Are Invasive Species Stressful? The Glucocorticoid Profile of Native Lizards Exposed to Invasive Fire Ants Depends on the Context

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ABSTRACT

Invasive species represent a substantial threat to native species worldwide. Research on the impacts of invasive species on wild living vertebrates has focused primarily on population-level effects. The sublethal, individual-level effects of invaders may be equally important but are poorly understood. We investigated the effects of invasive fire ants (*Solenopsis invicta*) on the physiological stress response of a native lizard (*Sceloporus undulatus*) within two experimental contexts: directly exposing lizards to a fire ant attack and housing lizards with fire ants in seminatural field enclosures. Lizards directly exposed to brief attack by fire ants had elevated concentrations of the stress hormone corticosterone (CORT), suggesting that these encounters can be physiologically stressful. However, lizards exposed for longer periods to fire ants in field enclosures had lower concentrations of CORT. This may indicate that the combined effects of confinement and fire ant exposure have pushed lizards into allostatic overload. However, lizards from fire ant enclosures appeared to have intact negative feedback controls of the stress response, evidenced by functioning adrenocorticotropic hormone responsiveness and lack of suppression of innate immunity (plasma bactericidal capacity). We review previous studies examining the stress response of wild vertebrates to various anthropogenic stressors and discuss how these—in combination with our results—underscore the importance of considering context (the length, frequency, magnitude, and types of threat) when assessing these impacts.

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Introduction

Global environmental change is becoming more frequent and severe, exposing natural populations to novel pressures (Palumbi 2001; Barnosky et al. 2012). The negative impacts of these changes on species are well documented; however, much of this research focuses primarily on the lethal consequences of environmental perturbations (Mooney and Cleland 2001; but see Sih et al. 2011). Understanding the sublethal implications of global environmental change—including physiological responses to these threats—is necessary in order to predict their long-term effects on populations (Hoffmeister et al. 2005; Strauss et al. 2006).

One sublethal impact that is receiving increasing attention is an individual's physiological stress response to a threat (Chrousos and Gold 1992; Wingfield et al. 1997). The hypothalamic-pituitary-adrenal (HPA) axis is the primary hormonal mediator of the vertebrate stress response and is regulated by negative feedback loops that maintain regulatory hormone concentrations within a narrow range (Norris 1997). In response to a stressor, glucocorticoids (GCs) released by the axis drive numerous suppressive or stimulatory effects within other body systems that assist in recovery from the stressor (Sapolsky et al. 2000) and/or a shift to a new homeostatic physiological state that can maximize benefits and prevent negative downstream effects (McEwen and Wingfield 2003; Romero et al. 2009). Repeated and/or persistent engagement of the HPA axis can lead to prolonged release of GCs, allostatic overload, disease conditions, and chronic stress conditions (McEwen and Wingfield 2003; Romero et al. 2009).

The role of the HPA axis and GCs in mitigating anthropogenic disturbances affecting wild vertebrate populations has recently become an area of active research. Most studies on this topic have focused on the endpoint of the HPA axis, that is, fluctuations in plasma GCs. GC profiles have been investigated under various regimes of anthropogenic stressors, such as pollutants, urbanization, habitat modification, and other disturbances (table 1). Many of these studies show higher baseline and stressor-induced GC concentrations in disturbed populations relative to undisturbed populations, and the consequences of these changes in GC profiles (e.g., downstream effects on reproduction, growth, or immunity) have occasionally been measured as well. However, some studies demonstrate the opposite

Table 1: Selected studies comparing baseline and acute glucocorticoid responses to anthropogenic disturbances in wild vertebrate populations

