Endocrine Stress Response of Eastern Fence Lizards to Fire-altered Landscapes

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Endocrine Stress Response of Eastern Fence Lizards to Fire-altered Landscapes

A Master's Thesis Presented to The College of Graduate Studies Austin Peay State University in Partial Fulfillment of the Requirements for the Degree Master in Biology

Mike Iacchetta

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To the College of Graduate Studies:

We are submitting a thesis written by Michael Iacchetta entitled "Endocrine stress response of Eastern Fence Lizards to fire-altered landscape." We have examined the final copy of this thesis for form and content. We recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Biology.

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ABSTRACT

MIKE IACCHETTA. Endocrine stress response of Eastern Fence Lizards to fire-altered landscapes (under the direction of DR. C. M. GIENGER)

Habitat alterations, such as fire, change the landscape's habitat structure and resource availability necessitating physiological responses to cope with the environmental change. Quantifying an endocrine stress response through measurement of glucocorticoids has become an increasingly common method for determining how organisms respond physiologically to challenges imposed by their environment. We tested the hypothesis that Eastern Fence lizards cope with fire effects on the environment by modulating their endocrine stress response. We measured the baseline and stress-induced plasma corticosterone (CORT) concentration of male Eastern Fence Lizards in a chronosequence of fire-altered habitats. Although vegetative canopy cover, leaf litter depth, and vegetation composition differed among habitat types (recently burned, recovering, and unburned), there was not a significant effect of habitat type on plasma CORT concentration or on body condition. Using a general linear model, we found no cumulative effect of habitat type, blood draw treatment (baseline or stress-induced), body temperature, body condition, or the time taken to collect blood samples on concentration of plasma CORT. Also, no individual factor in the model was a significant predictor of plasma CORT concentration while statistically controlling for other factors in the model. Low intensity burns, typical of prescribed fire, may not produce a challenging stressor necessary to elicit adjustments to the endocrine stress response in Eastern Fence Lizards. Instead, lizards may respond behaviorally to avoid prolonged periods of allostatic

overload by locating appropriate microsites within their environment that allow individuals to maintain longer periods of optimal performance.

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Introduction

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 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 that are secreted to initiate gluconeogenesis. Gluconeogenesis produces energy in the form of glucose (McEwen and Wingfield 2003, Romero 2004). Exposure to unpredictable environmental challenges stimulates increased secretion of glucocorticoids and breakdown of non-carbohydrate molecules to produce the energy required to respond and overcome stressful situations (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 is the major glucocorticoid secreted by reptiles to stabilize metabolic processes during stressful events (Greenberg and Wingfield 1987). The baseline concentration of corticosterone is that which is secreted in the absence of unpredictable environmental stressors (Romero 2004) and describes the typical stress load experienced by individuals during routine physiological function (Bonier et al. 2009). When an unpredictable stressor is imposed on an animal, the secretion of glucocorticoids increases to re-allocate energy stores to bodily function necessary to escape the stressful event. The stress-induced concentration of corticosterone is the concentration resulting from a response to unpredictable stressors imposed on an individual (i.e. prolonged handling of the animal), which measures acute stress or sensitivity to novel external stressors that are potentially life-threatening (Cash et al. 1997). The magnitude of the physiological response to environmental stressors can be determined by comparing baseline and stress-induced corticosterone 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 imposed on an animal will decrease the body condition, a measure of stored energy in fat reserves, through continual conversion of stored energy reserves into usable energy (Fokidis et al. 2011). Species may adapt to prolonged increases in glucocorticoid concentration caused by allostatic overload by suppressing immediately non-essential functions, such as reproductive activity and immune response, in the short-term, thereby increasing the likelihood of survival (McEwen and Wingfield 2003). Under chronically stressful situations, reproduction may be forgone completely in order to re-allocate essential nutrients and materials to functions proximally necessary for survival (Hews and Abell Baniki 2013), and a suppressed immune system can leave an individual more vulnerable to infectious diseases and parasitism (French et al. 2008, McCormick and Langkilde 2014). However, energy that is re-allocated to other functions of the body allows individuals to cope with stressors, such as predator-prey interactions or harsh environmental conditions (Lima 1998, Drake et al. 2012). If exposed to chronic stressors, individuals can potentially endure decreased health and reduced reproductive success (Bonier et al. 2009).

