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**THE CAUSES AND CONSEQUENCES OF ADAPTATION TO A NOVEL INVADER IN
THE EASTERN FENCE LIZARD (*SCeloporus undulatus*)**

A Dissertation in
Ecology
by
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ABSTRACT

Many environments are changing rapidly due to human impacts including habitat alteration, climate change, and the introduction of invasive species. This global environmental change has numerous direct and indirect effects on species that can exert novel pressures. In some cases organisms must adapt to these changes or face extirpation or extinction. Understanding the diversity and extent of these effects of global change, and the processes by which species adapt to it, is critical to future conservation and can provide important insights into ecological and evolutionary processes.

My dissertation addresses questions about the effects imposed by invasive species on native species and how native species adapt to associated novel pressures by using a system of native fence lizards (*Sceloporus undulatus*) subject to differing levels of exposure to an invasive predatory fire ant (*Solenopsis invicta*). In the southeastern United States, fence lizard populations have adapted to this novel predator via an increase in anti-ant behaviors, including twitching and fleeing which promote escape from and removal of attacking ants; longer hind limbs which support this behavior; and a heightened stress response.

Novel pressures induced by invasive species may include lethal (e.g. predation, injury) and sublethal (e.g. altered energy or habitat availability) effects. The nature of these effects may vary with characteristics of an organism (e.g., sex, age, size) and history of exposure to the invader. I explored how the effects of fire ants on fence lizards varied with life stage of the lizard and their evolutionary exposure (adaptation) to the ants.

I first investigated the effects of fire ants on the vulnerable life history stage of fence lizard eggs by creating artificial nests in the field and monitoring them daily. I found that fire ants do prey on fence lizard eggs in the field and may predate up to 60% of fence lizard nests in fire ant-invaded areas. Eggs from lizards from fire ant-invaded and -uninvaded sites were equally

susceptible to predation, suggesting a lack of adaptation to this threat. I next quantified the effects of fire ants on juvenile and adult lizards from fire ant-invaded and -uninvaded sites by placing them in large field enclosures with natural and reduced densities of fire ants for two weeks. Fire ants reduced survival of adult but not juvenile lizards, regardless of their evolutionary history with fire ants. Juvenile lizards grew less in enclosures with fire ants than in fire ant-free enclosures, but there was no effect of fire ant density on adult growth. This research shows that fire ants have both lethal and sublethal effects on lizards, and that these vary with lizard life stage.

I conducted field surveys and manipulations to examine the consequences of behavioral adaptations of fence lizards to fire ants. I exposed lizards from fire ant-invaded and -uninvaded sites to encounters with predatory fire ants and non-threatening native ants, and to simulated attacks by a native predator (a taxidermied American Kestrel). While lizards responded similarly to the native predator, lizards from fire ant-invaded sites showed a generalized increase in responsiveness to all ants. While this behavior is adaptive against fire ants, it may also attract attention from native predators as evidenced by my findings of higher injury rates of lizards at fire ant-invaded sites. At a geographic scale, I found that the presence of fire ants reverses natural latitudinal gradients in behavior, stress responsiveness, and morphology of fence lizards. This implies that fire ants are driving these traits away from historical values that evolved under fire ant-free conditions and suggests potential costs to adapting to fire ants.

As a whole, this work shows that invasive species can have broad impacts on native taxa, and that these impacts may vary with the life stage of affected native species and the degree to which they have adapted to the invader. Adaptive responses by native species may be strong enough to reverse pre-existing latitudinal clines in relevant traits over large portions of an affected species' range. However, while native species can adapt to novel invaders, these adaptations may incur also costs that should be considered when assessing population persistence and management in the face of these increasing perturbations.

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Chapter 1

Introduction

The world is a rapidly changing place. Climate change, anthropogenic habitat alterations, and the introduction of invasive species all contribute to the accelerating pace of global environmental change (Cox et al. 2000; Butchart et al. 2010). These novel and rapid changes cause diverse lethal and sublethal effects that may alter population demography, community composition, and ecosystem functioning (Didham et al. 2007). Faced with these novel evolutionary pressures, species must adapt to minimize lethal effects, mitigate sublethal costs, or, in the case of very strong pressures, avoid extirpation or extinction (Hoffmann and Sgrò 2011).

Invasions of non-native species are occurring with increasing frequency as the globe becomes increasingly connected (Hulme 2009). Invaders affect biological systems worldwide and are implicated in the extinction of numerous taxa (Mack et al. 2000; Clavero and García-Berthou 2005), and have serious negative economic impacts (Pimentel et al. 2005). Native species and communities have not co-evolved with invaders and so lack adaptations to effectively deal with the novel pressures that invaders can impose (Shine 2010; Pintor et al. 2011; Dorcas et al. 2012).

While the large-scale spread of invasive species is considered a dire threat to biodiversity, species invasions can also be treated as large scale (and otherwise unethical) “experiments” (Sax et al. 2005; Lach and Bui-Hooper 2010). Careful analyses of the progression of invaders can allow insights into ecological and evolutionary processes (Sax et al. 2007). For example, chronosequences of sites or populations invaded for differing amounts of time can allow researchers to assess the strength of the effects of invaders and the mechanisms and consequences of any resulting adaptation (Lankau et al. 2009; Dostál et al. 2013). Species invasions can also illuminate the processes driving large-scale biological patterns such as latitudinal gradients.

While these gradients may be long-standing features, they may also form quite quickly (within ≈ 50 generations) when species move into large geographic areas with novel conditions (Johnston and Selander 1964; Dlugosch and Parker 2008). While much previous research focuses on how abiotic characters such as temperature, season length, and productivity, drive gradients (De Frenne et al. 2013), determining how biotic factors affect these patterns has been more difficult. Studying how invasive species may alter latitudinal patterns in native species can help us understand the importance of biotic factors, and information about these potentially large-scale effects is key to understanding the diverse impacts of invaders.

Effects of Invasive Species

Invasive species can have both lethal and sublethal effects on native organisms. Lethal effects of invaders can include predation (Kiesecker and Blaustein 1997), poisoning via the introduction of novel toxins (Phillips and Shine 2004), and death due to parasitism (Wikelski et al. 2004; Koop et al. 2011) and disease (Crowl et al. 2008). Organisms may also suffer sublethal effects, including reductions in growth and body condition (Vogel and Pechmann 2010), and changes in nutrient and prey availability (Suarez et al. 2000; Davis et al. 2008), habitat use (Todd et al. 2008), and habitat structure (Crooks 2002; Croll et al. 2005). These effects may impose substantial novel pressures and elicit adaptive responses from native species. For instance, native fish from areas invaded by cane toad tadpoles, which are highly toxic, have evolved increased avoidance learning, allowing them to learn more quickly to avoid toxic prey (Caller and Brown 2013). In a similar vein, native mussels exposed to an invasive predator show inducible thickening of shell walls, an important defense, when presented with cues of this predator, but nearby mussel populations unexposed to the invasive predator show no similar inducible defenses (Freeman and Byers 2006).

Life stage may play an important role in how pressures affect populations or the ability of populations to respond to novel pressures. Many characteristics of organisms vary with life stage, including morphology, physiology, coping strategies, and other plastic responses (e.g., Arnqvist and Johansson 1998; see also Wright and McConnaughay 2002; Piersma and Drent 2003). In some cases, particular life stages, such as eggs or developing young, may be differentially vulnerable to predation or other lethal effects imposed by invaders (Sakai et al. 2001; Koop et al. 2011). In native species in which habitat use, diet, or other niche characteristics shift with development, sublethal costs imposed by invasive species may change across ontogeny (Phillips and Shine 2006; Correa et al. 2012). Understanding the diversity and strengths of effects imposed by invaders and how they may vary with the life stage of impacted native species is key to understanding how these pressures may drive rapid adaptation (Stearns 1992; Berthon 2015).

Adaptation to Invasive Species

While evolutionary responses were previously considered to occur primarily over long timescales, adaptation to novel pressures imposed by invasive species on ecological timescales is now both theoretically supported (Thompson 1998; Lambrinos 2004) and documented across many biological systems (Phillips and Shine 2004; Strauss et al. 2006). Indeed, invasive species themselves adapt rapidly to the novel conditions that they encounter in their invasive ranges (Dlugosch and Parker 2008; Kolbe et al. 2014). Adaptations can occur via multiple pathways such as evolution by natural selection, epigenetic mechanisms, and plastic responses which include learning, physiological responses, and acclimation (Reznick and Ghalambor 2001; Shimada et al. 2010; Schrey et al. 2013; Nunes et al. 2014). Adaptations have been documented in a wide variety of traits, including changes in behavior (antipredator, foraging; Kiesecker and Blaustein 1997; Orrock and Danielson 2004), physiology (stress and immune responses; Berger

et al. 2007; Graham et al. 2012; Van Zwol et al. 2012), and morphology (body shape, limb length; Seeley 1986; Losos et al. 2004; Langkilde 2009a).

Adaptations to novel conditions, while increasing fitness in the face of new challenges, may incur costs. For example, changes in behavior frequently carry opportunity costs (Lima and Dill 1990; Magurran et al. 1992), induced defenses may incur energetic costs (Van Buskirk 2000), and physiological adaptations may require prioritizing energy to one system at the expense of others (Clarke 2003; Wingfield 2013). These adaptations may also alter existing geographic patterns of intraspecific variation (Reznick and Ghalambor 2001; Shine 2010; Hurlbert and Liang 2012). In cases where novel pressures occur over only a part of a species' range or affect distinct parts of a range differently (e.g., polar warming), adaptation may vary geographically (Hurlbert and Liang 2012). As a result, large-scale environmental change, including invasive species, may disrupt existing latitudinal gradients (Rice and Sax 2005). Invasion-induced changes in traits that vary latitudinally will of necessity deviate from historical, coevolved baselines and may also entail costs (as above) (Stockwell et al. 2003).

Adaptation to novel selective pressures, whether at local or continental scales, can result in maladaptation (e.g., Bradley St. Clair and Howe 2007), which occurs when organisms express a trait or a value of a trait that does not confer optimal fitness (Crespi 2000). Maladaptation can also present as an evolutionary trap wherein formerly reliable environmental cues may be altered such that previously adaptive organismal responses to these cues result in negative fitness effects (Schlaepfer et al. 2005; Schlaepfer et al. 2010). For instance, snails from environments supporting predatory fish often respond to chemical cues from these predators by burrowing defensively; however, while snails recognize cues from invasive predatory crayfish, they continue to burrow in response, an ineffective defense which results in high predation and low fitness (Sih et al. 2013). As such, studies of presumptive adaptation to invasive species should avoid untested

assumptions of adaptationism and consider the possibility that these changes may incur fitness costs or even reduce fitness via maladaptation.

Study System and Research Objectives

In my dissertation, I broadly investigate how the lethal and sublethal effects of invasive species on native species vary with life stage and evolutionary exposure, how native species adapt to invaders at geographic scales, and the potential costs of these adaptations. To address these questions, I made use of a system encompassing an invasive predatory insect, the red imported fire ant (*Solenopsis invicta*; hereafter “fire ants”) and its impacts on a native reptile, the eastern fence lizard (*Sceloporus undulatus*). This system utilizes comparisons of populations of fence lizards exposed to fire ants for varying periods of time (up to 40 generations, and including unexposed populations) and allows inferences about the costs of exposure to fire ants and adaptation of fence lizards to these novel pressures.

Red imported fire ants are native to the Pantanal region of South America but are invasive worldwide (Wojcik 1983; Holway et al. 2002). Invasive fire ants have many deleterious ecological and economic consequences and are considered one of the world’s worst invasive species (Morrison et al. 2004). Fire ants were introduced into the United States in Mobile, Alabama in the 1930’s and have since spread to occupy large swathes of the southern U.S. (Lofgren et al. 1975). In the intervening 80 years post-invasion, large bodies of research have focused on ways to control fire ant populations and their effects on agriculture, invertebrate communities, and vertebrate species of economic interest (Allen et al. 2004; Tschinkel 2006). Fire ants recruit in large numbers to defend their mounds and attack prey, and possess a powerful and painful venomous sting capable of paralyzing much larger organisms (Wojcik et al. 2001; Allen et al. 2004). Invasive fire ants can achieve high population densities, especially in disturbed

environments (Macom and Porter 1996), and invasion can result in changes to native arthropod communities, though these may recover post-invasion (Gotelli and Arnett 2000; Morrison 2002). Fire ants have been implicated in diverse impacts on vertebrates, including direct predation on vulnerable life stages (e.g., eggs and nestlings) and sublethal and indirect effects on foraging patterns, habitat use, and growth and development (Wojcik et al. 2001; Allen et al. 2004).

The eastern fence lizard is a widespread vertebrate in the eastern U.S. whose range and microhabitat preferences overlap broadly with this invasive fire ant (Langkilde 2009b). Previous research has documented a variety of lethal and sublethal effects of fire ants on fence lizards and adaptation of fence lizard populations to these pressures (Langkilde 2009a). Fence lizards and fire ants occupy similar microhabitats (Langkilde 2009a), and fire ants find and recruit to attack lizards quickly within these habitats (Freidenfelds et al. 2012). Twelve attacking fire ants can immobilize, and subsequently kill, an adult fence lizard within one minute, and as few as three attacking fire ants can kill juvenile fence lizards (Langkilde 2009a). Ants make up a significant portion of the fence lizard diet (DeMarco et al. 1985; Mobley 1998), and lizards can also be envenomated when consuming this toxic prey (Langkilde and Friedenfelds 2010). Lizards from sites invaded by fire ants also have higher baseline levels of corticosterone (CORT), the primary stress hormone in reptiles, indicating that lizards may be stung frequently, and that there may be additional physiological costs to exposure to fire ant attacks (Graham et al. 2012)

Fence lizards have adapted in multiple ways to this novel threat with associated changes in behavior, morphology, and stress responsiveness. In areas uninvaded by fire ants, fence lizards' natural response to the presence of a predator is to remain motionless, relying on their cryptic patterning to prevent detection by native visual predators (Trauth et al. 2004). This strategy, however, can lead to injury or death if used in response to fire ants, which continue to attack and envenomate motionless lizards. In fence lizard populations invaded by fire ants, adult lizards show increases in flee behaviors in response to fire ant attack and also perform various

body twitches; these behaviors are effective at removing attacking ants and reducing further recruitment of ants (Langkilde 2009a; Freidenfelds et al. 2012). Fence lizards at fire ant-invaded sites also have relatively longer hind limbs, a trait associated with increased sprint speed (Miles 1994) and more vigorous twitching behavior, increasing the effectiveness of these behaviors for removing attacking ants (Langkilde 2009a). Fence lizards at fire ant-invaded sites also have higher physiological stress responsiveness, as evidenced by higher levels of CORT in response to a standardized stressor (Graham et al. 2012). This higher responsiveness may prime lizards to use flee and twitch behaviors more effectively (Langkilde unpubl. data) and could also enhance survival. For instance while lizards from fire ant-naïve populations respond to high stress by tending to hide under cover objects, lizards from fire ant-invaded sites with elevated CORT seek to flee upwards which may reduce risks of fire ant attack (Trompeter and Langkilde 2011). These behavioral, morphological and physiological adaptations to fire ants occur via many interacting pathways, including heritable changes, within lifetime selection, and plastic processes (Langkilde 2009a; Freidenfelds et al. 2012; Robbins and Langkilde 2012).

My thesis research assesses and quantifies the diverse effects of fire ants on fence lizards and examines the contexts underlying fence lizards' adaptation to this novel threat. In Chapter 2, I investigate potential impacts of fire ants on an understudied life stage of the eastern fence lizard, the egg, and determine how vulnerable this life stage may be to fire ant attack. In Chapter 3, I investigate and quantify the lethal and sublethal effects of fire ants on adult and juvenile fence lizards using a large-scale field enclosure experiment that manipulates lizards' exposure to fire ants. In Chapter 4, I examine whether fence lizards' behavioral adaptation to fire ant attack is generalized to other species and investigate costs that may be associated with this adaptation. Chapter 5 examines how fire ants may drive changes in latitudinal gradients of key traits in fence lizards and potential costs of these changes. As a whole, this research illuminates how impacts of invasive species may vary across the life histories of native species and how adaptation may both

support persistence of, and impose costs on, species faced with these novel environmental challenges.

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Chapter 2

Invasive fire ant (*Solenopsis invicta*) predation of Eastern Fence Lizard (*Sceloporus undulatus*) eggs

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Abstract

Invasive species are a threat to biodiversity, and understanding their impacts on native ecosystems is a research priority. Red imported fire ants (*Solenopsis invicta*) are invasive in the southeastern United States and have multiple effects on a variety of native species. In some species, particular life stages, including juveniles and eggs, may be especially vulnerable to fire ants, but research on these impacts has been limited. Fire ants occupy microhabitats used for nesting by Eastern Fence Lizards (*Sceloporus undulatus*) across much of their range. We examined the extent of fire ant predation on fence lizard eggs by constructing artificial nests at a fire ant-invaded location and monitoring them for predation for up to 20 days. During this period, 24% of nests were predated by fire ants, and survival curves suggest 61% of nests may be vulnerable to fire ant predation over the entire incubation period. Distance of nests to the nearest fire ant mound and canopy cover above nests were not significant predictors of predation, indicating that nest site choice by fence lizards may not be able to avert fire ant predation. Invasive fire ants likely represent a novel level of predation pressure on many species, and these effects should be accounted for in management and conservation strategies.

Introduction

Invasive species are a major threat to biodiversity worldwide and have important ecological and economic impacts (Wilcove et al., 1998). Non-native invaders have been implicated as causative agents of changes in nutrient cycling and ecosystem functioning, alterations in community structures, loss of ecosystem services, reductions in abundance in affected species, and species extirpations and extinctions (Pyšek and Richardson, 2010). Research into the mechanisms by which invasive species impact and interact with native species is key to understanding the extent and strength of their effects on ecosystems and making informed and accurate predictions and management decisions concerning invaders (Sax et al., 2005).

Ants are one of the most successful taxa of animal invaders and have diverse and extensive ecological impacts worldwide (Cole et al., 1992; Holway et al., 2002). These effects include changes in the abundance and distribution of native invertebrates and vertebrates and corresponding alterations to mutualisms, pollination, seed dispersal, and other ecological processes (Holway et al., 2002). Red imported fire ants (*Solenopsis invicta*) are native to South America and have become invasive in over seven countries, including in the southeastern United States (Tschinkel, 2006). Previous research has revealed both direct and indirect impacts of fire ants on native species at both local and biogeographical scales (reviewed in Allen et al., 1994; Gotelli and Arnett, 2000; Wojcik et al., 2001).

Many of the direct effects of fire ants on native species are the result of these ants responding quickly and in large numbers to defend their nest mounds or to potential prey, resulting in both sublethal and lethal interactions with organisms, including vertebrates (Allen et al., 2004; Tschinkel, 2006). Fire ant venom has both hemolytic and neurotoxic effects, and stings can lead to paralysis, infections, and loss of digits or vision (Wojcik et al., 2001; dos Santos Pinto et al., 2012). Encounters with fire ants can cause species to alter habitat use (Killion et al., 1995;

Holtcamp et al., 1997) and change activity levels (Pedersen et al., 1996), and can result in altered physiological (Graham et al., 2012) and behavioral responses to stress (Trompeter and Langkilde, 2011). Attacking fire ants can also overwhelm and kill vertebrates, and direct predation by fire ants has been observed on multiple species (reviewed in Allen et al., 2004).

Many native species have life stages that lack defenses, either behavioral or otherwise, to invasive fire ants, and thus are particularly vulnerable (reviewed in Allen et al., 2004). Fire ants pose the greatest threat to immobile individuals (such as the sick or young, Allen et al., 2004), and to species that respond to threats by becoming immobile (e.g., box turtles, Montgomery, 1996; deer fawns, Allen et al., 1997; hog-nosed snakes, Tuberville et al., 2000; and fence lizards, Langkilde, 2009a). Juveniles of some species, such as turtles or nestling birds, have limited mobility or opportunity to escape attack by foraging fire ants (Drees, 1994; Epperson and Heise, 2003), and hatchlings are particularly vulnerable after they have pipped their eggs but not yet emerged (Allen et al., 2004). Fire ants are also capable of penetrating intact eggs of some species (Moulis, 1997; Wojcik et al., 2001). Reptile eggs seem to be particularly vulnerable, and fire ants have been observed penetrating eggs of many reptile species in both the laboratory (Diffie et al., 2010) and field (Mount et al., 1981; Thawley, 2014). However, studies looking at the potential impacts of fire ants on nesting and reproductive success in lizards are rare (but see Chalcraft and Andrews, 1999, for effects of native *Solenopsis*).

