Endocrine stress response of Eastern Fence Lizards in fire-disturbed landscapes

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Abstract

Landscape disturbances can alter habitat structure and resource availability, often inducing physiological responses by organisms to cope with the changing conditions. Quantifying the endocrine stress response through measurement of glucocorticoids has become an increasingly common method for determining how organisms physiologically respond to challenges imposed by their environment. We tested the hypothesis that Eastern Fence Lizards cope with fire disturbance effects by modulating their secretion of corticosterone (CORT). We measured the baseline and stress-induced plasma CORT of male Eastern Fence Lizards in a chronosequence of fire-altered habitats (recently burned, recovering from burn, and unburned). Although habitat use by lizards differed among burn treatments, including differences in use of canopy cover, leaf litter, and vegetation composition, we did not detect a significant effect of fire-induced habitat alteration on plasma CORT concentration or on body condition. In addition, we found no effect of blood draw treatment (baseline or stress-induced), body temperature, body condition, or time taken to collect blood samples on concentration of plasma CORT. Low intensity burns, which are typical of prescribed fire, may not be a sufficient stressor to alter CORT secretion in Eastern Fence Lizards (at least during the breeding season). Instead, lizards may avoid allostatic overload using behavioral responses and by selecting microsites within their environment that permit thermoregulatory opportunities necessary for optimal performance and energy assimilation.

Key words endocrine response, fire ecology, habitat alteration, prescribed fire, reptile, Sceloporus undulatus

Quantifying the endocrine stress response through measurement of glucocorticoids has become a common method for determining how organisms physiologically respond to challenges imposed by the external environment (Romero and Wikelski 2001; Cash and Holberton 2005; French et al. 2008; Drake et al. 2012; Graham et al. 2012). Glucocorticoids are steroid hormones produced in the adrenal gland by way of the hypothalamus–pituitary–adrenal (HPA) axis, and are secreted to initiate gluconeogenesis, producing energy in the form of glucose (McEwen and Wingfield 2003; Romero 2004). Exposure to unpredictable environmental challenges stimulates increased secretion of glucocorticoids and the breakdown of non-carbohydrate molecules to yield energy to respond to stressful conditions (Korte et al. 2005; Landys et al. 2006). By this process, an organism can achieve an allostatic state (stability of physiological processes) in response to environmental change (McEwen and Wingfield 2003).

Corticosterone (CORT) is the major glucocorticoid secreted by reptiles to stabilize metabolic processes during stressful events (Greenberg and Wingfield 1987). The baseline concentration of CORT is that which is secreted in the absence of unpredictable environmental stressors (Romero 2004) during routine physiological function (Bonier et al. 2009). The stress-induced level of CORT can
then be considered a measure of acute stress, or sensitivity to novel external stressors that are potentially life-threatening (Cash et al. 1997). The magnitude of the physiological response to stressors can be determined by comparing baseline and stress-induced CORT levels (Dupoué et al. 2014).

Allostatic overload, or a prolonged imbalance of energy expenditure and energy input, requires an individual to adapt physiologically and behaviorally to survive (Romero 2004). Chronic stress on an animal may decrease body condition, a measure of stored energy in fat reserves, through continual conversion of stored energy to more immediately accessible gluconeogenic metabolites (Fokidis et al. 2011). Species may adapt to allostatic overload by the short-term suppression of immediately non-essential functions, such as reproductive activity and immune response, thereby increasing the immediate likelihood of survival (McEwen and Wingfield 2003). Under chronically stressful conditions, reproduction may be forgone completely and a suppressed immune system can leave an individual vulnerable to infectious diseases and parasitism (French et al. 2008; McCormick and Langkilde 2014).

Habitat alteration and resulting increases in allostatic load (the routine energy requirement to accomplish normal life-history tasks) of individuals (McEwen and Wingfield 2003; Romero 2004) have been associated with population declines of many reptile species (Gibbons et al. 2000; Tracy et al. 2006). Some habitat alterations, such as logging, vegetation removal, and fire change the landscape suitability for certain species, necessitating behavioral or physiological responses for survival. Fire modifies habitat structure by converting intact vegetative communities to an earlier stage of ecological succession by reducing leaf litter, removing canopy cover, and increasing mineralization of soil (DeBano et al. 1998). These disturbances can change the availability of essential resources including prey, refuge, nesting sites, and thermoregulatory opportunities (Russell et al. 1999). Since resource availability may change after fire-borne disturbance (Howey et al. 2016), the energy required for individuals to obtain resources may also change, which could in turn alter CORT secretion for maintenance of an allostatic state.