Habitat alteration is an important environmental stressor that can increase the allostatic load of an individual (McEwen and Wingfield 2003) and has been associated with declines in populations of many reptile species (Gibbons et al. 2000). Some habitat alterations, such as logging, vegetation removal, and fire temporarily change the landscape's suitability for certain species, necessitating physiological responses to cope with the environmental change and to increase survival. Fire modifies habitat structure by converting intact vegetative communities into an earlier stage of ecological succession by reducing leaf litter, removing canopy cover, and increasing mineralization of soil (DeBano et al. 1998). These habitat alterations can change the availability of vital resources including, prey distribution, refuge and nesting sites, and thermoregulatory opportunities (Russell et al. 1999). Since resource availability may potentially change after fire-borne disturbance, the amount of energy needed to obtain those resources may also change, which will in turn affect the level of corticosterone required to maintain an allostatic state.

The goal of this project was to determine whether a stress response in the corticosterone pathway is elicited by reptiles experiencing habitat alterations created by prescribed fire. The Eastern Fence Lizard is a species found throughout the Southeastern United States, commonly inhabiting areas where prescribed burning is implemented to restore imperiled disturbance-dependent habitat types, 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 on the environment by modulating their endocrine stress levels. More specifically, we sought to determine whether fire indirectly affects allostatic load, sensitivity to acute stressors, and body condition of lizards by manipulating microhabitat structure and requiring increased energy expenditure to obtain vital resources. We predicted that baseline and stress-induced corticosterone 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 microhabitats caused by fire. Since corticosterone secretion was assumed to increase in lizards recently affected by fire, we predicted that body condition would be lower in lizards recently affected by fire because changes to resource availability may require an individual to adapt by increasing energy expenditure necessary to obtain vital resources.

Methods

Study Site

Land Between the Lakes National Recreation Area (LBL) encompasses over 170,000 acres between Lake Barkley and Kentucky Lake (Tennessee and Kentucky). Wildfires were once a natural presence in the area, but fires have been suppressed since 1964 when the site was designated a National Recreation Area (Franklin 1994, O'Neill and Doyle 2002, Wright et al. 2002). Prescribed fire has been implemented at LBL as a land management practice with the intent to restore native oak-grassland habitat and encourage recreational activities by reducing the stand density of trees and fuel load on the forest floor (Jacobs and Lisowsky 2004). Prescribed fires are conducted periodically within designated experimental units throughout the recreation area.

Three sites with differing burn histories were sampled in the spring and summer of 2016. A 1,326-acre prescribed burn site at the South Bison Range in Stewart County, TN (burned March 2016), a 2,461-acre burn site at the Franklin Creek site in Trigg County, KY that has had seven years to recover from the most recent prescribed fire (burned 2010), and a 3,031-acre site adjacent to Franklin Creek (unburned for at least 50 years) were sampled. 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 the burned habitat was similar to the recovery and unburned habitats in dominant tree species, elevation, and soil type (Close et al. 2002).

Lizard Capture and Processing

Adult male *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 the mass and energy expenditure of each individual varied throughout the sampling period due to differences in clutch size, state of pregnancy (gravid vs. non-gravid), and stages of egg development among individuals . Lizards were captured using a handheld pole and noose at least 100 meters away from the boundary of each site to minimize the probability of movement between habitat types by lizards 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 & Weber Inc., NY) inserted 2 cm into the colon through the cloaca.

Lizards were randomly assigned to one of two treatments before capture: baseline blood draw or stress-induced blood draw. Lizards receiving a baseline blood draw had blood collected within three minutes of capture, whereas lizards assigned to the stress-induced treatment had blood collected after 30 minutes of confinement within a cloth bag (Romero and Reed 2005). Analysis of blood samples collected within three minutes of capture has been shown to be a reliable indicator of chronic stress load across multiple taxa (Romero and Reed 2005). Handling lizards within a cloth bag is used to experimentally induce a stress response permitting the measurement of elevated corticosterone concentration in lizards exposed to a common acute stressor (Romero and Wikelski 2002). We chose to induce 30 minutes of confinement handling stress because it has been shown to elevate corticosterone levels above baseline in other lizards (Moore et al. 1991, Seddon and Klukowski 2012) and more time was needed to capture lizards to increase our sample size.

