An invasive plant influences amphibian phenotypes: Field and laboratory experiments

By

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Dedication

To my grandfather Leonard Jones, an inspirational biologist and free spirit;
I know he would be proud.

To Lyn Kelsay, I never forgot his resonating philosophy about the work of science.

To Lee Hogenson, a dear Okie friend that dealt me reality in a foreign place when I needed it most.
Losing you during my PhD taught me a lot about what was important in life.

Thoughts of them all kept me grounded and motivated me when it counted.

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Abstract Reviews

Chapter 1 *Behavioral responses of tadpoles to predators and invasive plant extracts*

Invasive plants have many influences on the communities they invade, including transformation of the physical structure and chemical composition of native habitats. Such changes in habitats caused by exotic plants have the potential to alter key interactions, such as those between native predators and prey. Although rarely examined, invasive plants could alter predator-prey interactions by reducing habitat quality for prey, causing prey to accept higher predation risk as prey forage for important resources. For example, leaf litter from exotic Amur honeysuckle (*Lonicera maackii*) reduces the quality of aquatic habitats for larval American toads (*Anaxyrus americanus*). This reduction in quality causes toad tadpoles to swim to the surface more often, which places them at greater risk of predation. We use a factorial experiment to evaluate the potential for honeysuckle to affect the typical anti-predator behavior of toad tadpoles. Our design exposed tadpoles to three cue treatments: two chemical predator cues (central newts, *Notophthalmus viridescens*, and late-stage larval green frogs, *Lithobates clamitans*) and a water control. Exposure to cue treatments was crossed with three leachate treatments, where tadpoles were placed in leachate from honeysuckle leaf litter, leachate from native tree leaf litter, or a water leachate control. Leachate from honeysuckle significantly increased the frequency of tadpole visits to the surface of the experimental arena; frequency of surfacing was not affected by predator cues. Leachate from honeysuckle also caused tadpoles to move more readily (i.e. they exhibited a lower latency to move), whereas the presence of either chemical predator cue led to a higher latency to move. Total movement was not affected by honeysuckle leachate, but

was significantly lower in response to either predator cue treatment. For all three measures of tadpole behavior, there was no significant interaction between the effect of predator cue treatment and leachate treatment, illustrating that predator cues and leachate treatments have additive, though often antagonistic effects on tadpole behavior. These results suggest that, although their behavior indicates that honeysuckle leachate is physiologically stressful to toad tadpoles, this does not preclude the exhibition of typical anti-predator behaviors. Our work provides a mechanism capable of producing accelerated metamorphosis of tadpoles in honeysuckle leachate in field studies, and suggest that these differences may arise because tadpoles respond to risk from predators and also face increased physiological costs. When exposed to honeysuckle, toad tadpoles did not alter surfacing behavior to respond to predator chemical cues (no honeysuckle-by-predator effect). Increased surfacing frequency when reared in honeysuckle leaf extracts, which was not suppressed by predator chemical cues, may expose tadpoles to elevated risk of predation by surface predators in the presence of those invasive plants. Therefore, honeysuckle did not alter existing antipredator behaviors but induced a mal-adaptive response of increased surfacing, even in the presence of predator chemical cues.

Chapter 2 *Invasive plant alters habitat selection and promotes antipredator behavior of adult gray tree frogs*

Invasive plants reduce the quality of native habitats, which may influence habitat selection behaviors of native animals. Some animals assess the quality of breeding environments based on abiotic qualities (i.e. sunlight, temperature) or risk of predation. By transforming both the structural environment of forests and the chemical environment of aquatic habitats, invasive plants may influence the ovipositon site selection of native animals. However, native animals should select environments depending on the quality, which are altered by both predators and invasive plants. In this study, we investigate whether ovipositing adult gray tree frogs (*Hyla chrysoscelis/versicolor*) alter habitat selection and predator avoidance behaviors in the presence of a habitat-transforming invasive plant, Amur honeysuckle (*Lonicera maackii*). Across a forested landscape invaded by honeysuckle, we manipulate the presence of predatory spotted salamanders (*Ambystoma maculatum*) in experimental ponds (mesocosms) within forest invaded and uninvaded by honeysuckle shrubs and measure changes in both the amount and time of oviposition. In our experiment, tree frogs oviposit less in predator mesocosms within invaded areas but do not avoid predator mesocosms within uninvaded areas. In addition, tree frogs breed earlier in the season in the invaded areas. In contrast, within uninvaded areas, there is a trend of later oviposition in response to predators. Consequently, honeysuckle affected tree frog's risk sensitivity to predators, but only early in the season when tree frogs were attracted to honeysuckle areas. These results suggest that invasive plants change the antipredator behavior of native animals, which may be explained by early-season breeding when predator densities are lower. Therefore, our study shows how animals alter habitat selection behavior and shift breeding phonology in response to environments transformed by invasive plants.

Chapter 3 *Invasive plant litter induces phenotypic changes in tadpoles that coincide with antipredator traits*

One of the most important goals in conservation biology is to understand how native species respond to exotic invaders. Through changes in aquatic environments by leaf leachates, invasive plants can induce phenotypic trait changes, such behavior or development, in native tadpoles. However, native predators also induce changes in phenotypes like behavior, development and morphology. Not yet understood are how phenotypes induced by native predators correspond to

phenotypic changes induced by non-native plants. We manipulated wetland communities using outdoor mesocosms to investigate whether aquatic environments invaded by invasive Amur honeysuckle (*Lonicera maackii*) influenced phenotypic traits of green frog (*Lithobates clamitans*) and tree frog (*Hyla chrysoscelis*) tadpoles. By crossing honeysuckle leaf-litter treatments with different degrees of predation risk by larval dragonflies (*Anax junius*) (predators: caged, free-roaming, absent), we determined whether phenotypes induced by honeysuckle coincided with or altered phenotypes induced by predators. We measured behavioral, morphological and developmental phenotypes of both tadpole species and determined their survival from each treatment combination. When exposed to caged predators, green frogs decreased their presence near the surface while tree frogs increased their surface presence. Honeysuckle induced tree frog behavioral changes that were nearly identical to their antipredator responses (greater proportion visible and near the water surface). In response to predators, green frogs accelerated development and grew longer body sizes. Honeysuckle induced morphological changes of shallow tail depths in green frogs, which coincides with predator-induced tail depths from other studies. As expected, free-roaming predators were most lethal to both green and tree frogs compared to either caged predator or predator-free treatments. Neither honeysuckle nor the honeysuckle-by-predator interaction influenced mortality of either tadpole species. Therefore, we have no evidence that phenotypes induced by honeysuckle influence risk of predation. To our knowledge, this is the first study to show that an invasive plant can induce a morphological change in amphibians. Moreover, our results suggest that a widely invasive plant is capable of inducing phenotypic changes in tadpoles that are similar to antipredator responses. Therefore, invasive plants may induce traits with similar costs and consequences as those induced by predators.

Chapter 1

Behavioral responses of tadpoles to predators and invasive plant extracts

Caleb R. Hickman, John L. Orrock, and James I. Watling

ABSTRACT

Invasive plants have many influences on the communities they invade, including transformation of the physical structure and chemical composition of native habitats. Such changes in habitats caused by exotic plants have the potential to alter key interactions, such as those between native predators and prey. Although rarely examined, invasive plants could alter predator-prey interactions by reducing habitat quality for prey, causing prey to accept higher predation risk as prey forage for important resources. For example, leaf litter from exotic Amur honeysuckle (*Lonicera maackii*) reduces the quality of aquatic habitats for larval American toads (*Anaxyrus americanus*). This reduction in quality causes toad tadpoles to swim to the surface more often, which places them at greater risk of predation. We use a factorial experiment to evaluate the potential for honeysuckle to affect the typical anti-predator behavior of toad tadpoles. Our design exposed tadpoles to three cue treatments: two chemical predator cues (central newts, *Notophthalmus viridescens*, and late-stage larval green frogs, *Lithobates clamitans*) and a water control. Exposure to cue treatments was crossed with three leachate treatments, where tadpoles were placed in leachate from honeysuckle leaf litter, leachate from native tree leaf litter, or a water leachate control. Leachate from honeysuckle significantly increased the frequency of tadpole visits to the surface of the experimental arena; frequency of surfacing was not affected

by predator cues. Leachate from honeysuckle also caused tadpoles to move more readily (i.e. they exhibited a lower latency to move), whereas the presence of either chemical predator cue led to a higher latency to move. Total movement was not affected by honeysuckle leachate, but was significantly lower in response to either predator cue treatment. For all three measures of tadpole behavior, there was no significant interaction between the effect of predator cue treatment and leachate treatment, illustrating that predator cues and leachate treatments have additive, though often antagonistic effects on tadpole behavior. These results suggest that, although their behavior indicates that honeysuckle leachate is physiologically stressful to toad tadpoles, this does not preclude the exhibition of typical anti-predator behaviors. Our work provides a mechanism capable of producing accelerated metamorphosis of tadpoles in honeysuckle leachate in field studies, and suggest that these differences may arise because tadpoles respond to risk from predators and also face increased physiological costs. When exposed to honeysuckle, toad tadpoles did not alter surfacing behavior to respond to predator chemical cues (no honeysuckle-by-predator effect). Increased surfacing frequency when reared in honeysuckle leaf extracts, which was not suppressed by predator chemical cues, may expose tadpoles to elevated risk of predation by surface predators in the presence of those invasive plants. Therefore, honeysuckle did not alter existing antipredator behaviors but induced a mal-adaptive response of increased surfacing, even in the presence of predator chemical cues.

INTRODUCTION

Invasive species can cause significant changes in the native habitats they invade, altering the composition diversity, and structure of the habitats they invade (Mack et al. 2000, Levine et al. 2003, Orrock et al. 2008). These changes have the potential for widespread effects in native ecosystems, including the modification of interactions among native species within invaded habitats (White et al. 2006, Orrock et al. 2008, Vilà et al. 2011). Reduction of habitat quality may be one important mechanism whereby exotic plants might alter interactions among native predators and prey, since prey that are stressed for resources often exhibit greater risk-prone foraging (Lima and Bednekoff 1999, Brown and Kotler 2004). Despite the pervasive nature of exotic plants, the primacy of predator-prey interactions, and the importance of stress for altering decisions by prey, few studies have examined how reduced habitat quality by invasive plants alters anti-predator behavior of prey (Sih et al. 2011).

Exotic plants may commonly reduce the quality of native habitats by causing changes in the chemical environment (Vilà et al. 2011). These chemical changes may be particularly important in aquatic ecosystems adjacent to invaded habitats, as aquatic ecosystems inundated with leaves from invasive plants can negatively impact growth and survival of invertebrates (Canhoto and Laranjeira 2007, Going and Dudley 2008, Leonard 2008) and larval amphibians (Maerz et al. 2005a, Brown et al. 2006, Leonard 2008, Watling et al. 2011a). In addition to affecting growth and survival, the reduction in habitat quality caused by exotic plants can alter the behavior of larval amphibians: tadpoles of two species exhibited increased surfacing behavior in response to environments altered by invasive plant leaves (Watling et al. 2011a). Although the precise mechanism is not known, some plant extracts disrupt gill lamella function (Temmink et al. 1989), suggesting that tadpoles in invaded habitats experience greater oxygen stress, and this stress prompts greater surfacing (Wassersug and Seibert 1975). Stress-induced surfacing may pose a dilemma for larval amphibian prey, as the increased movement associated with surfacing is in direct opposition to the most effective anti-predator behavior, which is to exhibit reduced activity when predator cues are detected (Lima and Dill 1990, Ferrari et al. 2009a).

In this study, we determine if invasive plants cause risk-prone behaviors of native prey. We exposed native prey, American toad tadpoles (*Anaxyrus americanus*), to both chemical cues from predators and leaf extracts made from an invasive shrub, Amur honeysuckle (*Lonicera maackii*). Native to Asia, honeysuckle shrubs have invaded forests throughout the eastern and mid-western USA (Luken and Thieret 1996), overlapping throughout the range of American toads. Previous studies have shown that toad tadpoles experience decreased survival in the laboratory and accelerated development in mesocosms when exposed to extracts produced by honeysuckle leaves (Watling et al. 2011; Watling, Hickman, and Orrock 2011). Honeysuckle releases chemicals from roots and leaves (Dorning and Cipollini 2005; Cipollini and Dorning 2008) and changes the chemical environment of adjacent aquatic habitats (Poulette and Arthur 2012), where toad tadpoles develop. Toad tadpoles swim to the surface more frequently when reared in honeysuckle extracts (Watling et al. 2011), which may cause tadpoles to be more easily detected and consumed by predators (Moore and Townsend 1998, McIntyre and McCollum 2000). Therefore, predators in the honeysuckle extract environments may pose an elevated risk for toad tadpoles. In addition, toad tadpoles respond to predators with decreased movement (Petranka and Hayes 1998), which may decrease surfacing frequency (Baird 1983, Feder 1983) in contrast to honeysuckle effects on surfacing behavior. We predict that honeysuckle should cause risk-prone responses of tadpoles through two possible mechanisms: 1) altering antipredator behaviors of reduced movement (freezing), 2) inducing a novel but mal-adaptive surfacing response. If honeysuckle alters existing antipredator behaviors, then we expect a significant change in antipredator responses due to the combination of exposure to the honeysuckle extract and predator chemical cues. If honeysuckle induces mal-adaptive responses, then we do not expect toad tadpoles to respond to predator chemical cues with altered surfacing behavior (no honeysuckle-by-predator effect).

METHODS

Preparation of plant leachates

To determine the effects of honeysuckle on behavior, we prepared three leachates (honeysuckle, native, water) by the same process described by Watling et al. (2011). Leaf material was collected from several locations at August A. Busch Memorial Conservation Area (BMCA) in St. Charles County Missouri, both from honeysuckle shrubs and from leaf litter in forest dominated by native trees (oaks, *Quercus* spp., and shagbark hickory, *Carya ovata*). Leaves were dried to constant mass and leachate prepared by soaking 5 g leaves/1-l water for 48 hrs. Leaves were then strained from leachate solution to remove potential food material and to decrease the likelihood of decomposition effects of leaf material on dissolved oxygen (Canhoto and Laranjeira 2007, Earl et al. 2012). A control solution of treated water was prepared identically but without leaves. We introduced leaf leachate s and control solutions to 1.8-L development chambers and later to corresponding 500-mL test chambers.

Animal collection and maintenance

We collected American toad tadpoles (*Anaxyrus americanus*) (*N* = 63) at early stages (Gosner stage 25 or less, McDiarmid and Altig 1999) from five roadside pools between 7 April and 18 May, 2009, at BMCA. Toad tadpoles were exposed to honeysuckle leachate treatments in the lab by randomly assigning individual animals to plastic rearing chambers holding 1-l leachate treatments (see leachate collection). Tadpoles were fed commercial *Spirulina* algal discs *ad libitum* prior to transfer to individual testing chambers. Rearing and testing chambers were stored in a climate-controlled room at 12:12 light:dark photoperiod at 15°C. The purpose of the rearing environment was to stimulate any physiological and behavioral changes caused by the honeysuckle leachate s.