Eastern Fence Lizards (*Sceloporus undulatus*) are vulnerable to fire ants as both juveniles and adults (Langkilde, 2009a; Robbins and Langkilde, 2012), and fire ants can penetrate and consume fence lizard eggs during staged, field encounters (Newman et al., 2014). However, we currently lack knowledge about the prevalence of fire ant predation on lizard eggs in the field and factors that may influence this. We conducted a field study to: 1) quantify the predation pressure of fire ants on fence lizard nests and 2) determine if nest site conditions (canopy cover) and

location (distance from fire ant mound) affect levels of fire ant predation by constructing artificial nests in areas invaded by fire ants and monitoring their survival.

Materials and Methods

Egg predation trials were conducted at Solon Dixon Forestry Education Center, Escambia County, Alabama, USA where *S. undulatus* is known to nest successfully (C. Thawley, pers. obs.). We placed artificial nests in edge habitats where mixed pine/hardwood forests dominated by longleaf (*Pinus palustris*) and loblolly pine (*Pinus taeda*) graded into mowed field habitats and areas around roadsides and outbuildings. In these habitats fire ant mounds were spaced an average of 10 m apart (Langkilde, 2009b); mound densities were comparable to other disturbed, fire ant-invaded sites (Tschinkel, 2006). Trials were conducted from 26 June to 17 July 2013, a period during which *S. undulatus* eggs were naturally incubating at this site.

We obtained eggs for trials from female fence lizards caught across the eastern U.S. at sites with a history of fire ant invasion (AL, MS) or which have not yet been invaded by fire ants (AR, TN, PA, and NJ). Lizards were housed in tubs with moist sand allowing for nest construction, and enclosures were checked twice daily for freshly laid eggs. We incubated eggs in moist vermiculite (-200 kpa) for 7–27 days before placing them in artificial nests in the field.

Artificial *S. undulatus* nests were constructed by modifying the methods of Buhlmann and Coffman (2001) to allow for daily observations of nests without continual soil disturbance likely to attract fire ants. Nests were placed in open, sandy soils that *S. undulatus* prefers for nesting (Angilletta et al., 2009) and were constructed by digging 10 cm diameter holes 15 cm deep into the soil. We inserted clear, acrylic tubes (7 cm diameter, 30.5 cm length; Uline S-7017) with white vinyl endcaps (Uline S-14134) into these holes and placed six *S. undulatus* eggs (a realistic clutch size, Du et al., 2014; Thawley, pers. obs.) 7–10 cm below the surface and in

contact with the outer wall of the tubes (Fig. 2.1). Small pieces of plastic transparency were placed above the eggs to prevent soil entering the immediate space around them, and soil was carefully replaced around the eggs and tube. This allowed for a small amount of empty space to remain around the eggs and provided for viewing of the eggs from the interior of the tube. To prevent heating of the tubes and eggs to levels above soil temperatures due to sunlight entering the aboveground portions of the tubes, each tube was capped and filled with a plastic bag of soil to the ground level and lined with white plastic to reflect sunlight. If *S. invicta* entered an artificial nest during construction, we relocated the nest as a nesting female would likely do.

For each artificial nest, we measured distance to nearest fire ant mound as well as percent canopy cover using a spherical densitometer (Forestry Suppliers Inc., Model C). As pilot trials revealed that nine-banded armadillos (*Dasypus novemcinctus*) will dig next to observation tubes inserted into the ground and prey on lizard eggs, each artificial nest was surrounded by an enclosure 60 cm in diameter and made from galvanized fencing that did not exclude fire ants.

We monitored nests daily for 15–20 days by removing upper tube caps and soil inserts and lowering a small digital video camera (Pentax Optio W90) into each tube. Eggs were filmed from multiple angles, and video footage was analyzed in the field to verify the condition of eggs. If ants were found on eggs during videotaping, we allowed several hours for the attack to progress before returning and excavating eggs. To identify ants before all eggs had been consumed, we interrupted ant attacks to identify ant species and assess if excavated eggs had been penetrated. As these interruptions may have preempted ants penetrating some eggs that they otherwise would have, we considered nests as predated if \geq four of the six eggs were punctured. We therefore report predation of nests rather than of individual eggs.

We tested for effects of distance to nearest fire ant mound and canopy cover on whether or not nests were predated by fire ants using logistic regression. We generated estimates of nest predation for the entire incubation period by fitting survival curves to predation data collected

during our observational period (15–20 days) and extrapolating to an estimated incubation period of 56 days at this site (Thawley, pers. obs.). We tested the fit of exponential and Weibull survival curves to nest data, and selected the exponential curve as having the best fit (LRT; $\chi^2=0.63$, $df=1$, $P=0.43$). All statistical analyses were conducted using program R v3.1.1 (R Foundation for Statistical Computing, Vienna, Austria available online at <http://www.R-project.org>, 2014).

Results

Seven of the 25 artificial nests were preyed on over the 20-day course of the study. Of these, fire ants were confirmed as nest predators at five nests via egg excavation, and were observed via camera (but not collected) as predators of a sixth nest. In a seventh nest, fence lizard eggs were preyed on by a thief ant (*Solenopsis sp.*); this nest was omitted from further analyses. Of the six fire ant-predated nests, three contained eggs from female lizards from fire ant-invaded sites ($n=11$), and three contained eggs from females from fire ant-uninvaded sites ($n=13$).

The environmental and spatial variables we measured did not affect predation rates. Artificial nest sites had a mean of 37.5% canopy cover (range=9–62%), and were a mean of 4.3 m (range=1.5–10.5 m) from the nearest fire ant mound. Neither canopy cover nor distance to nearest fire ant mound were significant predictors of fire ant predation on nests ($\beta=-0.022$, $df=1$, $P=0.57$; $\beta=-0.076$, $df=1$, $P=0.32$, respectively). Extrapolating from an exponential survival curve based on the nest data provides an estimate of 61% nest mortality due to fire ants over the 56-day incubation period (Fig. 2.2).

Discussion

Invasive fire ants likely represent a strong predation pressure on Eastern Fence Lizard eggs. While juvenile and adult fence lizards are capable of behavioral avoidance of fire ants (Freidenfelds et al., 2012), fence lizard eggs have little defense against these predators. Fence lizard eggs are rich in protein and are present during late spring and summer, when fire ants actively seek out high protein foods to support growth of brood (Sorenson et al., 1983; Stein et al., 1990). These eggs are thus likely an attractive food source to fire ants. Our results indicate that up to 61% of lizard nests may be at risk of predation by fire ants, similar to levels of mortality found for turtle nests (45%; Buhlmann and Coffman, 2001). This result is based on extrapolation of a survival curve rather than monitoring eggs throughout incubation, and it may be inaccurate if fire ant predation rates vary significantly over time. Our estimate of predation may, in fact, be conservative, as pipped or hatched *Sceloporus* may remain underground until soil conditions favor emergence, extending the period when they are at risk of predation by fire ants (Buhlmann and Coffman, 2001). However, even within the first 20 days of incubation, 24% of nests were predated by fire ants, representing a significant mortality cost.

Although our estimates of predation on nests by invasive fire ants are high, these lizards likely experienced some historic level of nest predation by native fire ants (*Solenopsis geminata* and *Solenopsis xyloni*) prior to the invasion of *S. invicta*. These native fire ants have similar predatory modes to *S. invicta*, and are known to prey on hatchling birds (Kroll et al., 1973; Delnicki and Bolen, 1977), and likely on sea turtle eggs and hatchlings (Moulis, 1997; Wetterer, 2006). While fence lizards may have adapted to the historical presence of native fire ants, invasive fire ants likely pose a greater threat for several reasons. Colonies of *S. invicta* are larger than those of *S. geminata* and *S. xyloni* (Tschinkel, 1988), and *S. invicta* workers recruit aggressively to food sources (Morrison, 2000). After displacing native fire ants, *S. invicta* now

occurs at higher densities than native fire ant species historically did (Porter et al., 1988; Tschinkel, 2006). This increased density likely both increases the proportion of available habitat occupied by fire ants, especially in disturbed areas, and, within those areas, produces a stronger predation pressure on native species including fence lizards (Vinson, 1994).

While it could be possible for fence lizards to reduce fire ant predation by selecting nest sites with low or no fire ant presence, this is unlikely for several reasons. Our results show that neither canopy cover (up to 62%) nor distance from nearest fire ant mound (up to 10 m) had a significant effect on nest survival. While sites with high canopy density are avoided by fire ants (Tschinkel, 2006), fence lizards do not make use of these sites for nesting (Angilletta et al., 2009). Also, fire ant mounds are often densely spaced, being an average of 10 m apart (range 1–35 m) at this and other fire ant-invaded sites (Langkilde, 2009b). Fire ant colonies generally expand to occupy space until they contact workers from other colonies, ensuring that most microhabitats suitable for nesting by fence lizards are occupied by foragers from at least one fire ant colony (Adams, 2003; Tschinkel, 2006). Additionally, fence lizard nests represent microclimates preferred by fire ants; fence lizards nest in sandy soils at depths that are commonly occupied by foraging tunnels of fire ants (Markin et al., 1975; Angilletta et al., 2009). Fence lizard nests also maintain temperatures favorable for fire ant activity (Lofgren et al., 1975; Angilletta et al., 2009), and may represent a sheltered, thermally optimal food source when surface temperatures are unfavorable for foraging (Porter and Tschinkel, 1987). As such, fire ants likely occupy and forage in most edge and patchy habitats suitable for fence lizard nest habitat within fire ant-invaded areas.

Of the six nests predated by fire ants, three were from fire ant-invaded sites, and three were from uninvaded sites. While this is a small sample, it suggests that eggs produced by fence lizards at fire ant-invaded sites do not have special adaptations to prevent or deter fire ant attack. This result corresponds with patterns of thickness in fence lizard eggshells, which showed no

adaptive thickening of eggshells produced by lizards at fire ant-invaded sites (Goldy-Brown, pers. obs.).

Because estimates of nest failure are not available for *S. undulatus* in the absence of fire ants, we do not know the extent of the effects that fire ant predation on nests may have on fence lizard populations. However, estimates of nest failure rates in congeners, which include nest predation, range from 32% in *Sceloporus virgatus* (Vinegar, 1975) to 78% in *Sceloporus olivaceus* (Blair, 1960). Blair (1960) suggests that vertebrates, especially snakes and armadillos, account for the majority of nest predation, and may find lizard nests via scent left by mothers during laying. It is possible that fire ants may be replacing the predation pressure of other nest predators (such as armadillos which were excluded during this study). However, in our study, fire ants entered nests via underground tunnels, suggesting that they may detect nests via different modes than vertebrate predators. Also, predation rates we observed (24%) and extrapolated (61%) would encompass a large portion of the nest failure expected naturally in similar species. These observations suggest that fire ants may replace or potentially add to other sources of nest mortality.

We also lack data on whether fire ants may reduce abundance of *S. undulatus* at fire ant-invaded sites. While fire ant-induced mortality has been observed in many species (Allen et al., 2004), population level effects have only been demonstrated experimentally in two vertebrates: bobwhite quail and white-tailed deer (Allen et al., 1995; Allen et al., 1997; reviewed in Tschinkel, 2006). Ultimately, many fence lizard populations persist despite long-term (up to 80 years) invasion by fire ants, suggesting that they have compensated for fire ant-induced mortality via demographic processes or adaptation (Langkilde, 2009b). Large-scale, experimental efforts to determine impacts of fire ants on fence lizard populations would illuminate this issue.

In conclusion, fire ants may represent a strong and potentially novel pressure on fence lizards and other species, including some of conservation concern, which have life history

strategies that result in high vulnerability to predation by fire ants (e.g. egg-laying or delayed emergence from nests; Landers et al., 1980; Allen et al., 1994; Forsys et al., 2001). While fence lizard populations persist in fire ant-invaded areas despite potentially high predation of eggs (and possibly juveniles and adults; Langkilde, 2009a) by fire ants, other species may not be so fortunate. Indeed, fire ants have been surmised to contribute to enigmatic population declines in several reptile species, including Southern Hog-nosed Snakes (*Heterodon simus*) and Eastern Kingsnakes (*Lampropeltis getula*) (Tuberville et al., 2000; Winne et al., 2007). Our method could be adapted to studies of predation on many subterranean nesting species as it is simple to implement, inexpensive (<\$5/nest, assuming possession of a suitable camera), quick (<5 min/nest/day), and allows monitoring of many nest replicates. Future studies should experimentally verify both the ability of fire ants to prey on eggs in natural scenarios and the frequency at which they do so. These results, combined with models investigating how this predation may alter fitness of affected populations, will allow strong inferences about how fire ants may affect the persistence of species with vulnerable life stages.

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Figures

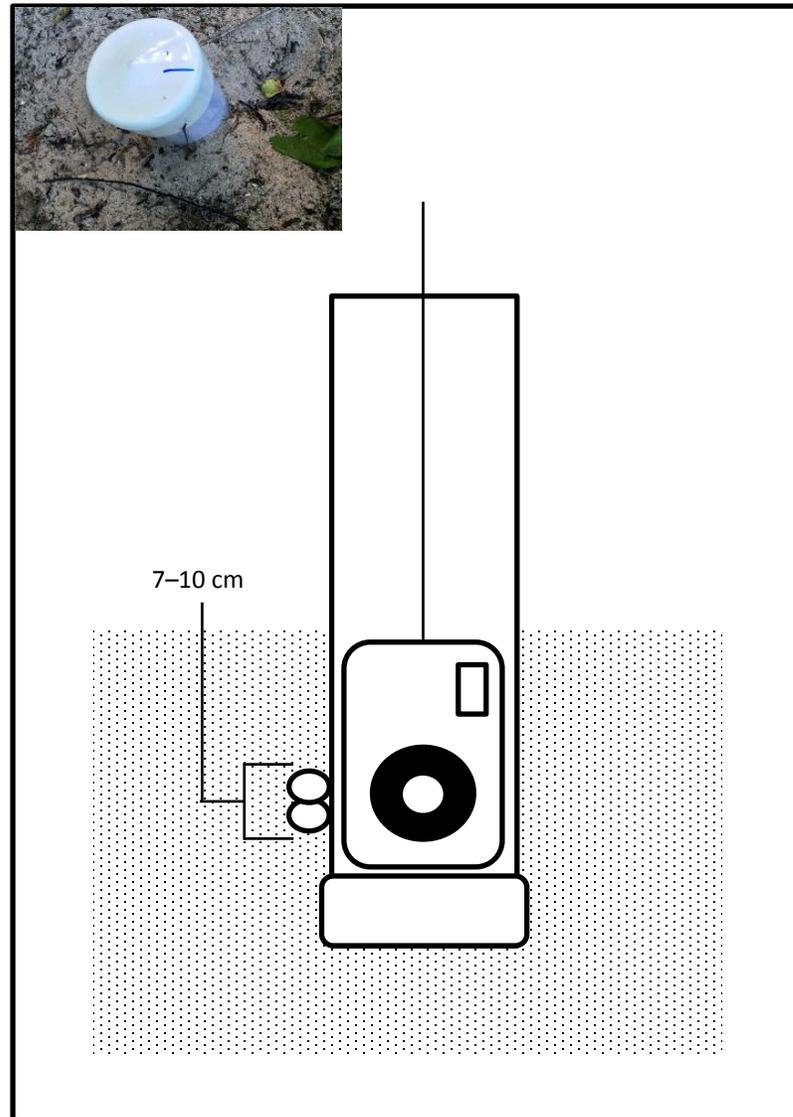


Figure 2.1. Artificial nests constructed using acrylic tubes placed 15 cm deep into soil (shown in stippling). Six fence lizard eggs were placed along the outer edge of the tube at 7–10 cm depth. A digital camera lowered into the tube allowed video recording of egg predation. Inset shows nest tube inserted into ground. Example video is available at <https://scholarsphere.psu.edu/files/qz20ss57q>

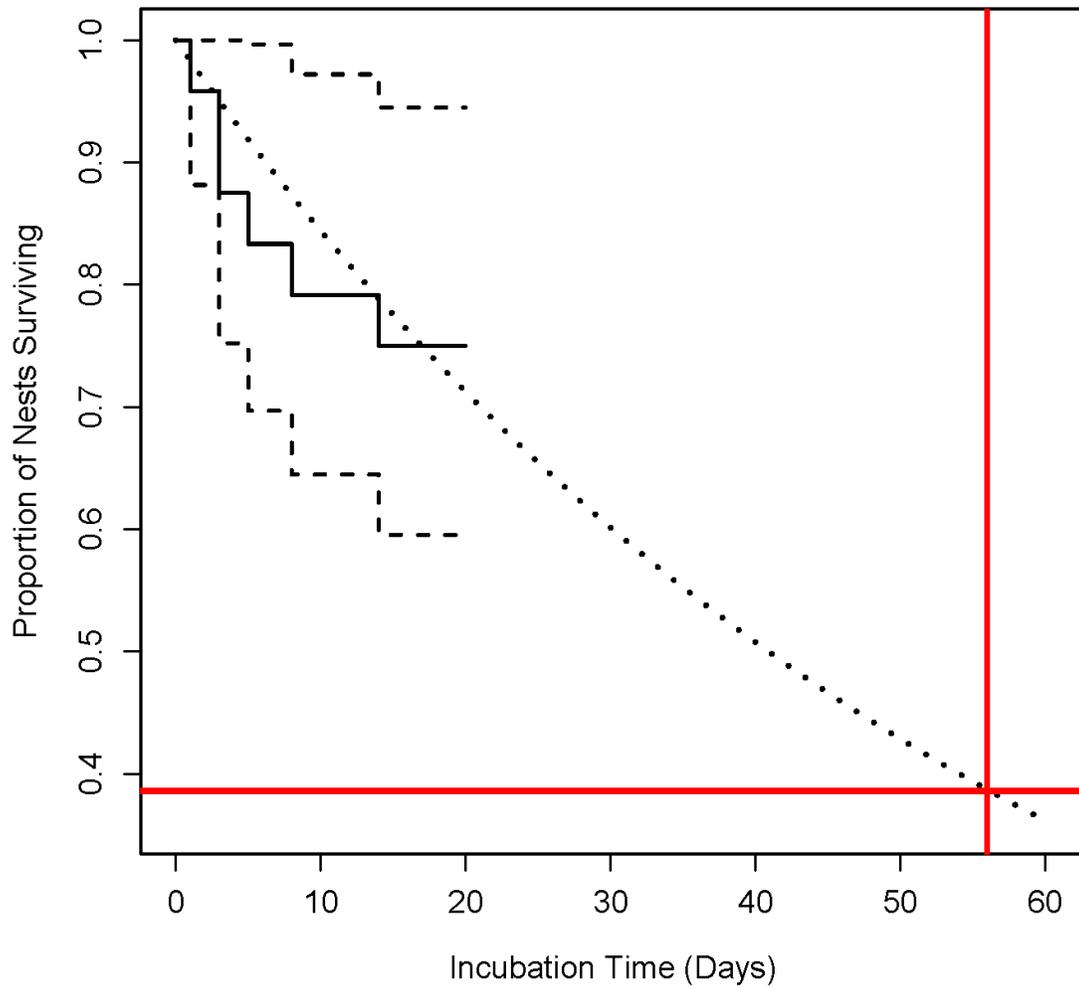


Figure 2.2. Survival of fence lizard nests over time. Solid line shows Kaplan-Meier curve fit to survival data (20 days) with 95% confidence intervals as dashed lines. Dotted line represents fitted exponential survival curve. Estimated incubation time at study site is 56 days (vertical red line) corresponding to an extrapolated predation rate of 61% (horizontal red line).