Blood samples were collected from the retro-orbital sinus using a 75μL 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 six hours. At the field station (Hancock Biological Station), blood samples were centrifuged at 6,000 rpm for five minutes, and plasma was decanted into uniquely labeled 1.5 mL microcentrifuge tubes. Samples were initially stored at -20°C at the field station for 1-10 days until they could be transferred for long-term storage in the lab at -80°C (Austin Peay State University).

The time of capture and the total handling time to acquire a blood sample were recorded for each individual. Measurement of total handling time began as soon as the lizard was restrained in the noose and stopped after measuring body temperature and completing the blood draw. Snout-vent length (SVL), tail length, and body mass were measured for each lizard in the field after blood collection. Each lizard was toe clipped for unique identification, and recaptured individuals were not used for analyses.

Habitat Assessment

At the site of each lizard capture, a square 1 m^2 habitat frame was centered on the ground over the location where each lizard was first sighted (Daubenmire 1959). To determine the vegetative canopy cover of a location, measurements were taken with a spherical densiometer in each of the four cardinal directions and averaged. Leaf litter depth was recorded at the four corners of the frame and averaged. To assess the general structural aspects of the vegetative community, vegetation composition was split into four categories: herbaceous plants, live woody plants, course woody debris, and other cover.

Percent coverage of ground vegetation within each category was estimated visually within the 1 m^2 frame.

Hormone Assay

Corticosterone (CORT) assays were conducted using an ELISA kit (Enzo Life Sciences 900-097), which has been previously used to determine CORT concentrations across multiple taxa (Klukowski 2011, Hopkins and Durant 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). The minimum detectable concentration of plasma CORT for the assay was 27 pg/mL.

Plasma aliquots of 10 μL were combined with 10 μL of steroid displacement reagent (SDR) and incubated for 10 minutes. Then, 280 μL of assay buffer were added to create a 30-fold dilution. Samples for each individual were run in duplicate. 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 minutes and the light absorbance was read at 405 nm using spectrophotometry. The color (absorbance of light) of the solution in each well signifies the competitive binding of endogenous steroid and alkaline phosphatase steroid for sheep antibody binding sites. 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 among sites were compared using a one-way ANOVA followed by post-hoc Tukey Kramer pairwise comparison of means. A linear regression of log-transformed SVL by log-transformed body mass was performed and the residuals from the best-fit line were used to create an index that estimates each lizard's body condition (BCI) (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 across habitat treatments including body temperature, BCI, and time of blood collection. A GLM was used with habitat type, type of blood draw, body temperature, body condition, and time of blood collection as predictor variables and concentration of plasma corticosterone as the response variable. The GLM allowed us to determine how each predictor variable affects the secretion of corticosterone while statistically holding all other predictor variables within the model constant (Whitlock and Schluter 2015). All analyses were conducted using JMP Pro 10 software.

Results

Habitat Structure

Vegetative canopy cover differed among sites (Table 1) with cover being 14-24% lower at sites in burned habitat (60.3 ± 3.7%) than those in recovering habitat (73.9 ± 3.3%) and unburned habitat (84.2 ± 3.5%). Canopy cover did not significantly differ between sites in recovering habitat and unburned habitat ($p = 0.09$). Leaf litter depth was different among sites (Table 1), and mean litter depth was ten-fold lower at sites in burned habitat (2.4 ± 3.7 mm) than sites in recovering habitat (22.4 ± 3.3 mm) and unburned habitat (25.5 ± 3.6 mm). There was no difference in the leaf litter depth of sites in recovering and unburned habitats ($p = 0.80$). Herbaceous cover was 13-18% higher in recently burned habitat than in recovery and unburned habitats. Although the percentage of live woody plant cover was 11-17% higher in recovery habitat than the other two habitats, the amount of live woody plants in locations did not significantly differ among habitat treatments. Coverage by course woody debris did not differ among habitat treatments with mean coverage between 22% and 33% (Table 1). Other cover primarily consisted of leaf litter, rock, and bare ground. The percent coverage of other cover was different among habitats (Table 1). Post-hoc Tukey Kramer results indicate that the mean of other coverage differed between the burned habitat (22.8 ± 4.9%) and the unburned habitat (41.3 ± 4.7%). The recovery habitat (30.1 ± 4.3%) did not differ from the burned and unburned habitats.