Central newts (*Notophthalmus viridescens*) are predators of toad tadpoles (Miller 1909, Petranka 1998), and late-stage larval green frogs (*Lithobates clamitans*) are potential intraguild predators that both compete with and consume other tadpole species (Schiesari et al. 2009). Newts and green frog tadpoles generally inhabit different microhabitats. Predatory newts often forage at the surface of aquatic habitats (Johnson 2000), while larval green frogs spend most of their time foraging on the bottom of aquatic habitats (Duellman and Trueb 1994). Predator newts (*N* = 19; mean = 1.88 g ± 0.58 SD) and late-stage larval frogs (*N* = 15; mean = 1.55 g ± 0.66 SD) were collected at BMCA 13-21 May 2009. To prevent diet cues from confounding response to predator-specific cues, we fed newts black worms (*Lumbriculus variegates*) and frogs *Spirulina* algal discs *ad libitum*.

Preparation of predator chemical cues

To collect predator chemical cues, we housed newts and larval frogs in 19.5-l aquaria filled with treated (dechlorinated and dechlorimined) water. Animals were kept together by species and not fed during chemical cue acquisition periods. To control for concentration of chemical cues between predator treatments, we adjusted the amount of water to wet mass of animals at 1g/200-mL. After 48 hours, animals were gently removed and any particulate matter filtered with cheesecloth. To preserve predator cues across multiple testing days, stirred solutions were poured into 50-mL aliquots and stored frozen (-20 ^{0}C), which does not affect tadpole responses to chemical cues from predators (Mathis et al. 2003, Hickman et al. 2004). We coded chemical cue aliquots so the treatments were blind to a single observer (CRH). We also tested toad tadpoles with a water control that was processed identically to predator chemical cues.

Experimental design

We performed a fully factorial and randomized 3X3 experiment with predator chemical cue (predator newt, predator frog, water control) following individual tadpole exposure for 21 days to the extraction treatment (honeysuckle, native control, water control). We transferred individual toad tadpoles from rearing chambers into separate 500-mL test chambers. Test chambers contained leachate solutions consistent with rearing environment. We determined that toad tadpoles would acclimate to test chambers and begin foraging within 300 s. Following acclimation periods, we introduced a constant volume of predator chemical cue (30mL) into randomly selected test chambers at a rate of ~1 mL/s by use of syringe attached to polyethylene tubing, as in Hickman et al. (2004). We dyed predator chemical cues with food coloring to assess the moment animals encountered predator chemical cues. Food coloring did not alter feeding on algae in previous observations, and 30mL of dyed predator chemical cue was a sufficient amount to disperse throughout the chamber. We recorded and later scored swimming movement and surfacing behaviors for each tadpole in 300-s periods after the introduction of predator chemical cues.

Behavioral measures

We calculated behaviors from digital video (Sony Handycam®, 60X optical, 200X digital zoom) to avoid disruption of animals by the observer. We defined swimming movement as forward propulsion following undulation of the tail. Latency to move was calculated as the time it took individual toad tadpoles to initiate movement after contact with dyed predator cues, and total movement was calculated as the total seconds of continued movement. We defined surfacing frequency as swimming movement to the top 2cm of the chamber with subsequent surface scores counted when animals dropped below the top 2cm and resurfaced (Moore and Townsend 1998; McIntyre and McCollum 2000). Tadpoles are capable of swimming directly to the surface with little overall movement. However, toad tadpoles must move before they can swim to the surface; therefore, honeysuckle leachate s or predator cues could influence total movement activity through altered hesitation in movement (measured by latency to move) or more surfacing events. We measured total movement time after the introduction of predator chemical cues to determine how the honeysuckle leachate-by-predator chemical cue interaction would influence overall activity.

Data analysis

To determine interdependence between responses (movement, surfacing frequency), we conducted a multivariate, nonparametric permutation analysis (Anderson 2001, McArdle and Anderson 2001). If multivariate results were significant, we performed univariate analyses to determine causes of each altered activity. To determine an effect of the leaf leachate environment and predator chemical cues on behavior, we used generalized linear mixed models with a Poisson distribution and denominator degrees of freedom estimated with the Kenward-Rogers method (Littell et al. 2006a). We examined main effects of the leaf leachates (honeysuckle, native control, water control) and predator cues (predator newt, predator frog, control), as well as their interaction, and modeled individual tadpoles as random effects to account for behavioral variability (Schielzeth and Forstmeier 2009). If we found a significant treatment effect, we compared effects of honeysuckle leachate and predator chemical cue treatments using simple linear contrasts. All mixed models were performed in SAS 9.2 (Littell et al. 2006a) and multivariate permutation analysis was performed in PRIMER 6 (version 6.1.11) with PERMANOVA+ add-in (version 1.0.1: PRIMER-E Ltd. Ivybridge, UK).

RESULTS

Multivariate analysis revealed a significant effect of the honeysuckle leachate environment (F = 3.51, df = 2, P = 0.037) and predator chemical cue (F = 3.89, df = 2, P = 0.026) but not a significant interaction between the two factors (F = 0.17, df = 4, P = 0.948).

The trade-off between responding to honeysuckle leachates and predator chemical cues could be indicated by the interaction between surfacing and movement. However, no significant interactions between plant leachates and predator chemical cues indicated that alterations of the chemical environment by invasive honeysuckle did not affect the antipredator responses of toad tadpoles, either via changing surfacing frequency or latency to move (no honeysuckle leachate-by-predator cue effect, Table 1). Total movement was not altered by the honeysuckle leachate environment (Table 1), but toad tadpoles reduced total movement in response to both predator chemical cues compared to the control (both $P < 0.025$, Fig. 1F). Plant leachate environment did not affect the total movement responses of tadpoles to predator chemical cues (no significant interaction term, Table 1). Toad tadpoles increased activity in response to the honeysuckle leachate environment and decreased activity in response to predator chemical cues, when compared to controls (Fig. 1). Latency to move and surfacing frequency were altered by the leaf leachate environment, while predator chemical cues altered latency to move but not surfacing (Table 1). Reduced activity in response to predators was evident from 32% of tadpoles that never moved during the 300-s observation period. Tadpoles moved twice as soon when reared in honeysuckle leachates compared with animals in the water control (latency to move: $t = -2.86$, $P = 0.006$). Movement did not differ between tadpoles exposed to the honeysuckle leachate compared with the native leaf leachate (Fig. 1A). Animals had intermediate latency to move when reared in the native leachate environment but their behavior was not significantly different than that of tadpoles reared in the water control and honeysuckle leachate environments (Fig. 1A). Toad tadpoles were motionless over twice as long when presented with either predator chemical cue compared with the control cue (latency to move: both $P < 0.025$, Fig. 1B). Toad tadpoles increased surfacing when raised in the honeysuckle leachate compared with either water control or native leaf treatments ($F_{2,52.32} = 3.30$, $P = 0.045$, Fig. 1C). Although there was a trend of reduced surfacing frequency in response to predators, surfacing was not significantly different in response to either predator chemical cue compared with the control ($F_{2, 52.19} = 1.92$, $P = 0.157$, Fig. 1D).

DISCUSSION

Native organisms must increasingly contend with novel stresses from exotic plants in addition to risks of predation from native predators. Our results provide evidence that the behavior of larval amphibians is sensitive to chemical changes in the aquatic environment caused by exotic plants, as well as sensitive to cues of predation risk. The three behaviors we assayed differed in how each was affected by exotic plant leachate and cues of predation risk: latency to move was reduced by exotic plants and increased by predator cues, whereas surfacing frequency only affected by the leachate treatment and total movement was only affected by cues of predation risk (Fig. 1). Our findings have several implications. First, exotic plants may affect the growth, development, and persistence of amphibians in natural settings because larval amphibians experience greater stress, greater predation risk, or both when they inhabit aquatic environments altered by invasive plants. Second, our results highlight how different behaviors may provide insight into how animals reconcile the interplay of predation risk and stresses created by exotic plants. Finally, our results illustrate that studies of anti-predator behavior must be designed with the potentially cryptic effects of invasive plants in mind.

Our work provides support for the recent studies that show how terrestrial invasive plants can have cryptic effects on the behavior of aquatic amphibians (Watling et al. 2011a); our findings also confirm that amphibians are aware of risk in their environment and alter their behavior in response to predators (Ferrari et al. 2009b). However, because invasive plants and predators place different constraints on amphibian behavior (i.e. leachate from exotic plants promotes increased movement for surfacing, while predation risk promotes reduced movement), our primary goal was to determine whether larval amphibians give greater priority to one or the other. Based on the changes in amphibian behavior from previous studies (Watling et al. 2011a), we predicted that amphibians exposed to exotic-plant leachate would exhibit reduced responses to predator cues because increased surfacing due to chronic physiological stress would take priority over reduced movement when presented with predation risk. However, even though this tradeoff did not occur, tadpoles may be under a greater risk of predation within exotic plant leachates.

In this study, leachates made from an invasive plant altered the behavior of native tadpoles resulting in potentially risk-prone responses. In nature, real costs may be realized when tadpoles must surface in predator-rich ponds invaded by honeysuckle. Either tadpoles suffer anoxic stress by forgoing surfacing in response to predators or risk predation when surfacing makes the predators aware of the tadpole location. For example, tadpoles increase surfacing frequency in response to low dissolved oxygen levels in aquatic environments (Wassersug and Seibert 1975). Under low oxygen conditions, a greater number of green frog tadpoles (*Lithobates clamitans*) are eaten by fishing spiders (*Dolmedes triton*) that are attracted to surfacing movement (Moore and Townsend 1998). Visually oriented predators more easily detect and consume tadpoles that swim to the surface of aquatic habitats (Moore and Townsend 1998; McIntyre and McCollum 2000). McIntyre and McCollum (2000) found that predation on *Lithobates* tadpoles was elevated in low oxygen environments, presumably because tadpoles that surfaced for oxygen were in closer proximity to predators that were themselves restricted to the surface by lack of oxygen. If predation risk is greater, American toad tadpoles are capable of adjusting their antipredator behaviors (Anholt et al. 1996) and toad tadpoles can be conditioned to respond to novel predators (Mirza et al. 2006). Small, obligate gill-breathing toad tadpoles in our experiment reduced non-surfacing movements in response to predator chemical cues but do not alter surfacing frequency in response to predators. A lack of antipredator behaviors associated with surfacing could be due to gill-breathing strategies of toad tadpoles. Under hypoxic conditions, small bullfrog tadpoles (*Lithobates catesbeiana*) with gills swim more frequently in the presence of free-roaming salamander predators (McIntyre and McCollum 2000). Alternatively, surfacing behavior is significantly reduced when large bullfrog tadpoles without gills are exposed to chemical cues produced by predatory dragonfly larvae (McIntyre and McCollum 2000). Lung-breathing tadpoles are capable of storing oxygen more efficiently than gill breathers (McDiarmid and Altig 1999). Gill-breathing tadpoles likely alter movement rather than surfacing in response to predators because, unless compromised respiration occurs, they rarely move to the surface regardless of predator presence (McDiarmid and Altig 1999). Obligate gill breathing tadpoles are able to adjust their metabolic rate when exposed to predation risk (Barry and Syal 2012), which may offset the costs of lower energy intake and the need to surface for increased oxygen uptake in honeysuckle environments. Our study shows how new threats can arise if invasive plants expose prey to dangerous situations for which there are no associated antipredator behaviors.

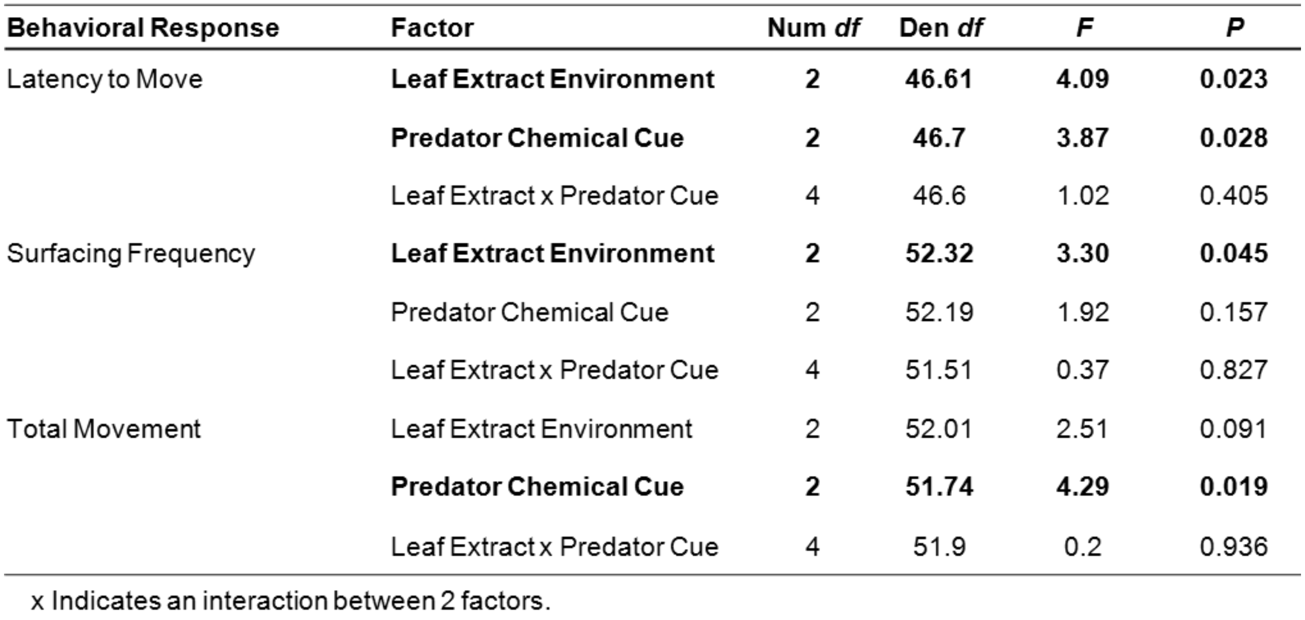
It is possible that tadpoles are trading off stress from the honeysuckle environment with risk of predation, but we failed to capture these changes in our experiment. An indication that a dilemma occurred for tadpoles comes from their latency to move response, which was very low in honeysuckle but relatively high in response to predators. Although latency to move reflects the onset of activity and potentially surfacing, it is difficult to link them together because activity to each behavior changes in time and space. For instance, tadpoles may have waited longer to move and then surface in response to predators and honeysuckle, followed by several visits to the surface. Therefore, we could be missing an important signal in our data and without a statistically significant change in behavior due to the honeysuckle-by-predator interaction; we cannot conclusively determine whether a tradeoff occurs. However, our data does capture a reduction in total movement in the presence of predators but with much variability in response to honeysuckle.