Study	Anthropogenic disturbance	Study species	Baseline GCs in affected population	Acute stress GCs in affected population	Other effects
Berger et al. 2007	Invasive species (cats and dogs)	Marine iguana (*Amblyrhynchus cristatatus*)	Similar	Higher	
Graham et al. 2012	Invasive species (fire ants)	Fence lizard (*Sceloporus undulatus*)	Higher	Higher	Immunity ectoparasites
Jessop et al. 2015	Invasive species (red fox)	Lace monitor (*Varanus varius*)	Higher	Higher	
Romero and Wikelski 2002	Tourism (presence of humans)	Marine iguana (*A. cristatatus*)	Similar	Lower	
Owen et al. 2014	Vehicles (proximity to road)	Copperhead snake (*Agkistrodon contortrix*)	Similar	Lower	
Fowler 1999	Tourism (presence of humans)	Megellanic penguin (*Spheniscus megellanicus*)	Lower	Higher	
Müllner et al. 2004	Tourism (presence of humans)	Hoatzin (*Opisthocomus hoazin*)	Similar	Higher	Hatching success
Creel et al. 2002	Vehicles	Wolf (*Canis lupus*), elk (*Cervus canadensis*)	Higher	NA	
Crino et al. 2011	Vehicles	White-crowned sparrow (*Zonotrichia leucophrys*)	Higher	Higher	Parental care predation
Hayward et al. 2011	Vehicles (proximity to road)	Northern spotted owl (*Strix occidentalis*)	Similar	NA	
Wasser et al. 1997	Vehicles (proximity to road)	Northern spotted owl (*S. occidentalis*)	Higher (males only)	NA	
Hayward et al. 2011	Vehicles (motorcycle noise)	Northern spotted owl (*S. occidentalis*)	Higher	NA	
Wasser et al. 1997	Habitat alteration (deforestation)	Northern spotted owl (*S. occidentalis*)	Higher (males only)	NA	
Homan et al. 2003	Habitat alteration (deforestation)	Spotted salamander (*Ambystoma maculatum*)	Lower	Lower	
Lucas et al. 2006	Habitat alteration (deforestation)	Carolina chickadee (*Poecile carolinensis*)	Higher	NA	
Wasser et al. 1997	Habitat alteration (forest fragmentation)	Northern spotted owl (*S. occidentalis*)	Higher (males only)	NA	
Suorsa et al. 2004	Habitat alteration (forest fragmentation)	Eurasian treecreeper (*Certhia familiaris*)	Higher	NA	
Homan et al. 2003	Habitat alteration (paved roads)	Spotted salamander (*A. maculatum*)	Higher	NA	
Baxter-Gilbert et al. 2014	Habitat alteration (paved roads)	Painted turtle (*Chrysemys picta*)	Similar	NA	
Franceschini et al. 2008	Pollution (polychlorinated biphenyls)	Tree swallow (*Tachycineata bicolor*)	Unpredictable	NA	
Norris et al. 1999	Pollution (metal contamination)	Brown trout (*Salmo trutta*)	Similar	Lower	
Wikelski et al. 2001	Pollution (oil spill)	Marine iguana (*A. cristatatus*)	Higher	NA	
Fowler 1995	Pollution (oil spill)	Megellanic penguin (*S. megellanicus*)	Higher (females only)	NA	
French et al. 2008	Urbanization	Tree lizard (*Urosaurus ornatus*)	Lower	Lower	Immunity
Fokidis et al. 2009	Urbanization	House sparrow (*Passer domesticus*), northern mockingbird (*Mimus polyglottos*), curve-billed thrasher (*Toxostoma curvirostre*), Abert's towhee (*Pipilo aberti*), canyon towhee (*Pipilo fuscus*)	Similar	Higher	
Partecke et al. 2006	Urbanization	European blackbird (*Turdus merula*)	Similar	Lower	
Schoech 2007	Urbanization	Florida scrub-jay (*Aphelocoma coerulescens*)	Lower	Higher	
Brearley et al. 2012	Urbanization	Squirrel glider (*Petaurus norfolcensis*)	Higher	NA	
Dickens et al. 2009	Translocation	Chukar (*Alectorris chukar*)	Lower	NA	HPA axis negative feedback
Holding et al. 2014	Translocation	Northern Pacific rattlesnake (*Crotalus oreganus*)	Similar	Similar	No difference in testosterone

Note. GC, glucocorticoids; HPA, hypothalamic-pituitary-adrenal; NA, not applicable.

finding, documenting reduced baseline or acute stress concentrations of GCs within affected populations or a rise in GCs without a concomitant effect on other physiological components (e.g., immunity; Graham et al. 2012; table 1). Lower baseline or acute stress concentrations of GCs in affected populations are often hypothesized to be the result of chronic stressors and downregulation of the HPA axis, which can depress GC concentrations (Rich and Romero 2005; Dickens et al. 2009). Perhaps because of their amenability for study or sensitivity to anthropogenic disturbances (or both), amphibians and reptiles—particularly lizards—are the subject of a substantial number of these studies (table 1).

Invasive species are a significant anthropogenic stressor confronting wild vertebrates. Introductions of nonnative species and pathogens are possibly the greatest persistent anthropogenic threats to wild vertebrates, besides outright habitat loss (Vitousek et al. 1997; Clavero and García-Berthou 2005). Invasive species are a direct cause of population declines and extinctions of native vertebrates, especially for narrowly distributed island endemics (reviewed in Fritts and Rodda 1998). Unlike loss of habitat, invasive species often have unpredictable and counterintuitive impacts on native species (Shine 2010), including numerous sublethal but pervasive effects (Mooney and Cleland 2001; Strauss et al. 2006) and occasionally unforeseen benefits (e.g., King and Tschinkel 2006; King et al. 2006). The often unpredictable ways stressors can influence the GC profiles of various species (see table 1) make studies on a variety of systems necessary to elucidate the proximate causes for these varied endocrine responses.