Covariate/Factor Analysis

Across the three habitat types, 73 lizards were measured for body condition and body temperature (Table 2). Body condition was similar among habitats (Figure 2), and the mean BCI (residual mass) for lizards in each habitat treatment differed by less than one one-hundredth of a gram. Body temperatures were similar among habitat treatments (Figure 2), and mean temperatures differed among habitat treatments by at most two tenths of a degree. Time of blood collection was similar among habitats (Figure 2), showing the duration of each blood draw was consistent throughout the experiment.

Corticosterone Response

A total of 68 plasma samples of unique individual lizards were analyzed for plasma corticosterone concentration. Mean plasma corticosterone concentration for baseline samples was 6,963 (± 1,700) pg/mL for lizards in burned habitat, 3,347 (± 1,437) pg/mL for lizards in recovery habitat, and 8,071 (± 1,621) pg/mL for lizards in unburned habitat ($F_{2,32} = 2.67$, $p = 0.08$). Mean corticosterone concentration for stress-induced samples are 5,781 (± 2,032) pg/mL for lizards in burned habitat, 4,984 (± 1,870) pg/mL for lizards in recovery habitat, and 7,303 (± 2,247) pg/mL for lizards in unburned habitat ($F_{2,30} = 0.32$, $p = 0.73$). The plasma corticosterone concentration did not significantly differ between lizards in the baseline treatment and lizards in the stress-induced treatment within any of the habitat types examined (Figure 1).

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 ($F_{6,61} = 1.00$, $p = 0.43$). Individual effects tests on habitat type ($F_2 = 1.97$, $p = 0.15$), blood draw treatment ($F_1 = 0.0001$, $p = 0.99$), body temperature ($F_1 = 0.24$, $p = 0.62$), body condition ($F_1 = 1.22$, $p = 0.27$), and time of blood collection ($F_1 = 0.0014$, $p = 0.97$) show that none of the factors in the model significantly influenced the plasma corticosterone concentration of Eastern Fence Lizards. Log-transforming the data to normalize the distribution did not change the significance of the factors within the model; therefore, we left corticosterone concentration untransformed for ease of interpretation. No single factor in the model was a good predictor of a change in corticosterone concentration while controlling for all other factors within the model.

Discussion

Although recently burned habitat differed from recovery and unburned habitats in structural and vegetative characteristics, there was no detectable difference in the plasma corticosterone stress response in Eastern Fence Lizards among habitat with different burn histories. The prescribed burn reduced leaf litter depth and vegetative canopy cover, but these alterations did not result in altered chronic stress of lizards, as measured by baseline corticosterone. Prescribed burns are implemented to create vegetative changes to the understory by allowing higher light levels to reach the forest floor, 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 produce drastic 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 the responses demonstrated by other lizards to the drastic vegetative alterations caused by wildfire (Lindenmayer et al. 2008, Pianka and Goodyear 2012).

Body condition of lizards did not differ among habitats, suggesting that energy stores were not used differently among lizards in the different treatments or that prey availability was similar across habitats. Other measured covariates with potential to affect the level of plasma corticosterone, time of blood collection and body temperature, did not differ among habitats and did not show a relationship with plasma corticosterone with all other variables held constant. In fact, body temperature and body condition were strikingly similar across habitat types (Table 2), indicating that lizards maintained similar physiological condition in different habitats. These results are contrary to those observed in some reptile and bird species responses to habitat alteration in which body condition was reduced during chronically stressful situations within the breeding season to compensate for the increased energetic cost incurred to cope with environmental stress (Romero and Wikelski 2001, Carsia and John-Alder 2003, Velando and Alonso-Alvarez 2003, Madliger et al. 2015).