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Chapter 3

Lethal and Sublethal Effects of an Invasive Predatory Ant on a Native Lizard Vary Across Ontogeny

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Abstract

Accelerating global change, including the introduction of non-native species, is imposing novel selective regimes on species worldwide. In addition to having practical application, studying these systems can increase our understanding of ecological and evolutionary processes that shape populations. Invasive species exert diverse pressures on native species. These can generate lethal effects via predation or poisoning and sublethal effects, such as changes in behavior, growth, and body condition, both of which may vary across ontogeny of the affected species. We examine the effects of invasive predatory fire ants (*Solenopsis invicta*) on juveniles and adults of the native Eastern fence lizard (*Sceloporus undulatus*). We placed fence lizards from fire ant-invaded and -uninvaded populations in large field enclosures and manipulated fire ant density to quantify lethal and sublethal effects of this predator. Juveniles, but not adults, changed their perch height in response to fire ant density. High fire ant densities reduced growth (in snout-vent-length) in juveniles but did not affect growth of adults or body condition of juveniles. Adult body condition was affected by fire ant density, but the nature of the effect varied with population history with fire ants. While juvenile survival was unaffected by fire ant density, likely due to anti-ant adaptations, adults suffered increased mortality in enclosures with high fire ant density. Survival did not significantly vary with population history with fire ants for either juveniles or adults. These effects may be due to direct predation or indirect mechanisms such as trade-offs associated with anti-ant behaviors or competition with fire ants for food. All documented effects varied across ontogeny, highlighting the importance of accounting for life stage when examining effects of invasive species. Understanding both lethal and sublethal effects of invasive species and how they vary with key developmental and ecological traits of native species is critical to a fuller understanding of the impacts of invasive species.

Introduction

As the rate of global change accelerates, species must adapt to evolutionarily novel conditions or face declines in fitness, extirpation, and potentially extinction (Pimentel et al. 2005; Deutsch et al. 2008). Understanding whether species can adapt swiftly to different types of change, and the processes underpinning rapid adaptation, can lead to a better understanding of evolution and inform conservation and management decisions (Sax et al. 2007). Biological invasions, an increasingly frequent component of global change, are a major conservation concern (Mack et al. 2000; Clavero and García-Berthou 2005) but can provide unique opportunities to study rapid adaptation in ecologically relevant contexts (Strauss et al. 2006).

Lethal effects of biological invasions are well-known and documented and can be caused by predation (Allen et al. 2004), disease (Lips et al. 2008), toxicity (Phillips and Shine 2004), and parasitism (Koop et al. 2011). Lethal effects of introduced predators are particularly well-studied (Snyder and Evans 2006; Salo et al. 2007). Native species that have not coevolved with invasive predators may suffer lethal effects due to a failure to detect or recognize the threat posed by novel predators or a lack of an appropriate response or defense (reviewed in Sih et al. 2010). Lethal effects reduce individual fitness via mortality and cause population declines (Doody et al. 2009).

Invaders can also have sublethal effects, but these are less understood. Induced changes in behavior are a frequent sublethal effect of biological invasions, as behavior is a highly plastic trait and allows rapid, within lifetime responses to novel conditions (Sih 2013). Sublethal effects of invaders on behavior occur in a variety of systems and include changes in foraging behavior, habitat use, and antipredator behavior (Pearl et al. 2003; Orrock and Danielson 2004; Rius et al. 2009). Reductions in growth and body condition (e.g., Wilder and Rypstra 2004; Matsuzaki et al. 2012) are another widely observed sublethal effect of invasive species. These may be caused by less efficient or reduced foraging and food acquisition as a consequence of behavioral changes to

avoid predation (Pangle and Peacor 2006; reviewed in Harvell 1990 and Lima and Dill 1990). Alternatively, lowered energy intake could result from reduced availability of food resources due to competition with the invader or habitat alteration (Smith 2005; Baxter et al. 2004). Reduced growth can delay time to maturity or result in smaller adult body size, both of which can reduce lifetime reproductive output (Peckarsky et al. 1993). Reduced body condition can lower the amount of time and energy for reproduction and can cause cumulative effects that increase mortality due to associated stress, starvation, or lowered immune responses (e.g., Persons et al. 2002; Amo et al. 2006). Thus, assessing effects of invaders on growth and body condition can integrate many sublethal effects (Wright and Gribben 2008).

Both lethal and sublethal effects of invaders can negatively impact fitness via reduced survival or reproduction (Gribben and Wright 2006, Dorcas et al. 2012). Sublethal effects can have large impacts on fitness and demography, even when compared to those caused by direct predation (Ives and Dobson 1987; Werner and Peacor 2003; Pangle et al. 2007). As such, it is critical to study both types of effects in order to obtain a comprehensive understanding of the pressures that invaders exert on native species, and how these may drive adaptation (Sih et al. 2010).

Lethal and sublethal effects of invaders can both vary strongly with life stage (Rius et al. 2009). Some stages, such as eggs and juveniles, may be particularly vulnerable to lethal effects due to an inability to escape a threat or behaviorally defend themselves (Tschinkel 2006; Iwai et al. 2010; Koop et al. 2011; Thawley, Chapter 2). Sublethal effects can also vary ontogenetically due to developmental differences in factors such as diet and habitat use. For instance, the presence of an invasive predator can result in certain life stages of native species altering their habitat use (D'Amore et al. 2009), or change prey and nutrient availability to different life stages of native predators, resulting in altered growth rates (Correa et al. 2012). Some life stages, however, may be constrained in their ability to respond to the effects of invaders (e.g., eggs which

cannot escape predators, or juveniles which require time or energy to reach a future stage) (Mills et al. 2004). As such, differential effects of invasive predators across ontogeny may determine demographic impacts, or whether adaptation of the species to these novel pressures is possible (Reznick and Ghalmabor, 2001; Carlson et al. 2014).

Adaptation can occur on ecological timescales and in response to novel pressures, including invasive species (Thompson 1998; Berthon 2015). Adaptive changes can ameliorate negative impacts of invaders and mitigate fitness loss due to lethal and sublethal effects (Losos et al. 2004; Phillips et al. 2004). For instance, mice alter their foraging behavior due to interactions with invasive ants, which serve as toxic competitors, while balancing risks of exposure to native predators (Orrock and Danielson 2004). Native clams have adapted both behaviorally, by burrowing deeper, and morphologically, by having longer siphons, to avoid predation by an invasive crab predator (Whitlow 2010). Within species, local adaptive responses in populations affected by novel predators can mitigate negative effects of invaders (Freeman and Byers 2006; Strauss et al. 2006). Such adaptation to novel change can support persistence of populations or species even in situations where negative effects of invaders might otherwise lead to extirpation or extinction (Shine 2010; Carlson et al. 2014).

Understanding the nature of invader-induced pressures, whether via lethal, sublethal, or a combination of the two effects, and how they vary across ontogeny, is important for determining how species may adapt to novel environmental threats (Mills et al. 2004; Phillips et al. 2004; Berthon 2015). A more complete understanding of these novel systems can yield important or unique insights into ecological and evolutionary processes (e.g., Shine et al. 2011). However, such studies rarely examine a broad spectrum of effects over multiple life stages within a single system. We address these issues using a system of native Eastern fence lizard (*Sceloporus undulatus*) populations with varying exposure to an invasive predator, the red imported fire ant (*Solenopsis invicta*). There is strong evidence that adaptive responses (behavioral and

morphological) have occurred in populations of native fence lizards exposed to invasion by fire ants, suggesting that these ants exert strong selective pressure on lizard populations (Langkilde 2009a). We examined the effect of fire ants on adult and juvenile fence lizards by conducting a large-scale enclosure experiment that addressed the following specific questions:

- 1) What sublethal effects, including changes in behavior, growth, and body condition, do fire ants have on fence lizards?
- 2) Do fire ants exert lethal effects on fence lizards, and if so, to what extent?
- 3) Do the effects of fire ants vary with life stage (adult vs. juvenile) or evolutionary exposure to fire ants of fence lizards?

Methods

Study system overview

The red imported fire ant is a widespread invasive species that was first introduced to the United States via Mobile, Alabama in the 1930's (Lofgren et al. 1975). These ants have since spread to 13 additional states and have caused significant economic and agricultural losses (Morrison et al. 2004; Code of Federal Regulations 2016). Additionally, fire ants can cause direct mortality of vertebrates due to their venomous stings, high population densities, and ability to recruit to prey or to defend their mounds in large numbers (Allen et al. 1994; Wojcik et al. 2001). Fire ants achieve their highest densities in edge and disturbed habitats (Tschinkel 2006) which are also the primary habitat for Eastern fence lizards (Trauth et al. 2004). Fence lizards encounter fire ants frequently in these habitats (Freidenfelds et al. 2012), yet do not modify their habitat use in the presence of this evolutionarily novel venomous predator (Langkilde 2009b). Fence lizards generally avoid native, visually-hunting predators, such as snakes and birds of prey, by remaining

still and relying on their cryptic coloration and patterning (Cooper et al. 2000; Jensen et al. 2008). This tactic is ineffective against fire ants, however, and as few as 12 attacking ants are capable of paralyzing and subsequently killing an adult fence lizard (Langkilde 2009a). Ants comprise a significant portion of the diet of fence lizards, especially juvenile lizards (DeMarco et al. 1985; Mobley, 1998), and lizards can be envenomated and killed when consuming fire ants (Langkilde and Freidenfelds 2010).

Previous research has shown sublethal effects fire ants on stress physiology of fence lizards and documented changes in traits of fence lizards to the threat of fire ants within ≈ 40 generations (Langkilde 2009a; Graham et al. 2012). Adult fence lizards from sites invaded by fire ants show increases in flee and twitch behaviors in response to ant attacks (Langkilde 2009a), behaviors which reduce contact with and envenomation by fire ants (Freidenfelds et al. 2012). Juvenile lizards from all sites twitch and flee at a high rate, potentially an adaptation to avoiding attack by native ants that pose a danger to these small animals (Langkilde 2009a). Lizards from fire ant-invaded populations also have relatively longer hind limbs, a trait that supports more effective removal of attacking fire ants by twitch and flee behaviors and is associated with higher sprint and burst speeds which can dislodge ants (Miles 1994; Langkilde 2009a).

Previous work in vertebrate species has shown that fire ants may cause differential sublethal and lethal effects in native species depending on the life stage of the organism affected. For instance, eggs are often vulnerable to fire ant attack (Chalcraft and Andrews 1999; Diffie et al. 2010), and at the site of the present study, up to 60% of fence lizards nests may be preyed upon by fire ants (Thawley, Chapter 2). Juveniles have lower body mass than adults and so may be more affected by the same dose of venom (Robbins and Langkilde 2012). Likewise, young animals may have reduced abilities to defend against or escape from fire ant attacks and so be more likely than adults to suffer reduced growth (e.g. Giuliano et al. 1996; Allen et al. 1997) or

mortality (e.g. Reagan et al. 2000; Epperson and Heise 2003; reviewed in Allen et al. 2004) following attack by fire ants.

Lizard capture and housing

We caught adult and juvenile fence lizards using nooses and via hand capture from a total of six sites: three replicate sites invaded by fire ants and three that remain fire ant-free. Fire ant-invaded study sites are Solon Dixon Forestry Education Center, Alabama (SD: 31.1649° N, 86.7034° W), Blackwater River State Forest, Florida (BWR: 30.9417° N, 86.8176° W), and Geneva State Forest, Alabama (GSF: 31.1172° N, 86.1648° W). Study sites uninvaded by fire ants are St. Francis National Forest, Arkansas (SF: 34.7121° N, 90.7255° W), Standing Stone State Park, Tennessee (SS: 36.4679° N, 85.4164° W), and Edgar Evins State Park, Tennessee (EE: 36.0778° N, 85.8324° W) (all coordinates in WGS84). Sites SD, BWR, and GSF were first invaded by fire ants approximately 72, 53, and 72 years prior to lizard capture, respectively (Callcott and Collins 1996). All six sites contain temperate mixed forests and ample disturbed edge habitats that are favored by both fence lizards and fire ants (Langkilde 2009b). Adult lizards were captured during the breeding season (May-July), and juveniles were captured soon (≤ 6 weeks) after hatching. After capture, lizards were placed in individual cloth bags and transported to our field station at Solon Dixon Forestry Education Center for housing and placement in enclosures.

All lizards were housed individually at our field station for at least one week to allow recovery from capture stress and ensure lizards were in similar condition prior to placement in enclosures. Lizard terraria (30 × 21 × 26 cm, L × W × D) each contained a shelter and water bowl and were lined with paper toweling. A broad-spectrum, 60-W incandescent lamp placed at one end of the terraria provided a light and a thermal gradient on a 12:12 hr cycle. Gravid female

lizards near to parturition were placed in terraria with sand moistened daily to allow for natural egg-laying. Lizards were fed a diet of crickets (*Acheta domesticus*) dusted with supplementary vitamins and minerals four times weekly (Herptivite and Ultrafine Calcium with Vitamin D; Reocal, Los Gatos, CA), were provided with water *ad libitum*, and were misted daily to maintain humidity. Prior to placement in enclosures, we measured lizards for snout-vent length (SVL, to the nearest mm) and mass (to the nearest cgm), and recorded their sex. Each lizard was given a unique mark on its dorsum with a black permanent marker to allow individual identification from a distance and reduce the need to disturb lizards in enclosures. Lizards were also provided with a unique toe-clip, a minimally stressful procedure (Langkilde and Shine 2006) to allow a backup individual identification in the case that dorsal marks were lost due to shedding of skin.

Enclosures

We constructed four large-scale (520 m²) outdoor enclosures at a fire ant-invaded site, the Solon Dixon Forestry and Education Center in AL. Enclosures were built near native populations of fence lizards in a disturbed field habitat interspersed with trees and were located within 200 m of each other. We constructed enclosures with aluminum flashing (61 cm in height) secured to metal poles and buried 14 cm below ground level to prevent escape of fence lizards via burrowing. Following construction we carefully searched each enclosure for native fence lizards to ensure the enclosures were unoccupied prior to introduction of experimental lizards.

Each enclosure contained four large trees used as perches by fence lizards. We supplemented perch sites in enclosures by adding three cover objects composed of combinations of wood pallets and hardwood and pine branches and logs. Prey and water were naturally available to lizards throughout the study. Enclosures were inspected each morning and after inclement weather to ensure they were not damaged. Before each study period, grass was mowed

prior to introduction of lizards to prevent growth onto enclosure walls and to allow easier detection of fire ants (see below). Enclosures have been used to study Sceloporine lizards successfully in many experimental contexts (e.g., Cox et al. 2005; Robbins 2010), and lizards placed in enclosures in other studies have thrived and grown at similar rates to nearby lizards outside of enclosures (Niewiarowski and Roosenburg 1993).

Two weeks prior to placing lizards in enclosures, we created high (natural) and low density fire ant treatments by eradicating fire ant mounds inside and within a 7m buffer around two of the enclosures using hot water treatments. During early morning, fire ant colonies move queens and brood into the mound to reach optimal temperatures, making them more vulnerable to eradication (Tschinkel 2006). During this time (0700-0900 hrs), we sledgehammered a rebar stake into mounds, creating an opening into which we poured up to 25 l of hot water (82-88°C). This technique has been used successfully to reduce fire ant densities and performs similarly to reduction treatments with poison baits (e.g., Amdro; Harlan et al. 1981) without the confounding effects of reducing native ant and arthropod densities (King and Tschinkel, 2006; Tschinkel and King, 2007). During 2012, pitfall trapping revealed that densities in fire ant treated enclosures were an average of 15% of those in enclosures with naturally occurring fire ant densities (Thawley, unpubl. data). Treated enclosures were monitored daily for fire ant activity, and any newly established colonies or fire ant foraging trails were eliminated. As this activity may have constituted a stressor for lizards in fire ant-treated enclosures, we inspected untreated enclosures daily to ensure that this potential stressor was equivalent across enclosures.

Groups of ten lizards from each site were placed into enclosures for a 14-day period followed by a three-day period during which surviving lizards were caught and removed from enclosures. We measured SVL and mass of all lizards prior to placement in enclosures and, for surviving lizards, following recapture from enclosures at the end of the study. Lizards were assigned to one of the four enclosures via a stratified random procedure to ensure similar sex

ratios and size distributions, and each enclosure contained only lizards from one site. For juvenile lizards, these densities are within the range of those observed in nature and lower than those used in previous enclosure experiments (Gerwien and John-Alder 1992; Robbins 2010). For adult lizards, densities were approximately double those reported in this genus under natural conditions (Vinegar 1975; Parker 1994), and we introduced additional perch and shelter sites to accommodate these higher densities. Enclosure trials occurred over a two-year period, with adult lizards from EE, SS, and BWR, and juvenile lizards from SD and SF tested in 2011, and adult lizards from SF, SD, and GSF, and juvenile lizards from BWR, EE, GSF, and SS tested in 2012.

We observed lizards for two periods daily: 1) in early morning at the beginning of the diel activity cycle for fence lizards and coinciding with our monitoring and eliminating fire ants (0700-0900 hrs) and 2) after the hottest part of the day (1500-1700 hrs). No observations were conducted during periods of rain or inclement weather when lizards were inactive. During monitoring, we used a spotting scope as necessary to individually identify lizards to assess survival and estimate their perch height to the nearest 0.5 ft. At the end of each two-week period, we thoroughly searched enclosures over a three-day period, capturing all detected lizards. We thus assume that all lizards surviving at the end of each period were caught and that lizards that were not caught had died.

Statistical Analyses

Our analyses focused on detecting effects of fire ant density (high or low), invasion status of lizards' population of origin (invaded or uninvaded), and the interaction between these factors which would indicate that lizards are affected differentially by fire ants based on their lifetime or evolutionary experience with this novel predator.

Behavior

We used linear mixed models to model observations of perch height which were $\ln+1$ transformed. Lizard identity was treated as a random effect to model the variance between multiple observations of the same lizard and thus account for pseudoreplication. Initial models revealed an interaction between life stage and sex which resulted in poor model fit. As such, we chose to model perch height for adult and juvenile lizards separately. Starting models of perch height tested for effects of fire ant density, invasion status, their interaction, and sex, and included terms accounting for the effects of size (SVL), site, and enclosure. We used likelihood ratio tests to compare nested models and select parsimonious final models. Finally, after constructing parsimonious models, we tested to see if perch height differed between lizards that died while in enclosures and those that survived.

Growth and Body Condition

We assessed growth of lizards (change in SVL) and changes in body condition while in enclosures for all surviving juvenile and adult male lizards. Body condition was calculated as the residuals from a linear regression of log-transformed body mass on log-transformed SVL (Schulte-Hostedde et al. 2005). As mass was significantly correlated with these two morphological measurements (all $p < 0.006$), we did not analyze it separately. We used general linear models to assess changes in SVL and body condition of juveniles and adult males while in enclosures. Change in SVL of adult males was transformed using an $\ln(y + 1)$ transformation.

We chose to exclude female lizards from analyses of growth and body condition. This study took place during the breeding season of all populations of *S. undulatus* used, when females invest energy in reproduction and not growth. Females in enclosures were reproductively active,

both producing and laying clutches throughout. Changes in female gravidity were strongly correlated ($\rho > 0.582$) with changes in mass and body condition, and females from different sites, which become gravid at different times and have different numbers of clutches (C. Thawley, pers. obs.) were at significantly different stages of their reproductive cycle based on assessment of gravidity ($p < 0.020$).

Starting models included effects of fire ant density, invasion status, their interaction, and sex, and included terms accounting for site, enclosure, and, in the case of body condition, SVL. Final models were selected using likelihood ratio tests.

Survival

We used Program MARK (v 8.0) to test for effects of sex, SVL, body condition (for juveniles only), invasion status, fire ant density, and their interaction as covariates on survival (Φ) and detection probability (p) of fence lizards in enclosures (White and Burnham 1999). Separate models were constructed for adults and juveniles due to known differences in ecology and responses to fire ants (Langkilde 2009a). We selected parsimonious models using quasilikelihood adjusted Akaike's information criterion corrected for small sample sizes ($\Delta QAIc_c$) (Burnham and Anderson 2002).