Animals can become habituated to repeated stressors, resulting in reduced GC responses to that particular stimulus (Walker et al. 2006). Habituation should be most prevalent when an initially stressful stimulus is not actually harmful or dangerous, for example, in response to repeated low-level shock stimuli (Pitman et al. 1990), handling of animals in captivity (McKnight et al. 1978), or the presence of humans in the environment (Romero and Wikelski 2002; Walker et al. 2006). If the novel stressor poses a real threat and the GC response triggers adaptive responses that increase an individual's chances of surviving this threat, we would not expect habituation via an attenuated GC response to exposure. Instead, we would expect an individual to retain their initial stress response to the novel threat or even to become sensitized to it, mounting a higher GC response with repeated or continuing exposure (Natelson et al. 1988; Pitman et al. 1988, 1990).

Comparing the GC responses of individuals from populations that have been exposed to invasive species versus those that are naive can provide important insights into how populations respond to invasions and whether they can become sensitized or habituated to this threat. However, the correlative nature of this approach makes it difficult to isolate the invader as the causal factor driving observed differences. Testing the direct impact of interactions between invasive and native species can be facilitated by staging encounters between these species under controlled conditions, although this can be a difficult proposition if truly natural conditions are required (e.g., this might require introduction of an invasive species, an ethically dubious method).

We use a study system in which these manipulations are tractable, that of interactions between invasive predatory fire ants (Solenopsis invicta) and native fence lizards (Sceloporus undulatus).

Using this system, we test whether and to what degree invasive species directly affect the GC response of a native species. We quantify changes in corticosterone (CORT; the primary GC of reptiles) concentrations of fence lizards following encounters with invasive predatory fire ants in two experimental contexts. We (1) test whether lizards directly exposed to fire ant attack in the field exhibit increased GC concentrations and (2) expose lizards to fire ants for an extended time period (2 wk) under seminatural conditions and attempt to determine whether this chronic stress treatment has downstream impacts on other systems by quantifying adrenocorticotropic hormone (ACTH) sensitivity and immune function. Consistent with most studies that have examined significant yet sublethal stressors (table 1), we predict that lizards exposed to fire ants under these conditions would have elevated baseline GC concentrations and—if this stress is chronic—decreased ACTH sensitivity and immune function. We further predicted that the lizards' history of exposure to fire ants would alter this suite of responses, potentially because of adaptation to this novel threat.

Material and Methods

Study System

The red imported fire ant (Solenopsis invicta) is a now widespread invasive species that was first introduced in the southeastern United States in the 1930s. This invader has since spread to 13 US states and seven additional countries worldwide (Tschinkel 2006; Code of Federal Regulations 2013). These venomous ants can cause direct mortality of vertebrates that do not escape quickly (e.g., Mueller et al. 1999; Allen et al. 2001; Langkilde 2009a) and are suspected of causing population declines in a number of vertebrate species (Wojcik et al. 2001; Holway et al. 2002; Allen et al. 2004). We have documented potential impacts of these ants on native eastern fence lizards (Sceloporus undulatus), evidenced by differences in survival, behavior, morphology, and GC profiles between lizards from sites invaded by fire ants versus sites that are free of fire ants (Langkilde 2009a, 2009b; Trompeter and Langkilde 2011; Graham et al. 2012; C. J. Thawley, unpublished data). Importantly, baseline and stress-induced concentrations of CORT were higher in lizard populations at sites that had been invaded by fire ants than at uninvaded sites (Graham et al. 2012).

Here we present a study designed to test whether these differences in CORT concentrations can be attributed to the presence of fire ants rather than environmental factors that may covary with their presence. We captured adult lizards (mean snout-vent length [SVL] = 65 mm, range = 56–75 mm; mean mass = 11 g, range = 6–17 g) from three replicate sites within and three replicate sites outside of the fire ant invasion range (for a map, see Graham et al. 2012). Fire ant–invaded sites, which were first invaded 54–70 yr ago, were Solon Dixon Forestry Education Center, Escambia County, Alabama (SD; 31°09′49″N, 86°42′10″W); Blackwater River State Forest, Florida (BWR;

30°56′30″N, 86°49′03″W); and Geneva State Forest, Alabama (GSF; 31°07′02″N, 86°09′53″W). Uninvaded sites were St. Francis National Forest, Lee County, Arkansas (SF; 34°43′50″N, 90°42′18″W); Standing Stone State Park, Tennessee (SS; 36°28′04″N, 85°24′59″W); and Edgar Evins State Park, Tennessee (EE; 36°4′40″N, 85°49′57″W). We transported lizards from these sites to our field station in southern Alabama (Auburn University Solon Dixon Forestry and Education Center) for use in this study.

