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THE EFFECT OF CORTICOSTERONE ON BEHAVIOR IN *SCELOPORUS UNDULATUS*

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Abstract:

Levels of the stress hormone, corticosterone (CORT) in the blood can be a great indicator of stress levels in lizards. This approach reveals that invasive species impose novel pressure on natives, elevating stress levels. Native species often behaviorally adapt to these pressures in order to increase their chances of surviving exposure to these non-native threats. We tested the hypothesis that eastern fence lizards (*Sceloporus undulatus*) that have been exposed to predatory invasive fire ants (*Solenopsis invicta*) for several generations exhibit different behavioral responses to experimentally elevated CORT than do lizards whose habitat has not yet been invaded. Lizards from the uninvaded site respond to elevated CORT levels by hiding more and moving and basking less, whereas those from the invaded site exhibit the opposite pattern; hiding less, moving more, and spend more time on the basking log when CORT levels are elevated. These differences in response may facilitate survival in these different environments. Within natural uninvaded populations, lizards would benefit from becoming less active and hiding in response to a stressful event, as this would reduce their vulnerability to predators and conspecifics. In contrast, within fire ant invaded sites, increased CORT levels occur following encounters with fire ants. Responding to elevated CORT levels by moving away and off the ground would increase the lizards' chances of surviving these encounters in the presence of this invader. The changes in basking behavior following elevated CORT levels appears to be due to changes in the propensity of lizards escaping up off the ground rather than having any thermoregulatory relevance. A second study revealed that lizards body temperatures were not affected by experimentally elevated

CORT levels, and this response did not differ between fire ant invaded and uninvaded sites. Together, these reveal that the behavioral response to physiological stress can be altered by the introduction of non-native species, allowing native species to persist in the face of this novel threat.

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General Introduction

Anthropogenic activities are increasingly disturbing natural environments. Perturbations, including habitat loss, increased tourism and pollution, and the introduction of non-native species, imposes novel selective pressures on natural communities. Individuals respond to threats by producing glucocorticoid stress hormones, which facilitate their fight or flight response (Sapolsky et al. 2000) and help enhance short-term survival by redirecting behavior and energy usage (Wingfield et al. 1998). Animals secret these steroid hormones when the hypothalamic-pituitary-adrenal (HPA) axis is activated by a stressor (Sapolsky et al. 2000). Corticosterone (CORT) is the major glucocorticoid in reptiles, amphibians, birds, and numerous rodents (Romero 2004). Circulating levels of CORT in the blood can provide a useful index of stress and can be used to monitor population health (Kort et al. 2005; Creel et al. 2002).

The physiological stress response evolved in order to increase survival (Norris et al. 1999; Hontela 1998). Stress hormones protect the body during and after stressors such as food shortage, negative social interactions, predator encounters, injury, and disease (Broom and Johnson 1993; Sapolsky 1992; Palumbi 2001). One important role of stress hormones is to allow animals to adaptively respond to a stressor by mobilizing energy to mount the appropriate behavioral response (Sapolsky 1992; Summers et al. 2000; Stratakis and Chrousos 1995). Therefore, we may expect that the behavioral response of individuals to elevated CORT levels should be under selection and evolve to increase an individual's survival under particular situations. This response will allow organisms to

cope and survive acute and chronic stressors, including the novel threats imposed by non-native species.

Fire ants (*Solenopsis invicta*) are an ecologically important, globally invasive species that co-occurs with (Porter and Tschinkel 1987; Trauth et al. 2004; Tschinkel 2006), and frequently attacks, native fence lizards (*Sceloporus undulatus*, Langkilde 2009a). Lizards that encounter fire ant attack exhibit elevated CORT levels (Langkilde and Freidenfelds unpubl. data). Like other anthropogenically-imposed perturbations (Romero and Wikelski 2001; Tarlow and Blumstein 2007; French et al. 2008), fire ants cause elevated physiological stress (CORT) levels within invaded populations (Langkilde and Freidenfelds, unpubl. data). Understanding the consequences stress has on the behavior of native species and how the nature of this response evolves following the introduction of a novel threat is critical for managing and predicting invaders and provides important insight into the evolution of the complex relationship between behavioral and physiological threat responses.