There are at least three potential explanations for the lack of a physiological stress response exhibited by Eastern Fence Lizards to landscape alterations caused by fire. First, low intensity burns, such as prescribed fire regimes used in this study site, may not produce a detectable behavioral or physiological stress response in Eastern Fence Lizards. Organisms respond to unpredictable or severe habitat disturbances and a limitation in resources by triggering the hypothalamus-pituitary-adrenal (HPA) axis to increase the secretion of glucocorticoids (Romero and Wikelski 2001, Newcomb Homan et al. 2003, Madliger et al. 2015, Meillère et al. 2015). The perception of noxious stimuli is necessary to induce a stress response (Romero 2004). Reptiles are ectothermic and their physiological processes are closely tied to their thermal environment. Structural components of the environment, which create sites for refuge, thermoregulation, and forage opportunities, can be considered vital resources because they can dictate the physiological performance of ectothermic species. Some major components of vegetative structure (i.e. woody debris and live woody plants) in microhabitats where lizards were encountered did not differ among habitat types, even though landscape features of the habitats were quite different structurally. Since the relative abundance of woody debris and live woody vegetation in lizard locations were not different among habitat treatments and composed a large proportion of the microhabitat structure lizards used, there may not have been a sufficient reduction in critical structural components of the environment necessary to elicit a stress response (Wingfield and Romero 2001). Lizards achieved similar body condition and body temperature in control and altered habitats, indicating that either lizards experienced similar conditions in those habitats or were able to locate microsites within altered habitats that allowed them to maintain similar physiological condition as those found within unaltered habitats. Since body condition was nearly identical among habitats, lizards could potentially find localities with similar food availability within their environment, reducing the need to mobilize energy reserves. Fire can increase the visibility of prey items by removing understory vegetation (Griffiths and Christian 1996), potentially increasing the detectability and acquisition of food resources, which may allow lizards to better cope with chronic stressors (Kitaysky et al. 1999). Ultimately, the prescribed fire in the burned habitat may not have altered critical vegetative structure to a level necessary to stimulate a stress response.

Second, lizards may use behaviorally plasticity to cope with stress imposed by habitat alteration, suppressing a hormonal response (Korte et al. 2005). Many lizard species can change their behavior when individuals encounter a stressor, including fleeing from non-native invading species sooner (Langkilde 2010), reducing strenuous social behaviors within changing landscapes (Schall and Sarni 1987), optimizing refuge-use strategies in situations where predation risk increases (Martin and Lopez 1999), or changing color after aggressive interactions with conspecifics (Greenberg and Crews 1990). The mean body temperatures of lizards were nearly identical among habitats and were within the range of body temperatures (33-36°C) that allows *Sceloporus undulatus* to achieve maximum sprint performance and maximum assimilation efficiency (Angilletta 2001, Angilletta et al. 2002). Some *Sceloporus* lizards alter the intensity of basking behavior at different elevations (Adolph 1990). By changing the duration of exposure to solar radiation, lizards can maintain their preferred body temperature longer, yielding longer periods of optimal performance. Maintaining optimal body temperatures in an altered landscape could be accomplished by changing how microhabitats are used. A previous study at our field site showed that North American Racer (*Coluber constrictor*) in burned sites were more active and more frequently located on the ground compared to unburned sites where snakes were found in more arboreal habitats (Howey et al. 2016). Eastern Fence Lizards are primarily arboreal and may maintain optimal body temperatures by altering the height of selected perches used for basking as the closely related Western Fence Lizard (*Sceloporus occidentalis*) does among different latitudes throughout its range (Adolph 1990). Vegetation removal and soil erosion resulting from the construction and presence of a road along coastal Argentina cause *Liolaemus* spp. to modify their use of vegetation patches that provide thermal refugia, protection, and food (Vega et al. 2000). Altering basking intensity or altering microhabitat use for basking may help lizards cope with the environmental stress caused by fire disturbance.

Lastly, the corticosterone response of lizards may be largely suppressed during the breeding season as a result of increased output of testosterone. 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, 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). Eastern Fence Lizards are short lived and have limited lifetime opportunities to reproduce (Tinkle and Ballinger 1972, Haenel et al. 2003). Therefore, stressors would need to be substantial enough to elevate baseline corticosterone levels beyond the stimulus threshold needed to inhibit reproductive activities during the breeding season, as shown in other species with a short window of opportunity to successfully reproduce (Zerani et al. 1991, Wingfield and Romero 2001, Moore and Jessop 2003, Wingfield and Sapolsky 2003). In *S. occidentalis*, individuals needed 240 minutes after capture to show a significant elevation in corticosterone during the breeding season, but only 60 minutes to show elevated corticosterone during the non-breeding season (Dunlap and Schall 1995). If chronic stress inhibits normal reproductive behavior and physiology, the individual risks the possibility of not having another opportunity to produce offspring (Moore and Jessop 2003). Natural selection may favor males that do not suppress reproductive activities during periods of chronic stress, as 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). The physiological prioritization of reproduction during the breeding season may have masked the stress caused by the prescribed fire.