Based on alternative metrics, experimental duration, and low statistical power, we may not have detected tradeoffs for animals exposed to predators and honeysuckle. Because the activity associated with surfacing in response to honeysuckle and antipredator behaviors of reduced movement is complex in time and space, we may be missing a key behavior that links these interactions. Animals switch their responses to predators depending on the level of danger and can modify their behaviors to predators to accommodate other factors in their environment, including increased competition, reduced resources and additional predation risk (Lima and Dill 1990, Ferrari et al. 2009b). However, studies of American toads do not often measure more than one type of behavior in response to predators (but see: Anholt, Skelly, and Werner 1996; Mirza et al. 2006). American toads reduce swimming movement in response to greater predator densities (Anholt et al. 1996) but this response depends largely on the size of tadpoles (Skelly and Werner 1990). Other larval toads (*Bufo bufo*) aggregate in response to predators (Watt et al. 1997). In our study, toad tadpoles may mix strategies so they can surface in response to honeysuckle and reduce movement in response to predators. However, total movement, similar to our metric, has been successfully used in numerous other studies to indicate how tadpoles reconcile decisions to respond to predation risk over other environmental pressures that may be biotic (competitors or other predators) or abiotic (chemical changes or high temperature) (Anholt et al. 1996, Petranka and Hayes 1998, Mirza et al. 2006, Smith et al. 2009). Regardless of the activity quantified, duration of an experiment may be important in determining cost of exhibiting particular behaviors. For example, surfacing in response to honeysuckle may come at a cost to foraging and allocation of energy, which is beyond the short period of our observations. In addition, there is evidence that short pulse experiments like ours may overestimate the intensity of antipredator behavior expected under field situations or when chronic exposure to high risk situations occurs, especially when other costs like foraging are at stake (Lima and Bednekoff 1999). Therefore, decisions to respond to predators and honeysuckle may vary under natural conditions and over longer time, which would require different metrics than we used to capture these interactions.

Amphibians have highly plastic behaviors so capturing responses that represent a population can be difficult. Our results provide a non-significant trend of altered movement behaviors in response to the honeysuckle-by-predator combination with relatively large effect sizes. However, statistical power to detect a difference may be limited, potentially because of the combination of low replication and high variation of behaviors among individual tadpoles. Therefore, we performed a retrospective power analysis to determine whether our design was able to detect significant changes in behavioral responses (Thomas 1997). To estimate power we calculated noncentrality and variance from our data and applied a two-tailed type I error rate ($\alpha = 0.05$) (Thomas 1997, Stroup 2002). Compared to surfacing in response to honeysuckle, our study had a low power to detect differences in surfacing responses to predators (38%) and a habitat-by-predator chemical cue effect (13%). Our study, however, had moderate power to detect changes in latency to move in honeysuckle (70%) but a low power to detect honeysuckle effects on latency to move in response to predators (honeysuckle-by-predator cues: 30%). Therefore, a low sample size may have precluded us from determining that honeysuckle could alter antipredator behaviors but we can be moderately confident that our study could detect altered movement in response to honeysuckle. Nevertheless, latency to move and total movement in response to predators and surfacing in response to honeysuckle had biologically relevant effect sizes compared to results published on American toad tadpoles (Lawler 1989, Petranka and Hayes 1998, Gallie et al. 2001, Smith and Awan 2009, Watling et al. 2011a). A reduction in power because of overall variability in behavior extends beyond this study, where an emerging issue of interest for conservation of animals is to understand how variability in behavior may aid animals in response to human-altered systems (Sih et al. 2004, 2010).

Our results highlight the importance of designing behavioral studies to determine the potentially cryptic effects of invasive plants on native animals. Amur honeysuckle caused risk-prone responses of larval American toads by inducing behaviors that should expose them to elevated risks of predation. In addition, toad tadpoles do not respond to predator cues with altered surfacing, which indicates that this invasive plant induces a behavior for which there may be no corresponding antipredator response. These results support a growing body of work that invasive plants have the potential for widespread effects in native ecosystems through modifications of interactions among native species (White et al. 2006, Orrock et al. 2008, Vilà et al. 2011). This study provides evidence that invasive plants can influence interactions by simply altering the ecosystems they invade.

Table 1: ANOVA table for activity behaviors when tadpoles were raised in the leaf leachate/extract environment followed by exposure to predator chemical cues. Bold indicates significant difference at alpha = 0.05.

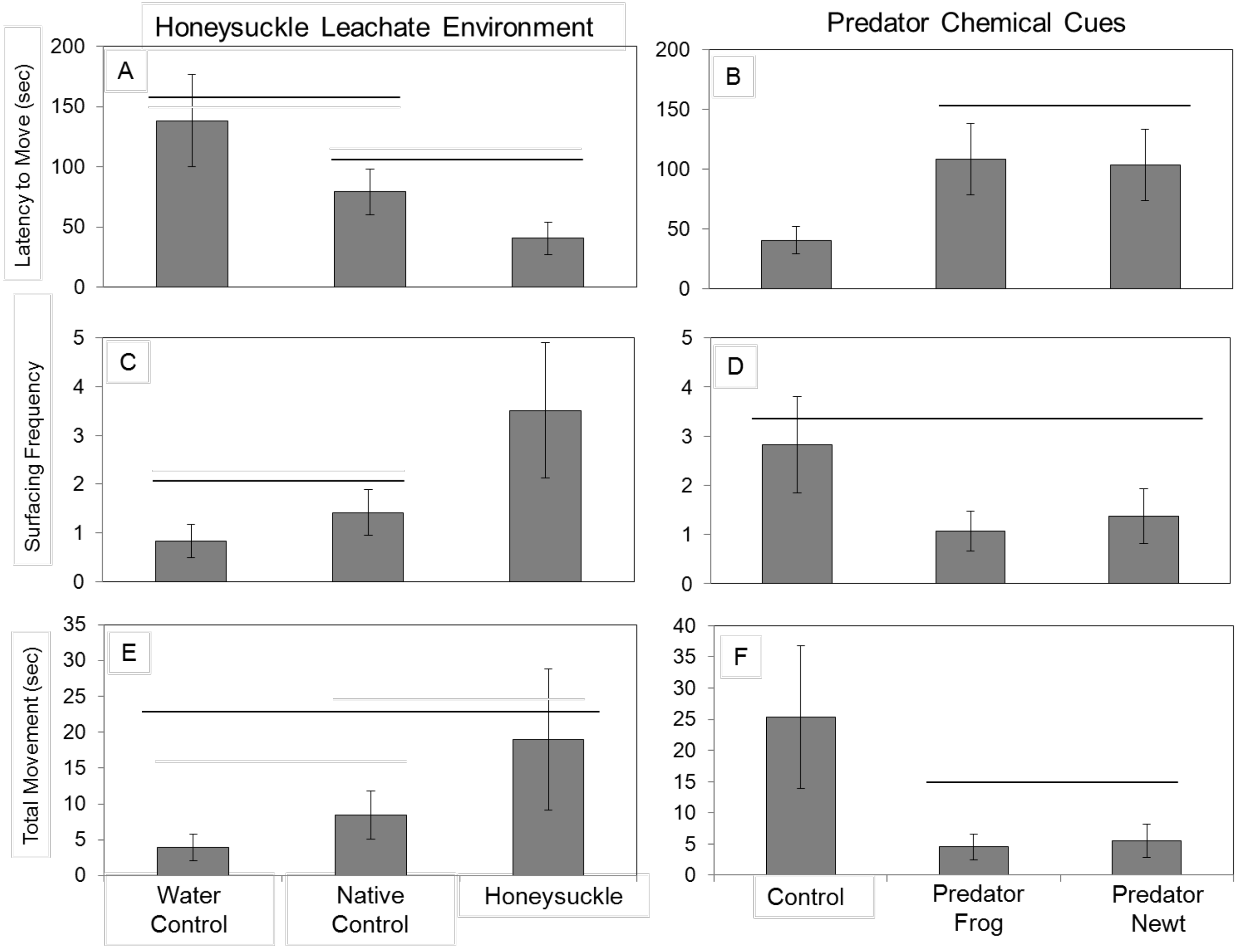


Behavioral Response	**Factor**	**Num *df***	**Den *df***	***F***	***P***
Latency to Move	**Leaf Extract Environment**	**2**	**46.61**	**4.09**	**0.023**
	Predator Chemical Cue	**2**	**46.7**	**3.87**	**0.028**
	Leaf Extract x Predator Cue	4	46.6	1.02	0.405
Surfacing Frequency	**Leaf Extract Environment**	**2**	**52.32**	**3.30**	**0.045**
	Predator Chemical Cue	2	52.19	1.92	0.157
	Leaf Extract x Predator Cue	4	51.51	0.37	0.827
Total Movement	Leaf Extract Environment	2	52.01	2.51	0.091
	Predator Chemical Cue	**2**	**51.74**	**4.29**	**0.019**
	Leaf Extract x Predator Cue	4	51.9	0.2	0.936

x Indicates an interaction between 2 factors.

FIGURE LEGEND

Figure 1: Panels show least square means (±1SE) that illustrate how treatment factors alter activity (latency to move: A, B; surfacing frequency: C, D; total movement: E, F). Total movement is a proxy for the combined changes in latency to move and surfacing. Activity is determined within each panel for separate factors compared with appropriate controls: predator chemical cues (control-water, frog predator, newt predator), the honeysuckle leachate environment (water-control, native, honeysuckle). Lines indicate no significant difference from pair-wise analysis.



Chapter 2

Invasive plant alters habitat selection and promotes antipredator behavior of adult gray tree frogs

Caleb R. Hickman, James I. Watling and John L. Orrock

ABSTRACT

Invasive plants reduce the quality of native habitats, which may influence habitat selection behaviors of native animals. Some animals assess the quality of breeding environments based on abiotic qualities (i.e. sunlight, temperature) or risk of predation. By transforming both the structural environment of forests and the chemical environment of aquatic habitats, invasive plants may influence the ovipositon site selection of native animals. However, native animals should select environments depending on the quality, which are altered by both predators and invasive plants. In this study, we investigate whether ovipositing adult gray tree frogs (*Hyla chrysoscelis/versicolor*) alter habitat selection and predator avoidance behaviors in the presence of a habitat-transforming invasive plant, Amur honeysuckle (*Lonicera maackii*). Across a forested landscape invaded by honeysuckle, we manipulate the presence of predatory spotted salamanders (*Ambystoma maculatum*) in experimental ponds (mesocosms) within forest invaded and uninvaded by honeysuckle shrubs and measure changes in both the amount and time of oviposition. In our experiment, tree frogs oviposit less in predator mesocosms within invaded areas but do not avoid predator mesocosms within uninvaded areas. In addition, tree frogs breed earlier in the season in the invaded areas. In contrast, within uninvaded areas, there is a trend of later oviposition in response to predators. Consequently, honeysuckle affected tree frog's risk

sensitivity to predators, but only early in the season when tree frogs were attracted to honeysuckle areas. These results suggest that invasive plants change the antipredator behavior of native animals, which may be explained by early-season breeding when predator densities are lower. Therefore, our study shows how animals alter habitat selection behavior and shift breeding phonology in response to environments transformed by invasive plants.

INTRODUCTION

Invasive plants are capable of impacting biodiversity of native animals (Siersma and Johnson 2007, Vilà et al. 2011). Effects of invasive plants on native populations and communities may be due to changes in structure or chemistry of native habitats (Crooks 2002, Vilà et al. 2011). Through structural changes, invasive plants act as ecosystem engineers (Jones et al. 1997, Crooks 2002) which are capable of influencing interactions among native organisms (Gribben et al. 2009). In addition to structural changes, invasive plants modify habitat quality by releasing water-soluble chemicals from roots and leaves which influences understory environments (Dorning and Cipollini 2005, Cipollini and Dorning 2008) and changes nutrient dynamics (Poulette and Arthur 2012). Moreover, it is unknown whether changes in populations and communities in response to exotic invaders are due to suboptimal choices made by native animals (Sih et al. 2010, 2011).

Evidence suggests that invasive plants have the potential to change habitat selection behaviors of native animals. Because invasive plants alter the quality of both aquatic and terrestrial habitats, they may influence decisions of organisms capable of moving between aquatic and terrestrial environments in their life cycle. Animals are capable of distinguishing between the quality of habitats for optimal reproduction or foraging (Fretwell and Lucas 1969, Morris 2006, Krivan et al. 2008). The attractiveness of a given habitat patch may be perceived relative to the quality and expected fitness provided by neighboring patches in a landscape (Fretwell and Lucas 1969, Morris 2006, Krivan et al. 2008). Therefore, distributions and abundance patterns may be generated behaviorally, with characteristics of the environment guiding decisions to colonize or disperse (Resetarits et al. 2005). However, it is unknown if invasive plants change habitats in ways that lead animals to make suboptimal oviposition choices.

In this study, we examine whether an exotic plant changes native ecosystems in a way that causes suboptimal oviposition site selection of a native amphibian. Amphibians are an ideal system for our study because they alter ovipositon in response to several biotic (e.g., predators, competitors, vegetation (Resetarits and Wilbur 1989, Binckley and Resetarits 2007)) and abiotic (e.g., temperature, salinity, pond depth (Crump 1991, Spieler and Linsenmair 1997, Viertel 1999) factors. These factors may be influenced by habitats transformed by invasive plants (Jones et al. 1997, Crooks 2002). Adult gray tree frogs (*Hyla versicolor*) are well known to avoid oviposition in sites with predators (Resetarits et al. 2005) but this effect is exacerbated when predators are combined with altered aquatic environments (i.e. pesticides) (Takahashi 2007). Aquatic environments exposed to invasive plant leaves can negatively influence amphibian hatching success, development, survival and behavior (Maerz et al. 2005a, 2005b, 2010, Watling et al. 2011a, 2011b, Adams and Saenz 2012, Cohen et al. 2012). Therefore, invasive plants are capable of negatively affecting the quality of amphibian breeding environments. To determine whether habitats altered by exotic plants influence habitat selection behavior, we measured tree frog oviposition site selection in forest invaded by Amur honeysuckle shrubs (*Lonicera maackii*). Because honeysuckle releases water-soluble chemicals from roots and leaves (Dorning and Cipollini 2005; Cipollini and Dorning 2008) that influences larval amphibian development, survival and behavior (Watling et al. 2011a, 2011b), we believe tree frogs will consider the aquatic environment low quality. In addition, we also determine whether honeysuckle influences avoidance of experimental ponds (mesocosms) containing predators. If tree frogs determine that honeysuckle environments are unfavorable for their offspring, then we predict that honeysuckle will exacerbate the effects of predators on oviposition behavior.

METHODS

Site characteristics

We conducted this study from May to July 2009 at August A. Busch Memorial Conservation Area (BMCA) in St. Charles County Missouri, USA. Native to Asia, Amur honeysuckle (*Lonicera maackii*) has invaded forests throughout the BMCA (Watling and Orrock 2010). The honeysuckle invasion at BMCA is extensive across the 12km^2 of secondary oak-hickory forest (*Quercus* & *Carya* sp.). Management history is relatively similar across the entire BMCA (J. Vogel, personal communication). Gray tree frogs (*Hyla chrysoscelis/versicolor*) are common at BMCA and readily breed in ephemeral pools, ditches and road ruts.

Design of the study system

To test oviposition responses in environments with natural variability of honeysuckle density, we chose sites in order to control biotic and abiotic variables that may influence our results. Distances between experimental areas were from 0.5 to 6 km to ensure that individual tree frogs did not colonize multiple locations at our site (Semlitsch 2008). We chose areas that included a pond surrounded by a radius of at least 50m of forested upland habitat known to harbor most of the within-season breeding of gray tree frogs in Missouri (Johnson and Semlitsch 2003). We retained experimental areas if we found evidence of breeding (tadpoles or eggs) when sampling ponds or mesocosms (10 total areas). At each area, we measured the honeysuckle density surrounding the edge of nearby focal ponds (pond size was not statistically different between treatments, *t*-test: $t = -0.53$, $df = 9$, $P = 0.609$). Honeysuckle was present throughout BMCA but only transformed the habitat at high densities so we separated areas into two categories based on the density of honeysuckle: five invaded areas (mean number of stems per 1m radius ± 1 SD =

5.24 ± 1.88) and five uninvaded areas (0.34 ± 0.57). Honeysuckle was present throughout BMCA, but we only considered forests invaded when densities were high and honeysuckle transformed the environment. Therefore, we separated areas into two categories based on the density of honeysuckle: five invaded areas (mean number of stems per 1m radius ± 1 SD = 5.24 ± 1.88) and five uninvaded areas (0.34 ± 0.57). Invaded and uninvaded areas had significantly different honeysuckle densities (t-test: $t = 6.51$, $df = 9$, $P < 0.001$). Leaf-out occurred from both native trees and honeysuckle by the start of our experiment.