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The effect of corticosterone on behavior in *Sceloporus undulatus*

Abstract

Animals respond to stressors by producing glucocorticoid stress hormones, such as corticosterone (CORT). This physiological stress response initiates the fight or flight response, facilitating the individuals survival in the face of a threat. To be adaptive, the nature of an animal's behavioral response to elevated CORT levels should be matched to the particular threats they face. As a result, an animal's behavioral threat response may differ in different populations. The novel pressures imposed by invasive species may select for a shift in the behavioral response of native species to elevated CORT levels. I tested whether population level exposure to the novel predatory threat imposed by the invasive fire ant (*Solenopsis invicta*) affects the behavioral response of the native eastern fence lizard (*Sceloporus undulatus*) to elevated CORT levels. Lizards from the uninvaded site that had their CORT levels experimentally elevated hid more, and moved and basked less, whereas those from the fire ant invaded site showed the opposite response; hiding less, and moving and basking more when their CORT levels were elevated.. These ants are small and forage primarily at ground levels, so moving away from stressful encounters and up off the ground (onto a basking log) may prove to be a more effective escape response within invaded sites than hiding, which likely provides a survival advantage for lizards faced with bird and snake predators within natural populations. This study suggests that invasive species may shape the relationship between the physiological and behavioral threat response of native species.

Introduction

When individuals are threated, they produce glucocorticoid stress hormones, which facilitate their fight or flight response (Sapolsky et al. 2000) and help enhance short-term survival by redirecting behavior and energy usage (Wingfield et al. 1998). Corticosterone (CORT) is the major glucocorticoid in reptiles, amphibians, birds, and many rodents (Romero 2004). Animals secret these steroid hormones when the hypothalamic-pituitary-adrenal (HPA) axis is activated by a stressor (Sapolsky et al. 2000). Therefore, circulating glucocorticoid levels in the blood can provide a useful index of stress, which can be useful in monitoring population health (Kort et al. 2005; Creel et al. 2002).

The physiological stress response evolved in order to increase survival (Norris et al. 1999; Hontela 1998). Stress hormones protect the body during and after stressors such as food shortage, negative social interactions, predator encounters, injury, and disease (Broom and Johnson 1993; Sapolsky 1992; Palumbi 2001). An important role of stress hormones is to allow animals to adaptively respond to a stressor by mobilizing energy to mount the appropriate behavioral response (Sapolsky 1992; Summers et al. 2000; Stratakis and Chrousos 1995). Energy is made available through glucogenesis, glycolysis, and lypolysis. Stress can cause energy to be redirected from certain functions causing metabolic shifts, appetite suppression, and impaired nutrient uptake (Davis et al. 1985; Barton et al. 1987) in addition to helping maintain homeostasis (Stratakis and Chrousos 1995). As a result, elevated glucocorticoid levels can allow animals to alter

 their locomotor activity, foraging, and dispersal to avoid the threat (Holmes et al. 1976; de Fraipont et al. 2000). This response can be adaptive, increasing an individual's chance of surviving these encounters.

Anthropogenic activities are increasingly disturbing natural environments. Perturbations, including habitat loss, increased tourism and pollution, and the introduction of non-native species, impose novel selective pressures on natural communities. The specific nature of the behavioral response generated by elevated levels of stress hormone may vary depending upon the environment the organism is in and the nature of the threat. For example, remaining hidden in a shelter following an encounter with a predator may increase an individual's chances of surviving if that predator is unable to enter the shelter, but may be maladaptive following the introduction of a novel predator that is able to pursue the animal into this refuge. Therefore, we may expect that populations exposed to novel threats may alter their behavioral response to elevated stress levels. I examined how the behavioral response of native fence lizards (*Sceloporus undulatus*) to elevated physiological stress levels changes following the invasion of the red imported fire ant (*Solenopsis invicta*).

The red imported fire ant originated in South America and has been introduced in over seven countries. This is a globally important invader, predicted to eventually colonize over 50% of the terrestrial surface of the earth (Morrison et al. 2004). Fire ants were introduced to the United States in the 1930s and their subsequent spread over the country has been well documented (Buren et al. 1974). Fire ants are generalist omnivores that forage widely for available food and attack animals in defense of their mounds (Barr et al. 1994; Holtcamp et al. 1997). They are able to forage high into tree canopies, but

forage much more effectively at ground level (Tedders et al. 1990, Langkilde 2009b).