To construct models in MARK, we created occurrence histories based on daily observations of lizards over their two-week periods in enclosures. For each model, we first optimized detection probabilities for each analysis, and then selected a parsimonious model for lizard survival using $QAIc_c$. To optimize detection probabilities, we selected from either a fully time-parameterized model or a simpler model including separate detection probabilities for each time period that enclosures contained lizards ($n=4$ and 3 time periods for adults and juveniles, respectively). As site, individual enclosure, and year were not fully crossed in our design,

modeling detection probabilities separately for each day or time period that enclosures were run allowed us to account for many proximate factors, such as weather, which would affect all enclosures similarly, and which are known to have strong impacts on activity in *S. undulatus* (Jones et al. 1987; C. Thawley, pers. obs.). We set detectability to zero for days when fence lizards were inactive and unobserved (i.e., those with heavy rains). Detection probabilities for the final day were set to one, as all surviving lizards were captured from enclosures.

Visual analysis of survival data indicated that daily survival rates were lower for adults and especially juveniles soon after introduction into enclosures, and then leveled off. As a result, we compared the fit of models with a constant survival rate and models that allowed survival rate to vary between an earlier and a later phase. Earlier phases were chosen as the first two and five days within enclosures for juveniles and adults respectively based on assessments of when half of mortality of fence lizards in enclosures had occurred.

We assessed goodness of fit of general models to the data using the median \hat{c} approach as implemented in MARK. The median \hat{c} approach generates a variance inflation factor, \hat{c} , an estimate of overdispersion, for general models (White and Burnham 1999). For adults, we assessed the fit of a model containing one value of Φ , a fully time parameterized model of detectability, and no covariates. For juveniles we assessed the fit of a general model that included two values of Φ (as above), a fully time parameterized model of detectability, and no covariates. Using these estimates of \hat{c} , values of AICc for working and final models were corrected to quasi-likelihood adjusted QAICc, along with coefficient estimates and their 95% confidence intervals, which we report in the results (Burnham and Anderson 2002).

A previous study found that juvenile fence lizards envenomated by fire ants suffered delayed mortality up to 11 weeks post-envenomation (Langkilde and Freidenfelds 2010). We therefore tested for delayed effects of exposure to high fire ant density in enclosures by housing surviving juveniles in the lab for up to 16 weeks after removal from enclosures. Lizards were

housed as above in mixed-sex groups of 3-4 lizards placed in correspondingly larger terraria (56 X 40 X 30 cm, L X W X H). We monitored lizards daily for survival and assessed the effects of fire ant treatment, invasion status of population, their interaction, sex, body condition, and size (SVL; pre-lab housing) on survival using a Cox proportional hazards regression. We used likelihood ratio tests to construct a parsimonious final model.

Aside from models constructed in MARK, all analyses were conducted using SPSS v.23 (IBM Corp., 2015) and in program R v3.1.2 (R Foundation for Statistical Computing, Vienna, Austria; available online at <http://www.R-project.org>, 2014).

Results

Behavior

Our final model for perch height of juvenile lizards in enclosures included effects of fire ant density, invasion status, sex, and size and random effects accounting for site and individuals. Juvenile lizards in enclosures with high fire ant density perched at heights significantly lower ($\beta = -0.944$, $F_{1,79.6} = 4.942$, $p < 0.029$) than juveniles in enclosures with low fire ant density. Larger juveniles used higher perches than smaller juveniles ($\beta = 0.012$, $F_{1,46.1} = 4.211$, $p < 0.046$). All other terms were not significant (all $p > 0.398$).

Our final model for perch height of adult lizards in enclosures included effects of fire ant density, invasion status, sex, and size, and random effects accounting for site and individual. Male lizards in enclosures perched significantly higher than females ($\beta_{\delta} = 0.242$, $F_{1,87.6} = 10.780$, $p < 0.001$) and larger adults tended to use higher perches than did smaller adults ($\beta = 0.015$, $F_{1,93.4} = 3.533$, $p < 0.063$) though this difference was not significant. All other terms were not significant (all $p > 0.538$).

In both adults and juveniles, lizards that died in enclosures did not show any differences in perch height from lizards that survived (all $p > 0.297$). However, our ability to detect any difference was low, as many lizards that died had very few observations or were never observed in enclosures prior to death.

Growth and Body Condition

Our final model for growth (SVL) of juveniles in enclosures included effects of fire ant density and invasion status and terms accounting for initial SVL ($p > 0.849$) and site ($F_{4,85} = 7.751$, $p < 0.001$). Juveniles in enclosures with higher fire ant density grew less than those in low fire ant density enclosures ($\beta = -1.283$, $F_{1,85} = 20.130$, $p < 0.001$), and juveniles from fire ant-uninvaded sites grew less than lizards from fire ant-invaded sites ($\beta = -1.308$, $F_{1,85} = 21.556$, $p < 0.001$) (Fig. 3.1a).

Our final model for change in body condition of juveniles in enclosures included effects of fire ant density and invasion status and terms accounting for initial SVL ($p > 0.675$), sex ($\beta_{\text{♀}} = 0.022$, $F_{1,82} = 6.374$, $p < 0.014$), and site ($F_{4,82} = 3.229$, $p < 0.016$). Fire ant density, invasion status, and their interaction had no effect on change in body condition in juvenile lizards (all $p > 0.238$).

Our final model for growth (SVL) of adult males in enclosures included effects of fire ant density and invasion status and terms accounting for initial SVL ($\beta = -0.056$, $F_{1,33} = 20.621$, $p < 0.001$) and site ($p > 0.080$). Fire ant density, invasion status, and their interaction had no effect on change in SVL in adult males (all $p > 0.655$). While the $\ln(y + 1)$ transformation of SVL growth improved residual distribution in this model, some evidence of non-normality still persisted.

Our final model for change in body condition of adult males in enclosures included effects of fire ant density ($p > 0.536$), invasion status ($p > 0.631$), and their interaction ($\beta = 0.052$,

$F_{1,32}=5.984$, $p<0.020$), and terms accounting for initial SVL ($p>0.672$) and site ($p>0.236$), although this model only explained a small proportion of the variance in body condition (adjusted $R^2=0.11$). While males from fire ant-invaded sites tended to have higher body condition in enclosures with high fire ant density when compared to those in low density enclosures, lizards from uninvaded sites showed the opposite pattern, with higher body condition in enclosures with low fire ant density (Fig. 3.1b).

Survival

Models for juvenile and adult survival both performed well. The median \hat{c} approach, which assesses model goodness of fit, generated an estimate of $\hat{c}=1.133$ for the general model for juvenile survival and an estimate of $\hat{c}=1.083$ for the general model for adult survival, indicating low levels of overdispersion (White and Burnham 1999).

For juvenile lizards, detectability was modeled with a fully time-dependent model with a negative effect of sex (female) on detectability ($\beta_{\text{♀}}=-0.278$; 95% CI:(-0.536, -0.019)). The best performing model of survival included two phases (Days 1-2 and Days 3-15) with an identical positive effect of body condition on survival during both phases ($\beta=0.109$; 95% CI:(0.021, 0.196)). Juvenile lizards had a lower estimated daily survival rate during the first phase ($\Phi_1=0.907$; 95% CI:(0.825, 0.952)) followed by a higher rate of survival ($\Phi_2=0.989$; 95% CI:(0.980, 0.994)) over the remainder of the two week enclosure periods (Fig. 3.2a). An effect of fire ant density on survival was not included in the best model of juvenile survival.

For adult lizards, detectability was best modeled with a fully time-dependent model. Detection probability was significantly reduced in adult female compared to male lizards ($\beta_{\text{♀}}=-0.688$; 95% CI:(-0.952, -0.424)). High fire ant density also reduced detectability ($\beta=-0.690$; 95% CI:(-0.951, -0.430)), and detectability increased with SVL ($\beta=0.047$; 95% CI:(0.019, 0.075)). For

adults, the best performing model of survival included a negative effect of high fire ant density on survival ($\beta=-1.245$; 95% CI:(-2.090, -0.401)). The estimated daily survival rate in enclosures with low fire ant density was (0.990; 95% CI:(0.979, 0.995)), while the survival rate in enclosures with high fire ant density was significantly lower (0.965; 95% CI:(0.947, 0.977)) (Fig. 3.2b). An effect of invasion status of source population on detection probability or survival was not included in the best model.

When housed in the lab for up to 16 weeks after placement in enclosures, larger juveniles ($\beta=-0.184$, $\chi^2_1=5.696$, $p<0.017$) and juveniles from fire ant-uninvaded sites had lower survival ($\beta=-1.242$, $\chi^2_1=5.966$, $p<0.015$). Juvenile lizards exposed to high fire ant densities in enclosures did not experience delayed mortality ($\beta=0.591$, $\chi^2_1=1.351$, $p>0.242$).

Discussion

Our research explores the effects of fire ants on different life stages of the same species in an ecologically relevant context. Juvenile and adult fence lizards have distinct ecologies and different responses to invasive fire ants and are thus subject to differing effects of fire ants. In this study, both stages altered their behavior in response to high fire ant densities. While juvenile lizards reduced their perch height, this remained unchanged in adults, which alter their activity in a way that changes their detectability. High fire ant density reduced growth (of SVL) in juveniles but not body condition, while in adults, body condition was affected by fire ant density but growth rate was not. Lastly, while fire ants did not reduce survival in juvenile lizards, adult lizards experienced higher mortality at high fire ant densities. As such, fire ants likely exert differential selective pressures across these different life stages which can, in turn, drive ontogenetic variation in adaptation. Despite strong evidence of adaptation in fence lizards to the presence of fire ants (Langkilde 2009a; Graham et al. 2012), we detected only equivocal evidence

of amelioration of sublethal effects of fire ants on lizards due to their evolutionary or lifetime history with fire ants and no mitigation of lethal effects.

These findings are based on a study conducted in large-scale, semi-natural field enclosures. While these enclosures allowed us to manipulate fire ant density experimentally and conduct observations that would otherwise have not been possible in the field, lizards may have responded to enclosures differently to field conditions in important ways. 1) Juveniles experienced lower survival rates during the first two days of being placed in enclosures than during the remainder of the experimental period, potentially indicating that juveniles were more vulnerable to predators or other forms of mortality during a period of acclimation to this novel environment. Mortality was likely unrelated to food availability as juveniles in enclosures grew at rates comparable to a free-living population in nearby Mississippi (Parker 1994). This mortality is not unexpected as juveniles in natural environments suffer high mortality during early life (Tinkle and Ballinger 1972; Parker 1994), and this effect did not vary with treatment. 2) While densities of juvenile lizards in enclosures were within natural levels, stocking densities of adults were double those found in nature (Vinegar 1975; Parker 1994). We increased perch density within enclosures with supplemental perch objects to support occupancy by these lizards over the two-week observational period, but behavior may have been affected. There was, however, evidence that lizards behaved normally in our enclosures. Lizards displayed behavioral patterns similar to wild lizards; as in the field, adult males perched higher and had higher detectability than females (Pounds and Jackson, 1983; Thawley, pers. obs.); larger lizards were more detectable and tended to have higher perches (Pounds and Jackson, 1983; Haenel et al. 2003); and lizards ate prey items, exhibited courtship behavior, and maintained individual territories (Haenel et al. 2003). As such, we believe that our findings are relevant to fence lizard populations in natural environments.

Sublethal Effects

Fire ants infrequently forage off the ground (Jusino-Atresino and Phillips 1994; Langkilde 2009b), so we expected that lizards in enclosures with high fire ant density may have used more arboreal microhabitats to avoid attacks, a tactic used by lizards in response to other terrestrial predators (Losos et al. 2004) and by other species specifically in response to fire ants (Forys et al. 2001). Indeed, while stressed lizards from fire ant-uninvaded sites tend to hide under cover when they flee, lizards from invaded sites move up off the ground when stressed, a potentially adaptive response to this primarily terrestrial ant (Trompeter and Langkilde 2011). However, in this study the presence of fire ants in enclosures had no effect on adult perch height. Fire ants did affect juvenile perch height, but in the opposite way to our predictions; juveniles in enclosures with high densities of fire ants perched lower than those in enclosures with lower fire ant densities, though this absolute difference was small (mean of 23.6 vs. 30.1 cm). The biological relevance, if any, of this difference is unclear. We also found no differences between perch height of lizards that died in enclosures versus lizards that survived. However, our ability to detect an effect of perch height on survival was low, as we were able to record at least one observation of perch height for only 55% of lizards that died in enclosures. We believe it is unlikely that availability of perches at different heights influenced these results, as multiple perch objects, including supplemental ones, were placed in enclosures, and lizards were observed using perches up to ≈ 10 m in height. Additionally, this lack of change in perch height corresponds to field data that also show no difference in adult perch height between fire ant-invaded and -uninvaded sites (Langkilde 2009a), suggesting that alterations in perch height may not be employed by fence lizards to reduce encounters with fire ants.

Adult lizards in high fire ant density enclosures had lower detectabilities, suggesting that lizards altered their activity patterns in response to fire ants in some way that we did not quantify.

High fire ant densities are known to increase defensive activity and alter foraging activity and habitat use in other vertebrates (Killion and Grant 1993; Pedersen et al. 1996; Pedersen et al. 2003; Orrock and Danielson 2004; Ligon et al. 2012). It is possible that, in response to fire ants, fence lizards reduced activities, such as foraging, at ground level or lower heights during which lizards are quite detectable by human observers. Lizards may also have moved high into trees and out of our sight, reducing detectability and preventing quantification of a shift in perch height. Such changes in behavior may have sublethal consequences such as reduction in energy intake or increases in energy expenditure, which could affect growth and body condition.

Juvenile growth was significantly reduced in high fire ant density enclosures (Fig. 3.1a). This effect of fire ants on growth has been observed in other species, though only following direct exposure to fire ants under laboratory conditions (Giuliano et al. 1996; Allen et al. 1997a). In previous studies with fence lizards, envenomation by fire ants did not lead to reductions in growth; however, this was following low exposure to fire ants at or near birth (Langkilde and Freidenfelds 2010). Fire ants may have reduced juvenile growth in the present study via several pathways. 1) Juveniles react to fire ants and native ants by fleeing and twitching (Langkilde 2009a) and thus may expend more energy, have reduced time to forage and obtain energy for growth, and even flee from potential prey (ants) in the presence of these predators (Pedersen et al. 1996). 2) Fire ants can reduce arthropod abundance and diversity and alter community composition, particularly of native ants (Vinson, 1994; Epperson and Allen 2010; Lach and Hooper-Bùi 2010), a major prey of juvenile fence lizards (DeMarco et al. 1985; Mobley 1998). As such, they may reduce prey available to lizards or even compete directly with lizards for prey (Ligon et al. 2011). Competition for prey with fire ants has been linked to changes in abundance in other species (Allen et al. 2001), and could have led to the observed reductions in fence lizard growth. Reductions in growth can have serious impacts on fitness, including increased time to reach maturity and reduced reproductive output, especially in species such as fence lizards for

which fecundity increases with size (Tinkle and Ballinger 1972; Angilletta et al. 2004). Growth was significantly lower in juveniles from sites uninvaded by fire ants, possibly a reflection of differences in intrinsic growth rates between sites (reviewed in Angilletta et al. 2004), or a result of lizards from uninvaded populations experiencing more divergent climatic conditions from natal sites.

While juvenile growth in SVL was reduced in enclosures with high fire ant density, body condition of juveniles was unaffected. This pattern is similar to that observed in food-restricted juveniles of *Amphibolurus muricatus* (Radder et al. 2007), and may indicate that some juvenile lizards do not invest energy in lengthwise growth at the expense of body condition or are subject to allometric growth constraints.

Growth rates in enclosures did not differ in adult males. This is not surprising as absolute adult growth in males was low for this 2-week period (mean=0.48 mm). However, the effect of fire ants on body condition of adult males varied as a function of the invasion status of the source population, suggesting that fence lizards varied in this sublethal effect of fire ants based on their evolutionary or lifetime history with fire ant exposure (Fig. 3.1b). Lizards from fire ant-invaded populations tended to have higher body condition in enclosures with high fire ant density and lower condition when few fire ants were present, while the opposite was true for fence lizards from uninvaded populations which had comparatively higher body condition in enclosures with low fire ant density. Ants are an important food source for fence lizards (DeMarco et al. 1985; Mobley 1998), and field-caught adult lizards from fire ant-invaded sites eat fire ants at a significantly higher frequency than lizards from uninvaded sites (Robbins and Langkilde 2012). If lizards from fire ant-invaded sites made more use of fire ants as prey, better exploiting this abundant and potentially valuable food source (Robbins and Langkilde 2012), this behavior could lead to higher body condition.

These sublethal effects, if consistent over longer periods of time, could result in future mortality. Effects of fire ants on growth and body condition might accumulate over a breeding season (Ligon et al. 2011) and, if our lizards had been left in enclosures longer, these effects may have become more pronounced and have affected survival. Our data indicated higher mortality of juveniles with lower body condition, suggesting that this could have important fitness consequences. Reductions in growth and body condition can likewise increase mortality when combined with other stressors or threats (Amo et al. 2006).

Lethal Effects

Naturally high densities of fire ants did not affect survival of juvenile fence lizards (Fig. 3.2a). This result is not unexpected as juvenile lizards from both fire ant-invaded and -uninvaded populations twitch and flee at high rates in response to fire ant attacks (Langkilde 2009a). This is likely an adaption to surviving encounters with native ants, which may pose a threat to small juvenile lizards, and allows juvenile lizards to effectively avoid attack by fire ants (Langkilde 2009a). In addition, while a previous study demonstrated that juvenile fence lizards envenomated by fire ants suffered delayed mortality (Langkilde and Freidenfelds 2010), we found no effect of exposure to high densities of fire ants in enclosures on subsequent survival of juvenile lizards in the lab.

Adult lizards, however, suffered higher mortality in high fire ant density enclosures than in enclosures with reduced fire ant density, evidence that fire ants can impose strong selective pressure on fence lizard populations. While 86% of adult lizards in enclosures with low fire ant density survived, only 60% survived in enclosures with high fire ant density over 2 weeks. *S. invicta* has been linked to reduced survival in many species (e.g., Todd et al. 2008; Long et al. 2015; reviewed in Allen et al. 2004) and observed to cause direct mortality in multiple vertebrates

(e.g., Freed and Neitman, 1988; Allen et al. 1997a), including this species of fence lizard (Langkilde 2009a). The present study provides important insight into the rate and degree of these lethal effects.

We had expected that mortality due to fire ants might be modulated by evolutionary and lifetime history of lizards with fire ants, as lizards from fire ant-invaded sites display adaptive increases in twitch and flee behaviors which allow removal of, and escape from, attacking fire ants (Langkilde 2009a). However, an interaction between invasion status and fire ant density was not selected for inclusion in our best-performing model. Visual inspection of survival curves (Fig. 3.2b), however, suggests a potential interaction between these factors, as lizards from fire ant-uninvaded sites suffered a large decrement to survival in enclosures with high fire ant density while lizards from uninvaded sites were less affected. Indeed, a candidate model including an interactive effect of fire ant density and invasion status on survival was among the top-performing models ($\Delta\text{QAICc}=3.71$). These results are suggestive, and may be better supported with larger sample sizes.

Surprisingly, adult fence lizards from fire ant-invaded sites tended to have lower survival than lizards from uninvaded sites when in enclosures with low densities of fire ants (Fig. 3.2b). While fleeing and twitching are effective defenses against fire ants, these behaviors may also attract native avian and ophidian predators which were present in enclosures, resulting in attacks and potentially lower survival (Thawley, Chapter 4). Supporting this, lizards from fire ant-invaded sites showed higher injury rates in the field than lizards from uninvaded sites (Thawley, Chapter 4). This effect has also been proposed to explain reductions in recruitment of white-tailed deer (*Odocoileus virginianus*) fawns which may suffer increased attacks from native, visually-hunting predators when they break crypsis in response to fire ant stings (Allen et al. 1997b; Mueller et al. 2001).