Mesocosms

To determine the effects of honeysuckle on habitat selection behavior we followed established methods to measure gray tree frog (*Hyla chrysoscelis*) oviposition behavior in mesocosms (Resetarits 1989, Binckley and Resetarits 2007). We deployed rectangular mesocosms measuring 23 × 14.5 × 5.5 cm (L × W × H) and inoculated them with ~20L of water containing bacteria, zooplankton and phytoplankton from adjacent ponds. We strained the pond water through a fine mesh net to limit transfer of competitors and predators. To simulate similar sized vernal pools near our mesocosms, we added 4.6 and 11.7L of local soil and dry leaf-litter respectively to each mesocosm. We included paired predator treatments in adjacent mesocosms (presence or absence of predators) within each area and distributed two pairs of mesocosms along the pond edge at two locations separated by >20m (40 mesocosms total). Data were pooled among predator and no-predator treatments at each site. Larval spotted salamanders (*Ambystoma maculatum*) that are known to cause reduced egg laying in gray tree frogs (Resetarits and Wilbur 1989) were used as predators in our experiment. Predatory salamanders were collected from five ponds across the

BMCA. After allowing mesocosms to settle for two days, we added predatory salamanders (10 per mesocosm). We replaced salamanders that doubled in size and absorbed their gills with younger animals from areas with later breeding.

From May to August 2009, we monitored mesocosms on every third day or following a rain event until no oviposition occurred for three consecutive weeks. In Missouri, gray tree frog eggs float on the surface within the first 24 hours following deposition and hatch in three-four days (Johnson 2000). We gently collected floating eggs or yolked larvae from mesocosms followed by dip-net sampling. Dip-net sampling continued in each mesocosm until ten consecutive sweeps with 20 x 15 cm nets resulted in no captures. To prevent transfer of predator chemical cues into predator-free mesocosms, we assigned nets for exclusive use for each treatment. We stored eggs and larvae in plastic containers for transport to the laboratory where we counted eggs and estimated the day of deposition based on known developmental timelines (McDiarmid and Altig 1999).

Analysis

To determine how honeysuckle influenced tree frog oviposition response to predators, we compared the total eggs and oviposition events per treatment (predator, predator-free) within each habitat specific area (invaded and uninvaded). To measure temporal changes in tree frog oviposition behavior, we analyzed the mean Julian day when oviposition occurred and weighted residuals sum of squares by the number of eggs deposited. We fit the total number of eggs and oviposition events with separate generalized linear mixed effects models assuming negative binomial or Poisson distributions, respectively, using SAS Proc GLIMMIX. Denominator degrees of freedom were estimated with the Kenward-Rogers method (Littell et al. 2006b). We modeled habitat areas (invaded and uninvaded) as fixed effects and experimental area as a random effect. We fit Julian date of deposition with a mixed effect model (Proc MIXED) and Gaussian distribution. Because tree frogs deplete their eggs over time and can decide how many eggs they lay, we also weighted deposition Julian date by the number of eggs laid, where we scale each deposition date using a loss function (WEIGHT statement SAS 9.2). We also calculated Julian date deposition following removal of egg depositions less than 30 because they are not representative of a full clutch size (McDiarmid and Altig 1999).

RESULTS

Besides tree frogs, other colonizers included adult scavenger beetles (Hydrophillidae), backswimmers (Notonectidae) and damselfly naiads (Zygoptera). Leeches (Glossiphoniidae), whirligig beetle (Gyrinidae), and scavenger beetle larvae (Hydrochara) were also observed, and likely colonized via propagules in local soil or water transplanted into mesocosms.

Number of eggs deposited

From May to July 2009, tree frogs deposited 5,771 eggs. There was a significant honeysuckle-by-predator effect on the number of eggs deposited (Table 1). When in invaded plots, tree frogs laid significantly fewer eggs in the predator treatment (Fig. 1A). Tree frogs laid a greater number of eggs in predator-free treatments within invaded plots than either treatment (predator or predator-free) within uninvaded plots (Fig. 1A), which did not have significantly different egg numbers from one another ($t = 0.59$, $df = 5.59$, $P = 0.575$). Although tree frogs deposited over twice the number of eggs in uninvaded than invaded areas, there was not a significant difference of mean density between treatments (Table 1).

Oviposition frequency

There were 15 oviposition events on 11 nights. Across the 10 areas, tree frogs colonized five uninvaded and two invaded areas. There was a marginal significance of the combination of density and mesocosm treatment on oviposition frequency (Table 1). This interaction occurred because there were significantly more oviposition events in predator-free treatment in areas with invaded (t = 2.51, P = 0.023) but there was no difference between mesocosm treatments in uninvaded areas (t = 1.14, P = 0.672) (Fig. 1B). Invaded areas had a marginally lower number of oviposition events compared to uninvaded plots (P = 0.059, Table 1) and there were no oviposition events in predator mesocosms within invaded areas (Fig. 1B).

Egg deposition over time

Tree frogs did not colonize any predator mesocosms in the invaded areas resulting in a lack of degrees of freedom to determine how density interacts with predator treatments to influence date of deposition (Table 1, Fig. 1C). Therefore, we do not interpret the interaction between mesocosm and density treatments. Tree frogs did not, however, alter oviposition date in response to predators in the overall model (Table 1) but tree frogs oviposited later, to a marginal degree, in response to predators when compared in uninvaded areas only ($F_{1,8}$ = 4.78, P = 0.06, Fig. 1C). Tree frog oviposition was significantly earlier in invaded areas when weighted by the number of eggs deposited, to a lesser but still significant degree when we removed low egg counts, and not significantly different when the number of eggs were not considered in the analysis (Fig. 2).

DISCUSSION

In this study, we used a large-scale field experiment to determine whether tree frogs alter habitat selection in response to invasive plants. Oviposition responses to predators were strongest in invaded forest with fewer eggs at a lower oviposition frequency. In addition, honeysuckle altered the timing of egg deposition where tree frogs deposited much earlier in invaded compared to uninvaded areas. Following transformation of environments they invade, exotic plants may alter interactions between prey and their predators simply by influencing the quality of the habitat. Because invasive plants are a pervasive threat to habitat quality across a landscape, our work reveals how populations and communities of native species are influenced by invasive plants through altered habitat selection behaviors. In addition, our results suggest a potential for an ecological trap for native amphibians that are attracted to poor quality environments created by invasive plants (Schlaepfer et al. 2005).

Response to predators and habitat context

Predator avoidance only occurred in invaded areas, which further supports that invasive plants alter the quality of native environments, thereby influencing the habitat context for colonizing animals. In invaded areas, tree frogs deposited fewer eggs and had fewer oviposition events in response to the predator treatment. Conversely, tree frogs did not avoid predators in uninvaded areas. Even though tree frogs are well-known for avoiding predators (Resetarits and Wilbur 1989; Resetarits et al. 2005), a lack of anti-predator behavior is possible. Vonesh and Buck (2007) also found that tree frogs did not avoid salamander predators and dismissed this result as a function of a low predator density compared to previous studies (Resetarits and Wilbur (1989): 4.7 salamanders m^{-2}; their study: $0.32m^{-2}$). However, we cannot make the same conclusion because our mesocosms had over 30 times the salamander density of the previous studies mentioned by Vonesh and Buck. Studies vary in experimental conditions that could inadvertently alter habitat selection depending on relative habitat quality (context). Changes in habitat quality influence prey response to predators if a habitat is associated with other risks (Resetarits and Binckley 2009) or all nearby habitats are deemed equally unfavorable (Fretwell and Lucas 1970). If a favorable habitat is adjacent to another, less favorable habitat that influences perception, animals may avoid the suitable habitats associated with the less favorable habitat (Resetarits and Binckley 2009, Resetarits et al. 2005). For example, animals may avoid suitable habitats that are associated with a greater density of predators or competitors (Resetarits et al. 2005). Alternatively, colonizers must choose less favorable habitats if preferred habitats are non-existent or co-opted by incompatible species (Resetarits et al. 2005). Therefore, it is possible that the quality of nearby ponds or even cues provided by the terrestrial environment will influence decisions to colonize mesocosms with predators. Unlike uninvaded forest, the areas invaded with honeysuckle may provide an enemy-free space so tree frogs are capable of avoiding mesocosms with predators without influence by low quality environments nearby. If elevated predator and competitor populations decrease the quality of uninvaded areas, then tree frogs adopt a strategy to colonize all available breeding pools because they are likely all unfavorable. By attracting tree frogs for early breeding, we believe the invaded forest provides favorable conditions for responding to predators. Tadpoles of the common frog (*Rana temporaria*), for example, do not respond to predators if their eggs are deposited 20 days later than those that have antipredator responses early in a breeding season (Orizaola et al. 2012). Honeysuckle, therefore, decreases tree frog exposure to predators, at least temporarily, by attracting tree frogs to these habitats at a time when predation risk and competition is low.

Egg deposition over time

Even though there were fewer deposition events, invaded areas attracted tree frogs to breed, on average, nearly a month earlier than tree frogs in uninvaded areas. Earlier in the season, competition and predation may be lower in honeysuckle, which could explain altered tree frog breeding phenology. Insects and amphibians avoid laying eggs in habitats with closed forest canopies (Binckley and Resetarits 2007) and therefore likely avoid habitats invaded with honeysuckle that leafs-out before most native plants (Collier et al. 2002). Consequently, early leaf-out of honeysuckle may prevent an elevated density of competitors and predators of tree frogs. Deviations in competition and predation within breeding environments alter tree frog oviposition (Marsh and Borell 2001; Resetarits and Wilbur 1989; Blaustein and Kotler 1993; Spieler and Linsenmair 1997; Resetarits and Wilbur 1989). Therefore, honeysuckle may temporarily provide enemy-free space until competitors and predators colonize the less-favorable honeysuckle environment or density effects alter the quality of the habitat and tree frog decisions to colonize invaded areas (Fretwell and Lucas 1969).

Changes in abiotic environment also influence habitat selection. With a novel layer of dense vegetation in the understory of forests, invasive shrubs alter light penetration (Luken et al. 1997, Collier et al. 2002), reduce soil temperatures (Herrera 1997, Watling et al. 2011c) and increase humidity (Chen et al. 1999). In a study of amphibian communities at our site, green frog

(*Lithobates clamitans*) populations were greater in invaded areas, which had lower daily mean (16.92°C) and maximum (21.93°C) temperatures compared with uninvaded areas (Watling et al. 2011c). Unlike sympatric species, green frog prevalence in honeysuckle was partially attributed to preference of relatively cool temperatures created by honeysuckle that coincided with green frog thermal maxima (Watling et al. 2011c). An alternative but less plausible explanation for shifts in breeding phenology is that honeysuckle causes changes in the microclimate that attracts tree frogs to breed early in the season. However, gray tree frogs in our study have a relatively high thermal maxima (38.7°C, John-Alder, Morin, and Lawler 1988) compared to sympatric amphibians at our site (20 and 30°C, Knowles and Weigl 1990). In addition, maximum temperatures or changes in humidity are likely negligible when tree frogs colonized during the spring. Gray tree frogs in Missouri are most active when temperatures are greater than 16°C (Johnson 2000) and there is some evidence that thermal physiology of anurans is phenotypically plastic (Navas et al. 2008). Regardless of the mechanism that attracts tree frogs to honeysuckle, tree frogs may not detect all of the threats associated with the honeysuckle invasion, like accelerated drying of vernal pools (Boyce et al. 2011) or increased predator densities (Pearson 2009).

Conclusions and implications

Our results provide evidence that invasive plants may change amphibian populations by influencing habitat selection through suboptimal decisions. In our study, tree frogs bred earlier and had stronger predator avoidance responses when exposed to invaded areas, which may still provide costs to tree frogs. For anurans, late but not early arrival into breeding ponds reduces competition with early Spring-time colonizers (Lawler and Morin 1993). Early breeding can be very dangerous to anurans where eggs laid early take longer to develop and risk being exposed to freezing (Loman 2009). In addition, honeysuckle likely attracts tree frogs to environments with a cue that conveys a safe habitat (i.e. decreased predation) but may expose tree frogs to other threats. For example, aquatic environments with leaves from honeysuckle cause elevated mortality, accelerated development and altered behavior of larval anurans (Watling et al. 2011a, 2011b). Environments invaded by honeysuckle also cause rapid drying of ponds from elevated transpiration (Boyce et al. 2011) which can result in a cost to amphibians of either desiccation or early metamorphosis. Even though we did not determine survival or measure population growth, our results suggest the potential for honeysuckle to either provide an ecological trap for tree frogs that are attracted to environments that threaten survival (Schlaepfer et al. 2002) or decrease predation risk and facilitate antipredator behaviors that benefit survival (Rodriguez 2006).

Table 1: ANOVA table from number of eggs deposited in mesocosms, oviposition frequency (number of colonization events) and date of deposition in response to factors of honeysuckle density, mesocosm (predator, predator-free) and the density-by-mesocosm interaction. Because there were no depositions in the predator mesocosms within honeysuckle, we could not estimate the honeysuckle-by-predator effect. Bold text represents factors that have at least a marginal difference. x indicates crossed factors.

Response	Effect	*Num DF*	*Den DF*	*F*	*P*
Num of Eggs	**Mesocosm**	**1**	**16**	**15**	**0.001**
	Density	1	16	0.4	0.534
	Density x **Mesocosm**	**1**	**16**	**14.16**	**0.002**
Oviposition frequency	**Density** x **Mesocosm**	**1**	**16**	**7.7**	**0.013**
	Density	**1**	**16**	**4.1**	**0.059**
	Density x **Mesocosm**	**1**	**16**	**4.42**	**0.051**
Deposition Date	Mesocosm	1	12	2.74	0.124
	Density	**1**	**12**	**33.33**	**<0.001**

Figure Legends

Figure 1: Panels show least square means (± 1 SE) of (A) total eggs, (B) number of oviposition events and (C) date of deposition into predator free or predator present mesocosms when tree frogs were breeding in invaded or uninvaded areas. Bars above treatments indicate no significant difference between treatments and asterisk * indicates significant difference.

Figure 2: Mean Julian date of deposition (± 1 SE) in invaded and uninvaded areas where the response variable was (A) not weighted, (B) removed if low egg counts ($N < 30$) occurred, and (C) weighted by the number of eggs laid on a deposition event, where deposition dates are scaled by a loss function (WEIGHT statement SAS 9.2).