They are thought to pose a major ecological threat to native communities. They often

become the most dominant ant in the area they invade (Wojcik et al. 2001; Allen et al.

2004; Allen 1997; Holway et al. 2002; Suarez 2005; Langkilde 2009a) and attack native

species, such as neonatal American alligators (Allen et al. 2004), gopher tortoises

(Landers et al. 1980), three-toed box turtles (Montgomery 1996), bobwhite quails (Allen

1997), and fence lizards (Langkilde 2009a).

Red imported fire ants occur at high densities within their invaded range (up to

179 mounds/ha within the sites used in this study, Langkilde 2009a). They share habitat

with fence lizards (Langkilde 2009b), and readily attack lizards they encounter in the

field, recruiting rapidly to these attacks in high numbers (Langkilde 2009a). Fence

lizards' tough epidermis provides little protection, as the fire ants pry up lizards' scales

and insert their stingers into the soft underlying skin (Langkilde 2009a). Fire ant venom

acts on the neuromuscular system (Blum et al. 1958; Allen et al. 2004) and as few as 12

fire ants can kill an adult lizard within a minute (Langkilde 2009a). Attack by fire ants

causes elevated CORT levels in *S. undulatus* (Langkilde and Freidenfelds unpubl. data).

These attacks are likely to be frequent and there is evidence that lizards from invaded

sites have higher field CORT levels than those from uninvaded sites (Langkilde and

Freidenfelds unpubl. data).

Fire ants impose a different threat to lizards as compared to native predators, such

as birds and snakes, due to their smaller size, presence of venom, and foraging strategy

(Holtcamp et al. 1997, Haight and Tschinkel, 2003). Therefore, the behavioral response

that promotes the survival of these lizards within natural populations exposed to naturally

occurring threat likely would not favor the survival of these lizards when faced with fire ants. There is growing evidence that the introduction of non-native species drives evolved changes in native species behavior, physiology, and development (Strauss et al. 2006; Suarez et al. 2005; Case and Bolger 1991; Suarez and Case 2002; Wauters et al. 2002). The invasion of non-native species allows researchers to evaluate how native species adapt to enduring threats, and understand the evolution of these important traits. It is likely that invasive species should select for individuals who are better able to tolerate invasion and the associated physiological stress, and may cause shifts in the behavioral response of native species to elevated stress levels (Meyers and Bull 2002; Pace 2007; Avitsur et al. 2002). We tested whether the behavioral response of fence lizards to elevated CORT levels in natural populations differs from that of lizards from populations that have evolved in the presence of the persistent novel threat presented by invasive fire ants.

Methods

Study animals

The lizards used in this study were hatched from eggs obtained from females collected from two sites: the Solon Dixon Forestry Education Center, Escambia county, Alabama (31.15°N, 86.71°W) and St Francis National Forest, Lee County, Arkansas (34.73°N, 90.70°W). These sites are similar in elevation and habitat, but differ in the presence of invasive fire ants; the site in Alabama was invaded by fire ants ~70 years

ago, while the site in Arkansas has not yet been invaded. Lizards from both sites were raised under the same conditions: in same sex groups of 4 or 5 housed in plastic terrariums 33 × 33 × 48 cm (L × W × H), with a paper towel substrate on half of the enclosure and a thin layer of sand lining the other half. Enclosures were furnished with a water dish and a plastic log was provided for basking and shelter. The log was positioned directly beneath a 60-watt incandescent light bulb, which provided heat for 8 hours per day, allowing the lizards to thermoregulate naturally. Fluorescent lights illuminated the room and were set on a 10:14 light:dark schedule.

Assessment of corticosterone response to stress

In order to ensure that we tested the behavioral response of lizards to realistic elevations of CORT, we first determined typical plasma CORT levels following the application of a stressor in this species. Thirty-six lizards were randomly assigned to one of 6 different treatment groups, according to the time after the stressor that plasma CORT levels were measured: 0 (control), 5, 30, 60, 90, or 120 minutes post-stressor. Sex was determined by the presence of enlarged post-anal scales (Cox et al. 2005). Each treatment contained 3 males and 3 females, with the exception of the control treatment, which contained 2 females and 4 males due to an initially mis-assigned sex. Lizards were subadults (3 - 4 months old, 2.39 ± 0.08 SE g) at the time of this study.