Taken together, these results demonstrate that sublethal and lethal impacts of fire ants on fence lizards vary strongly across fence lizard ontogeny, a pattern observed in other native species. Differences in predation by fire ants vary with ontogeny in songbirds, for which nestlings are more vulnerable than eggs (Stake and Cimprich 2003; Campomizzi 2008) and cottontail rabbits, for which recently born nestlings are more vulnerable than older, furred nestlings (Hill 1969). If the impacts of invasive fire ants vary so dramatically with life stage in fence lizards, which hatch with the same body plan and similar behavioral repertoire to adults (Roggenbuck and Janssen 1986), divergent effects of invaders across ontogeny are likely in many species, including those that undergo complex shifts in morphology and ecology across development (e.g., many insects, amphibians; Werner and Gilliam 1984; Moran 1994).

In conclusion, invasive species may produce diverse lethal and sublethal effects on native species, which can vary across ontogeny and have important demographic and fitness consequences (Pangle et al. 2007). Researchers should thus consider a variety of ecological contexts, including life history and developmental factors, when attempting to determine the lethal and sublethal impacts of invasive species. Field observations, experimental manipulations, and population modeling in multiple invasive systems will shed important light on how ontogenetic differences in effects of invasive threats affect demography, population persistence, and evolutionary processes.

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Figures

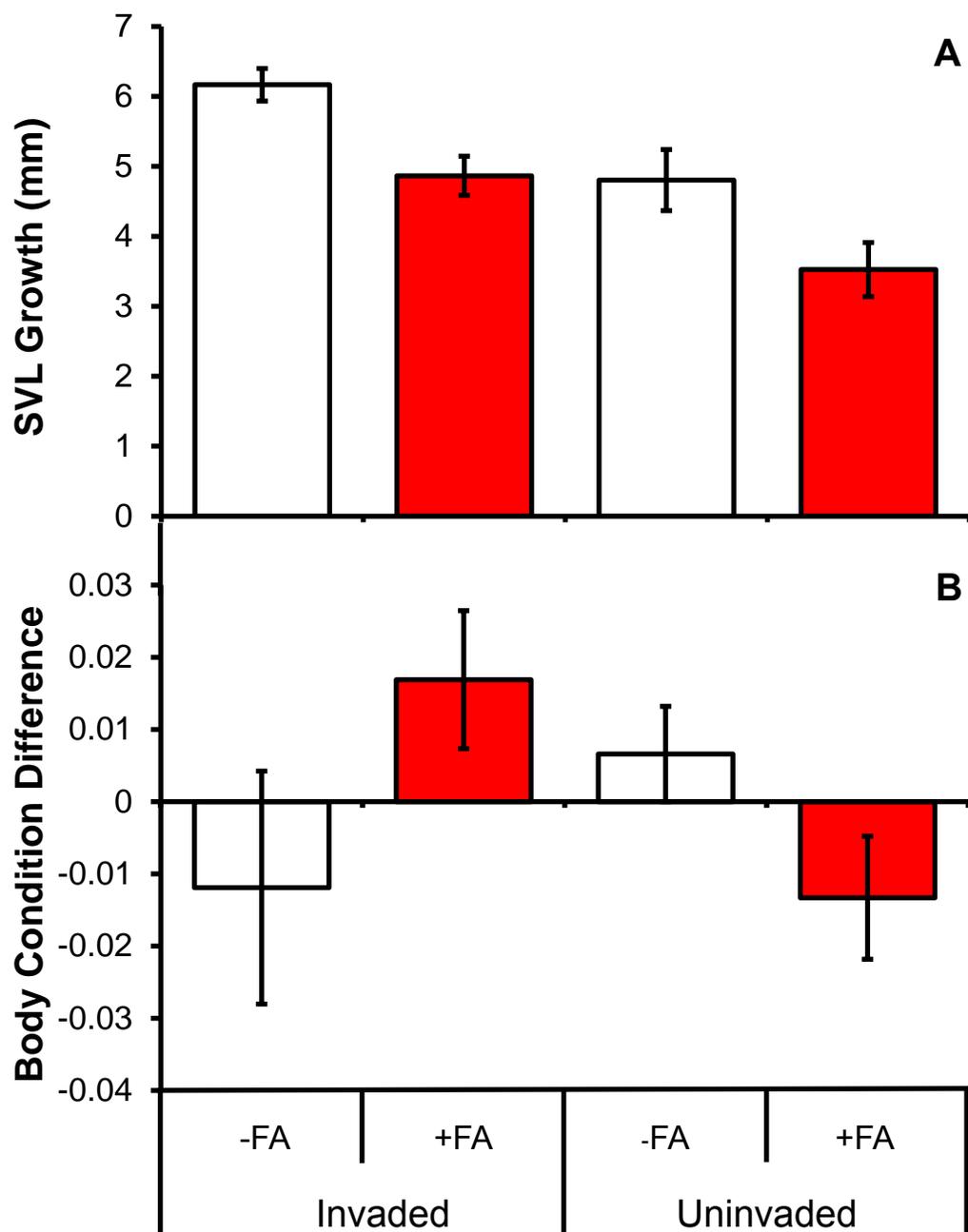


Figure 3.1. Change in snout-vent-length (SVL) in juvenile lizards (A) and body condition in adult lizards (B) from fire ant-invaded and uninvaded sites while in enclosures with reduced (white) and high (red) fire ant density. Error bars ± 1 standard error.

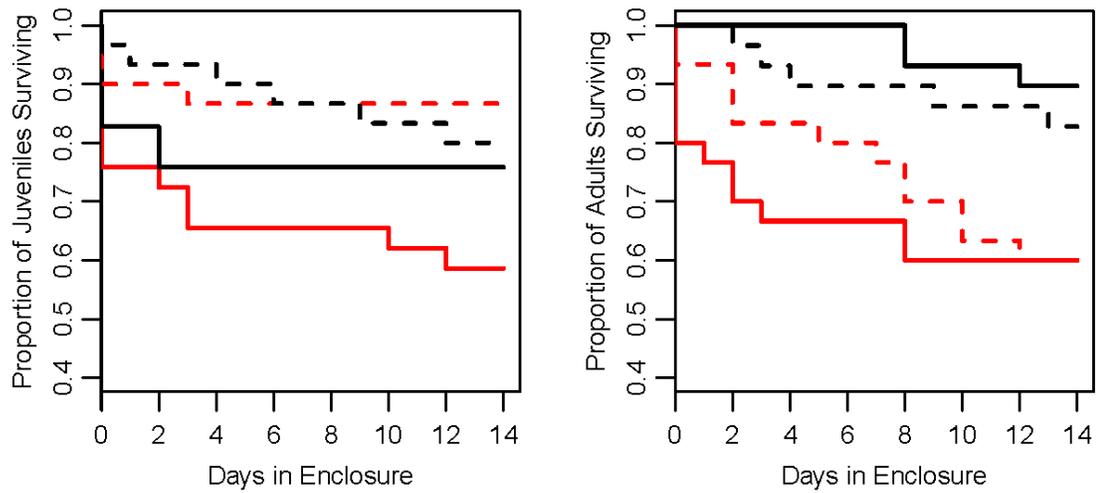


Figure 3.2. Kaplan-Meier survival curves based on live observations of juvenile (A) and adult (B) fence lizards from fire ant-invaded (dashed lines) and -uninvaded (solid lines) populations placed in enclosures with reduced (black) and high (red) fire ant density for two weeks.

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Chapter 4

Attracting unwanted attention: generalization of behavioral adaptation to an invasive predator carries costs

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Abstract

Many behavioral responses of animals to environmental cues are governed by general “rules of thumb”. Animals that face novel conditions as a result of global environmental change may alter these behavioral rules in order to persist. However, adaptation of generalized rules to novel pressures may cause a species to be maladapted to original conditions, such as predators, that remain in its environment. Invasive fire ants (*Solenopsis invicta*) are novel predators of Eastern Fence Lizards (*Sceloporus undulatus*). Lizards from fire ant-invaded sites break crypsis to flee from fire ants at higher frequencies than fire ant-naïve lizards. This shift promotes survival of attacks by these invasive ants, but could result in increased attacks by native visual predators. Generalization of this increase in anti-predator behavior to native species could further increase this cost. We tested whether lizards’ increased propensity to flee from fire ants is generalized to native ants and a predatory bird. We found that increased behavioral responsiveness to fire ants was generalized to two native ants, but not to a perceived avian predator. We also found that lizards from populations invaded by fire ants had higher injury rates in the field, a cost likely indicating greater attempted predation. We propose that generalized anti-ant behavior may improve survival in the presence of fire ants but increase attacks by native visual predators. This study suggests that generalized rules can be maladaptive under novel conditions, and highlights the challenges of assessing the costs and benefits of adaptations to rapid anthropogenic change.

Introduction

The complex suites of behaviors used by many organisms are often a product of both specific and general underlying rules. For example, individuals can choose to mate with, or treat as a predator, anything that smells, tastes, looks and behaves a specific way (Jennions and Petrie 1997; Frid and Dill 2002). This can be as general as responding to looming shapes or objects (Carlile et al. 2006) or objects of a particular color or texture (Gwynne and Rentz 1983) or size (Arak 1983, Marco et al. 1998), or as specific as distinguishing based on a particular combination of multiple specific cues (Hankiston and Morris 2003)

Behavioral rules are generally only as complex as required to be adaptive within the selective environment (Schlaepfer et al. 2002), and general rules may evolve more quickly and easily than more complex ones (Orr 2000). While relying on specific rules reduces the risk of making errors, general rules for behavior are valuable as they allow for responses to many different environmental cues and obviate the costs of discriminating specific cues (Sih 2013, Carthey and Banks 2014). These rules are strategies that work on aggregate over multiple iterations in diverse or uncertain situations (Lima and Dill 1990), and their use may lead to more predictable outcomes in highly variable environments (Heiner 1983).

However, reliance on general rules can incur costs as the chances of responding inappropriately are higher than if rules are more specific. For example, males with indiscriminate mate choice could waste time and energy, and lose mating opportunities, by attempting to mate with unsuitable partners or objects (e.g., anurans mating with dead or heterospecific individuals, or shoes; Meshaka 1996), and parents caring for any offspring they encounter could bear the costs of raising unrelated individuals (e.g., hosts of nest parasites; Payne 1998).

The fitness consequences of generalized behavioral responses are a focus of much recent research and theory, especially in the context of rapid environmental change (Schlaepfer et al.

2005, Sih 2013, Carthey and Banks 2014). Novel or rapidly changing selective regimes can result in maladaptation or evolutionary traps (Crespi 2000; Schlaepfer, Sherman and Runge 2010), including in response to environmental change (Bradley St. Clair and Howe 2007, Sinervo et al. 2010; Kerby and Post 2013). Indeed, general rules of behavior can be especially costly when the environment changes and organisms apply these rules in the wrong contexts. For example, an organism could attempt to mate with an invasive species (Gröning and Hochkirch 2008) or man-made object that fulfils their general rules for mate identification (Alcock 2013), reducing fitness. Generalized responses to novel predators and prey can also reduce survival. For instance, generalist predators preying upon toxic invasive Cane Toads (*Rhinella marina*) can be poisoned (Shine 2010), and New Zealand mudsnails react to invasive crayfish by burrowing, an ineffective defense against these novel predators (Sih 2013). Accelerating global environmental change provides an excellent opportunity to examine the costs and benefits of generalized behaviors, especially in the case of adaptive responses to the novel conditions (Sinervo et al. 2010; Carthey and Banks 2014).

To fully understand the consequences of behavioral adaptations to novel selective pressures, we need to know:

- 1) How behavior has changed in response to novel environmental conditions.
- 2) If behavioral changes are specific to novel environmental conditions (e.g., to an introduced predator only), or generalized to similar stimuli (e.g., to other ecologically similar species) or even more broadly (e.g., to all potential predators).
- 3) If behavioral changes are costly (e.g., cause decreased survival or wasted time and energy), as we might expect if changes in behavior are generalized across multiple situations/species.

Anti-predator behavior is an excellent system with which to address these questions. It is expected to be under strong selection in many situations including environmental change such as

the introduction of novel predators (Abrams 2000). For example, native tadpoles (*Lithobates aurora*) that are naïve to invasive predatory bullfrogs (*Lithobates catesbeiana*) suffer higher mortality than do tadpoles that have adapted to these invaders by reducing activity levels to avoid predation (Kiesecker and Blaustein 1997). Even when successful, anti-predator behavior is known to carry a variety of costs, including higher energy usage, lower foraging rates/opportunities, and reduced ability to defend territories (Lima and Dill 1990, Martín et al. 2009). If adaptations to novel predators alter general anti-predator behavioral rules, applying these rules to interactions with native predators may also incur these costs and lower fitness. When a trade-off between costs and benefits of novel behavior is not optimally balanced, maladaptation, potentially as an ecological or evolutionary trap, may occur (Schlaepfer, Runge and Sherman 2002, Schlaepfer et al. 2005, Sih 2013).

We address the three questions posed above using a system involving change in anti-predator behavior of native lizards (*Sceloporus undulatus*) in response to invasive predatory fire ants (*Solenopsis invicta*). Previous work gives us insight into the first question; following invasion by fire ants, fence lizard populations largely switch from relying on crypsis (an adaptive response to predominantly visual predators; Martín et al. 2009) to flight during encounters with these invaders (Langkilde 2009a). In this study, we use staged encounters to test if this shift in behavioral response to fire ants is generalized to two native ant species, and to a native avian predator, revealing the specificity of this behavioral adaptation. We also look for evidence of greater frequency of injuries (predation attempts) in lizards from populations showing increased responsiveness to fire ants. Such injuries would indicate a potential fitness cost of this behavioral change.

Materials and Methods

Study System

Red imported fire ants (*S. invicta*) are native to South America and were introduced to Mobile, AL in the southern United States in the 1930's (Tschinkel 2006). This species has since spread throughout the southeastern U.S. where it overlaps with the range of the eastern fence lizard (*S. undulatus*, Conant and Collins 1998). Both fence lizards and fire ants favor disturbed, edge habitats (Langkilde 2009b), and interact frequently in nature (Freidenfelds et al. 2012), although fence lizards do not change their habitat use in the presence of fire ants (Langkilde 2009b). Fire ants recruit in high numbers to potential prey, including small vertebrates (Allen et al. 1994; Wojcik et al. 2001), and as few as 12 attacking fire ants are capable of paralyzing and killing an adult fence lizard (Langkilde 2009a). Ants make up a significant portion of the diet of fence lizards (DeMarco et al. 1985; Mobley 1998), and lizards can also be envenomed when consuming fire ants (Langkilde and Freidenfelds 2010), which become the numerically dominant ant within invaded areas (Vinson 1994; Epperson and Allen 2010).

Previous research in this system has shown that fence lizards have adapted both morphologically and behaviorally to the presence of invasive fire ants within 70 years (≈ 40 generations; Langkilde 2009a). Fence lizards are well-camouflaged and generally rely on crypsis to survive in the presence of visual predators including lizards, snakes, and birds of prey (Cooper et al. 2000; Jensen et al. 2008). While lizards from areas not yet invaded by fire ants (uninvaded sites) flee from $\approx 50\%$ of fire ant attacks, lizards from areas invaded by fire ants for long periods of time (invaded sites) flee with at a significantly higher rate ($\approx 85\%$; Langkilde 2009a). These flee behaviors reduce contact with and envenomation by attacking fire ants (Freidenfelds et al. 2012).

Study Sites and Animals

We captured lizards from six study sites in the southeastern United States: (1) Solon Dixon Forestry Education Center, Escambia Co., Alabama (31.16°, -86.70°), (2) Geneva State Forest, Geneva Co., Alabama (31.12°, -86.17°), (3) Historic Blakeley State Park, Baldwin Co., Alabama (30.74°, -87.91°), (4) Saint Francis National Forest, Lee Co., Arkansas (34.71°, -90.73°), (5) Edgar Evins State Park, DeKalb Co., Tennessee (36.08°, -85.83°), and (6) Standing Stone State Park, Overton Co., Tennessee (36.47°, -85.42°) (all coordinates in WGS84). Sites 1, 2, and 3 were first invaded by fire ants approximately 74, 55, and 82 years ago, respectively, while sites 4, 5, and 6 have never been invaded by fire ants (Callcott and Collins 1996). All sites are characterized by temperate mixed forests and abundant edge and disturbed habitats where both fence lizards and fire ants are found at their highest densities (Langkilde 2009b; Langkilde, unpublished data). Only adult fence lizards were collected for behavioral trials, as juvenile lizards flee from fire ant attack at high rates regardless of population of origin (Langkilde 2009a). Following capture, lizards were measured for mass (to the nearest cg) and snout-vent length (SVL, to the nearest mm), and their sex recorded. They were housed in mixed sex groups (3-4 lizards per enclosure) in plastic enclosures (56 X 40 X 30 cm, L X W X H) furnished with a water dish, shelter, and a 60-W incandescent lamp at one end of the enclosure, which created a thermal gradient to allow lizards to thermoregulate. Overhead lights were set to a 12:12 light:dark schedule. Lizards were provided water *ad libitum* and were fed crickets (*Acheta domesticus*) dusted with supplementary minerals and vitamins four times weekly.

We use these lizards to test whether the observed difference in behavioral response to fire ants following invasion is generalized to native species - native ants that provide similar stimuli to fire ants but do not pose a lethal threat (Buschinger and Maschwitz, 1984; Wilson, 2003;

Thawley, personal observation), and an ecologically distinct avian predator to which lizards should respond by maintaining crypsis (Parker 1994).

Behavioral Response to Ants

We tested lizards from invaded (n=55) and uninvaded sites (n=43) for their fleeing response to fire ants and two species of native ants (*Dorymyrmex sp.* and *Pheidole sp.*) between 2011 and 2014. While these native ants will recruit to lizards, they generally have lower densities, recruit less aggressively, and have less potent venom (or lack venom altogether) (Wilson 2003, Tschinkel 2006), resulting in lower encounter rates and danger to fence lizards. Behavioral tests followed a protocol described in Langkilde (2009a). Briefly, we tethered lizards to pegs with 1 m lengths of cotton thread tied loosely around their necks. Pegs were placed in the ground 30 cm from a target ant mound, allowing the lizard to flee from and escape ant attack during the trials but preventing the lizards escaping into the surrounding landscape. Temperature of mounds and lizards was determined using a Raytek MT-6 infrared thermometer (Raytek Corporation, Santa Cruz, California, USA) accurate to $\pm 0.2^{\circ}\text{C}$. The surfaces of target mounds were gently disturbed by researchers to bring ants to the surface, and lizards were placed onto an undisturbed portion of the focal mound such that disturbed ants could find the lizard. We began trials when the first ant from the target mound contacted the focal lizard, and trials ended after 60 seconds or when a lizard fled beyond the boundary of the ant mound. During trials, we recorded the time after the start of the trial at which the lizard fled from the mound and the average number of ants on the lizard. All lizards were tested against all three ant species on consecutive days, with the order of ant species being randomized.

We analyzed these data for differences in the flight response of lizards to the three species of ant using a generalized linear mixed model with a logit link and a binomial

distribution. Our initial model included invasion status (invaded or uninvaded), sex, ant species, trial order, year, and interactions (invasion status*treatment, invasion status*sex, and invasion status*order) as fixed effects; site nested within invasion status as a random effect; snout-vent-length (SVL), mass, lizard temperature, mound temperature, and number of attacking ants as covariates; and a random effect for individual identity to account for repeated measures from individual lizards. Satterthwaite's Approximation was used to determine denominator degrees of freedom for F-tests (Keselman et al. 1999).

Behavioral Response to an Avian Predator

American Kestrels (*Falco sparverius*) are common predators of sceloporine lizards across the United States (Knowlton and Stanford 1942; Craig and Trost 1979), and have previously been used in anti-predator behavioral studies with these species (Fine 1999). In 2013, we tested the behavioral responses of lizards from three fire ant invaded sites (n=54) and three sites uninvaded by fire ants (n=35) to a simulated avian attack by placing lizards in an experimental arena with a perch, exposing them to a simulated predator, and filming lizard behaviors for later analysis. We used a preserved adult American Kestrel on loan from the Penn State Ornithological Collection (Federal Salvage Permit MB028785-0; PA Permit SAL00436) as a predator model. The kestrel was positioned in an attack posture with wings slightly spread, and we attached cardboard eyes to better simulate a living predator, as lizards use the appearance of eyes to gauge the danger presented by a predator (Hennig 1977). The kestrel bore an inconspicuous wire harness attached to a fixture on the ceiling directly above the test arena with clear monofilament line, allowing a clearance of 10 cm between the kestrel and the top of the lizard's perch site. A blind hid observers and the kestrel from the focal lizard prior to a simulated attack trial. Pilot data revealed that this setup produced a behavioral response from the focal lizard in $\approx 50\%$ of trials.