Experiment 1: Direct Exposure

We collected adult lizards (27 male, 29 female) from one fire ant–invaded site (SD; 14 males, 14 females) and one uninvaded site (SF; 13 males, 15 females) in September 2009 for the direct exposure experiment. Lizards were transported in cloth bags to our field station in Alabama and housed individually in a laboratory for 1 wk before stimulus exposure. Terraria (30 cm × 21 cm × 26 cm) were lined with paper toweling and furnished with a shelter, water bowl, and a broad-spectrum incandescent lamp as a heat source (on a 12L:12D cycle). Lizards were fed crickets (*Acheta domesticus*) every other day and provided ad lib. access to water.

Lizards from each site were assigned to either fire ant ($n = 28$) or control ($n = 28$) treatments balanced for site and sex. Sublethal fire ant exposure followed the methods of Langkilde (2009a, 2009b). Briefly, an active fire ant mound was located and gently disturbed to encourage a small number of ants (≈4–8) to emerge. Lizards were removed from their terraria and fitted with a 50-cm cotton-thread tether tied loosely around their necks and anchored next to the mound. This tethering prevented them from escaping (particularly important for lizards tested away from their source population; for more details, see Langkilde 2009b) but did not otherwise hinder movement. Lizards were placed on the ground near the mound and tapped on the tail to encourage them to run onto the mound. To ensure consistent exposure of lizards to fire ants, lizards that fled off the mound before 60 s were returned to the mound for the 60-s duration of the trial. This handling does not appear to have elevated CORT concentrations, because lizards from the fire ant treatment that fled and were returned to the mound did not have higher final CORT than did lizards from the fire ant treatment that did not flee ($F_{1, 26} = 2.64$, $P = 0.12$). The mean number of ants that attacked each lizard in this experiment was 3.8 ± 0.22 SE, ensuring that a 60-s exposure was nonlethal (Langkilde 2009b). Control lizards were handled in the same manner as the lizards exposed to fire ants, except that they were placed on a patch of soil with no fire ants present. After this sublethal fire ant exposure or control handling, lizards were immediately returned to their terraria.

To determine the effects of fire ant exposure on fence lizard CORT concentrations, adult lizards were bled serially at 10, 30, 60, and 90 min after exposure to the stimulus (fire ants or control). One of us (T. Langkilde) performed the stimulus exposure, while another (N. A. Freidenfelds) obtained blood samples, thus ensuring that blood samples were collected blind to treatment.

Blood samples (10 μL) were obtained from each lizard from the retro-orbital sinus using microhematocrit tubes (13450-216, VWR). Blood was obtained within 3 min of first disturbing each lizard to prevent handling-induced increases in sample CORT (Romero and Reed 2005; handling time of the lizard did not correlate with plasma CORT concentrations in this study ($P > 0.63$) and did not change the outcome of the statistical models). Samples were then stored on ice packs for no more than 1 h before centrifugation (Zip-Spin, LW Scientific). Plasma was drawn off and aliquoted into labeled 600-μL microcentrifuge tubes and immediately frozen ($-20°C$) until assay (<5 mo).

Experiment 2: Large Enclosure

Lizards used for enclosure trials were collected from all six sites (SD, GSF, BWR, SF, EE, SS) during the breeding season (May–July 2011 and 2012). Twenty lizards from each site (10 male, 10 female) were housed individually in terraria as described for experiment 1 and allowed to acclimate for 1 wk. Each lizard was identified with a numeral placed on the dorsum with a black permanent marker, allowing identification from a distance via the naked eye or a spotting scope. Lizards were randomly assigned to one of four enclosures via a stratified protocol to ensure equal sex ratios in each, and lizards from each site were kept in the same enclosures.

Enclosures were constructed from aluminum flashing (61 cm high) sunk 14 cm below the soil surface and secured with metal poles and zip ties (Robbins 2010). These were located within 200 m of one another in a field interspersed with trees near native populations of fence lizards within the fire ant invasion zone at the Solon Dixon Forestry and Education Center in Alabama. Each enclosure measured 520 m² and contained four trees suitable as fence lizard habitat; additionally, we introduced three cover objects to each enclosure, consisting of wooden pallets, branches, and logs to ensure a wide availability of perching sites and shelters. Suitable prey for lizards was naturally available; lizards were observed eating prey (mainly arthropods) on numerous occasions, and they did not lose weight or experience lower body condition during their time in enclosures (C. J. Thawley, unpublished data). Before introducing lizards into the enclosures, each enclosure was searched for native lizards, and fire ants were eradicated from half (two) of the enclosures, including from a 5-m buffer zone around the enclosures. Fire ants were killed by locating a mound, piercing the brood chamber with a length of rebar, and filling the chamber and mound with boiling water (Tschinkel and King 2007). Fire ants were eradicated for 2 wk before introducing lizards and were monitored daily throughout the trial, and any new mounds were eradicated (Tschinkel and King 2007). Enclosures with natural levels of fire ants were also inspected daily to ensure that lizards in all enclosures experienced similar levels of potential observer-induced stress. Pitfall trapping conducted in 2012 indicated that enclosures in which fire ants were eradicated had fire ant densities averaging 15% of those in enclosures with natural, unmanipulated densities (C. J. Thawley, unpublished data); this is similar to suppression levels achieved by a similar study (Harlan et al.