Lizards were given treatment-specific marks by applying different colors of non-toxic nail polish (colors #762, #616, #763, #964, Pure Ice, Bari Cosmetics, NY, USA) to their backs. This allowed for easy identification of treatment when retrieving lizards for blood sample collection. Five lizards, one from each treatment (except the control

lizards), were taken from their home terraria and placed together in an enclosure of similar dimension to their home enclosure. The enclosure was empty, and a 60-watt light bulb suspended above the arena supplied heat. Lizards were chased around the enclosure with a small paintbrush for 5 minutes, a technique that is known to result in elevated CORT levels in other lizard species (Langkilde and Shine 2006). Immediately after the application of this stressor, lizards were returned to their home cages. After the time interval assigned by their treatment, lizards were removed from their home cages and a 15µL blood sample was obtained from the post-orbital sinus using heparinized capillary tubes. The lizards assigned to the control treatment were not exposed to the stressor. Blood samples were obtained for control lizards immediately upon capture from their home enclosure at the same time as lizards from the experimental treatment groups were being sampled. Blood samples were obtained within 3 minutes of catching a lizard (mean 105 ± 0.003 SE seconds). As a result, we do not expect handling to have affected plasma CORT levels (Romero and Reed 2005). CORT levels do not vary significantly during the most active period of the day for this species (between 10:00 and 16:00) ($F_{3,31}$ = 4.45, P = 0.97; Langkilde unpubl. data). Therefore, all trials were conducted between 12:00 and 14:00 to minimize any diel effect on CORT levels.

Blood samples were centrifuged and the plasma was kept at -20°C until assayed. CORT was measured by enzyme immunoassay using Corticosterone Enzyme Immunoassay Kits (Immunodiagnostic Systems Ltd., Fountain Hills, AZ, USA). All samples had detectible levels of CORT and all samples were run on the same plate.

Effect of corticosterone on behavior

I manipulated circulating levels of corticosterone using a non-invasive method modified from Meylan et al. (2003). Corticosterone (minimum 92%; Sigma-Aldrich, Inc., St. Louis, MO) was delivered transdermally to the lizards in a mixture of the steroid hormone and sesame oil (8 µg corticosterone dissolved in 5 µL sesame oil). This dose of CORT elevated circulating plasma CORT levels to ~77 ng/mL (Langkilde unpubl. data), approximating the plasma CORT levels in lizards following the application of a chase stressor (above; Fig. 1). Control lizards were given only sesame oil. I used 20 lizards in each treatment group (control and CORT); ten (5 males and 5 females) from each site. Lizards were subadults (7-8 months old) at the time of this study.

Prior to each behavioral experiment, lizards were removed from their group housing and placed individually into an identical enclosure for one week to allow them to acclimate to these new conditions and for their CORT levels return to baseline levels (Langkilde and Shine 2006; Langkilde unpubl. data). To prevent my presence from affecting the lizard's behavior, all observations were conducted from behind an opaque blind. The blind was left in place throughout the study, permitting the lizards to become acclimated to its presence.

Prior to the start of each trial, the vials containing control oil and oil plus CORT were vortexed for 5 seconds to ensure they were thoroughly mixed. To ensure my observations were blind to the treatment each lizard received, my assistant loaded a pipette with the appropriate oil solution (control or CORT) for each lizard and handed it to the observer. The lizard was removed from the experimental enclosure and 5 µL of control oil or oil plus CORT was applied to its dorsal surface. The lizard was then

returned to the experimental enclosure. The total handling time (removal from enclosure and oil application) was less than 32 seconds (21.60 ± 0.99 SE). The lizard was left undisturbed for 15 minutes to allow the sesame oil (and CORT in the treatment group) to be absorbed into the bloodstream (Langkilde unpubl. data), before observing its behavior.