In all surveyed habitats, lizards did not demonstrate a significant hormonal response to handling confinement in a cloth bag. The minimum duration of time needed to increase the CORT levels in *Scelopours undulatus* when under the cloth bag handling treatment has not been determined. In some studies, Eastern Fence Lizards responded significantly to handling confinement lasting a minimum of one hour (Dunlap 1995, Dunlap and Wingfield 1995, Carey Webb 2014). Other studies have determined that baseline CORT levels are maintained after ten minutes of handling confinement (Tylan et al. 2016). Many other species of reptiles and birds sampled during the breeding season respond quickly to handling stress, which had elevated corticosterone after 30 minutes to one hour of handling time (Moore et al. 1991, Romero and Wingfield 1998, Schuett et al. 2004, Romero et al. 2006, Herr et al. 2017). Our results provide some evidence that thirty minutes of handling time may not have been sufficient to induce increased plasma corticosterone during the breeding season in male Eastern Fence Lizards. Handling stress treatments on Eastern Fence Lizards may need to be a minimum of one hour long in duration to produce an acute response to the cloth bag treatment.

We provide evidence to reject the hypothesis that corticosterone concentration is modulated in Eastern Fence Lizards when low intensity fires alter the landscape. 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. In congruence with other studies, baseline glucocorticoids may not be solely sufficient measures for determining stress responses to habitat alterations (Madliger et al. 2015), at least for certain species. 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 allow better understanding of other ways in which Eastern Fence Lizards tolerate disturbance caused by habitat alteration.

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Table 1: Mean and standard error of habitat characteristics among different habitat treatments. Variables with statistically significant differences and their respective p-values are in bold type. Post-hoc Tukey Kramer pairwise comparisons are superscript next to the mean values. Different letters between mean values indicates a significant difference between the mean values.

	Burned Habitat (n = 22)		*Recovery Habitat (n = 28)*		*Unburned Habitat (n = 24)*			
Variable	*Mean*	*SE*	*Mean*	*SE*	*Mean*	*SE*	*ANOVA results*	*p-value*
Canopy Cover (%)	60.3^{A}	3.7	73.9^{B}	3.3	84.2^{B}	3.5	$\mathbf{F_{2,71} = 11.00}$	**<0.0001**
Leaf Litter Depth (mm)	2.4^{A}	3.7	22.4^{B}	3.3	25.5^{B}	3.6	$\mathbf{F_{2,71} = 11.76}$	**<0.0001**
Herbaceous Cover (%)	22.0^{A}	2.8	8.4^{B}	2.5	3.3^{B}	2.7	$\mathbf{F_{2,71} = 12.08}$	**<0.0001**
Woody Stem (%)	28.7^{A}	5.7	40.1^{A}	5.1	23.1^{A}	5.5	$F_{2,71} = 2.72$	0.07
Woody Debris (%)	26.4^{A}	5.1	21.5^{A}	4.5	32.3^{A}	4.9	$F_{2,71} = 1.34$	0.27
Other (%)	22.8^{A}	4.9	30.1^{A}	4.3	41.3^{B}	4.7	$\mathbf{F_{2,71} = 3.81}$	**0.03**