1981). Lizards remained in enclosures for 2 wk, at which time enclosures were thoroughly searched for 3 d to ensure that all surviving lizards had been captured.

These trials were run over 2 yr, using lizards from EE, SS, and BWR in 2011 and from SF, SD, and GSF in 2012. In both years, blood samples obtained from surviving lizards ($n = 56$) immediately on capture were used to measure baseline CORT at the end of the 2-wk trial. We used the same blood sampling protocol as in experiment 1 but obtained 20–40 μL of blood to allow for additional tests. In the second year, we additionally measured two potential indicators of chronic stress for lizards from three sites (SD, SF, GSF): CORT responsiveness to ACTH ($n = 24$) and immune function ($n = 18$). After collecting the baseline blood sample, we placed each lizard in a cloth bag for 30 min and then took a second blood sample to determine CORT response to captive handling (hereafter referred to as stress induced, following Graham et al. 2012). We then injected each lizard with 10 μL 0.8 IU ACTH (porcine pituitary, Sigma A6303, Sigma-Aldrich, St. Louis) intraperitoneally (1-cm³ insulin syringe, 29-gauge needle) to determine how capable lizards were of mounting a CORT response. We did not include control animals injected with saline only because of sample size limitations, but preliminary studies determined that this dose of ACTH results in significantly elevated concentrations of CORT in fence lizards compared with lizards injected with saline (change in CORT after 30 min, ACTH injected vs. saline solution control: $t_8 = 16.6$, $P = 0.004$). We returned the lizards to their bags and, after 60 min, obtained a third and final blood sample. For this experiment, we compared CORT concentrations immediately before injection with ACTH to the sample taken 60 min later. We tested for CORT responsiveness to ACTH injection, which can change under chronic stress conditions in reptiles (Romero and Wikelski 2010). For example, an animal habituated to a stressor should still show a CORT response to ACTH; however, this may be reduced or absent in an animal that is physiologically desensitized or exhausted by chronic stress conditions (Cyr and Romero 2009).

Since prolonged exposure to increased concentrations of CORT associated with stress can have downstream negative effects on immunity (Sapolsky et al. 2000), in 2012 (for lizards from SD, SF, and GSF) we also tested baseline levels of a component of innate immunity: bactericidal capacity of lizard plasma. For this, we used a bacterial killing assay (Millet et al. 2007). This assay involves combining dilutions of lizard plasma with known amounts of bacteria and comparing the colony counts of these experimental samples to untreated controls to calculate the percentage of bacteria killed by the lizard blood. For more detailed methods involving this assay, see Graham et al. (2012).

Corticosterone Assays

Plasma CORT was measured by enzyme immunoassay (EIA) using commercially available kits (Corticosterone High Sensitivity EIA Kits, Immunodiagnostic Systems, Fountain Hills, AZ). Kits were previously validated for use with *S. undulatus* by assessing parallelism and quantitative recovery (Trompeter

and Langkilde 2011). This study determined that average CORT recovery for these kits ranged between 100% and 106%. Plasma was initially diluted 90% with assay buffer (5 μL plasma + 45 μL buffer) so that samples would fall within the detectable range of the assay's standard curve (Trompeter and Langkilde 2011). Each sample was run in duplicate using instructions provided with the kits. Coefficients of variation were calculated by comparing the values of control samples provided in the kits run in duplicate on each of the 10 plates used in this study: the mean intra-assay coefficient of variation within these kits was 3.02% (range = 2.01%–4.11%), and the mean interassay coefficient of variation among the kits (calculated by comparing across 10 plates) was 7.2% (range = 2.22%–15.34%).