I recorded each time a lizard performed a behavior during the thirty-minute trial. These behaviors were divided into five broad categories: movement, basking, eyes closed, vigilance, and hiding (Table 1). In addition, I recorded the time the lizard spent in each of three areas within the enclosure: on the basking log, under the basking log, and on the substrate.

Statistical analyses

Behavioral data were characterized as the number of times lizards performed a behavior within each category, standardized by the number of behaviors they performed within a trial. Statistical tests used were ANOVA and Fisher's PLSD post-hoc tests. Data on frequency of movement, hiding, time spent under the basking log, and time spent on the substrate were natural-log($x + 1$) transformed and data on time spent under the basking log were natural-log(gamma) transformed, to meet the assumptions of parametric tests. All data were analyzed using SPSS (version 17.0) (SPSS Inc., Chicago IL, USA).

Results

Assessment of corticosterone response to stress

Plasma corticosterone concentrations varied across time after we applied the

stressor (ANOVA with time since chasing as the factor and corticosterone concentration as the dependent variable: $F_{5,30} = 4.49$, $P < 0.01$; Fig. 1). Fisher's PLSD post-hoc tests reveal that plasma corticosterone concentrations increased significantly when tested 10 minutes after the stimulus was applied, and had recovered to background levels after 2 hours (Fig. 1).

Effect of elevated corticosterone levels on behavior

Experimental elevation of plasma CORT levels affected lizard behavior, but this effect depended upon the individual's site of origin (treatment * site, $F_{5,28} = 3.62$, $P = 0.01$). Fisher's PLSD post-hoc tests reveal that this result is driven by the fact that lizards from Arkansas (where fire ants have not yet invaded) responded to elevated CORT levels by moving less, hiding more, and basking less, whereas those from Alabama (invaded by fire ants ~70 years ago) responded by moving more and hiding less, but did not change their basking behavior (site: move, $F_{1,32} = 6.36$, $P = 0.02$; hide, $F_{1,32} = 4.35$, $P = 0.046$; bask, $F_{1,32} = 10.55$, $P < 0.01$. All other behaviors, $P > 0.05$) (Fig. 2). The experimental elevation of plasma CORT levels affected the lizards' use of the enclosures, but this effect depended upon their site of origin (treatment * site, $F_{3,30} = 4.25$, $P = 0.01$). Fisher's PLSD post-hoc tests reveal that this result is driven by the fact that lizards from the uninvaded Arkansas site spent less time up on their basking log when their CORT levels were elevated, whereas those from the fire ant invaded Alabama site spend more time on the log when they had elevated CORT levels (on log, $F_{1,32} = 13.31$, $P = 0.001$) (Fig. 3).

Males and females significantly differed in their behavior (MANOVA with treatment, sex and site as factors and the frequency of behavior within each category as the dependent variables: sex: $F_{5,28} = 2.80$, $P = 0.04$). Fisher's PLSD post-hoc tests reveal that this result is driven by the fact that males closed their eyes more often than did females ($F_{1,32} = 14.67$, $P = 0.001$; all other behaviors, P > 0.05). A lizard's sex did not significantly impact the affect of either treatment or site on its behavior (sex*treatment, sex*site, sex*treatment*site, P > 0.05). Males and females did not significantly differ in their use of the enclosure (MANOVA with treatment, sex, and site as factors and the time spent in each area as the dependent variables: P = 0.326). There was no significant interaction between sex and any other factor (sex*treatment, sex*site, sex*treatment*site, P > 0.05).

Discussion

This study supported the hypothesis that lizards from invaded and uninvaded sites had different behavioral responses. I found that the lizards from the uninvaded site hid more and showed decreased movement and basking when they have elevated CORT levels. In contrast, those lizards from the fire ant invaded site showed the opposite response, moving more, hiding less and spend more time on their basking log when their CORT levels are elevated. Hormonal responses, such as increases in CORT can play an important role in survival by facilitating an adaptive response to a stressor. However, the response that promotes survival may vary depending upon the nature of the threat. This

study reveals that fence lizards modify their behavior when CORT levels are experimentally elevated, and that the nature of this behavioral response differs for lizards from fire ant invaded versus uninvaded populations.

