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# Red imported fire ant predation on eggs of the eastern fence lizard

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**Abstract.** Red imported fire ants were introduced to the United States in the early 1900s. These invasive ants can impact wildlife, attacking and envenomating some native species and outcompeting them for food. Eastern fence lizards (*Sceloporus undulatus*) have developed novel behaviours and morphologies that have allowed them to survive in the face of these venomous ants. However, we know nothing of the effect of fire ants on fence lizard eggs – a potentially important life history stage. We tested whether fire ants could penetrate fence lizard eggs in the field, examining intact eggs, and those that had been previously damaged and had yolk on the outside (which should attract fire ants). We found that fence lizard eggs are vulnerable to fire ants, even when intact. Further studies on the susceptibility of reptilian eggs to fire ant predation, and incorporation of these data into population models, could shed light on potential population-level impacts of this globally-important invader.

**Keywords.** Invasive species; life history; oophagy; reptile; *Sceloporus undulatus*; *Solenopsis invicta*

## Introduction

Invasive species, whether introduced accidentally or intentionally, have caused significant ecological and economic impacts across a variety of habitats (Pimental, Zuniga and Morrison, 2005). Invasive social insects can negatively impact native biota (Gilliam, 1993; Beggs, 2001; Goulson, 2003; O'Dowd, Green and Lake, 2003). Within the United States alone, approximately 4,500 arthropod species have been introduced, including many social insects (Pimental, Zuniga and Morrison, 2005). Understanding the impact of social insects on native flora and fauna is critical for predicting and managing these invasions.

The red imported fire ant, *Solenopsis invicta* (Buren, 1972), is included on the International Union for the Conservation of Nature (IUCN) Invasive Species Specialist Group list of the top 100 world's worst invasive species (Lowe et al. 2000). This ant was accidentally introduced to the southeastern United States in the early 1900s, and its subsequent spread across 11 states has been well documented (Callcott and Collins, 1996). Red imported fire ants (hereafter referred to as fire ants) are effective predators: they attain high densities, recruit in large numbers, and have powerful venom (Jetter, Hamilton and Klotz, 2002).

The impacts of this invasive ant on native vertebrate populations are not well understood (Tschinkel 2006), but there is evidence that fire ants do impact some native species they encounter (Allen, Epperson and Garmestani, 2004). While adults of many species are assumed to have characteristics that should prevent or ameliorate the negative effects of fire ants (including the ability to move away from attack, and larger body size; but see Langkilde 2009a), earlier life stages, such as mobility-limited juveniles or eggs, are assumed to be vulnerable to attack (Conners 1998a; Allen, Epperson and Garmestani, 2004). Fire ants prey upon eggs and hatchlings of several bird and reptile species (reviewed in Allen, DeMarais and Litz, 1994; Chalcraft and Andrews, 1999; Allen, Epperson and Garmestani, 2004).

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The eastern fence lizard, *Sceloporus undulatus* (Bosc and Daudin, 1801), co-occurs with fire ants, the presence of which results in alterations to the behaviour and morphology of these lizards (Langkilde, 2009a). Adult fence lizards from fire-ant invaded sites show higher frequencies of flee and twitch behaviours and have longer hind limbs, which allows them to avoid and remove attacking fire ants better than lizards from sites without fire ants (Freidenfelds, Robbins and Langkilde, 2012).

We know nothing, however, about the impacts of fire ants on fence lizard eggs; a potentially important life history stage that may be particularly vulnerable to fire ants. Fence lizards are known to lay eggs in fire ant infested areas (Thawley, pers. obs.). Fence lizards tend to lay eggs in warm, sunny locations when soils are moist (i.e. after rainfall) in order to increase egg viability and developmental rate (Warner and Andrews, 2002), and these habitats are also preferred by fire ants for mound thermoregulation and colony growth (Hung and Vinson, 1978). Additionally, fence lizard nesting sites and fire ant foraging tunnels occur at similar soil depths (Markin, O'Neal and Dillier, 1975; Angilletta, Sears and Pringle, 2009). However, we do not know whether fire ants recognize fence lizard eggs as potential food sources or whether they are able to penetrate fence lizard eggs. We tested this by placing fence lizard eggs in artificial nests and recording fire ant recruitment to intact eggs, and damaged or yolk-covered eggs that mimic nests that have experienced prior predation (e.g., as is common by raccoons, *Procyon lotor*; Allen et al., 2001).