The goal of this project was to determine whether a stress response in the CORT pathway is elicited by reptiles experiencing habitat disturbance created by prescribed fire. The Eastern Fence Lizard Sceloporus undulatus is found throughout the eastern United States, commonly inhabiting areas in which prescribed burning is implemented to restore imperiled disturbance-dependent vegetative communities, such as oak savannahs and pine savannahs (Waldrop et al. 1992; Harper and Ford 2016). We tested the hypothesis that Eastern Fence Lizards cope with fire effects by modulating their secretion of CORT. More specifically, we sought to determine whether fire indirectly affects allostatic load, sensitivity to acute stressors, and body condition of lizards. We predicted that baseline and stress-induced CORT would be higher in lizards inhabiting recently burned habitat than lizards in recovering and unburned habitat as an adaptive mechanism for coping with disturbance and the redistribution of suitable microhabitats. We also predicted that body condition would be lower in lizards recently affected by fire, as changes to resource availability may require increasing energy expenditure to obtain critical resources, thus reducing energy stores (body fat).

Materials and Methods

Study site

Land Between the Lakes National Recreation Area (LBL) encompasses approximately 170,000 acres between Lake Barkley and Kentucky Lake (Tennessee and Kentucky, USA). Wildfires were historically a natural and frequent occurrence in the area with a typical 4–8 year fire interval (Guyette et al. 2012), however, fires have been largely suppressed since 1964 when the site was designated a National Recreation Area (Franklin 1994; O’Neill and Doyle 2002; Wright et al. 2002). In the last decade, prescribed fire has been implemented at LBL as a land management practice with the intent to restore native oak-grasslands by reducing the stand density of trees and fuel load on the forest floor.

Three sites with different burn histories were sampled in the spring and summer of 2016. We sampled lizards and vegetation on 1,326-acre prescribed burn site at the South Bison Range in Stewart County, TN (burned March 2016), a 2,461-acre burn site in the Franklin Creek area of Trigg County, KY (6 years since burning in 2010), and a 3,031-acre site adjacent to Franklin Creek (unburned for at least 50 years). These sites were considered burned, recovery, and unburned habitats, respectively. The burned site was 19.5 km south of the recovery and unburned sites, but was similar in topography, soil type, and dominant tree species (Close et al. 2002; Hromada et al. 2018; Wild and Gienger 2018).

Lizard capture and processing

Adult male Eastern Fence Lizards Sceloporus undulatus were located using haphazard visual encounter surveys from late April until mid-July 2016. To eliminate sex-related differences in stress physiology and body condition during the breeding season, only male lizards were captured and sampled (Carsia and John-Alder 2003). Females were not sampled because mass and energy expenditure potentially varies throughout the sampling period due to differences in state of reproduction (gravid vs. non-gravid), clutch size, and stage of egg development among individuals (Anigilletta et al. 2001). Lizards were captured using a handheld pole and noose at least 100 meters away from the boundary of each site to minimize the likelihood of lizard movement between habitat types and to mitigate the effect of other disturbances, such as road traffic. Immediately upon capture, body temperature was measured using a Schultheis cloacal thermometer (Miller and Weber Inc., NY) inserted 2 cm into the colon through the cloaca.

Lizards were randomly assigned to 1 of 2 treatments before capture: baseline blood draw or stress-induced blood draw. Lizards receiving a baseline blood draw had blood collected within approximately 3 min of capture, whereas lizards assigned to the stress-induced treatment had blood collected after 30 min of confinement within a cloth bag placed in the shade (Romero and Reed 2005). Analysis of blood samples collected within 3 min of capture has been considered a reliable indicator of chronic stress load across many taxa (Romero and Reed 2005). Confinement is used to experimentally induce a stress response, permitting the measurement of elevated CORT as a function of exposure to a standardized acute stressor (Romero and Wikelski 2002). We chose to induce 30 min of confinement handling stress because it has been sufficient to elevate CORT levels above baseline in S. undulatus (Graham et al. 2012), and in other lizards (Moore et al. 1991; Seddon and Klukowski 2012).