Figure 1

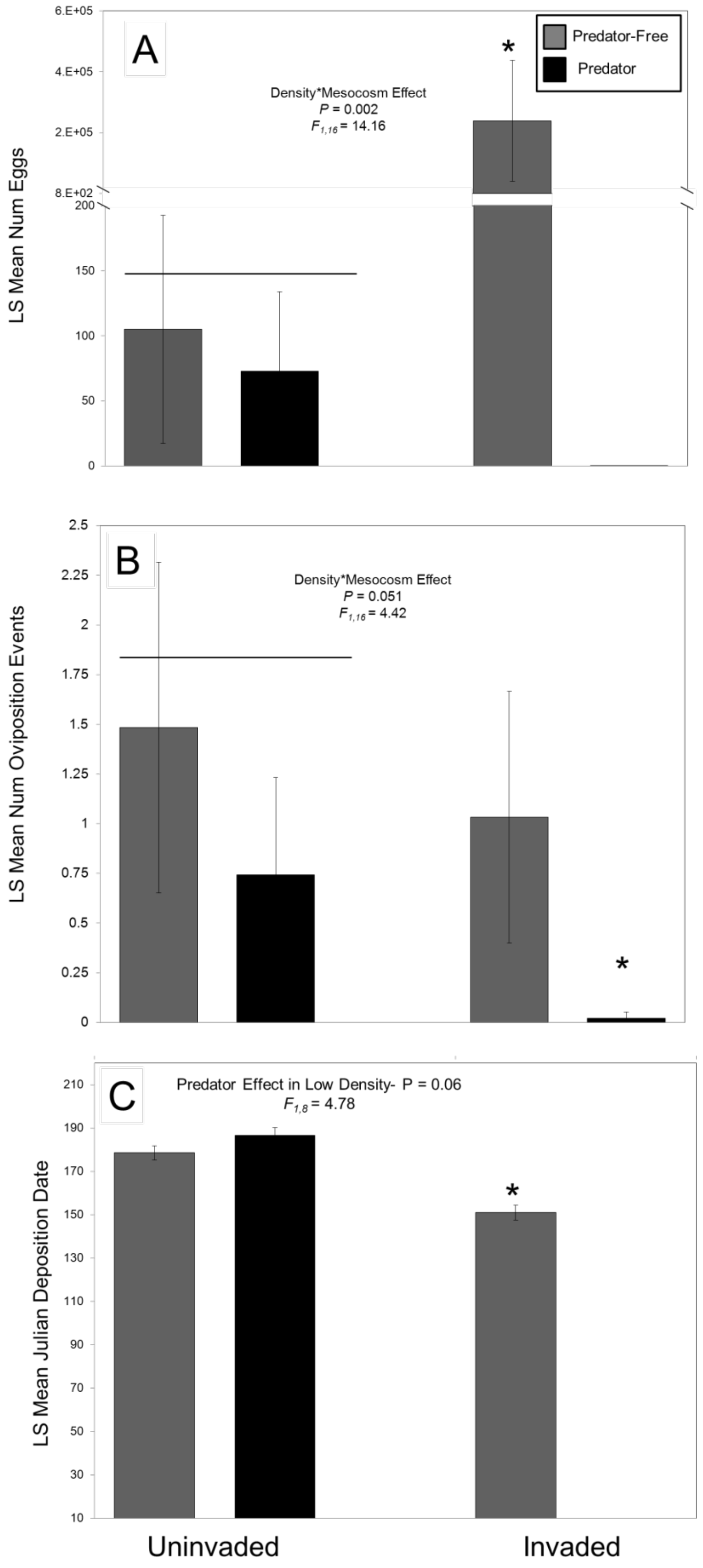
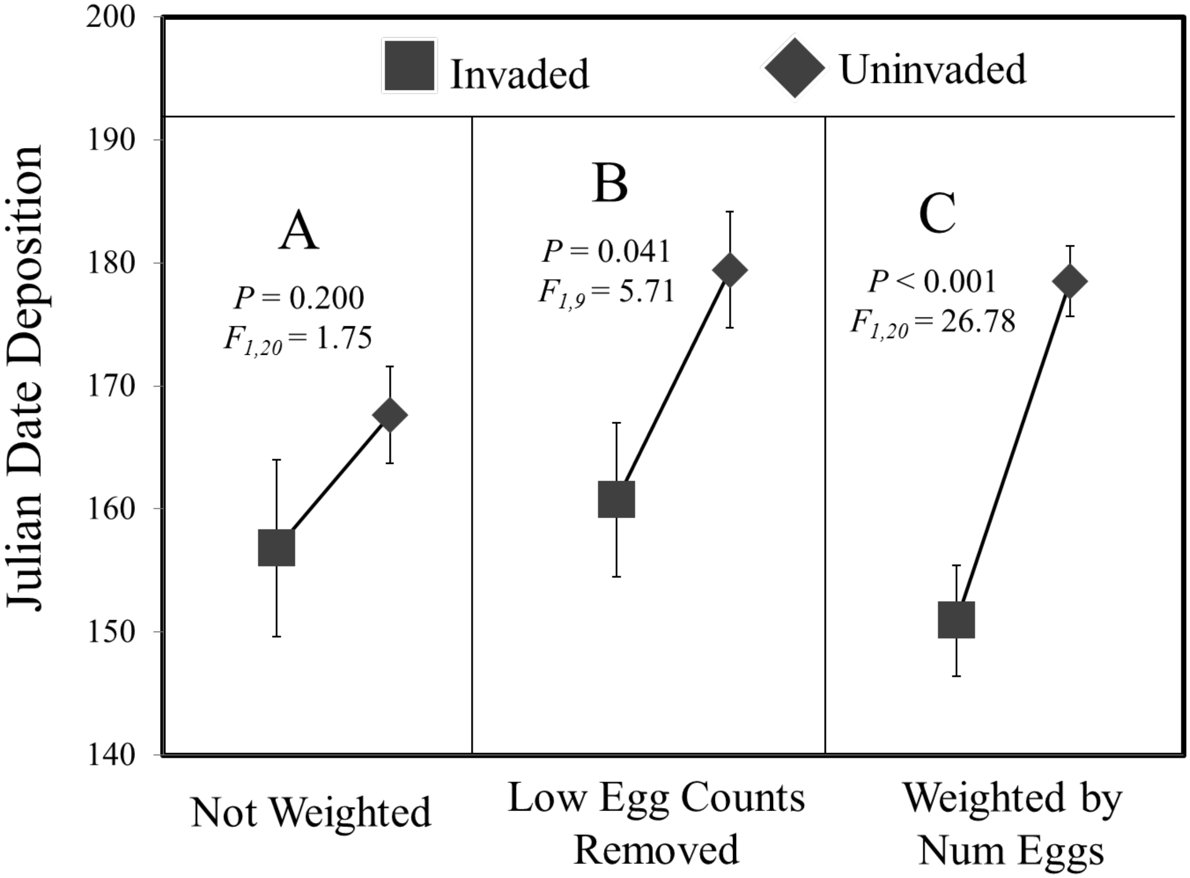

A
6.E+05
4.E+05
2.E+05
8.E+02
200
150
100
50
0
LS Mean Num Eggs
Predator-Free
Predator
Density*Mesocosm Effect
P = 0.002
$F_{1,16}$ = 14.16
*
B
2.5
2.25
2
1.75
1.5
1.25
1
0.75
0.5
0.25
0
LS Mean Num Oviposition Events
Density*Mesocosm Effect
P = 0.051
$F_{1,16}$ = 4.42
*
C
210
190
170
150
130
110
90
70
50
30
10
LS Mean Julian Deposition Date
Predator Effect in Low Density- P = 0.06
$F_{1,8}$ = 4.78
*
Uninvaded
Invaded

Figure 2


200
190
180
170
160
150
140
Julian Date Deposition
■ Invaded
◆ Uninvaded
A
$P = 0.200$
$F_{1,20} = 1.75$
B
$P = 0.041$
$F_{1,9} = 5.71$
C
$P < 0.001$
$F_{1,20} = 26.78$
Not Weighted
Low Egg Counts Removed
Weighted by Num Eggs

Chapter 3

Invasive plant litter induces phenotypic changes in tadpoles that coincide with antipredator traits

Caleb R. Hickman and John L. Orrock

ABSTRACT

One of the most important goals in conservation biology is to understand how native species respond to exotic invaders. Through changes in aquatic environments by leaf leachates, invasive plants can induce phenotypic trait changes, such behavior or development, in native tadpoles. However, native predators also induce changes in phenotypes like behavior, development and morphology. Not yet understood are how phenotypes induced by native predators correspond to phenotypic changes induced by non-native plants. We manipulated wetland communities using outdoor mesocosms to investigate whether aquatic environments invaded by invasive Amur honeysuckle (*Lonicera maackii*) influenced phenotypic traits of green frog (*Lithobates clamitans*) and tree frog (*Hyla chrysoscelis*) tadpoles. By crossing honeysuckle leaf-litter treatments with different degrees of predation risk by larval dragonflies (*Anax junius*) (predators: caged, free-roaming, absent), we determined whether phenotypes induced by honeysuckle coincided with or altered phenotypes induced by predators. We measured behavioral, morphological and developmental phenotypes of both tadpole species and determined their survival from each treatment combination. When exposed to caged predators, green frogs decreased their presence near the surface while tree frogs increased their surface presence. Honeysuckle induced tree frog behavioral changes that were nearly identical to their antipredator responses (greater proportion visible and near the water surface). In response to predators, green frogs accelerated development and grew longer body sizes. Honeysuckle induced morphological

changes of shallow tail depths in green frogs, which coincides with predator-induced tail depths from other studies. As expected, free-roaming predators were most lethal to both green and tree frogs compared to either caged predator or predator-free treatments. Neither honeysuckle nor the honeysuckle-by-predator interaction influenced mortality of either tadpole species. Therefore, we have no evidence that phenotypes induced by honeysuckle influence risk of predation. To our knowledge, this is the first study to show that an invasive plant can induce a morphological change in amphibians. Moreover, our results suggest that a widely invasive plant is capable of inducing phenotypic changes in tadpoles that are similar to antipredator responses. Therefore, invasive plants may induce traits with similar costs and consequences as those induced by the risk of predation.

INTRODUCTION

A long-standing concern in conservation is how native animals respond to and recover from the invasion of exotic species (Elton 1958). In addition to rapid evolution, native species may use phenotypic plasticity in response to exotic invaders (Hendry et al. 2008). Native species can react to an exotic invader through changes in physiology (Price-Rees et al. 2012), behavior (Greenlees et al. 2010, Sih et al. 2010, Freidenfelds et al. 2012) and/or morphology (Phillips and Shine 2004, Langkilde 2009, Wright et al. 2012). If invasive species induce trait changes in native animals (Strauss et al. 2006, Carroll 2007), these changes can either come at a compromise or they may facilitate traits that are also beneficial for responding to native environmental conditions (Schlaepfer et al. 2005, Rodriguez 2006, Wright et al. 2012). For example, native fence lizards (*Sceloporus undulatus*) grow longer hind limbs and flee in response to invasive fire ants (*Solenopsis invicta*) (Langkilde 2009). The lizards' response to fire ants also increases hormones for stress (Trompeter and Langkilde 2011), thereby reducing the lizards' ability to respond to native predators. Alternatively, an invasive plant (*Bryophyllum* spp.) can facilitate survival of a native omnivorous lizard (*Tiliqua scincoides*) to become more tolerant to invasive cane toad (*Rhinella marina*) toxins if lizards are first exposed to chemicals released by the invasive plant (Price-Rees et al. 2012).

Invasive plants may affect traits of native species by changing the quality of the chemical environment of native ecosystems (Vilà et al. 2011). For example, invasive plants can secrete secondary compounds from roots and leaves that alter the chemical environment of nearby aquatic ecosystems via soil water or surface water pathways (Gordon 1998, Ehrenfeld 2003). Leaves from invasive plants in terrestrial environments can fall into aquatic ecosystems and negatively impact growth and survival of invertebrates (Canhoto and Laranjeira 2007, Going and Dudley 2008, Leonard 2008) and larval amphibians (Maerz et al. 2005a, Brown et al. 2006, Leonard 2008, Watling et al. 2011a). Previous studies have shown that by altering the chemical environment, invasive plants cause changes in larval amphibians including increased movement activity, reduced hatching success, decreased growth and accelerated development (Brown et al. 2006, Watling et al. 2011a, 2011b, Adams and Saenz 2012). However, studies have never determined whether litter from invasive plants induces phenotypic changes that represent compromises or facilitation with phenotypes used in response to native environmental pressures.

Organisms benefit from having plastic traits if environments are altered or new risks arise (Zhivotovsky et al. 1996, Pujol-Buxó et al. 2013). Different environments generate selective pressures that may favor multiple phenotypes simultaneously. Animals may exhibit multiple phenotypes that represent compromises or facilitations between one another (Gomulkiewicz & Kirkpatrick 1992). Larval amphibians are a model for understanding what environmental characteristics drive phenotypic plasticity in animals. Some phenotypes of tadpoles induced by particular predators, like tail shape, come at a compromise to phenotypes induced by competitors (Relyea 2004a, Relyea and Auld 2005) or other predators (Lass and Spaak 2003, Relyea 2004a). In a recent study, an herbicide induced morphological changes in American toad tadpoles (*Anaxyrus americanus*). The morphological changes in the toad tadpoles were in the same direction and of the same magnitude as those induced by a native predator (Relyea 2012). Tadpole phenotypes can be induced by both the quality of plant litter (Stoler and Relyea 2013) and presence of predators (Relyea 2004b), which may have very similar or very different effects on phenotypes when combined.

The goal of this study was to determine the consequence of trait changes induced by invasive plants when tadpoles must also respond to predators. To achieve this goal, we reared two tadpole species (green frogs, *Lithobates clamitans*, and tree frogs, *Hyla chrysoscelis*) in aquatic habitats with leaf-litter from an exotic shrub, Amur honeysuckle (*Lonicera maackii*) and exposed tadpoles to a predator (larval dragonflies, *Anax junius*). Following exposure to predators and the honeysuckle environment, we measured behavior, morphology, development and survival of each tadpole species. Honeysuckle causes changes to aquatic environments that result in mortality as well as elevated surfacing activity and accelerated development of some tadpoles (Watling et al. 2011a, 2011b). Larval dragonfly predators (*Anax*) also cause tadpoles to alter their behavior, morphology and development (VanBuskirk 2002, Fraker 2009). However, predator induced traits of tadpoles are very plastic and may be compromised or reinforced by traits induced by competitors or other predators (Relyea 2001a, 2004a, Warkentin and Touchon 2008, Fraker 2009, Nunes et al. 2012). Similar to predator induced morphologies, tadpoles vary their tail depth in response to the quality of leaf litter in their aquatic environment (Stoler and Relyea 2013). In environments invaded by honeysuckle, tadpoles should also encounter predators on a regular basis. Therefore, it is beneficial for tadpoles to respond to predators despite the changes caused by honeysuckle. American toads (*Anaxyrus americanus*) exposed to honeysuckle leachates are able to reduce swimming movements in response to predators despite the tendency to surface frequently in honeysuckle (Hickman, Chapter 1). Honeysuckle also induces accelerated development in tadpoles (Watling et al. 2011b), where development can coincide with morphological changes in response to predators (Hossie and Murray 2012). In our study, we determine whether honeysuckle would induce phenotypic changes and whether these changes would coincide or conflict with phenotypes induced by predators. Therefore, we measured 1) how honeysuckle would influence phenotypes (including behavior and morphology), 2) how phenotypes relate to those induced by predators, and 3) whether there is an elevated mortality for tadpoles exposed to the combination of predators and honeysuckle. Because honeysuckle induces behavioral changes, alters the quality of the aquatic environment and induces accelerated development in some tadpoles, we believe that honeysuckle should induce phenotypic changes that rival those induced by predators. Since studies suggest honeysuckle-induced changes are species-specific (Watling et al. 2011a), we believe tree frogs and green frogs will have different phenotypic responses which may only occur in the most plastic traits. Because there is some evidence that tadpoles can maintain antipredator traits in the presence of traits induced by honeysuckle (Hickman, Chapter 1), we predict that honeysuckle will not expose tadpoles to increased risk of predation.