## Materials and Methods

In summer 2012, we collected gravid female fence lizards from the Solon Dixon Forestry Education Center of Auburn University, Andalusia, Alabama, USA. We kept these lizards in plastic tubs (56 x 40 x 30 cm, L x W x D) furnished with a shelter site, a heat lamp to provide a temperature gradient, and moistened sand for nesting. Lizards were provided with water *ad libitum* and fed crickets (*Achetus domesticus*) 4 times weekly. Female lizards created nests in the moist sand, and egg clutches (N=2) were collected from the tubs. Eggs were kept in moistened vermiculite for at least 28 days prior to the experiment to allow eggshells to harden.

We used 12 eggs collected from 2 "nesting" female fence lizards. We randomly assigned eggs to one of three treatments: punctured, dipped in egg yolk, and intact (n = 4 per treatment). The "punctured" eggs were punctured with a pin to simulate damage from a predator and assess whether fire ants are more easily able to access and consume eggs whose first line of defense, the shell, has been damaged. The "yolk-dipped" eggs were dipped in chicken egg yolk to determine whether fire ants are more attracted to eggs that are intact but have cues that may indicate food (which an intact egg may not; Allen et al., 2001), such as would be the case if some eggs in a clutch had been damaged and spilt yolk onto adjacent intact eggs. "Intact," unmanipulated eggs were included to determine whether fire ants would recruit to and could penetrate intact fence lizard eggs.

We located four fire ant mounds of approximately the same size (13-15 centimetres in diameter) in sunny, sandy areas typical of where fence lizards construct their nests (Angilletta, Sears and Pringle, 2009). Mounds were on average 4.5 meters apart (range: 3–6 m). We constructed three artificial nest holes by digging 10 cm-deep holes in the sand (Angilletta, Sears and Pringle, 2009) 15 cm from the centre of each mound and equally spaced. We placed eggs into individual nylon mesh bags (similar to those used by Chalcraft and Andrews, 1999) and used stratified random sampling to assign one egg from each of the three treatments to be buried in the holes around each mound. After 24 hours, we retrieved the mesh bags and their contents. We checked intact and yolk-dipped eggs for fire ant penetration by examining them for puncture holes (Conners, 1998b). All penetrated eggs were checked for fire ant presence and content loss by visual estimation (weighing the difference of the egg contents was not feasible because variable amounts of soil adhered to the egg due to presence of yolk from treatment and/or puncturing). We also recorded occurrences of any eggs that were entirely missing from the mesh bag (presumably consumed). We determined egg viability by observing how many of the yolk-dipped eggs and intact eggs were unpenetrated at the time of retrieval.

## Results and Discussion

The results of our observations reveal that fire ants are able to recognize even intact fence lizard eggs as a potential food source and are able to penetrate them. This accords with the ability of fire ants to penetrate eggs of other reptiles, including lizards (Mount, Truth and Mason, 1981; Chalcraft and Andrews, 1999). Unlike eggs of the rough green snake (*Opheodrys aestivus,* Conners, 1998b), fence lizard eggs that exhibited puncture marks were not leaking fluids; they retained

**Table 1.** Average percentage of fence lizard egg contents lost, and the percentage of eggs that appeared viable following fire ant exposure, for the three treatments.

| Treatment | Average % Contents Lost | % Viable |
|---|---|---|
| Punctured | 29% (100, 0, 0, 15) | N/A |
| Yolk-dipped | 25% (50, 0, 50, 0) | 25% |
| Intact | 47.5% (100, 15, 25, 50) | 0% |

Numbers in parentheses represent the individual percentage content loss for Mounds 1-4, respectively. Eggs with 100% content loss were completely consumed by fire ants.

the majority of their amniotic fluids. One intact fence lizard egg and one punctured egg were entirely missing at the end of the trial (24 hours); they were presumably completely consumed by fire ants, as the ground was undisturbed. Other ant species may be able to penetrate fence lizard eggs. However, given the proximity of our experimental eggs to these fire ant mounds, potential native predators (including ants) were likely excluded from the eggs by the fire ants. Additionally, the red imported fire ant (*S. invicta*) was the only ant species found on all 12 of the eggs, supporting the notion that the observed predation was due to these fire ants.