To commence a behavioral trial, we measured the focal lizard's temperature as described previously and placed it in an experimental arena (56 X 40 X 30 cm, L X W X H) furnished with a sand substrate and a hollow, half-log shelter in the center of the arena which lizards could perch on and seek refuge beneath. We standardized each focal lizard's position by placing it lengthwise on the half-log perch, facing the direction from which the avian predator would appear, ensuring that all four feet were in contact with the perch. If lizards fled from the perch prior to the beginning of a trial, they were returned to the perch. For each trial, we recorded video using a small webcam positioned within the blind and connected to a laptop computer, and released the kestrel from a known starting position behind the blind. A small light, out of the lizard's visual range, was simultaneously triggered to indicate the release of the kestrel on the video. The kestrel was allowed to pass over the perch four times (twice from each direction) before the trial ended. Each day we randomly selected one previously untested lizard from each enclosure for a trial to prevent stress associated with prior lizard handling in the same enclosure from influencing results, and each lizard was tested only once. All trials were conducted within one minute of first handling the focal lizard to ensure that elevated stress levels due to handling did not affect flight responses (Romero and Reed 2005).

We played back resulting video footage using Media Player Classic v1.5.2 (mpc-hc.org) and measured three behavioral variables for each trial: (1) whether or not a lizard responded behaviorally to the simulated attack, (2) latency to response (time between kestrel release and initiation of a response by the focal lizard), and (3) the strength of the behavioral response. Response strength was assigned to one of four levels: 0 - no reaction, 1 - weak reaction; the lizard ducked its head or flattened its body to the perch, but did not move more than two of its feet, 2 - strong reaction; the lizard moved three or more of its feet and substantially shifted its position (usually to the side of the half-log), but remained on the perch, and 3 - flight; the lizard completely left the perch, either seeking shelter beneath it or moving onto the sand substrate.

We tested for differences in probability of responding to simulated avian attack using logistic regression with invasion status (invaded or uninvaded) and sex as fixed effects and SVL and lizard temperature as covariates. For the subset of lizards that did respond to the simulated avian predator (n=52), differences in latency to respond were assessed using a general linear model with invasion status and sex as fixed effects and SVL and lizard temperature as covariates. We tested for differences in strength of response using ordinal multinomial logistic regression with invasion status and sex as fixed effects and SVL and lizard temperature as covariates.

Injury Rates

We recorded evidence of injury for all lizards caught from populations at the same fire ant-invaded and -uninvaded sites used for behavioral trials in 2011-2014 (n=589). We noted observations of any injuries, including missing limbs or parts of limbs, broken or regenerated tails, and scars. Injuries, including broken or regenerated tails, have been used as proxies for predation pressure in lizards (Parker 1994, Arnold 1988 but see Angilletta et al. 2004). We did not consider missing digits as injuries, as digits can often be lost outside of predatory interactions due to intraspecific competition between male lizards, aggressive courtship by males of female lizards, or during shedding (Schoener and Schoener 1980). Because this data was observational, we could not address the proportion of lizards in these populations which were killed by predators or avoided detection or injury altogether.

We tested for differences in rates of injury in lizards using generalized linear mixed model with a logit link and a binomial distribution. Our initial model included invasion status (invaded or uninvaded), sex, interactions (invasion status*sex and invasion status*SVL), and year as fixed effects, site nested within invasion status as a random effect, and SVL and mass as covariates. Parsimonious final models were produced by removing non-significant fixed effects

and covariates in a stepwise process using likelihood ratio tests. All analyses were conducted using SPSS v.21 (IBM Corp., 2012).

Results

Behavioral Response to Ants

Our final model included invasion status, sex, ant species, and trial order as fixed effects; site nested within invasion status as a random effect; and SVL and lizard temperature as covariates (Sex: $F_{1,93}=4.505$, $p<0.036$; SVL: $F_{1,95}=6.935$, $p<0.01$; Lizard Temperature: $F_{1,285}=7.522$, $p<0.006$; Trial Order: $F_{2,285}=3.924$, $p<0.021$). Year, interactions between fixed effects, mass, mound temperature, and number of attacking ants did not significantly explain variation in the behavioral response of lizards and so were omitted from the final model. Lizards from fire ant-uninvaded sites fled less frequently from both encounters with native ants and invasive fire ants (Invasion Status: $\beta_{\text{Uninvaded}}=-1.469$, $F_{1,4}=13.799$, $p<0.018$; Fig. 4.1A). Lizards from both invaded and uninvaded sites showed differences in frequency of flee responses to different species of ants (Ant Species: $F_{2,285}=9.111$, $p<0.001$; Invasion Status*Ant Species: $F_{2,283}=1.484$, $p<0.229$; interaction not included in final model), with post-hoc tests showing that lizards fled significantly less frequently from *Pheidole sp.* than the other two species ($\beta_{\text{Pheidole}}=-1.404$, $p<0.001$).

Behavioral Response to Avian Predators

Lizards responding to the simulated avian predator showed a broad suite of normal defensive responses including jumping off or sheltering underneath the perch log, shifting to the

side of the perch, flinching, flattening the body, and/or ducking the head. Lizards from fire ant-invaded and -uninvaded sites were similarly likely to respond to the model avian predator ($\beta_{\text{Invaded}}=0.238$, Wald $\chi^2_1=0.027$, $p=0.577$) (Sex: Wald $\chi^2_1=0.038$, $p=0.845$; SVL: Wald $\chi^2_1=1.527$, $p=0.216$; Lizard Temperature: Wald $\chi^2_1=0.227$, $p=0.634$), had a similar latency to respond ($F_{1,51}=0.882$, $p=0.352$; Sex: $F_{1,51}=0.139$, $p=0.711$; SVL: $F_{1,51}=0.090$, $p=0.765$; Lizard Temperature: $F_{1,51}=0.001$, $p=0.982$), and their response was of similar strength (Wald $\chi^2_1=0.027$, $p=0.868$; Sex: Wald $\chi^2_1=0.017$, $p=0.897$; SVL: Wald $\chi^2_1=0.188$, $p=0.665$; Lizard Temperature: Wald $\chi^2_1=0.059$, $p=0.809$).

Injury Rates

The final model for injury rates included invasion status and sex as fixed effects, site nested within invasion status as a random effect, and SVL as a covariate; interactions between fixed effects and lizard length did not significantly explain variation in the data and were omitted from the final model. Lizards from fire ant uninvaded sites had lower injury rates than did lizards from invaded sites ($\beta_{\text{Uninvaded}}=-0.778$, $F_{1,585}=13.799$, $p<0.001$; Sex: $\beta_{\text{♀}}=-0.911$, $F_{1,585}=19.718$, $p<0.001$; SVL: $\beta_{\text{SVL}}=0.607$, $F_{1,585}=13.166$, $p<0.001$; Fig. 4.2).

Discussion

This study reveals that the increased use of fleeing behavior by fence lizards following the invasion of fire ants is not restricted to encounters with this invader, but rather is generalized to native ants. This altered responsiveness is not extended, however, to avian predators. Higher injury prevalence in lizards from fire ant invaded sites suggests that lizards may suffer increased predation attempts as a cost of this behavioral shift.

These results extend previous findings that fence lizards from fire ant-invaded areas flee from fire ants at a higher rate than do lizards from uninvaded sites (Langkilde 2009a; Freidenfelds et al. 2012) by showing that these lizards are also more likely to flee from native ants (Fig. 4.1A). Despite the lesser threat of these ants, lizards from populations exposed to fire ants show an increased rate of fleeing to all types of ants included in our trials, suggesting that this response is generalized.

This generalization could be due to the sensory cues used by lizards to respond to ants. Visual diagnosis of ant species can be difficult even for humans (Ward 2010), and lizards often initiate flight after ants crawl on their bodies but prior to being stung (Thawley, personal observation), suggesting that lizards may respond to tactile cues that should be similar across ant species as opposed to visual stimuli. Lizards also respond to cues of ant contact simulated via “tickling” with fishing line (Thawley, personal observation). If lizards cannot distinguish fire ants from native ants before being stung, fleeing from all ants may be the only currently available behavioral strategy. Lizards from both invaded and uninvaded areas, do, however, flee less frequently from one type of native ant (Fig. 4.1A), suggesting that lizards may be able to distinguish ant species. These ants may provide the same types of cues, but vary in the amount of cue they produce (e.g. tactile contact), allowing differential responses under some circumstances. Additionally, previous research shows that fence lizards from fire ant-invaded sites, but not uninvaded sites, avoid the scent of fire ants (Langkilde 2009b), suggesting that fence lizards possess at least one pathway allowing identification of fire ants. If fence lizards can detect relevant cues allowing discrimination of fire ants from native ants, future evolution may allow differential responses to different types of ants (Sih et al. 2011).

We found no evidence that the differences in behavioral response to ants between lizards from invaded versus uninvaded sites are generalized to a simulated avian predator. Lizards have a long evolutionary history with native avian predators, which are a threat regardless of fire ant

presence. Elevated rates of fleeing from ants may not be generalized to these predators because they are ecologically distinct from ants and provide different cues. Further research on what cues (tactile, visual, chemosensory) lizards use to detect predators would shed light on how they may evaluate avian predators (e.g., Cantwell and Forrest 2013) or distinguish between dangerous and non-threatening ants (see Suarez et al. 2000 for an example of a lizard distinguishing between ant species).

This generalized response to ants is likely beneficial on average. In invaded areas, where fire ants are the most abundant ant in disturbed areas (Morrison 2002, King and Tschinkel, 2008), fence lizards encounter fire ants frequently, and fleeing from all ants as if they were fire ants may be an adaptive strategy that allows for escape prior to being attacked by large numbers of fire ants (Freidenfelds et al. 2012). However, fleeing breaks crypsis, a common reaction by prey to decrease detection by predators (Lima and Dill 1990) and a fence lizard's first line of defense against visual predators. As such, this behavior may represent a trade-off between the immediate risk of fire ant predation and the potential risk of detection by a visual predator (Martín et al. 2009). Because lizards that are adapted to fire ants additionally break crypsis when they encounter native ants, which are not a threat, they may further increase their risk of predation by visual predators, increase their energy expenditure, lose feeding opportunities by fleeing from potential prey (native ants), and suffer reduced ability to defend territories (Lima and Dill 1990, Parker 1994, Mobley 1998).

Our field data support the existence of a predation cost to this anti-ant behavior. Lizards at invaded sites show higher injury rates, a proxy for predation pressure, indicating that they may suffer greater attack by non-ant native predators. Males, which choose perch sites for territorial defense and courtship opportunities (Pounds and Jackson 1983; Thawley, personal observation) that likely expose them to greater predation risk (Parker 1994), show higher injury rates than females, supporting the idea that injury rates reflect predation attempts. These injury rates may

also be due to additional behavioral factors, as fence lizards from fire ant-invaded sites spend less time seeking refuge underneath cover objects when stressed in the lab (Trompeter and Langkilde 2011). Together with the increased propensity to break crypsis when encountering ants, these data suggest that these lizards are indeed more exposed to visual detection by predators.

The observed differences in injury rates could be explained by factors other than behavioral differences. Increased injury rates at invaded sites could reflect differences in the predator community, including a greater diversity or absolute abundance of predators in southern regions (Pianka 1970). However, populations further north than those at our study sites do not show lower injury rates (Thawley, unpublished data), arguing against a latitudinal cline in predation pressure. Fire ants might alter the community of predators of fence lizards at fire ant-invaded sites, perhaps increasing the occurrence of native predators. We find this unlikely, however, as recorded interactions between fire ants and vertebrates are generally negative (Allen et al. 2004). In fact, fire ants have been found eating the eggs of the North American racer (*Coluber constrictor*), a common ophidian predator of fence lizards (Thawley 2014).

Furthermore, as our data are only observational, and injuries indicate survival of a predatory encounter, these injured lizards represent only a subset of lizards co-occurring with predators. For instance, our data cannot assess the proportion of populations which are consumed by predators (and therefore undetectable), escape attackers without injury, escape detection by predators altogether, or are detected but remain unattacked. For example, lizards from fire ant-invaded sites might avoid mortal attacks by native predators at higher frequencies than lizards from uninvaded sites, resulting in an increased injury rate *and* an increased survival rate. However, we consider this possibility unlikely as lizards from invaded and uninvaded sites flee from avian predators at similar rates and with similar strengths and latencies to react (Fig. 4.1B). Additionally, in the closely-related common side-blotched lizard (*Uta stansburiana*), lizards with

tail injuries suffer higher rates of mortality in the field, suggesting that increased injuries likely bear their own fitness costs (Wilson 1992).

Together, these data suggest that this generalized behavioral reaction to ants may be maladaptive during interactions with native ants. Maladaptation due to biological invasions may be common, as previously adaptive responses to native species can actually reduce fitness when employed against invaders (Schlaepfer et al. 2002, Schlaepfer et al. 2005, Sih 2013). In this case, fleeing from fire ants is an effective defense and should improve lizard survival and fitness, even in the presence of native predators (Freidenfelds et al. 2012). However, generalized flight from ants, including non-lethal native ants, is maladaptive and likely results in increased attacks, lost energy intake, and potentially lower fitness. Future adaptations may support more optimal responses, such as fleeing only from dangerous ants, as selection should favor the incorporation of new cues or other refinements of generalized behavioral rules that promote higher fitness (Schlaepfer et al. 2010).

Future research into changes in behavior triggered by novel threats should focus not only on the existence of responses to novel environmental pressures, but also whether adaptations are generalized to other contexts including existing threats. Some research suggests that generalized adaptations may better allow for adaptive responses to novel conditions when compared to more specific adaptations, which may be inappropriate or ineffective in novel situations (Carthey and Banks 2014, but see Sih 2013). However, our research shows that generalized adaptations are also vulnerable to being maladaptive under some types of rapid environmental change. Indeed, as anthropogenic change accelerates, disentangling the multiple drivers of behavioral, and other adaptations, as well as assessing their costs and benefits, is likely to become increasingly challenging (Caro and Sherman 2011). Understanding whether novel adaptations are generalized to multiple contexts will be critical for determining the long-term consequences of adaptive responses to environmental change and for managing affected species.

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Figures

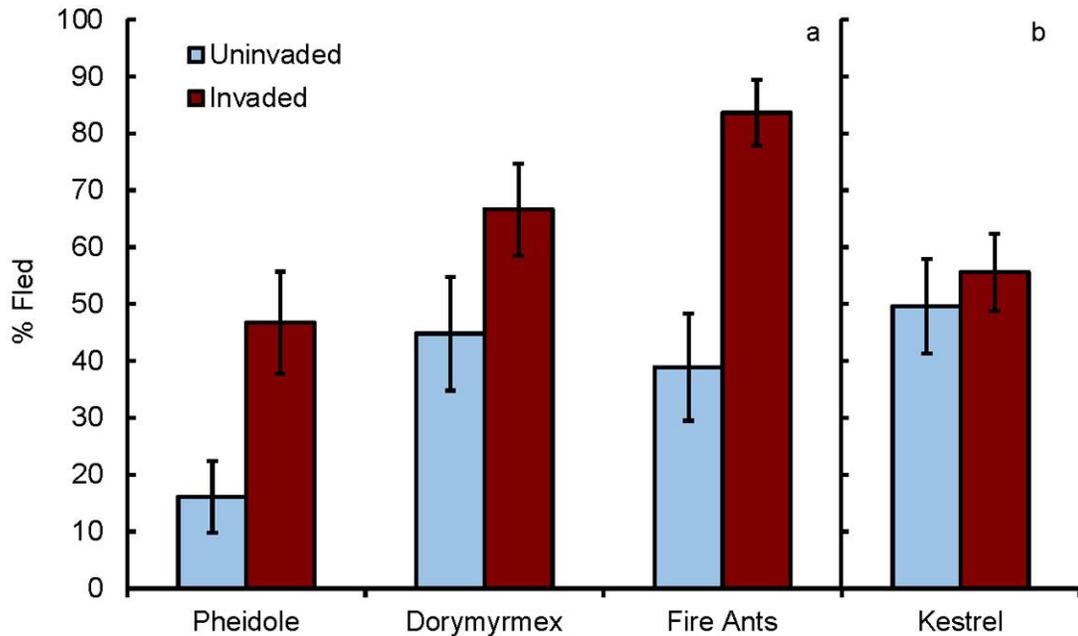


Figure 4.1. Percent of eastern fence lizards (*Sceloporus undulatus*) from fire ant-invaded (dark red) and -uninvaded (light blue) populations a) fleeing from attacks by invasive fire ants and two genera of native ants (estimated marginal means calculated for SVL=6.4 cm and lizard temperature of 30.7°C) and b) fleeing from simulated attacks by an avian predator (estimated marginal means calculated for SVL=6.5 cm and lizard temperature of 33°C). Error bars represent ± 1 standard error.

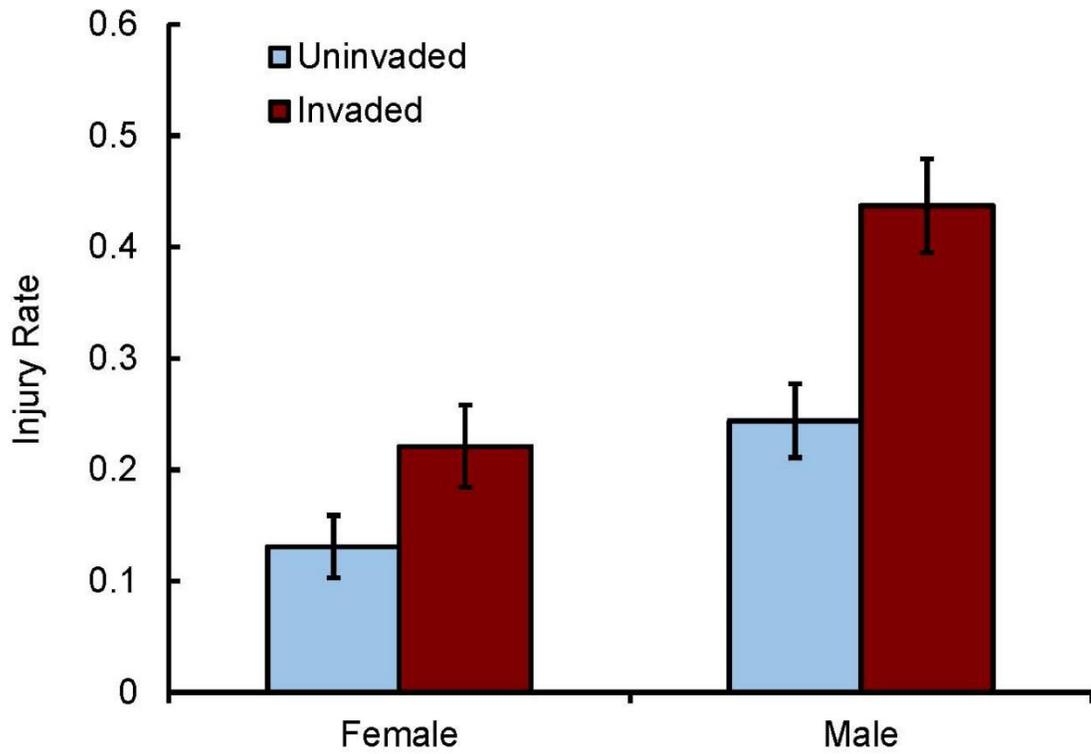


Figure 4.2. Female and male fence lizards from fire ant-invaded (dark red) sites had higher injury rates than lizards from uninvaded (light blue) populations ($P < 0.001$). Estimated marginal means calculated for $SVL=6.36$. Error bars represent ± 1 standard error.