Data Analysis

Plasma CORT collected from lizards in the direct exposure experiment was compared across bleed times (10, 30, 60, 90 min) using a repeated-measures ANCOVA with treatment (exposure to fire ants vs. controls), invasion status of source population (invaded vs. uninvaded), and lizard sex as main effects and SVL as the covariate. For the enclosure experiment, we tested for differences in potential indicators of prolonged exposure to elevated CORT (baseline CORT, stress- and ACTH-induced CORT, and bactericidal capacity) using separate ANCOVAs with treatment (fire ant vs. no fire ant), invasion status of the source population (invaded vs. uninvaded), and lizard sex as the main effects and SVL as a covariate for this analysis. Year was included as a factor in analyses of baseline CORT; we could not also include site since not all sites were tested each year, resulting in incomplete separation of the data set. However, in an ANCOVA omitting year, site was not a significant predictor of CORT concentrations ($P = 0.20$). We included baseline CORT and SVL as covariates for analysis of stress- and ACTH-induced CORT and analyses of immunity (percent bacteria killed). Except for sex effects on percent bacteria killed, sex and SVL did not significantly explain variation of any of the variables tested, and so these were omitted from the final analyses (sex: $P > 0.12$; SVL: $P > 0.11$; retaining these did not qualitatively alter our results). Baseline CORT did not significantly explain variation in percent bacterial killing and so was omitted from the final analysis ($P > 0.73$). Plasma CORT concentrations and SVL were log transformed, and bacterial killing scores were angular transformed as needed before analyses to meet assumptions of parametric tests.

Results

For fence lizards in the direct exposure experiment, CORT concentrations increased over time in both control and treatment groups (likely because of the presence of the observer, multiple bleeds, and handling in the controls), but the increase in CORT was significantly greater for lizards directly exposed to fire ants than for controls (time × treatment: $F_{3, 48} = 2.87$, $P = 0.046$; fig. 1, *top*). This pattern held irrespective of the source population's invasion status (uninvaded or invaded;
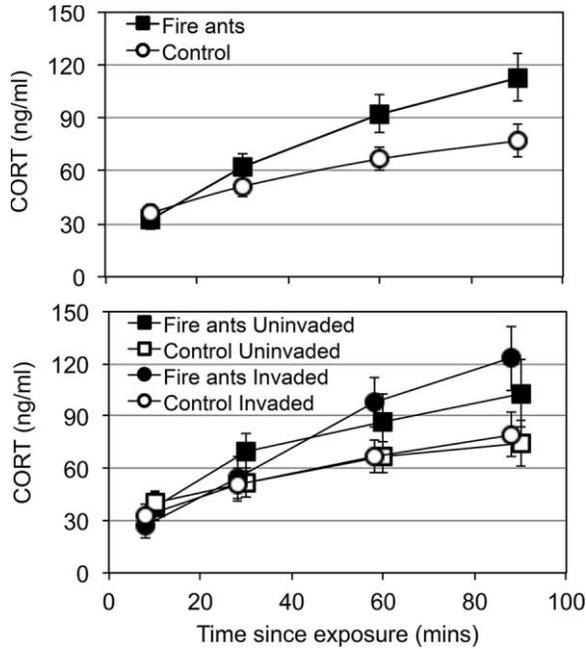
Figure 1. Plasma corticosterone (CORT) concentrations of lizards repeatedly sampled after 60-s exposure to fire ants (filled symbols, $n = 28$) or control handling (open symbols, $n = 28$; *top*) and with fire ant–invaded (circles) and uninvaded (squares) source populations indicated separately (*bottom*). There was a significant treatment effect (see "Results"), but the invasion history of the lizards (invaded vs. uninvaded populations) did not significantly influence CORT concentrations. Bars represent means ± 1 SE.

time × treatment × invasion status: $F_{3, 48} = 0.93$, $P = 0.43$; time × invasion status: $F_{3, 48} = 1.42$, $P = 0.25$; fig. 1, *bottom*).

Fence lizards exposed to fire ants in the enclosure experiment had significantly lower baseline CORT values compared with those in enclosures that were free of fire ants (treatment: $F_{1, 74} = 4.71$, $P = 0.04$; fig. 2). Invasion status of the source population did not affect baseline CORT concentrations or the effect of treatment on baseline CORT (invasion status: $F_{1, 74} = 1.75$, $P = 0.19$; treatment × invasion status: $F_{1, 74} = 1.02$, $P = 0.32$; year: $F_{1, 74} = 5.55$, $P = 0.02$). The lizards' CORT response to handling and ACTH injection was not affected by invasion status or enclosure treatment (stress-induced CORT: treatment: $F_{1, 19} = 1.13$, $P = 0.72$; invasion status: $F_{1, 19} = 0.29$, $P = 0.60$; treatment × invasion status: $F_{1, 19} < 0.001$, $P = 0.97$; baseline CORT: $F_{1, 19} = 1.82$, $P = 0.19$; ACTH-induced CORT: treatment: $F_{1, 19} = 0.59$, $P = 0.45$; invasion status: $F_{1, 19} = 1.09$, $P = 0.31$; treatment × invasion status: $F_{1, 19} = 0.79$, $P = 0.39$; baseline CORT: $F_{1, 19} = 0.38$, $P = 0.54$; fig. 3).