A caveat of this study is that, although it was conducted in the field using actual lizard eggs and fire ant habitat, we created artificial "nests" for the eggs. These likely did not exactly mimic natural nests, and may have altered the ability of fire ants to locate the eggs. Fire ants are attracted to disturbances (Allen et al., 2001; LeBrun, Plowes and Gilbert, 2012), and thus may have been attracted to our artificial nests. This is likely also true of disturbance caused by lizards as they dig their nests (Allen et al. 2001), but we may have unintentionally provided additional surface cues that attracted fire ants.

Applying yolk to the outside of the eggs was intended to simulate attractants associated with damage to neighboring eggs, and we expected this to attract fire ant predation. We also expected that puncturing eggs should have increased fire ant predation, as we provided access through the protective shell, such as may occur through a partial predation event that damages the eggs. Contrary to these expectations, the intact eggs lost more contents than did the penetrated and yolk-dipped eggs (Table 1), suggesting that eggs that are penetrated or get covered in yolk due to separate predation events are not likely to suffer greater fire ant predation risk, and that fire ants are easily able to penetrate the eggs protective shell. It is possible that some of the content loss in the punctured fence lizard eggs may have been a result of leakage or evaporation while underground but, given the loss of mass in the intact eggs, we attribute the majority of the content loss to fire ant predation.

We consider it likely that none of the eggs in this experiment would have hatched successfully, given that all eggs had either been penetrated or were covered with fire ants when retrieved. Although eggs with tiny punctures maintained the majority of their amniotic fluids within the 24-hour period, it is probable that fire ants would have fully consumed the eggs if the observational period had been allowed to continue. Even the sole unpenetrated egg would likely have eventually been penetrated as it was covered by fire ants when we recovered it. Thus it seems that, while adult fence lizards have developed morphological and behavioural adaptations to improve their chances of escaping fire ants (Langkilde, 2009a), fence lizard eggs in the vicinity of fire ant mounds are likely to be vulnerable to predation by fire ants.

It is possible that fence lizards might have strategies to reduce fire ant predation of eggs. Lizards may actively select to nest in areas where fire ants are absent or occur at lower densities. However, adult fence lizards, which are frequently attacked by fire ants within invaded sites (Freidenfelds, Robbins and Langkilde, 2012), do not spatially avoid fire ants; likely because they (like eggs) require the same habitat as fire ants (open and sunny) and the costs of moving into closed-canopy habitat may outweigh any benefits (Langkilde 2009b). Simulated fence lizard nests that are as far as 5.75 m from the nearest fire ant mound are susceptible to fire ant attack (Thawley, unpubl. data), which is often as far as a lizard can get from a fire ant mound (fire ant mounds are typically ~10 m from the next nearest fire ant mound; Langkilde, 2009b). Lizards from fire ant invaded sites may reduce the risks of egg predation by producing eggs with thicker shells that may be less easily penetrated by fire ants; however, our data does not support this (Goldy-Brown, unpubl. data). Furthermore, fence lizards have an evolutionary history with native fire ants, including subterranean species, and so may have adaptations that have allowed them to survive invasion by the invasive fire ants.

Future research testing for potential adaptations against egg predation by fire ants, evidence of lizards being pre-adapted to invasive fire ants, and population-level effects of fire ant predation of fence lizard eggs, would be valuable in determining the ecological and evolutionary impacts of invasive fire ants on native lizards. It is possible that increased egg predation will have no effect on the population or maybe even increase population sizes if, for example, hatchling survival is density-dependent (Tinkle, Dunham and Congdon, 1993). Our results support the need to consider the predation pressure of fire ants across all life stages when assessing or predicting the effect of fire ants on the persistence of herpetofaunal populations.

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