Blood samples were collected from the retro-orbital sinus using a 75 µL heparinized capillary tube (Phillips and Klukowski 2008; Langkilde 2010; Klukowski 2011; Graham et al. 2012). Blood samples were pipetted into microcentrifuge tubes and stored on ice in the field for up to 6 h. At a nearby field station (Hancock Biological Station, Murray, KY), blood samples were centrifuged at 6,000 rpm for 5 min, and plasma was decanted into uniquely labeled 1.5 mL
Habitat assessment
At the site of each lizard capture, a square 1-m² habitat frame was placed on the ground in the location where the lizard was first sighted (Daubenmire 1959). To assess the general structural aspects of the vegetative community, vegetation composition was split into 4 categories: herbaceous plants, live woody plants, coarse woody debris, and other. Percent of ground cover for each category was estimated visually within the 1-m² frame. Leaf litter depth was recorded at the 4 corners of the frame and averaged. Overhead canopy cover was measured with a spherical crown densiometer.

Hormone assay
CORT assays were conducted with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Enzo Life Sciences 900-097) that has been widely used to determine plasma CORT concentrations across multiple taxa (Hopkins and Durant 2011; Klukowski 2011; Rivers et al. 2014). The kit was tested through parallelism between a serial dilution of a pooled sample of lizard plasma and a serial dilution of standard stock solution. We determined that an optimal dilution of plasma was 1:30, which is congruent with other studies using small sample volumes (Phillips and Klukowski 2008; Klukowski 2011). Plasma aliquots of 10 μL were combined with 10 μL of steroid displacement reagent (SDR) and incubated for 10 min. Then, 280 μL of assay buffer were added to create a 30-fold dilution. Samples for each individual were run in duplicate and averaged. Six out of 70 blood samples contained less than 10 μL of plasma and were subsequently assayed without duplication. Instead, plasma volumes of 3.33 μL were run in singlet and kept at a 1:30 dilution with assay buffer and SDR. Excess reagent was washed away with a 5% p-nitrophenyl phosphate solution. The enzyme reaction was stopped after 60 min, and the light absorbance was read at 405 nm using a spectrophotometer (BioTek Synergy HT). The standard curve of a serial dilution of the stock solution was used to estimate the concentration of CORT in each plasma sample (Klukowski 2011). Calculations of intra- and inter-assay variation were 2.1% and 13.8%, respectively (Davies 2013).

Statistical analyses
Habitat structural and vegetative characteristics were compared among sites using a one-way analysis of variance (ANOVA) followed by Tukey-Kramer pairwise comparisons of means. As an index of each lizard’s body condition (BCI), we used a linear regression of log-transformed SVL by log-transformed body mass and the residuals from the best-fit line were used for comparisons (Jakob et al. 1996). One-way ANOVAs were used to compare baseline CORT and stress-induced CORT of lizards among habitats and to compare potential covariates influencing CORT secretion including body temperature, BCI, and time of blood collection. A General Linear Model (GLM) was used with habitat type, type of blood draw, body temperature, BCI, and time of blood collection as predictor variables and concentration of plasma CORT as the response variable. The GLM allowed us to determine how each predictor variable affected the secretion of CORT while statistically holding all other predictor variables within the model constant (Whitlock and Schluter 2015). Analyses were conducted using JMP Pro 12.

Results
Habitat structure
Canopy cover differed among habitat used by fence lizards in the different treatments (F_{2,71} = 11.00, P < 0.0001). Fence lizards used sites with more open canopy in the burned habitat than they did in either recovery or unburned habitat, but canopy cover did not differ between recovery or unburned treatments (Figure 1). Leaf litter depth was different among habitat treatments (F_{2,71} = 11.76, P < 0.0001) with less leaf litter in burned habitat than recovery or unburned (Figure 1). Herbaceous cover was different among habitat treatments with a higher percentage of herbaceous cover in burned habitats (F_{2,71} = 12.08, P < 0.0001) compared with either recovery or unburned (Figure 1). Percentage of woody stems and woody debris did not differ among habitat treatments (F_{2,71} = 2.72, P = 0.07; F_{2,71} = 1.34, P = 0.27, respectively), but percentage of other cover was different with unburned sites having more cover than burned or recovery.

Covariate/factor analysis
Across the 3 habitat types, 73 lizards were measured for body condition and body temperature. Body condition was similar among habitats (Figure 2), and the mean BCI (residual mass) for lizards in each habitat treatment only differed by about one-hundredth of a gram (F_{2,70} = 0.02, P = 0.98). Body temperatures were similar among habitat treatments (Figure 2), and mean temperatures differed among habitat treatments by at most two-tenths of a degree (F_{2,70} = 0.05, P = 0.95). Time of blood collection was not different among habitats (Figure 2; F_{2,65} = 1.14, P = 0.33).