We did not find downstream effects of treatment on immunity. Plasma bactericidal capacity of lizards was not affected by the presence or absence of fire ants in enclosures (treatment: $F_{1, 13} = 1.06$, $P = 0.32$). There was not an effect of the source population's invasion status nor an interaction with treatment (invasion status: $F_{1, 13} = 3.36$, $P = 0.09$; treatment × invasion

status: $F_{1, 13} = 0.04$, $P = 0.85$; sex: $F_{1, 13} = 7.29$, $P = 0.02$ [female > male]; fig. 4).

Discussion

We found that fence lizard GC profiles were affected by exposure to fire ants, but the direction of change was context dependent. Not surprisingly, directly exposing fence lizards to fire ant attack resulted in elevated concentrations of CORT. This acute CORT response triggered by fire ants may occur frequently in lizards living within the fire ant invasion zone; fire ants quickly locate and attack fence lizards in the field (within 132 ± 29 s [± SE; Freidenfelds et al. 2012], whereas native ants do not (only one of 18 lizards was even found by native ants within 6 min; T. Langkilde, unpublished data). In addition to attacking lizards for mound defense, these ants actively search for and sting lizards as much as 4 m from the nearest mound (the maximum distance from a mound at these sites; Freidenfelds et al. 2012). This suggests that elevated baseline CORT concentrations recorded in field-caught lizards from fire ant–invaded sites (Graham et al. 2012) are likely due to recent encounters with fire ants. The CORT response to fire ant attack observed in this study did not differ significantly between lizards from fire ant–invaded versus uninvaded populations. This expands on findings that wild-caught lizards from fire ant–invaded sites had higher stress-induced CORT concentrations in response to handling than did lizards from uninvaded sites (Graham et al. 2012). This same pattern of lizards from fire ant–invaded sites having an increased CORT response to handling—but not to exposure to fire ants—compared with lizards from uninvaded sites was also observed in lab-reared lizards (G. L. McCormick, unpublished data). The threats posed by handling versus fire ants are ecologically very different, which may explain these differences in responsiveness, and this pattern is worth exploring further.

Contrary to our predictions, longer-term (2 wk) exposure of lizards to fire ants within our outdoor enclosures resulted in reduced baseline CORT concentrations. The lower baseline CORT could indicate that fire ant–exposed lizards were less
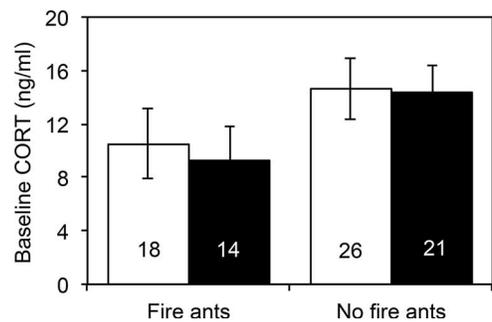

Figure 2. Baseline plasma corticosterone (CORT) concentrations of lizards from uninvaded (open bars) and fire ant–invaded (filled bars) sites after 2 wk of being maintained within field enclosures from which fire ants were eradicated or present. Bars represent means ± 1 SE; numbers indicate samples sizes.
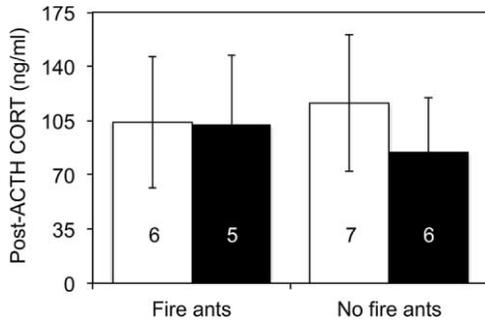
Figure 3. Plasma corticosterone (CORT) concentrations of lizards from uninvaded (open bars) and fire ant–invaded (filled bars) sites following injection with adrenocorticotropic hormone (ACTH) after 2 wk of lizards being maintained within field enclosures from which fire ants were eradicated or present. Bars represent means ± 1 SE; numbers indicate samples sizes.

stressed. Starlings exposed to frequent and unpredictable stressors showed reduced levels of GCs, which was attributed to downregulation of the HPA axis (Rich and Romero 2005). The elevated CORT concentrations of lizards that are directly exposed to fire ants (fig. 1) and of wild-caught lizards from fire ant–invaded sites (Graham et al. 2012) suggest that the stress caused by fire ant exposure—in combination with the potential stress imposed by semicaptive novel environments— may have driven lizards into allostatic overload (McEwen and Wingfield 2003; Romero et al. 2009). We did not, however, find any difference in lizard CORT responsiveness to handling or ACTH between treatments, arguing against a complete downregulation of HPA functioning or physiological exhaustion (e.g., Cyr and Romero 2009), and no evidence of immune suppression. The goal of our study was not to completely disentangle the possible contributions of all components of HPA regulation; however, future studies using similar methods to Rich and Romero (2005) and Dickens et al. (2009) may unravel this complicated issue. In particular, studies incorporating treatments with dexamethasone should be used to confirm whether downregulation of the HPA axis is occurring.