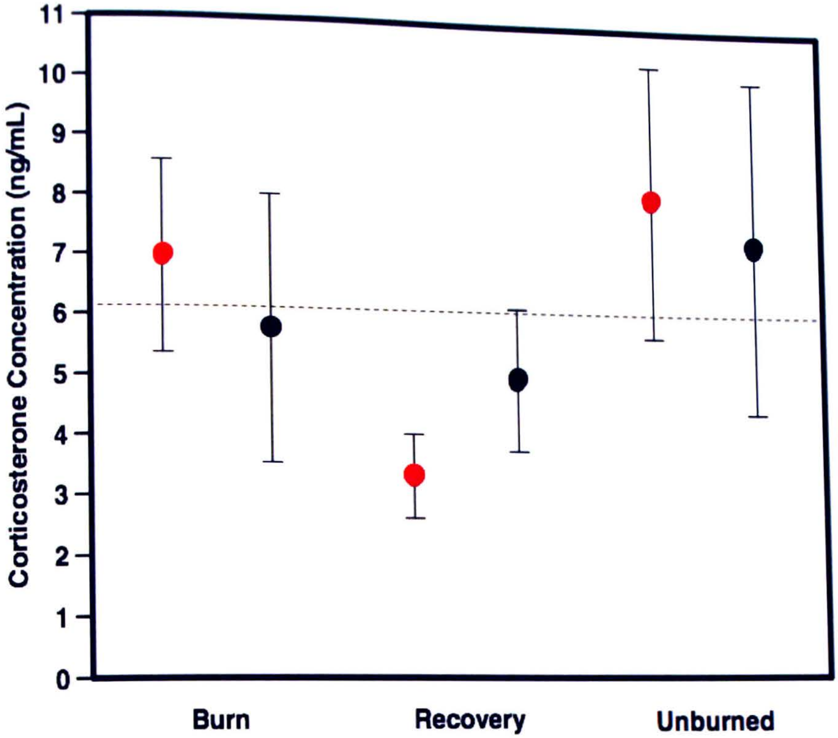

Figure 1: The dot plot compares the plasma corticosterone concentration (ng/mL) between baseline plasma samples and stress-induced plasma samples. The error bars encapsulate one standard error from the mean. Black dots represent the mean baseline CORT concentration. Red dots represent the mean stress-induced CORT concentration. The dotted line represents the grand mean of all lizards in each treatment.

Table 2: Mean and standard error for body temperature, body condition, and bleed time of *S. undulatus* among different habitat treatments. Also, the F-statistic and p-value of each one-way ANOVA are presented.

	Burned Habitat			*Recovery Habitat*			*Unburned Habitat*			*ANOVA*	
Variable	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*n*	*results*	*p-value*
Body Temp. (°C)	34.18	0.48	22	34.38	0.42	28	34.22	0.47	24	$F_{2,70} = 0.05$	0.95
BCI (g)	-0.0004	0.02	22	-0.0024	0.02	28	0.0032	0.02	24	$F_{2,70} = 0.018$	0.98
Time of Blood Collection (sec)	172.91	13.81	21	199.70	12.18	27	181.15	14.15	20	$F_{2,65} = 1.142$	0.33

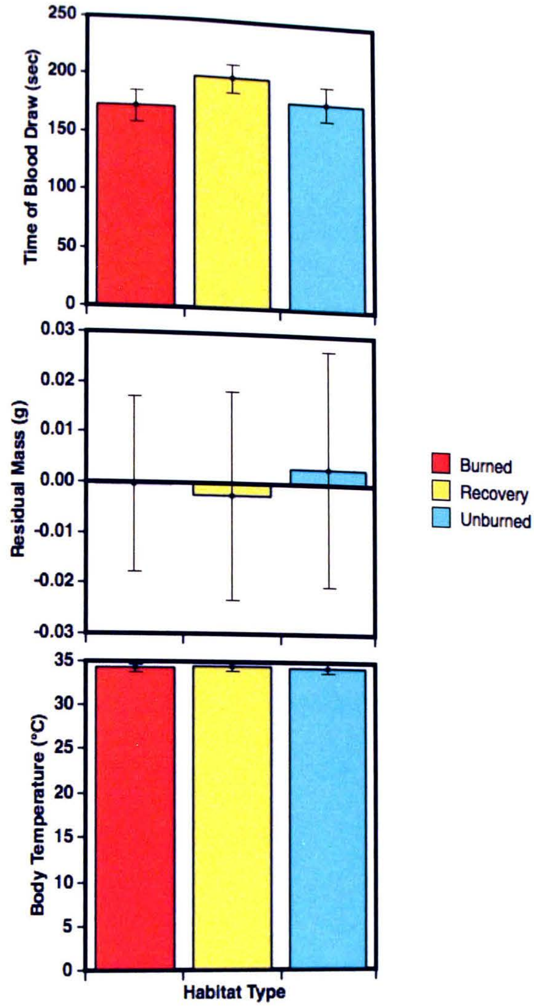

Figure 2: The bar chart shows mean bleed time, residual mass, and body temperature among habitat types. Error bars are one standard error from the mean. For lizards given the baseline blood draw treatment, bleed time was recorded as the amount of time elapsed between capture of the lizard to the termination of blood extraction. For lizards given the stress-induced blood draw treatment, bleed time was recorded as the amount of time elapsed between removal from the cloth bag to the termination of blood extraction. The residual mass is drawn from the best-fit line of the regression of SVL and mass.