METHODS

Amur honeysuckle background

Originally from Asia, Amur honeysuckle (*Lonicera maackii*) is a shrub that has invaded temperate forests throughout the eastern and mid-western USA (Luken and Thieret 1996). In addition to over-shading, honeysuckle outcompetes other plants by releasing allelopathic chemicals from their roots and leaves (Dorning and Cipollini 2005; Cipollini and Dorning 2008). Honeysuckle leaf leachates decrease survival and accelerate development of tadpoles (Watling et al. 2011; Watling, Hickman, and Orrock 2011) and causes tadpoles to increase movement to the water surface (Watling et al. 2011a). Although the specific mechanism is unknown, leachate s from some plants disrupt gill lamella function (Temmink et al. 1989), which may impact resilience of larval amphibians to hypoxia and induce stress responses. In previous studies, honeysuckle environments in laboratory and field did not influence dissolved oxygen or total phenolics compared to native species (Hickman unpublished; Watling et al. 2011b). However, the presence and/or concentration of specific phenolic compounds may be responsible for impacts on amphibians (e.g. apigenin and luteolin in honeysuckle, Cipollini et al. 2008). In addition, honeysuckle leaf-litter reduces the soil and water quality of native environments (Poulette and Arthur 2012, McNeish et al. 2012), which may influence tadpole morphologies (Stoler and Relyea 2013).

The mesocosm experiment

We constructed 76 mesocosms and two predator cages for each mesocosm. Mesocosms consisted of 68L plastic tubs measuring 19.75 x 16 x 15.5 cm (L x W x H) positioned into four rows under a canopy of oak and hickory forest (*Quercus spp.* & *Carya spp.*) at Washington University's Tyson Research Station in St. Louis CO. Missouri. The entire array was covered by 95% ± 1.01 (mean ± 1SE) canopy, measured with a concave spherical densiometer (Halverson et al. 2003). Plastic tops were cut so that the edges would close tightly on a fiberglass mesh to prevent colonization of other organisms, but allow air, sunlight and precipitation to enter. We deployed six treatment combinations represented by a cross between two habitat treatments (honeysuckle and native leaf environments) and three predator treatments (caged predator, free-roaming predator, predator-free control) replicated 12 times for 72 experimental units. In addition, we created four sampling mesocosms represented by each honeysuckle-by-predator (caged and free-roaming) treatment in order to determine survival in the first 24-hour period (which was 100%) and estimate when to terminate the experiment based on predator-related mortality.

We stocked mesocosms with habitat made up of soil and leaves from five sites that were dominated by native (*Quercus spp.* & *Carya spp*.) and invaded (Amur honeysuckle, *L. maackii*) vegetation. Leaves were dried, homogenized in a single container and 200 grams were added per mesocosm. Soil was also homogenized from five locations for invaded and native habitats but not fully dried in order to retain any soil microbes that would be important for decomposition or nutrient cycling in local habitats. We filled mesocosms with 40L of well water and inoculated each with 500mL of pond water homogenized from five open canopy sources without honeysuckle nearby to establish controlled levels of primary productivity. Mesocosms were allowed to accumulate resources such as plankton for 10 days before introducing tadpoles.

We collected partial egg masses of green frogs (*Lithobates clamitans*) (N=4) between 7 to 9 June 2010 and tree frogs (*Hyla chrysoscelis/versicolor*) (N=5) from 9 to 11 June 2010 at Tyson Research Station and held them in captivity until tadpoles lost yolk sacs and were free swimming. On 18 June 2010, we homogenized clutches by species to prevent sibling effects and added larvae of 25 green frogs (*Lithobates clamitans*) and 25 tree frogs (*Hyla chrysoscelis/versicolor*) into each mesocosm. These densities correspond to those found in nature (W. Resetarits, personal communication). On 19 June 2010 we deployed larval dragonfly predators (*Anax junius*) into PVC cages (two cages/mesocosm, ~33X16cm ea.; N=104 predators) and mounted cages vertically inside all predator mesocosm treatments (including free-roaming and no predator treatments) to promote chemical distribution of predator cues throughout the water column. Predators (lethal and free-roaming) were caged for 2 days prior to the start of the experiment to provide tadpole prey an opportunity to detect predator chemical cues and time to establish refuge before direct predation could occur. On 21 June, we released *Anax* from cages allocated to the free-roaming treatment. To ensure predation cues were present, we fed caged

Anax a focal tadpole from each species eight times over 26 days resulting in the consumption of 32 tadpoles in each caged predator mesocosm. If *Anax* perished, we replaced them within a day.

Behavioral measurements

One of our main objectives was to determine whether honeysuckle affected tadpole behaviors. Because free-roaming predators consumed a large number of tadpoles and low numbers of tadpoles are difficult to observe, we excluded the free-roaming predator treatment from analysis of behavior. We lightly pried off lids of mesocosms and tadpoles were provided five minutes to re-acclimate prior to observations. Each mesocosm was observed for 180s.We counted the number of tadpoles from each species that were 1) visible and 2) near the surface in each mesocosm and averaged observations made on 6, 9 and 12 July. Visible tadpoles were considered active and inactive tadpoles were assumed to be under leaf-litter. We defined the surface as the top one-third of the mesocosm. After we emptied mesocosms at the end of the experiment, we used the final count of tadpoles to determine the proportion we observed visibly active and near the surface within each mesocosm.

Morphological & developmental measurements

Because invasive plants, including honeysuckle, influenced growth and development of tadpoles (Watling et al. 2011b, Cohen et al. 2012, Rogalski and Skelly 2012, Cotten et al. 2012, Martin and Blossey 2013) and the most commonly measured phenotype of tadpoles is morphology, one of our main objectives was to determine if honeysuckle affected tadpole morphology and development. Free-roaming predators consumed most of the tree frogs in our experiment, so we were unable to include the free-roaming predator treatment in our analysis for tree frogs.

Soon after collection and preservation of surviving tadpoles at the end of the experiment, we photographed animals with a digital camera for measurement of physical characteristics. From images, we made morphological measurements using ImageJ (Version 151 1.6.0_20, NIH). We measured (1) total length from the tip of the nose to the end of the tail; (2) tail depth residuals from total length where tail depth is the widest area from the top to the bottom of the mid-tail; (3) the proportion of the total length that is tail (prop tail). We used reliable subsamples to ensure tail fins were mounted in order to measure tail depth. We scored gosner developmental stages (McDiarmid and Altig 1999) and averaged subsamples of randomly selected individuals per species (depending on survival) from each mesocosm (n = 10). Size of tadpoles did not correspond to density within any treatment.

Survival

We ended the experiment and collected survival data from 14 to 17 July 2010. To locate tadpoles we sorted out leaves, dip-netted and sifted mesocosm contents through fine-meshed nets. We euthanized tadpoles as they were collected from the mesocosms with an initial treatment of Orajel© on head and body followed by submersion into solution of MS-222. We mounted tadpoles on a profile to display tail fins and stored them frozen until image capture.

Statistical analysis

We estimated behaviors (mean proportion active or at surface) and the number of tadpoles of each species that survived in response to native and honeysuckle leaf environments crossed with the free-roaming predator, caged predator and predator-free control treatments using univariate analysis of variance (ANOVA). To determine how honeysuckle leachate s and predators influenced morphology, we used both univariate (ANOVA) and multivariate analysis of variance (MANOVA). MANOVAs detect overall effects of treatments on response vectors and ANOVAs were used to determine which specific responses accounted for the overall differences among treatments in the MANOVAs. For significant univariate effects of honeysuckle, we conducted Tukey's post-hoc pairwise comparisons to determine differences between treatments.

RESULTS

Behavior

Predators did not influence the number of green frogs visible but honeysuckle induced a marginally significant increase in the number of green frogs visible (Table 1). There was no honeysuckle-by-predator effect on the number of green frogs visible (Table 1). Caged predators caused a significantly lower number of green frogs to be near the surface but honeysuckle alone did not alter the proportion of green frogs at the surface (Table 1). The non-significant predator-by-honeysuckle interaction indicates that the observed negative effects on surface behavior by predators and honeysuckle were either additive or there was too much variation to detect a difference in the magnitude of change (Table 1, Fig. 1). Non-significant differences of the honeysuckle-by-predator interaction on surface behavior was unlikely due to variability in surface behavior because variances between honeysuckle and native were not significantly different (Levene's test: $F_{1,48} = 0.45$, $P = 0.506$).

For both honeysuckle and caged predator treatments, there were significantly more tree frog tadpoles visible (Table 1). Because predators and honeysuckle induced visibility in the same direction, the combination of honeysuckle and predators did not alter the trend of increased visibility of either treatment (no significant honeysuckle-by-predator interaction, Table 1). Similarly, we observed significantly more tree frogs near the surface in response to both honeysuckle and caged predator treatments (Table 1). The non-significant interactions were unlikely due to elevated variability because the variance of the proportion visible (Levene's test: $F_{1,48} = 0.756$, $P = 0.389$) and surface behaviors (Levene's test: $F_{1,48} = 0.701$, $P = 0.407$) between honeysuckle and native environments were not significantly different.

Morphology & Development

Following normalization procedures, tadpole density did not influence development or size within treatments; therefore, our results are not a consequence of predator-related thinning. Only green frogs responded to both predators and honeysuckle with changes in morphology and development (Table 2). In green frogs, honeysuckle induced significantly smaller tail depth and free-roaming predators induced longer total lengths (Fig. 2). Green frogs accelerated development in response to free-roaming predators compared to caged predators (pairwise: $t = -3.01$, $df = 67$, $P < 0.01$). However, green frog development in the predator-free control treatment was intermediate between but not significantly different from development in caged and free-roaming predator treatments (pairwise: both $P > 0.124$). Overall, tree frog tadpoles responded to caged predators with a marginally longer total length than tadpoles reared in predator free mesocosms (Table 2, Fig. 2).

Survival

Both green frogs and tree frogs suffered more mortality in response to free-roaming dragonfly predators (Table 3). Only two tree frog tadpoles survived the free-roaming predator treatment and green frogs lost an average of 78 percent of mesocosm populations to free-roaming predators. In response to caged predators, tree frog survival was greater compared to tree frogs not exposed to predators (pairwise: $t = -2.58$, $df = 70$, $P = 0.012$, Fig. 3). Survival of neither tadpole species was influenced by honeysuckle and honeysuckle did not affect the differences in survival of tadpoles among predator treatments (All $P > 0.459$).

DISCUSSION

Our results show that invasive plants are capable of inducing phenotypic changes in amphibians in the same direction and magnitude as phenotypes induced by predators. In our study, we exposed green frog and tree frog tadpoles to treatment combinations of aquatic environments with predation risk and invasive plant leaves. Caged predator cues influenced behavior of green frogs and tree frogs. The honeysuckle leaf treatment induced changes in morphology of green frogs and behaviors of tree frogs that were similar to their responses to predators. Honeysuckle, however, had no influence on tree frog or green frog survival, which indicates that phenotypic changes induced by honeysuckle did not influence their susceptibility to either consumptive or non-consumptive effects of predators.

Predator induced traits

In our experiment, green frog and tree frog behaviors were similar in response to caged predators but caged predators induced only a single morphological character. Predator-induced behaviors (Kats and Dill 1998, Richardson 2001b, Ferrari et al. 2010, Takahara et al. 2012) and morphologies (Relyea 2001b, 2003b, 2004a, VanBuskirk 2002) occur in a variety of larval amphibians to reduce risk of predation. Common behavioral responses of tadpoles to predator chemical cues are reduced movement, increased refuge use, increased swimming and avoidance (Kats and Dill 1998, Richardson 2001b). Similar to previous studies, green frogs responded to predators with reduced activity near the surface (McIntyre and McCollum 2000, Relyea 2001b, Relyea and Yurewicz 2002) with a smaller proportion of tadpoles visible (McIntyre et al. 2004, Smith et al. 2008). In contrast, tree frogs became more active in response to predators (more visible and at surface), which corresponds to several tree frog species that use these behaviors as preparation for fleeing (Richardson 2001b). Though surface activity (i.e. vertical migration) is a well-studied behavior for several aquatic organisms escaping predation (e.g. Crustaceans, Insects, Fish: Kats and Dill 1998, Lass and Spaak 2003), this behavior has received less attention in amphibian studies (Moore and Townsend 1998, McIntyre and McCollum 2000). African clawed frogs (*Xenopus laevis*), for example, decrease surfacing frequency in response to the physical presence of a surface-swimming predator (garter snake, *Thamnophis couchi*) except when exposed to a benthic predator (snapping turtle, *Chelydra serpentina*) (Baird 1983). Therefore, different responses to predators may be due to the threat-sensitive nature of the interaction (Helfman 1989), where predation risk and associated responses vary greatly among larval anurans (Laurila and Kujasalo 1997, Richardson 2001a, Relyea 2004a). Differences between green frog and tree frog phylogeny and ecology explain their distinctive morphologies and behaviors when exposed to predators, including *Anax* used in our study (Richardson 2001b).

In contrast to other studies, neither tree frogs nor green frogs increased tail depth in response to predators in our study. Tree frogs generally respond to predators by deepening their tails (Relyea and Werner 2000b, Relyea 2001b, 2002a, 2003a) regardless of ontogeny (Relyea 2003a). However, tree frog tail depth can be significantly smaller in response to certain predators (e.g. *Belostoma*) (Relyea 2001b) or when tadpoles densities are greater (Relyea 2002a). Our mesocosms were at higher densities than previous studies (1 tadpole/ 0.74 L in our study versus 1 tadpole / 0.6 L in others), which may explain why tree frogs did not induce larger tail depths in response to predators. Green frogs, on the other hand, may not respond to *Anax* (the predator in this study) with a change in tail depth, but green frogs do reduce tail depth in response to fish, amphibian and beetle predators (Relyea 2001b). If tadpoles allocate energy to construct a larger body rather than tail depth, then a possible function is that the larger body provides increased surface area for digestion and continued growth when faced with competition (Relyea 2001b). However, body size alone (inversely related to proportion of the body that is tail) was not different in response to predators or the honeysuckle environment. Green frogs in this experiment exhibited a longer total length in the presence of the free-roaming predator and tree frog total length was greater in the presence of the caged predator treatment. Our morphological results match previous results from asymmetric competition under predation risk because green frogs grew larger with fewer tree frog competitors in the free-roaming predator treatment (interspecific competition/high predation risk) while tree frogs grew larger, to a marginal degree, with more conspecifics in the caged predator treatment (intraspecific competition/low predation risk) (Relyea 2004a, Smith et al. 2004). Tree frog responses to predators were likely weak because they encountered elevated competition, which is known to reduce antipredator morphologies (Relyea and Auld 2005), especially if predation risk is low (Relyea 2004a).