Overall, the CORT responses of lizards across three contexts (direct exposure, field enclosures, and free living) suggest that (1) direct encounters with invasive fire ants increase CORT concentrations (fig. 1); (2) the stress of being exposed to fire ants for a longer period (2 wk) combined with the potential stress of being held in seminatural enclosures may drive lizards into allostatic overload, resulting in lower baseline CORT concentrations (fig. 2); and (3) in free-living lizard populations (Graham et al. 2012), frequent encounters with fire ants result in higher baseline CORT concentrations in lizards from fire ant–invaded sites. These altered GC profiles are likely adaptive (Boonstra 2013). Lower baseline CORT levels may facilitate a larger reactive scope and thus a potentially stronger CORT response to a subsequent acute stressor (Romero et al. 2009), a pattern we have observed in wild-caught lizards from invaded populations (Graham et al. 2012). Experimentally elevated CORT concentrations increased the frequency of antipredatory behaviors (twitch and flee) in

fence lizards to subsequent fire ant encounters, suggesting that CORT can prime escape from and avoidance of fire ants (T. Langkilde, unpublished data). Thus, the ability to flexibly modulate HPA axis activity to promote strong CORT responses could promote survival. The reduced baseline CORT concentrations might reflect proper functioning of the negative feedback system, resulting in a mild reduction of CORT synthesis. This could prevent the animal from entering into a state of allostatic overload when encountering fire ants and prevent negative downstream effects on the HPA axis or pathological consequences. This is consistent with our observations that these lizards did not differ in body mass or immune function at the end of the enclosure trials (C. J. Thawley, unpublished data) and of wild-caught lizards from invaded and uninvaded sites having similar immune profiles (Graham et al. 2012). However, the possibility that lizards from fire ant–invaded sites are more vulnerable to negative effects of new stressors because of the existing fire ant–imposed stress should be investigated because it has important implications for understanding the long-term effects of novel environmental stressors.

Our study joins others that have examined GC responses to diverse anthropogenic disturbances and determined that GC concentrations are variable in affected populations relative to unaffected reference populations and that these changes are context specific (table 1). In some cases, population disturbances cause higher concentrations of baseline GCs than in reference populations (Graham et al. 2012). However, cases in which affected populations had similar and lower GC values are also reported (fig. 2; table 1). Stress-induced GC concentrations between affected versus reference populations (usually after experimental captive confinement) also vary, with about half of the affected populations studied exhibiting higher acute GC concentrations and about half exhibiting lower GC concentrations (table 1).

Differences in the duration of stressors (acute vs. persistent), types of stressors (potentially lethal vs. simply noxious), and selective forces on HPA axis function (within lifetime vs. evolved change) may be responsible for the diverse experimental outcomes of studies on the effect of anthropogenic
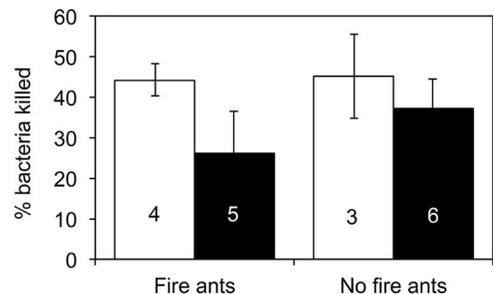

Figure 4. Percent bacteria killed by plasma from lizards from uninvaded (open bars) and fire ant–invaded (filled bars) sites after 2 wk of being maintained within field enclosures from which fire ants were eradicated or present. Bars represent means ± 1 SE; numbers indicate sample sizes.

stressors on HPA function (table 1). This variation in results probably also signifies species-specific differences in responses to anthropogenic stressors. The results of our study further suggest that the idiosyncratic outcomes of this body of research may reflect the different experimental contexts of the anthropogenic stressor studied (table 1). For example, within our study system, we found evidence of many of the potential outcomes listed in table 1: CORT concentrations of lizards in response to fire ant exposure were lower or higher, depending on the experimental context, and these results may describe habituation, exhaustion, or downregulation of the HPA axis under these contexts (e.g., Rich and Romero 2005).

Our results contribute to the growing evidence that anthropogenic disturbances—including the introduction of nonnative species—lead to different GC profiles among populations (table 1). The effects of such disturbances on the HPA axis—and of downstream influences on other life-history or physiological components—are likely to be unpredictable (e.g., see Dickens et al. 2009). Examining fitness consequences of multiple physiological responses to global environmental change will reveal their ultimate costs or possible adaptive benefits (e.g., Love et al. 2013). This study also highlights the need to consider the context in which endocrine responses and downstream effects are studied if we are to obtain a clearer picture of the physiological processes through which vertebrates respond to altered environments.

Acknowledgments

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