CORT response
A total of 68 lizards were analyzed for plasma CORT concentration. Baseline CORT did not differ among treatments and was 6.96 (± 1.70) ng/mL for lizards in burned habitat, 3.35 (± 1.44) ng/mL for lizards in recovery habitat, and 8.07 (± 1.62) ng/mL for lizards in unburned habitat (F_{2,32} = 2.67, P = 0.08). Stress-induced CORT was also not different among treatments with 5.78 (± 2.03) ng/mL for lizards in burned habitat, 4.98 (± 1.87) ng/mL for lizards in recovery habitat, and 7.30 (± 2.25) ng/mL for lizards in unburned habitat (F_{2,30} = 0.32, P = 0.73). The plasma CORT concentration did not differ between lizards in the baseline treatment and lizards in the stress-induced treatment within any of the habitat types examined (Figure 3).

GLM analysis
In our experiment, there was no cumulative effect of habitat type, blood draw treatment, body temperature, body condition, and time of blood collection on plasma CORT levels (full GLM; F_{6,61} = 1.00, P = 0.43). Individual effects tests on habitat type (F_{2,68} = 1.97, P = 0.15), blood draw treatment (F_{1,68} = 0.0001, P = 0.99), body temperature (F_{1,68} = 0.24, P = 0.62), body condition (F_{1,68} = 1.22, P = 0.27), and time of blood collection (F_{1,68} = 0.0014, P = 0.97) indicate that none of the factors in the model significantly influenced
the plasma CORT concentration of Eastern Fence Lizards. Log-transforming the data did not change the significance of the factors within the model, therefore, we left CORT concentration untransformed for ease of interpretation.

Discussion

Although recently burned habitat differed from recovery and unburned habitats in several structural and vegetative characteristics, including reduced leaf litter, reduced vegetative canopy cover, and relative vegetative composition, there was no detectable difference among habitats in the CORT stress response in Eastern Fence Lizards. There are at least 3 potential explanations for this.

First, low intensity burns, such as the prescribed fire regimes used in this study site, may not alter habitat sufficiently to produce a detectable physiological stress response. Prescribed burns are implemented by resource managers to change forest structure by removing vegetative cover, increasing sunlight penetration, promoting growth of herbaceous vegetation, and decreasing the density of woody vegetation (Bowles and McBride 1998; Pavlovic et al. 2006; Lettow et al. 2014). Unlike prescribed fires, intense wildfires may

![Figure 1. Differences in use of habitat features by lizards in burned, recovery, and unburned habitats. Asterisks indicate group habitat characteristics with a significant pairwise difference from other habitats.](image-url)
produce much more pronounced alterations of habitat, usually scorching crown canopy, eliminating understory debris and woody vegetation, and creating patches of severely scorched soil (Whelan 1995). Thus, the response of lizards in our study to prescribed fire are potentially less pronounced than responses demonstrated by other lizards to the more drastic habitat alterations caused by wildfire (Lindenmayer et al. 2008; Pianka and Goodyear 2012).

Structural components of the environment, which provide sites for refuge, thermoregulation, and forage opportunities, are important resources as they can dictate the physiological performance of ectotherms (Huey 1991). Some major components of vegetative structure (i.e. woody debris and live woody plants) measured in the microhabitats lizards used, there may not have been a sufficient alteration in critical structural components of the environment to elicit a response (Wingfield and Romero 2001).

Second, lizards may use behavioral plasticity to cope with stress imposed by habitat alteration, preventing or suppressing a hormonal response (Korte et al. 2005). Many lizards change their behavior when encountering a stressor, including fleeing from invading non-native species (Langkilde 2010), reducing social interactions when infected by parasites (Schall and Sarni 1987), or altering refuge-use strategies when facing increased predation risk (Martin and Lopez 1999). The mean body temperatures of lizards were nearly identical among habitats, and were within the range (33–36°C) that allows S. undulatus to achieve maximum sprint performance and maximum energy assimilation efficiency (Angilletta 2001; Angilletta et al. 2002). Either lizards experienced similar thermal conditions among habitats or lizards were able to locate and utilize microsites within the altered habitats that allowed them to maintain a similar physiological condition as lizards in unaltered habitats. We think the latter is more likely, and Wild and Gienger (2018) showed that opportunities for lizard thermoregulation were markedly different among the 3 habitats. They found that lizards in the burned habitats could achieve body temperatures that permitted optimal locomotor performance for 9.3 h/day, whereas recovery and unburned habitats had more restricted thermal opportunities and had environmental temperatures that only permitted optimal performance for 7.0 and 6.7 h/day, respectively. The habitats clearly differ in thermal quality, but lizards achieve nearly identical mean body temperatures, likely as the result of differential microsite selection and behavioral thermoregulation.