A comparison between phenotypic responses to invasive plants and predators

Honeysuckle and predators induced similar phenotypic changes in tadpoles. Compared to controls, honeysuckle induced changes in tree frog behaviors with a direction and magnitude that were nearly identical to but independent of the behavioral changes induced by caged predators. A marginally greater proportion of green frogs were visible in response to honeysuckle. In contrast, green frogs avoided the surface when exposed to caged predators. Invasive plant leachate s may compromise respiratory ability of tadpoles (Maerz et al. 2005a, Watling et al. 2011a), causing tadpoles to migrate near the surface where oxygen content is greatest. In a previous laboratory study on individual tadpoles, both leopard frogs (*Lithobates blairi*) and American toads (*Anaxyrus americanus*) increased trips to the surface in honeysuckle leachate s but tree frogs did not (Watling et al. 2011a). Elevated surfacing frequency (vertical migration) is advantageous to organisms that must swim to the surface for respiration and back to refuge to avoid predators at the bottom of aquatic habitats (Kats and Dill 1998, Lass and Spaak 2003). In response to honeysuckle, more tree frogs were visible and remained near the surface, which may explain why tree frogs did not increase their surfacing frequency in response to honeysuckle in a previous study (Watling et al. 2011a). Our results correspond to a study by Lawler (1989) which described that tree frogs respond to predator chemical cues by reducing movement while floating near the surface of aquatic habitats. In contrast, green frogs and other ranids make trips to the surface to obtain oxygen when lungs develop (Wassersug and Feder 1983, Crowder et al. 1998, McDiarmid and Altig 1999). Green frogs that make trips to the surface were likely in the open-area and visible but spent very little time at the surface in response to predators. Therefore, like a previous study, green frogs may increase surfacing frequency when exposed to honeysuckle (Watling et al. 2011a) while retaining their antipredator traits (Hickman, Chapter 1).

In addition to behavioral changes, litter from invasive Amur honeysuckle induced a shallow tail depth in green frogs, which corresponds to their response to predators (Relyea and Werner 2000a, Relyea 2001b, Relyea and Yurewicz 2002). Like their response to honeysuckle in this experiment, green frogs reduce tail depth when exposed to fish, amphibian and beetle predators (Relyea 2001b) but they did not respond to *Anax* in our experiment. Without a predator that induces a trait to match those induced by honeysuckle (tail depth), we were unable to determine how the combination of honeysuckle and predators would influence the same morphological trait for green frogs. However, a deeper tail can result in a cost of energy allocation to growth and maintenance of body size (Relyea 2000, Relyea and Auld 2005). Therefore, changes in tail depth may be a proxy for compromises in energy allocation and correspond to density-induced morphologies (Relyea 2002b).

A recent study found that some morphological changes of tadpoles depend on the quality of the leaf-litter introduced into water (Stoler and Relyea 2013). For instance, wood frog tadpoles (*Lithobates sylvatica*) decreased tail depth when exposed to tree species with low leaf-litter nitrogen compared to trees with high litter nitrogen (Stoler and Relyea 2013). Honeysuckle accelerates the loss of nitrogen in native forests and leaf-litter from honeysuckle monocultures decompose and lose nitrogen significantly faster than native forests (Poulette and Arthur 2012). In addition, honeysuckle leaf-litter decomposes and loses organic matter faster than native leaf-litter in aquatic environments (McNeish et al. 2012). Stoler and Relyea (2013) found that the increase in leaf-litter nutrient content may generate phenotypic responses that are similar to traits induced by greater competitor density but not all phenotypes correspond to changes in nutrients. Honeysuckle-induced morphological changes may be due to decreases in nutrients by low-quality leaf litter, which is known to induce changes in body size and tail length (Stoler and Relyea 2013). Higher densities also decrease nutrients and because there was no relationship between growth and density of tadpoles within honeysuckle or predator treatments, these morphological changes were independent of density effects (Relyea and Werner 2000b).

Honeysuckle induced morphologies may not be generalizable across amphibian species because honeysuckle did not alter any tree frog morphologies. Reasons for these results are unclear and warrant more research, such as development of nutrient and chemical profiles that correspond with both plant density and origin on the induction of tadpole morphologies. In addition, the similar responses to honeysuckle and predators may be due to a general stress hormone that induces similar developmental pathways for phenotypic changes. Even though we cannot be certain of the adaptive benefit of the shared responses to honeysuckle and predators, neither tree frog nor green frog survival was influenced by honeysuckle or the combination of honeysuckle and predation risk in our study.

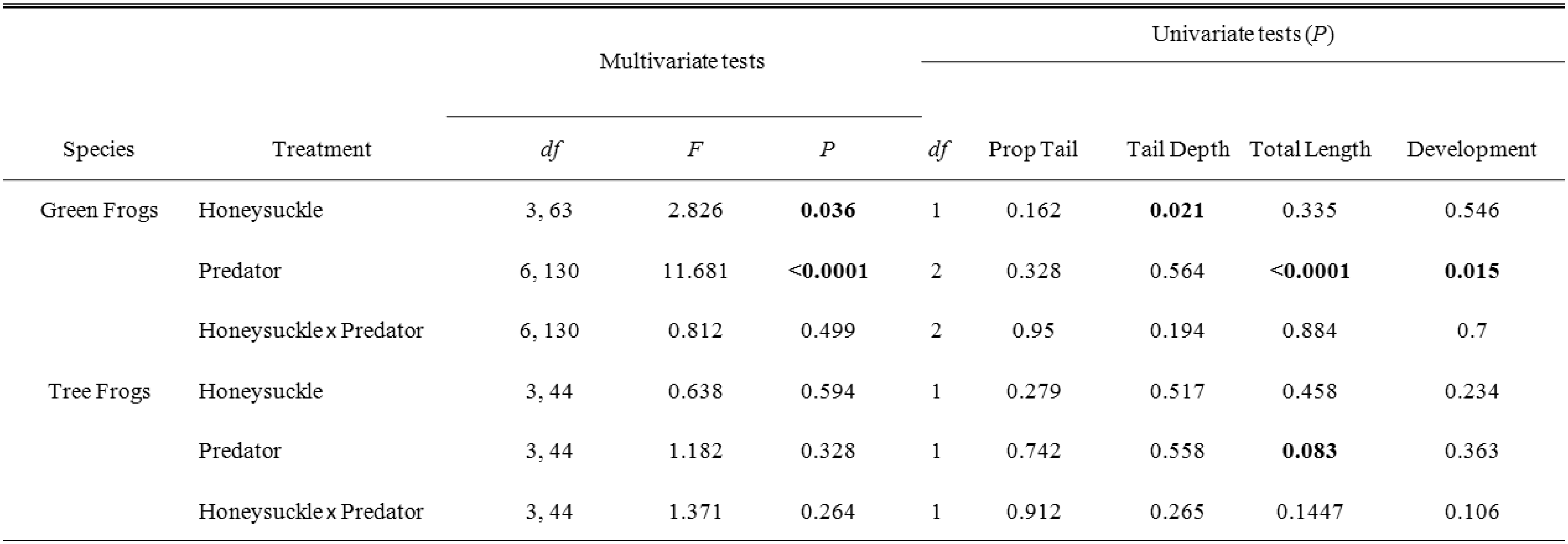
Conclusions

Our results demonstrate that a widely abundant plant invader can have unexpected consequences similar to those induced by predation risk. This work contributes to emerging research that shows native species may use established phenotypic plasticity to respond to exotic invaders (Hendry et al. 2008). This is the first study to show that larval amphibians alter morphology when exposed to invasive plants. Moreover, our results support a growing body of evidence on how invasive plants affect behavior and development of amphibians (Watling et al. 2011a, 2011b). Given the growing evidence of invasive plant effects on amphibians, it is reasonable to expect that other invasive plants decrease the quality of aquatic environments and influence amphibian phenotypes as well (Maerz et al. 2005c, 2005b, Brown et al. 2006, Watling et al. 2011a, 2011b, 2011c, Martin and Murray 2011, Adams and Saenz 2012, Cohen et al. 2012, Cotten et al. 2012). Because invasive plants induce changes analogous to predators, which influence metapopulation dynamics in tree frogs (Resetarits et al. 2005), then consequences of invasive plants may occur via changes in habitat selection on a landscape scale.

Table 1: Tree frog and green frog tadpole behaviors in response to honeysuckle and native leaf environments crossed with predators (caged and predator-free for tree frogs and free-roaming, caged and predator-free for green frogs). x - indicates crossed factors.

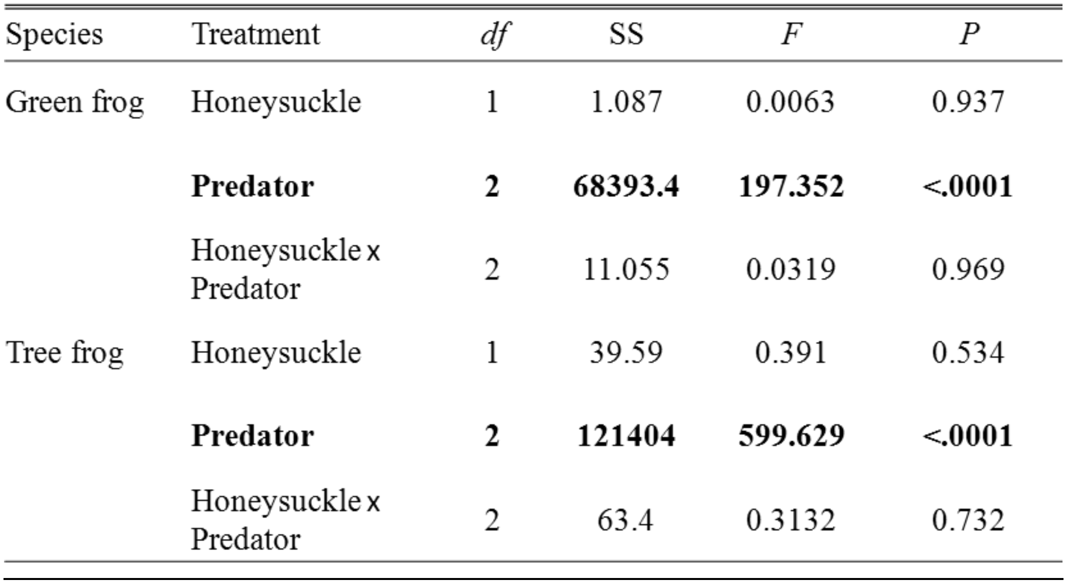
Species	Response	Treatment	*df*	SS	*F*	*P*
Green Frog	Proportion Visible	Honeysuckle	1	0.014	3.017	0.089
		Predator	1	0.008	1.731	0.195
		Honeysuckle x Predator	1	0.003	0.605	0.44
	Proportion at Surface	Honeysuckle	1	0.0003	0.314	0.578
		Predator	**1**	**0.006**	**6.631**	**0.013**
		Honeysuckle x Predator	1	0.001	1.175	0.284
Tree Frog	Proportion Visible	**Honeysuckle**	**1**	**0.123**	**10.612**	**0.002**
		Predator	**1**	**0.092**	**7.947**	**0.007**
		Honeysuckle x Predator	1	0.004	0.355	0.554
	Proportion at Surface	**Honeysuckle**	1	**0.015**	**4.116**	**0.048**
		Predator	**1**	**0.029**	**7.868**	**0.007**
		Honeysuckle x Predator	1	<0.001	<0.001	0.992

Table 2: Multivariate and univariate analysis results for the effects of honeysuckle and native leaf mesocosms crossed with predator treatments on the morphology and development of tree frog and green frog tadpoles. Sample sizes were too low to measure free-roaming predator effects on tree frogs. Only analysis of green frog morphology incorporated all predator treatments (free-roaming, caged and predator-free). x - indicates crossed factors.



		Multivariate tests			Univariate tests (*P*)				
Species	Treatment	*df*	*F*	*P*	*df*	Prop Tail	Tail Depth	Total Length	Development
Green Frogs	Honeysuckle	3, 63	2.826	**0.036**	1	0.162	**0.021**	0.335	0.546
	Predator	6, 130	11.681	**<0.0001**	2	0.328	0.564	**<0.0001**	**0.015**
	Honeysuckle x Predator	6, 130	0.812	0.499	2	0.95	0.194	0.884	0.7
Tree Frogs	Honeysuckle	3, 44	0.638	0.594	1	0.279	0.517	0.458	0.234
	Predator	3, 44	1.182	0.328	1	0.742	0.558	**0.083**	0.363
	Honeysuckle x Predator	3, 44	1.371	0.264	1	0.912	0.265	0.1447	0.106

Table 3: ANOVA of green frog and tree frog survival following exposure to honeysuckle leaf environment and predator treatments. x - indicates crossed factors.



Species	Treatment	*df*	SS	*F*	*P*
Green frog	Honeysuckle	1	1.087	0.0063	0.937
	Predator	**2**	**68393.4**	**197.352**	**<.0001**
	Honeysuckle x Predator	2	11.055	0.0319	0.969
Tree frog	Honeysuckle	1	39.59	0.391	0.534
	Predator	**2**	**121404**	**599.629**	**<.0001**
	Honeysuckle x Predator	2	63.4	0.3132	0.732

FIGURE LEGENDS

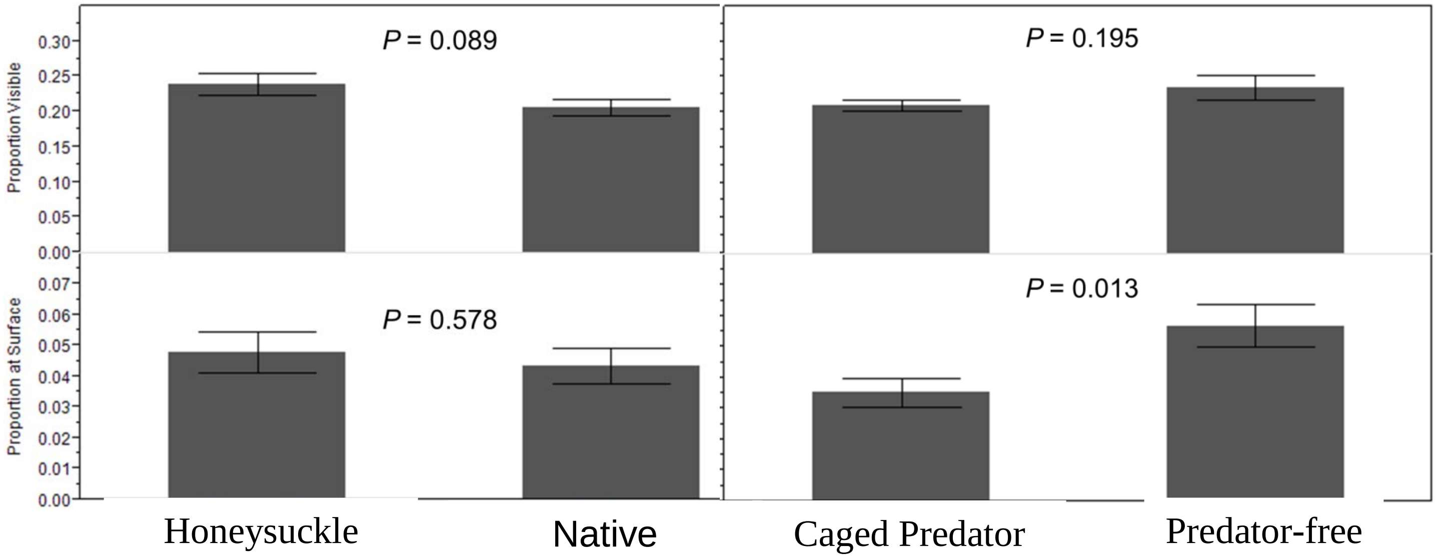
Figure 1: Effects of caged predator chemical cues and honeysuckle leaf environment on the proportion of green frog tadpoles visible and near the surface within mesocosms. Proportions were averaged across 3 observations per mesocosm, and means (±SE) from 12 predator-free and 13 caged predator mesocosms per treatment are shown.