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Chapter 5

An invasive species alters latitudinal clines of multiple traits in a native species

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Abstract

The study of latitudinal gradients and clines has furthered understanding of patterns in biodiversity, abundance, and life history traits and informed key ecological theory and models. Understanding the processes driving latitudinal clines has become increasingly important in light of accelerating global change. Many studies have focused on the role played by abiotic factors, especially temperature, in generating clines, but biotic factors, including the introduction of non-native species, may also drive clinal variation. We assessed the impact of invasive predatory fire ants on latitudinal clines in multiple fitness relevant traits, including morphology, stress responsiveness, and antipredator behavior, in a native fence lizard. In areas invaded by fire ants, a latitudinal cline in morphology is significantly different from both the cline found in museum specimens from historical populations and that found in current populations uninvaded by fire ants. Similarly, clines in stress responsiveness and antipredator behavior differ significantly between the portions of the fence lizard range invaded and uninvaded by fire ants. Changes in these traits are adaptive and together support increased and more effective antipredator behavior that allows escape from attacks by this invasive predator. This research shows that novel biotic pressures can change latitudinal clines in multiple traits within a single species on ecological timescales. As global change increases, a greater understanding of novel abiotic and biotic pressures and how affected organisms adapt to them across space and time will be central to predicting and managing our changing environment.

Introduction

Latitudinal gradients or clines are key features of biogeography that are found across taxa and ecosystems (Roy et al. 1998; Hawkins et al. 2003; Qian and Ricklefs 2007; Fuhrman et al. 2008). These patterns describe how biological factors such as diversity, abundance, and species' traits vary across space and in association with biotic and abiotic factors in the environment (Willeg et al. 2003). The study of latitudinal clines has provided insight into how key organismal traits, such as body size and fecundity, impact fitness (Jonsson and L'Abée-Lund 1993; Cardillo 2002). Latitudinal clines are incorporated into theory and development of models explaining life-history variation and the role of plasticity and adaptation in shaping traits (Niewiarowski 1994; Reznick and Ghalambor 2001). Some latitudinal clines are quite well known, such as those described by Bergmann's, Allen's, and Gloger's Rules, and considered to be driven by abiotic factors such as temperature and season length (Meiri and Dayan 2003; Millien et al. 2006). Most research has focused on the role abiotic factors play in structuring latitudinal clines, with temperature receiving particular attention (Chen et al. 2011; De Frenne et al. 2013). However, biotic factors including the novel pressures produced by invasive predators, competitors, and pathogens, have received increasing study (Merino et al. 2008; Schemske et al. 2009; Moles et al. 2011).

Studies of biogeographical impacts of abiotic and biotic factors have taken on a new importance in this era of increasingly rapid anthropogenic change (Araújo and Luoto 2007). Changes including warming, increasing climatic variability, and invasion of non-native species (Butchart et al. 2010), produce novel selective regimes (Parmesan 2006; Sax et al. 2007). Some species may not be able to successfully adapt, especially those from polar (Kerby and Post 2013; Mills et al. 2013) or alpine regions (Dullinger et al. 2012). However, many species do adapt to novel selective regimes (Strauss et al. 2006) and biogeographical patterns, including latitudinal

clines, can be altered in the process. For example, clines in genetic variation, body size, and phenology have shifted to track changes in abiotic conditions (Millien et al. 2006; Gardner et al. 2009; Yang and Rudolf 2010). Similarly, species introduced to novel environments recreate clines found in their native range, and develop new clines, within 50 years (Johnston and Selander 1964; Huey et al. 2000; Lee 2002). Studying effects of rapid environmental change on clines can help us understand novel ecological pressures and how organisms may adapt to them (De Frenne et al. 2013).

Much research on how global change alters latitudinal clines has focused on abiotic climate change as a driver (e.g., Rombouts et al. 2009; Edeline et al. 2013). However, biological invasions can provide valuable insights into the role of biotic factors in driving the formation and alteration of latitudinal gradients and clines (Bruno et al. 2005). Invasive species occur over large geographical extents and can apply strong selective pressures to native species (Salo et al. 2007; Sax and Gaines 2008). This provides an opportunity to assess how rapid changes in biotic factors may affect existing clines. Additionally, while most studies of changes in biogeographical clines address one focal trait of interest, under some conditions, including biological invasions, multiple traits within a species can shift simultaneously (e.g., Shine et al. 2011; Langkilde 2009). Here, we examine the role of novel predation pressure, a biotic factor, in altering latitudinal clines in multiple traits in a single species using a system of invasive predatory ants and native lizards.

Red imported fire ants (*Solenopsis invicta*) were introduced to Mobile, AL, USA in the 1930's and have spread throughout the southeastern United States (Fig. 5.1). They prey upon many native species including Eastern fence lizards (*Sceloporus undulatus*) and exert novel selective pressures (Allen et al. 2004, Langkilde 2009). Lizards, and especially fence lizards, have been valuable organisms for studying ecogeographical rules (Niewiarowski 1994; Angilletta et al. 2004; Du et al. 2010). Fence lizard populations differ in a suite of traits, including antipredator behavior, stress responsiveness, and morphology, according to whether or not they are from

populations that have been invaded by fire ants (Langkilde 2009; Graham et al. 2012). More southern populations of fence lizards, which have been invaded by fire ants for longer periods of time (Fig. 5.1), are more behaviorally responsive to fire ants and have correspondingly longer hind limbs and higher stress responsiveness, adaptations which support increased survival of fire ant attacks (Langkilde 2009; Graham et al. 2012), as compared to more northern fire ant-naïve populations. For these three traits, we examined whether these patterns represent pre-existing latitudinal clines or instead are due to changes in pre-existing clines as a result of novel selection by invasive fire ants.

Methods

Eastern fence lizards were captured via noose and by hand at 13 sites (7 invaded by fire ants, 6 uninvaded) across the latitudinal range of this species from 2006-2014, and measurements of morphology, stress responsiveness, and antipredator behavior were taken at subsets of these (Fig. 5.1, see Table 5.1 for details).

We measured hind-limb length and snout-vent length (SVL) in fence lizard (*S. undulatus*) museum specimens (n=165, from 83 unique sites) collected from across the latitudinal range of this species at sites uninvaded by fire ants and prior to invasion at sites currently occupied by fire ants occupied sites (Fig. 5.2). All preserved lizards were measured by one observer (T. Langkilde). In 2006-2014, we measured hind limb length and SVL of field-caught lizards (n=1450). Relative hind limb length was calculated by dividing lizard hind limb length by SVL and was standardized by year to account for observer bias; observers were consistent within years. Values of hind limb length are not directly comparable between live and preserved specimens due to measurement approaches required for preserved specimens.

We measured baseline and stress responsive levels of CORT in breeding females. During the breeding season in May-July, 2011 and 2013, blood samples were obtained from fence lizards (n=95) via the retro-orbital sinus within two minutes of first disturbing each animal (baseline samples). Lizards were then subjected to a standardized stressor (confinement in a cloth bag) for 30 minutes, and an additional blood sample was taken (post-stressor sample). Blood samples were stored on ice until centrifugation, when plasma was pipetted off and stored at -20°C until hormone quantification.

CORT was quantified using commercially available enzyme immunoassay (EIA) kits (Corticosterone High Sensitivity EIA Kits, Immunodiagnostic Systems Ltd.) previously validated for use in this species (Trompeter and Langkilde 2011). Plasma samples were diluted 90% with assay buffer so that levels of CORT fell within the detectable range of the assay's standard curve. Each sample was run in duplicate. Coefficients of variation were calculated using control samples provided in the kits. Mean intraassay coefficient of variation was 2.30% (range: 1.71-3.36%) and the mean interassay coefficient of variation among the seven plates was 5.36%.

In 2006-2013, captured lizards (n=487) were placed in cloth bags and transported to areas with active fire ant mounds for behavioral testing. We gently disturbed fire ant mounds and exposed lizards to sublethal fire ant attack for up to one minute. We recorded whether they fled from the attack or adopted crypsis by remaining motionless (for details of the methodological approach, see Langkilde 2009).

As abiotic conditions are often theorized to drive formation and maintenance of latitudinal clines, we assessed whether relevant climatic variables chosen *a priori* could be driving observed patterns in lizard traits. For each study site, we extracted values of selected variables from the BioClim database at a 2.5' resolution (<http://worldclim.org/bioclim>), allowing us to integrate conditions over each site and the surrounding area. Because climatic variables are often strongly correlated, we removed variables from our *a priori* set that had high correlations

with other variables (≥ 0.9) to construct a more parsimonious set of climatic variables. Our final set of variables included annual mean temperature (bio1), mean diurnal range (bio2), precipitation in the warmest quarter (bio18), and elevation (elev). We also conducted a principal components analysis using all 19 BioClim variables to generate an integrative metric of climatic conditions. We extracted the first principal component (pca1), which comprised 71.4% of the variance.

We used a linear mixed model to assess the effect of latitude on relative hind limb length of museum specimens with fixed effects for sex, latitude, and the interaction between sex and SVL; a random effect for site; and SVL as a covariate. We used a linear mixed model to assess the effect of latitude on morphology with fixed effects for latitude, invasion status, sex, and the interaction between sex and SVL; a random effect for site nested within invasion status; and SVL as a covariate. CORT responsiveness was calculated as the difference between stressed and baseline CORT levels, and CORT concentrations were inverse hyperbolic sine transformed to meet assumptions of normality. We constructed a linear mixed model to assess the effect of latitude on CORT responsiveness with fixed effects for latitude, invasion status, and year; a random effect for site nested within invasion status; and SVL as a covariate. Body condition was not significantly related to CORT responsiveness ($p > 0.36$) and was not included in the final model (LRT: $\chi^2 = -1.8$, $df = 1$, $p = 0.50$). We used a generalized linear mixed model with a logit link and a binomial distribution to assess the effect of latitude on behavior with fixed effects for latitude, invasion status, sex, and the interaction between sex and invasion status; random effects for site nested within invasion status and year; and SVL as a covariate. Parsimonious final models were constructed using likelihood ratio tests to eliminate unnecessary fixed effects. In each model, we separately estimated the slope and intercept of the trait's relationship with latitude at sites invaded and uninvaded by fire ants and compared the slopes using t-tests. If the slopes were significantly different, we inferred that the presence of fire ants affected the relationship of the target trait with latitude. To test for potential abiotic factors driving observed patterns in lizard

morphology, stress responsiveness, and behavior, we introduced each climatic predictor singly into each final model and tested whether it was a significant predictor of the trait of interest. Climatic predictors were introduced singly to avoid issues with multicollinearity between abiotic factors. All analyses were conducted using SPSS version 23 (IBM Corp.).

Results and Discussion

Here we show the first evidence that an invasive species has altered latitudinal clines in multiple traits of a native species. Evidence from natural history collections shows a positive relationship between relative hind limb length and latitude in fence lizard specimens collected prior to fire ant invasion across the full latitudinal range of this species (Fig. 5.3; $\beta=0.0017$, $F_{1,86.5}=5.546$, $p<0.021$). We find that same positive relationship between hind limb length and latitude in present-day fence lizard populations uninvaded by fire ants (Fig. 5.4c; $\beta=0.0031$, $F_{1,7.4}=9.692$, $p<0.016$; difference in slopes: $t=-1.101$, $p>0.271$). This cline is reversed, however, in populations invaded by fire ants (Difference in slopes: $t=4.444$, $p<0.001$), which have proportionally longer limbs at lower latitudes (Fig. 5.4c; $\beta=-0.0044$, $F_{1,8.5}=9.949$, $p<0.013$). Longer limbs are a heritable trait (Langkilde 2009) that is adaptive in these populations as it supports higher sprint speeds (Miles 1994) and allows twitch behaviors that remove a greater number of attacking fire ants (Langkilde 2009). This pattern of increasing hind limb length at lower latitudes, where fence lizard populations have longer histories of invasion with FA, thus matches the prediction that this adaptive trait should increase with evolutionary time that fence lizard populations have been exposed to fire ants.

In areas uninvaded by fire ants, CORT responsiveness shows a positive, but non-significant trend with latitude ($\beta=0.121$, $F_{1,8.23}=2.528$, $p=0.149$); however, this relationship is significantly different in populations from fire ant-invaded areas (Fig 5.4b; Difference in slopes:

$t=2.423$, $p=0.017$), where populations at the lowest latitudes have the highest levels of CORT responsiveness ($\beta=-0.269$, $F_{1,12.78}=3.640$, $p=0.079$). The lack of a significant latitudinal cline in CORT responsiveness in fire ant-uninvaded areas concurs with other work in this genus (Hews and Abell Baniki 2013). Increased CORT responsiveness is expected in fire ant-invaded areas where it can support more effective escape from fire ants (Trompeter and Langkilde 2011) and prime lizards to flee more readily from future attacks (T. Langkilde, unpubl. data).

In areas of the range of fence lizards not yet invaded by fire ants, fence lizard populations show a decreasing incidence of cryptic behavior with increasing latitude (Fig. 5.4a; $\beta=-0.312$, $F_{1,8}=20.080$, $p<0.001$). This pattern coincides with theoretical predictions and observations in other taxonomic groups that antipredator behaviors decrease with latitude (Díaz et al. 2013). In areas invaded by fire ants, this latitudinal cline is reversed (Difference in slopes: $t=5.824$, $p<0.001$), and crypsis is used least at low latitude populations longest invaded by fire ants (Figure 5.4a; $\beta=0.393$, $F_{1,8}=13.78$, $p<0.001$). Increases in fleeing behavior (i.e., decreases in crypsis) are adaptive and allow escape from attacking fire ants, and this pattern matches predictions that adaptive changes in lizard behavior should be greater in populations exposed to fire ants for longer periods of time (Langkilde 2009).

Our selected set of climatic variables did not significantly predict observed patterns in morphology (all $p>0.059$), CORT responsiveness (all $p>0.361$), or behavior (all $p>0.369$), indicating that these patterns are likely not driven by abiotic factors.

Our results show that adaptation to novel selection pressures can occur simultaneously across multiple pathways and encompass traits in multiple systems (morphology, physiology, and behavior). These adaptive shifts in traits are linked, as changes in stress responsiveness and hind limb length support increased flight behaviors, allowing an integrated organismal response to a single novel biotic threat. These results reveal that pre-existing latitudinal clines and the continuing presence of selective regimes that drove their formation may not preclude adaptation

to environmental change even if novel pressures run counter to pre-existing ones. In fact, latitudinal clines in a variety of traits are labile (Umina et al. 2005; Gardner et al. 2009). Variability, whether heritable or plastic, is a precondition for adaptive responses (Ayrinhac et al. 2004; Hoffmann et al. 2005). The existence of a latitudinal cline can indicate that enough variation exists within a species for a given trait to allow adaptation to a range of environmental conditions. Conversely, lack of variation in key traits, especially across gradients of important environmental factors, could be used to identify species of concern which may be unlikely or unable to adapt to environmental change (Bell 2013).

Both biotic (e.g., invasive species, habitat alteration) and abiotic (e.g., climate change) factors are changing increasingly rapidly as a result of human activities (Butchart et al. 2010). Whether species can adapt quickly enough to these novel pressures to persist or will face extirpation or extinction is one of the most pressing current ecological questions (Carlson et al. 2014). Our research demonstrates that adaptation to novel selective pressures can occur in multiple traits over a broad portion of a species' range within a short time horizon (<80 years, \approx 40 generations; Langkilde 2009), a period similar to those observed for clinal shifts in response to climate change or species' introductions to novel areas (Johnston and Selander 1964; Hoffman and Sgro 2011). As fire ants continue to invade more northern lizard populations, we expect trait values in these populations to respond by shifting from historical norms, providing a further powerful test of the ability of invasive species to reshape clines in this system. This work shows that biotic factors, such as the presence of a novel invader, can serve as powerful selective pressures over large geographical extents, and, in this case, not just shifting but actually reversing clines. Given that biotic factors can quickly alter latitudinal clines, future studies should address if biotic factors may mediate or be the primary cause of changes in patterns currently attributed to abiotic drivers such as climate change. As species introductions accelerate and novel ecological communities become more common (Moyle and Light 1996; Strauss et al. 2006), biotic

interactions may have an increasingly important role to play in structuring latitudinal gradients and clines.

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Figures

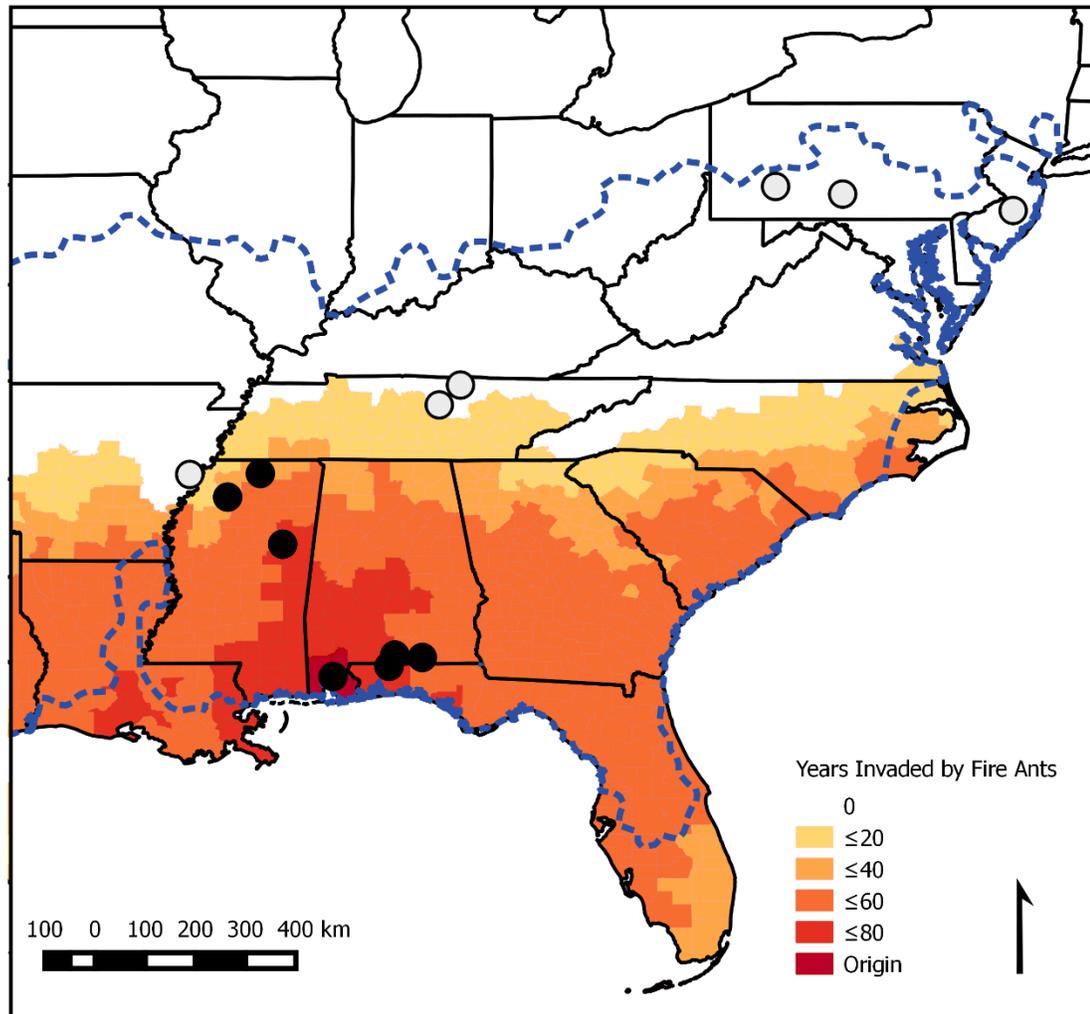


Figure 5.1. Geographical distribution of invasive fire ants (*S. invicta*) and fence lizards (*S. undulatus*). Orange shading indicates the history of invasion by fire ants; darker shaded areas were invaded longer ago. The range of *S. undulatus* is encompassed by the broken dark blue outline. The populations of fence lizards used in this study are indicated by black (fire ant-invaded) and grey (-uninvaded) dots.