Lastly, the CORT response of lizards may be largely suppressed during the breeding season as a result of increased testosterone (Klukowski 2011). The peak secretion of testosterone occurs during the breeding season (April-June), resulting in increased agonistic behaviors such as territorial defense and advertisement to conspecifics (Phillips and Klukowski 2008; John-Alder et al. 2009; Klukowski 2011). Although these displays may be energetically expensive, stress hormones are secreted at a significantly lower rate during the reproductive season than the post-reproductive season (Dunlap and Schall 1995). Thus we may expect a dampened stress response for the lizards measured here, primarily sampled during the reproductive season. Eastern Fence Lizards are short lived and have
limited opportunities to reproduce (Tinkle and Ballinger 1972; Haenel et al. 2003). Therefore, environmental stressors would need to be substantial enough to elevate baseline CORT beyond the stimulus threshold needed to inhibit reproductive activities during the breeding season (Zerani et al. 1991; Wingfield and Romero 2001; Moore and Jessop 2003; Wingfield and Sapolsky 2003). In S. occidentalis, individuals needed 240 min after capture to show a significant elevation in CORT during the breeding season, but only 60 min to show elevated CORT in the non-breeding season (Dunlap and Schall 1995). Natural selection may favor male lizards that do not suppress reproductive activities during periods of chronic stress, as has been shown in other species with short windows of opportunity to sexually reproduce (Wingfield et al. 1992; Romero et al. 2000; Boonstra et al. 2001; Wingfield and Sapolsky 2003). Ultimately, the physiological prioritization of reproduction during the breeding season may have masked any stress response induced by habitat disturbance due to prescribed fire.

Other measured factors (covariates) with the potential to affect levels of plasma CORT, including time of blood collection and body temperature, also did not differ among habitats and did not significantly influence plasma CORT. In fact, body temperature and body condition were strikingly similar across habitat types (Figure 1), indicating that lizards maintained similar physiological condition in different habitats. Differences in body condition would suggest that energy stores were used differently among lizards in the different treatments or that prey availability and acquisition was similar across habitats. In other reptile and bird species, body condition is routinely reduced during chronic stress (especially during the breeding season) to compensate for the putative increased energetic costs of coping with the stressor (Romero and Wikelski 2001; Carsia and John-Alder 2003; Velando and Alonso-Alvarez 2003; Madliger et al. 2015).

In all surveyed habitats, lizards did not demonstrate a significant hormonal response to handling confinement in a cloth bag (stress-induced treatment). The minimum time needed to significantly elevate CORT levels in Sceloporus using the typical cloth bag stress-induced treatment has not been determined. In some studies, Eastern Fence Lizards responded significantly to handling confinement lasting 30 min (Graham et al. 2012), whereas others required a minimum of 1 h (Dunlap 1995; Dunlap and Wingfield 1995). Many other species of reptiles and birds sampled during the breeding season quickly respond to handling stress, typically demonstrating elevated CORT after 30–60 min of handling time (Moore et al. 1991; Romero and Wingfield 1998; Schuett et al. 2004; Romero et al. 2006; Herr et al. 2017), and some species do not respond at all (Cree et al. 2000). Our results provide some evidence that 30 min of handling time may not have been sufficient to induce increased plasma CORT during the breeding season in male Eastern Fence Lizards. Our baseline plasma CORT values for S. undulatus (means across habitats range from 3.75–8.07 ng/mL) are similar to those of Graham et al. (2012) (means ~7–10 ng/mL), Klukowski (2011) (median = 1.7–6.8 ng/mL across breeding seasons) and John-Alder et al. (2009) (means ~ 4–17 ng/mL across months of the year).

Although a pronounced gradient in habitat quality was present among burned, recovery, and unburned habitats, Eastern Fence Lizards did not modify their glucocorticoid levels in response to habitat alteration. Baseline glucocorticoids may not be solely sufficient measures for determining stress responses to habitat disturbance (Madliger et al. 2015), at least for certain species, or during certain times of the year. Further research is needed to determine whether other ecological factors may induce a stress response, such as food availability, environmental temperatures, and population density. Quantifying behavioral responses in the field may also provide a better understanding of other ways in which Eastern Fence Lizards tolerate disturbance caused by habitat alteration.

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