Figure 2: Morphological (Total Length, Prop Tail (adjusted tail length from total length), Adjusted Tail Depth (tail depth residuals against total length) and developmental (Gosner) data for both green frogs and tree frogs. Rearing treatments of honeysuckle and native environments combined with predator treatments of free-roaming predator, caged predator and predator-free control. P-values represent slices and letters are pairwise comparisons (Tukey HSD).

Figure 3: The number of green frogs and tree frogs survived following exposure to honeysuckle and native leaf environment crossed with predator treatments (free-roaming, caged and predator-free) after 26 days.

Figure 1

Green Frogs



Tree Frogs

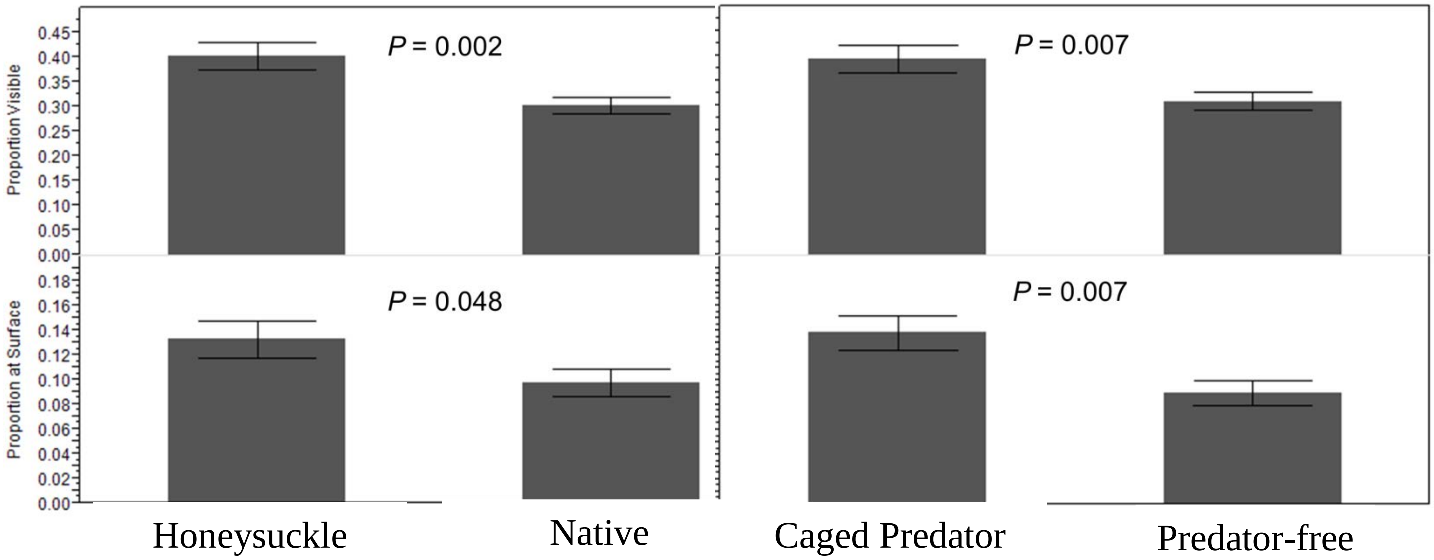
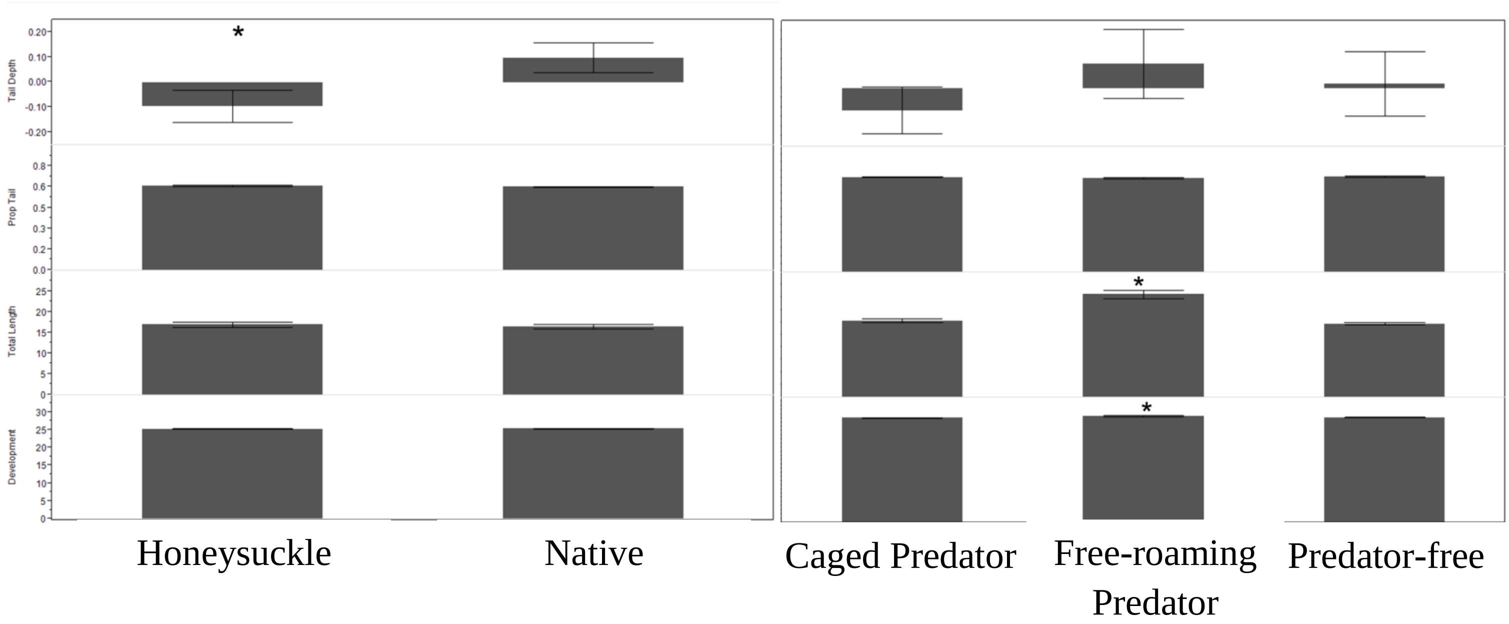
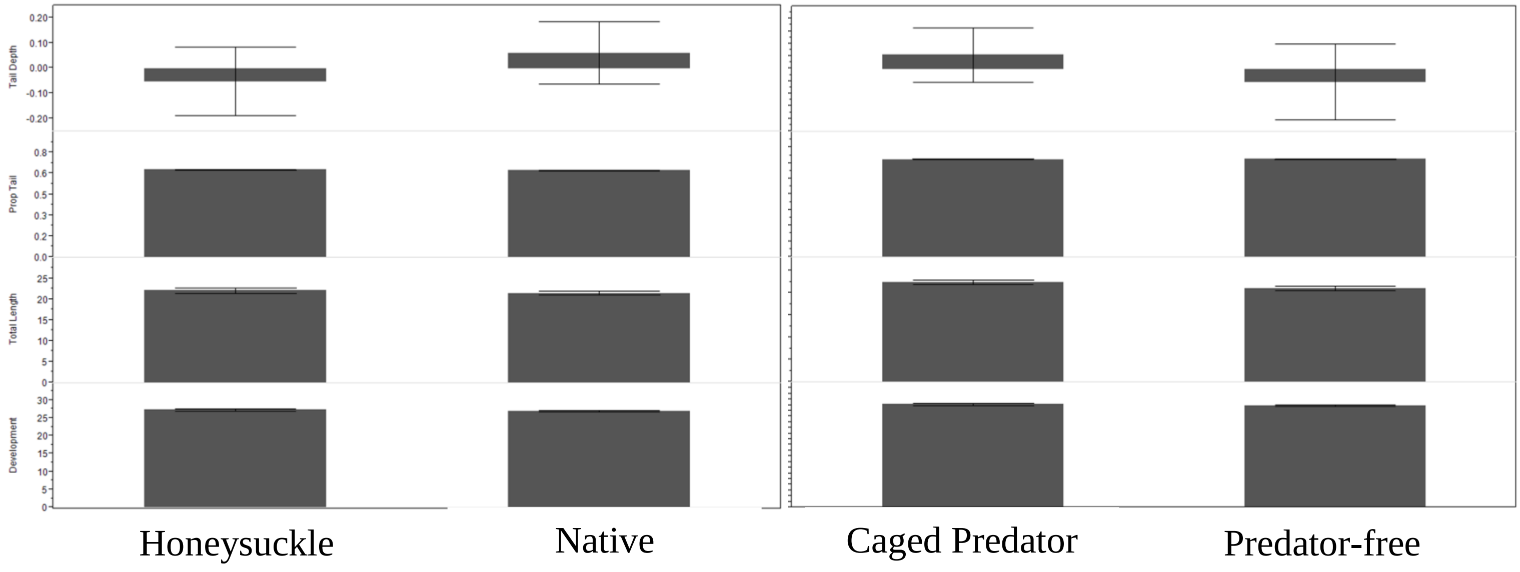


Figure 2



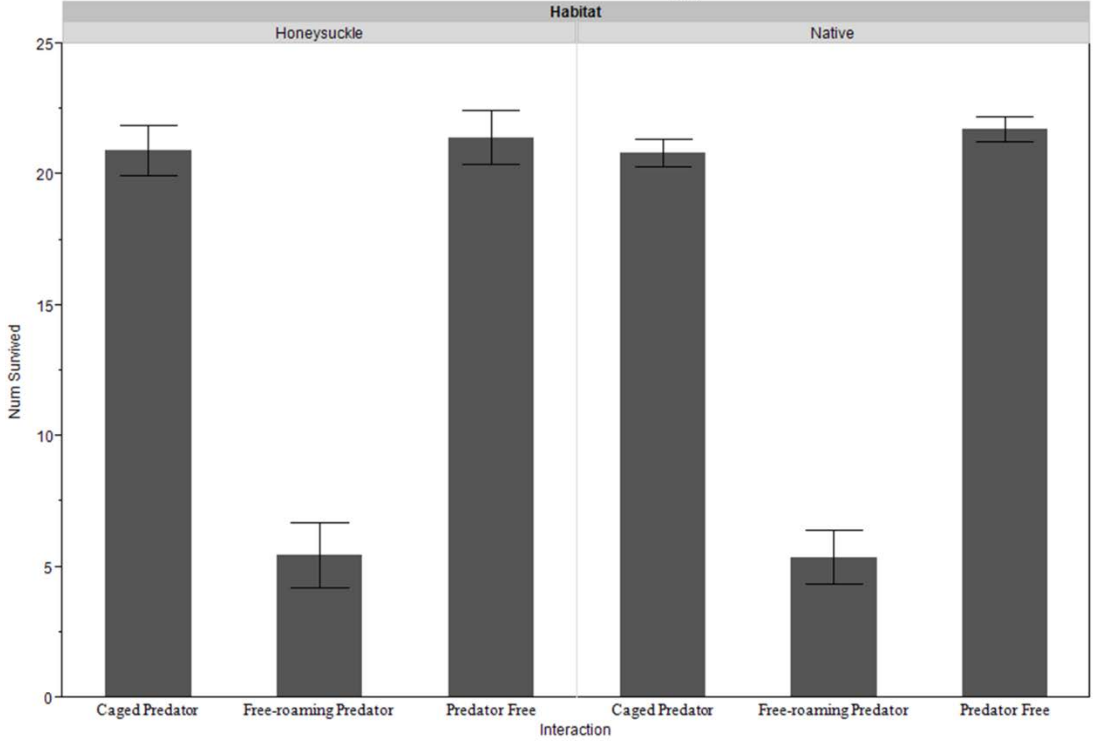
Green Frogs



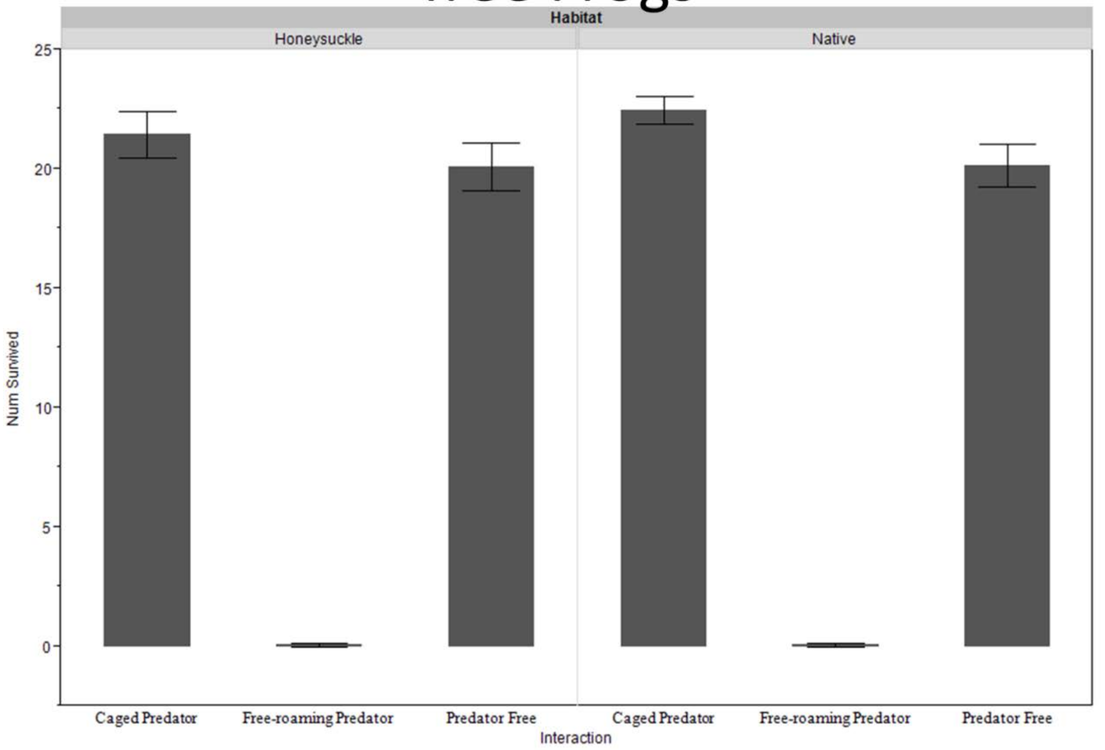
Tree Frogs

Figure 3

Green Frogs



Tree Frogs



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