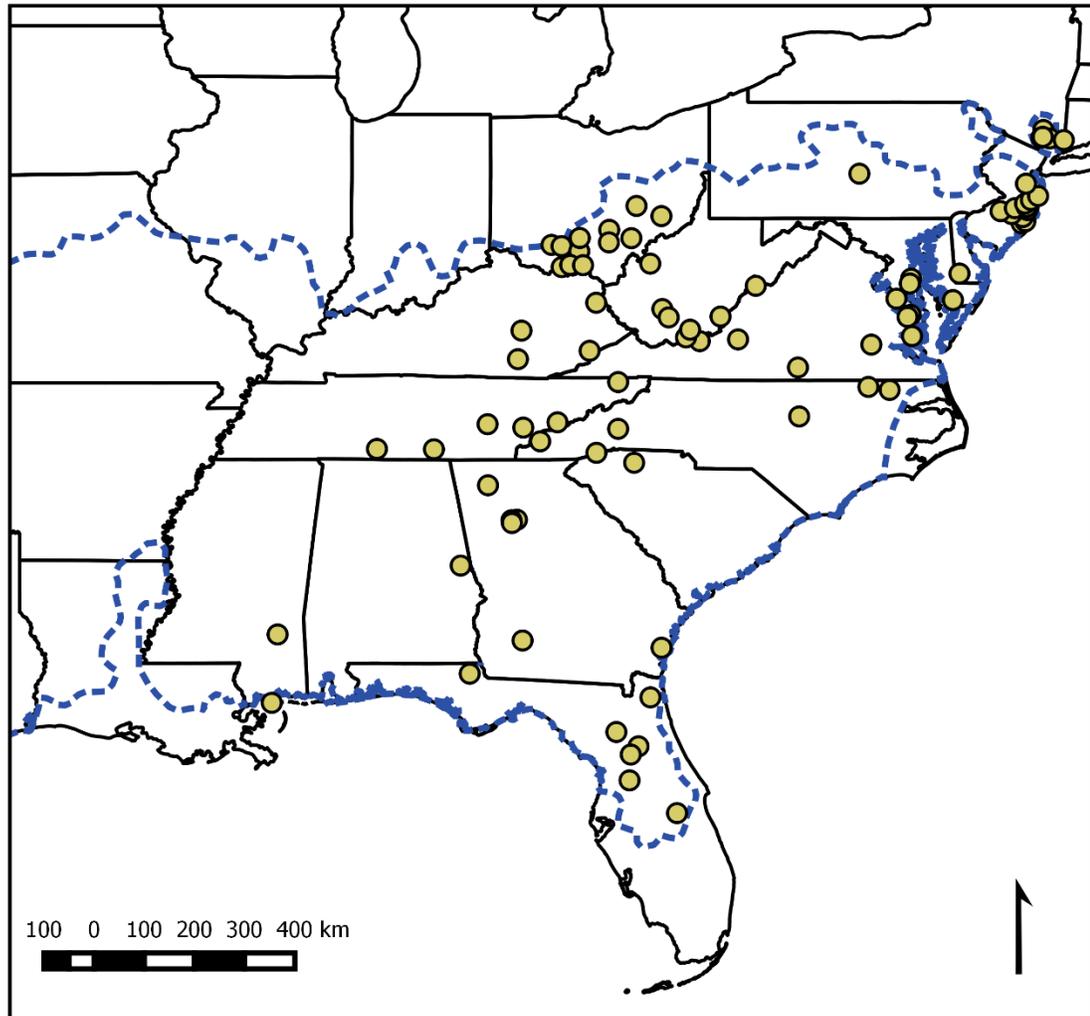


Figure 5.2. Locations of preserved specimens of *S. undulatus* used to determine historical pattern of hind-limb length. All specimens were collected prior to invasion of sites by *S. invicta*. The range of *S. undulatus* is encompassed by the broken dark blue outline.

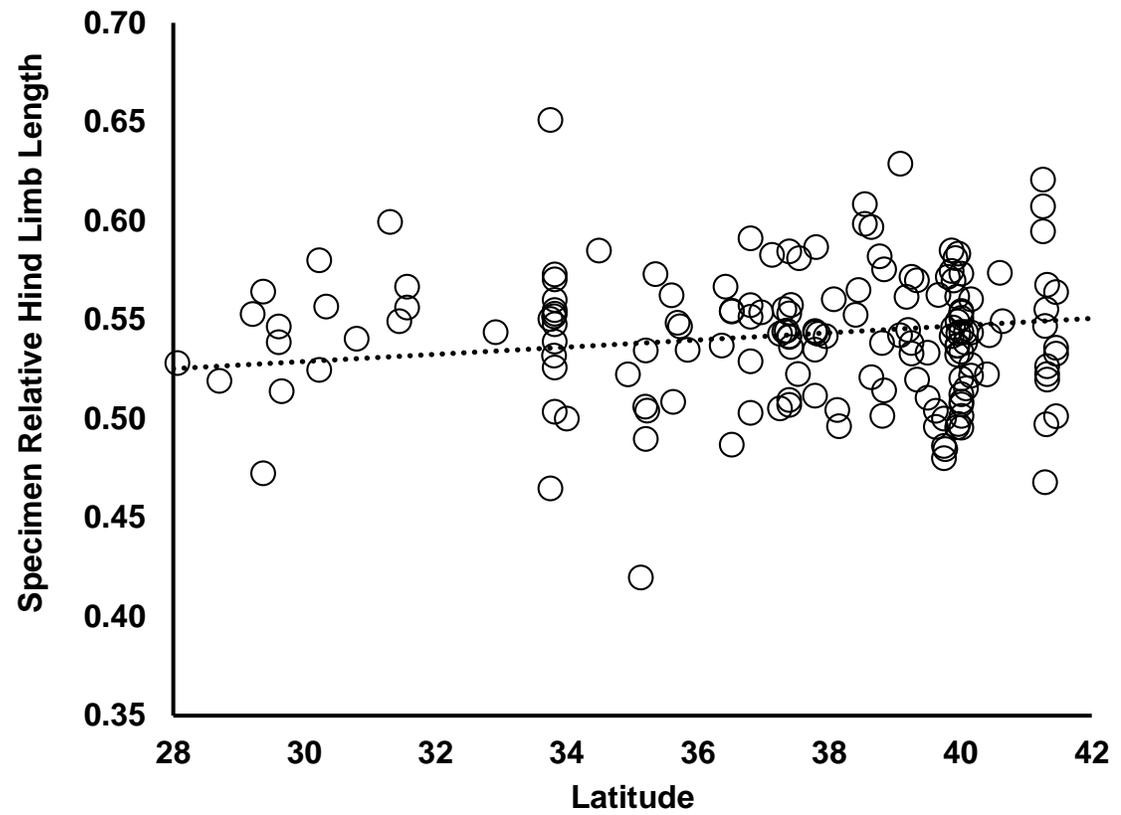


Figure 5.3. Relative hind limb length of preserved museum specimens of fence lizards (*S. undulatus*) collected prior to fire ant invasion or from fire ant-uninvaded areas increases with latitude.

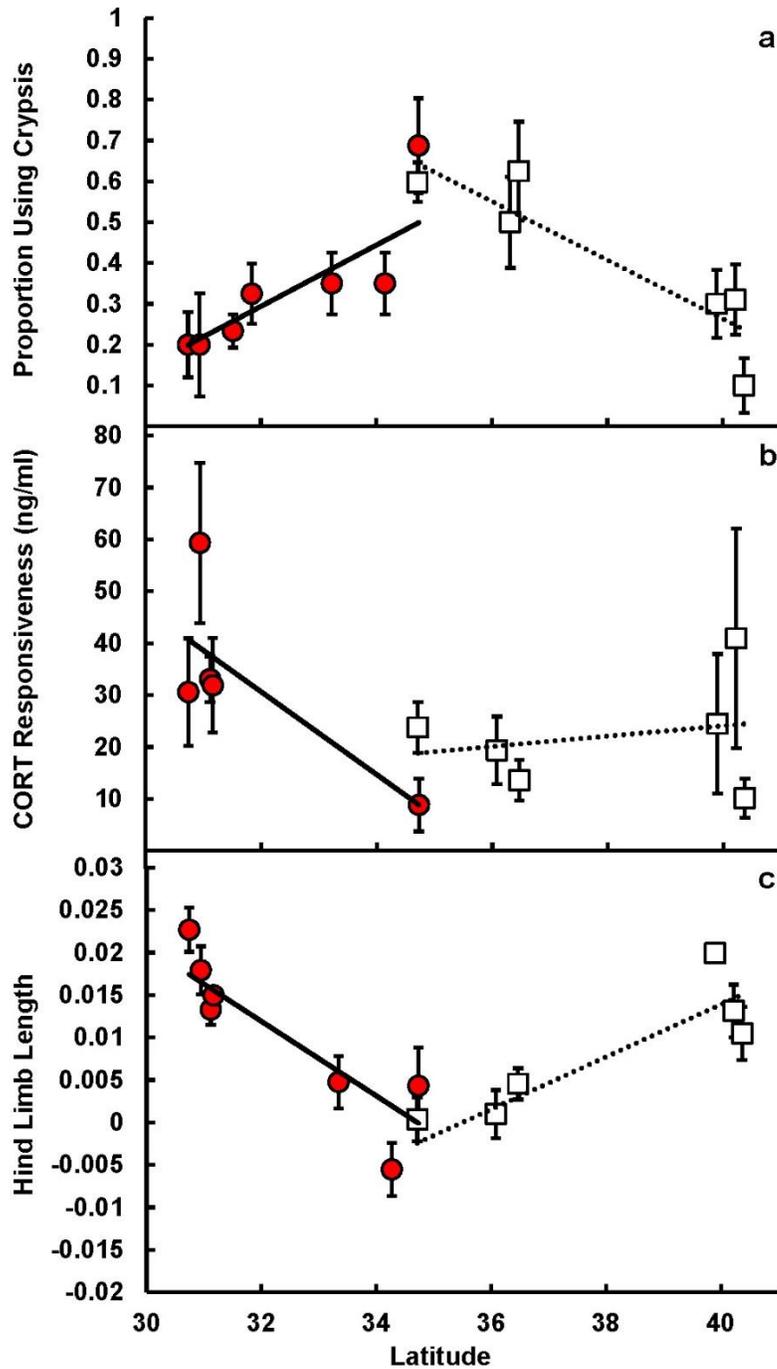


Figure 5.4. Trait values in fence lizards as a function of latitude and invasion by fire ants. A, Antipredator behavior (use of crypsis) of fence lizards decreases with latitude in fire ant uninvaded areas but increases in invaded areas. B, Stress responsiveness of female fence lizards is significantly different in fire ant -invaded and -uninvaded areas. C, Relative hind limb length of fence lizards increases with latitude in fire ant-uninvaded areas but decreases with latitude in invaded areas. Lines of best fit for invaded (solid) and uninvaded sites (broken) are shown, with those for uninvaded sites extrapolated to lower latitudes. Points are site means ± 1 standard error.

Tables

State	County	Invasion Status	Latitude	Longitude	Data Type
AL	Baldwin	Invaded	30.74	-87.91	M,S,B
FL	Santa Rosa	Invaded	30.94	-86.82	M,S,B
AL	Geneva	Invaded	31.12	-86.16	M,S,B
AL	Escambia	Invaded	31.16	-86.70	M,S,B
MS	Winston	Invaded	33.34	-88.90	M,B
MS	Panola	Invaded	34.27	-89.98	M,B
MS	Marshall	Invaded	34.73	-89.34	M,S,B
AR	Lee	Uninvaded	34.71	-90.73	M,S,B
TN	Dekalb	Uninvaded	36.08	-85.83	M,S,B
TN	Overton	Uninvaded	36.47	-85.42	M,S,B
NJ	Burlington	Uninvaded	39.90	-74.58	M,S,B
PA	Huntingdon	Uninvaded	40.22	-77.92	M,S,B
PA	Westmoreland	Uninvaded	40.37	-79.24	M,S,B

Table 5.1. Sites from which eastern fence lizards (*Sceloporus undulatus*) were sampled. Datum for all coordinates is WGS84. Data type indicates if population was used for analyses of morphology (M), stress responsiveness (S), or behavior (B).

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Chapter 6

Conclusion

Taken all together, my dissertation research reveals that the effects of invasive species can be both diverse and subtle. In this system, I showed that fence lizards experience the expected lethal effects of invasive fire ants as well as a variety of sublethal effects. Both types of effect varied with life stage of fence lizards, likely resulting in differential fitness consequences of fire ants on fence lizards at different life stages.

Fire ants prey on fence lizard eggs, but we have no evidence of sublethal effects of fire ants on this life stage. In contrast, juveniles do not have reduced survival in the presence of high fire ant densities, likely due to their uniformly high responsiveness to ants. They do, however, suffer decreased growth, which could have consequences including delays in sexual maturity (Haenel and John-Alder 2002) and reduced clutch sizes (Haenel and John-Alder 2002; Du et al. 2014). This decrease in growth may be due to increased energy use to escape from fire ants, a loss of foraging time due to responding to fire ants, a reduction in availability and consumption of non-toxic prey due to the presence of fire ants. These impacts could reduce lifetime reproductive output and fitness in fence lizards. Adult lizards, including those from fire ant-invaded sites, experience lethal effects of fire ants. Behavioral adaptations to these ants may mitigate some of this mortality (Boronow and Langkilde 2010). However, this increased responsiveness to ant attacks is generalized to native ants and may increase susceptibility to native predators. Fire ant invasion can also have sublethal effects on adults, including changes in body condition. Any reductions in body condition could be related to changes in energy intake or expenditure, which could reduce energy available for mating and producing eggs, potentially resulting in decreased reproductive output (Madsen and Shine 1996; Litzgus et al. 2008; but see Doughty and Shine

1997). As such, fire ants may affect fitness of adult fence lizards via both lethal and sublethal effects.

In addition to the effects described above, there is evidence that exposure to fire ants may impact fence lizards in other diverse ways, including changes in perch height and activity patterns (Thawley, Chapter 3), feeding behavior (Herr et al., In Press), ectoparasite load (Graham et al. 2012), immune function (McCormick et al., In Review), levels of circulating glucocorticoids (Graham et al. 2012), and behavioral responses to stress (Trompeter and Langkilde 2011). Future studies may explore how these effects can vary with life stage and investigate their relation to fitness.

In this study system, I have found a diverse array of effects of an invasive species that vary strongly across ontogeny of the native species. However, fence lizards have a relatively simple life history when compared to many organisms. Fence lizards hatch from eggs as miniature versions of adult lizards, their prey is similar to adults, only smaller (DeMarco et al. 1985), and their behavioral repertoire reflects that of adults (including performing adult behaviors such as push-ups within 4 weeks of hatching (Thawley, pers. obs.). Thus, differential impacts of fire ants may be more pronounced in species for which nestlings (e.g., cottontail rabbits) or hatchlings (e.g., vireos) are altricial (i.e. largely defenseless) for a period of time (Hill 1969; Campomizzi 2008). For other taxa that have life stages with vastly different ecologies, such as amphibians or holometabolous insects, impacts of invaders may vary considerably across an individual's lifetime. For instance, amphibian species [e.g., the Houston toad (*Anaxyrus houstonensis*)] may be relatively immune to impacts of fire ants during aquatic life stages but can be vulnerable during metamorphosis and after to terrestrial life stages (Freed and Neitman 1988; Brown et al. 2012). Likewise, vulnerability of an endangered butterfly, the Schaus swallowtail (*Papilio aristodemus*), to fire ant predation varies with mobility of life stage. While adults can escape fire ants via flying, larvae experience high predation but can escape by moving higher into

trees, and immobile eggs suffer total mortality in some scenarios (Forys et al. 2001). Future investigations of both lethal and sublethal impacts of invaders should account for potential variability across life stages either via empirical studies, as above, or in theoretical models specifically designed to address differential effects on life stages.

Empirical studies of the effects of global change on species may be limited by scale, practical or logistical considerations, or the number of hypotheses they can simultaneously test. Theoretical modeling can complement empirical studies, allowing quantification of how environmental changes can impact reproduction and fitness in multiple scenarios (van Groenendael et al. 1988). Multi-stage models, which support incorporation of differential effects across life stages, allow investigation of how effects of the environment, including the presence of predators, affect population growth. This approach has proven valuable in other systems (Rubbo et al. 2006), including those involving invasive species (Jongejans et al. 2006). Using the field studies presented in Chapters 2 and 3 along with relevant information from the extensive demographic literature in the genus *Sceloporus* (e.g. Tinkle and Ballinger 1972; Vinegar 1975; Manteuffel and Eiblmaier 2010), I have parameterized a preliminary multi-stage model of fence lizard populations. This model will be used to examine the strength of potential demographic effects of fire ants on fence lizard populations as well as differences in pressures imposed by fire ants across life stages. My preliminary results suggest that fire ants can reduce the intrinsic population growth rate of fence lizard populations, a proxy for fitness (Caswell 2001), and that survival of eggs and juveniles may have the largest impact on population growth. Ultimately, I will use this model to examine whether fire ant predation imposes differential selective pressures on different life stages and how pressures imposed by fire ants vary between populations that are recently invaded by fire ants as opposed to those that have adapted to fire ant attacks.

While my work has demonstrated the potential fitness consequences of fence lizard adaptations, such as increased anti-ant behaviors, our knowledge of how adaptations of lizards to

fire ants are passed from generation to generation is still incomplete. Hind limb length is heritable in other lizard species (Kolbe and Losos 2005), and estimates of heritability of this trait are high in this species (Langkilde 2009). Increased behavioral responsiveness of lizards to fire ants, however, does not appear to be learned within lizard lifetimes (Langkilde 2010), and preliminary data indicate that this behavior is influenced by a combination of between and within-lifetime effects (Robbins, unpubl. data). As such, epigenetic changes, which allow for within lifetime adaptation (via environmental induction) and transmission of adaptation to future generations (Jablonka and Raz 2009), are a potential mechanism that could explain observed patterns of adaptation.

Epigenetic changes induced by environmental threats are known to affect phenotypes, including behavior, in many systems, and may both underlie behavioral adaptations in fence lizards and serve as a pathway for inheritance of these adaptations (Franklin et al. 2010). Epigenetic processes, such as changes in DNA methylation, can modify gene function and expression without altering the underlying DNA sequence (Bossdorf et al. 2008) and may be a critical mechanism allowing response to environmental changes (Verhoeven et al. 2010). For instance, epigenetic variation has been proposed as a way for invasive species subject to low genetic variation (via bottlenecks, etc.) to adapt to new environments (Pérez et al. 2006, Schrey et al. 2012). Epigenetic changes may also promote more permanent evolutionary change via genetic assimilation, whereby a novel, epigenetically-induced phenotype is selected for genetically such that it becomes fixed and independent of the inducing agent (Richards et al. 2012).

Epigenetic variation may be induced by stressful conditions, and these phenotypic changes within the lifetime of an individual may be heritable across generations in certain situations (Jablonka and Raz 2009). Our previous research shows that fire ant exposure increases stress in fence lizards in both lab and field settings (Graham et al. 2012), suggesting that epigenetic changes could serve as a pathway for inheritance of these adaptations (Lindqvist et al.

2007). As such, studies of this potential avenue for heritability of adaptations to fire ants in lizard populations that have been invaded by this novel predator. Preliminary data from a collaboration I am involved with show that epigenetic marks of lizards vary between fire ant-invaded and uninvaded populations of fence lizards (Schrey et al., In Review). Future work will address how these marks vary across the fence lizard's range and whether they are linked to increased behavioral responsiveness to fire ants.

In conclusion, while environmental change threatens species and ecological communities, these rapid changes offer opportunities to gain key ecological insights. Biological invasions provide opportunities to examine how evolutionarily novel pressures, including predation and competition, impact native species and the adaptive processes by which affected organisms respond. Characterization of both the effects of novel change and adaptations to it may defy simplification, as they may vary with life stage of the organisms involved and the scale at which these changes occur. As global change is predicted to increase in the near future, studies of these systems will only become more important, as they provide for stronger conservation and management decision-making and support more effective prediction of future ecological dynamics.

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