could have subtle sub-lethal effects that might go unnoticed but are still important ecologically. Given the recent laboratory studies results indicating lower amphibian survival when exposed to Chinese tallow and other invasive plant species, more investigation to assess the impact of invasive plants is warranted and more attention should be given to sub-lethal impacts, as they pertain to fitness.

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