Adaptive advantages of CORT-induced behavioral responses can be conditional. For example, hiding, may be adaptive when being pursued by a visual a predator who uses motion to find prey (Edmunds 1974), but maladaptive when being pursued by a predator who relies on olfactory cues. Fleeing to refuge instead might also be costly as it interrupts normal activity and potentially moves lizards farther away from resources (Blumstein 2002; Cooper and Perez-Mellado 2004; Amo et al. 2007). My observed population differences in behavioral response may be the result of differential selective pressures. These two sites have similar habitats and elevations (Langkilde 2009b). However, an obvious difference between these sites is the fact that one was invaded by fire ants ~70 years (~35 lizard generations) ago whereas the other has not yet been invaded. The observed difference in the behavior of fence lizards from the invaded site may represent an adaptive shift towards behavior that is more likely to promote survival following stressful encounters with this novel predator (Magurran 1999). While it may be adaptive for lizards from uninvaded populations to hide under logs when exposed to a stressor, lizards that seek refuge under logs following stressful encounters with fire ants (causing elevations in CORT levels) would be vulnerable to fire ant attack. Instead, these lizards would be most likely to survive fire ant encounters if they flee and climb following a stressful encounter, as fire ants forage less effectively above ground level (Langkilde 2009b). Fence lizards are vulnerable to predators such as snakes, raptors and other predatory birds, carnivorous mammals, and other lizards (Crowley 1985). If they

hide, they reduce the likelihood of being exposed and can be in locations where their predators are too large to enter. Lizards that co-occur with predatory birds, but not with ants do not want to be up off the ground because it would be easier for birds to spot them, especially if they moved because visual predators would then be able to sight lizards that would look camouflaged if they remained motionless. This shift from the "hide" response exhibited by fence lizards in uninvaded sites following a stressful encounter to one of "flee and climb," is reflected in the increased level of movement observed in lizards from the invaded site with elevated CORT levels. Alternatively, the differences in behavioral stress response could also be innate due to genetic shifts from selective pressures (Dalesman et al. 2006; Hendrick and Kortet 2006).

Population differences in lizards' propensity to bask may be the result of a shift in behavioral response to elevated CORT (e.g. climbing instead of hiding). However, it may also be the result of changes in the impact of CORT on thermoregulatory behavior, as found in other species (Preest and Cree 2008). Understanding how CORT affects thermoregulation of fence lizards would shed light on the mechanism behind the observed shift in their use of the basking log with changes in CORT levels.

The documented change in behavioral response to elevated CORT levels may be the result of selection or a plastic response to the novel selective pressures imposed by fire ant invasion. There could possibly be other site-differences driving these changes, such as differences in native predator assemblage. There is no evidence to suggest that such differences exist, but this would be an important avenue for further research. In order to understand whether the observed shifts in response are actually adaptive, we need to quantify the survival relevance of the observed stress-responses for lizards from

invaded and fire-ant naïve sites. For example, do lizards that respond to elevated CORT levels by fleeing off the ground have a higher chance of surviving stressful encounters within invaded sites (where frequently elevated CORT levels as a result of frequent encounters with fire ants), whereas hiding following a stressor promotes survival within natural uninvaded sites? In addition, do lizards within invaded sites, which frequently experience elevated stress levels, exhibit additional adaptations to this stress (e.g. increased tolerance and reduced effects such as suppressed immune function)? Expanding this research to other species that exhibit different behavioral responses to stressors, as well as other adaptations to increased stress, would provide important insight into the evolution this critical survival strategy.

Acknowledgments

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Table 1. Behaviors recorded during trials and the categories they were assigned to.

| Category	Behavior	Reference
Move	Walk	Lizard uses legs to transport itself (Greenberg 1977)
	Turning About	Lizard moves body, but stays in the same location (Liu et al. 2009)
	Push Up	Lizard uses forelimbs to pump itself toward the light (source of heat) (Greenberg 1977)
Bask	Bask	Lizard rests on top of log below the light (source of heat)
Close Eyes	Close Eyes	Lizard closes lids over eyes (Greenberg 1977)
Vigilance	Move Head	Lizard moves only its head (Langkilde et al. 2003)
	Looking Around	Lizard moves its eye to look around, but does not move head (Liu et al. 2009)
Hide	Hide	Lizard retreats to refuge under log
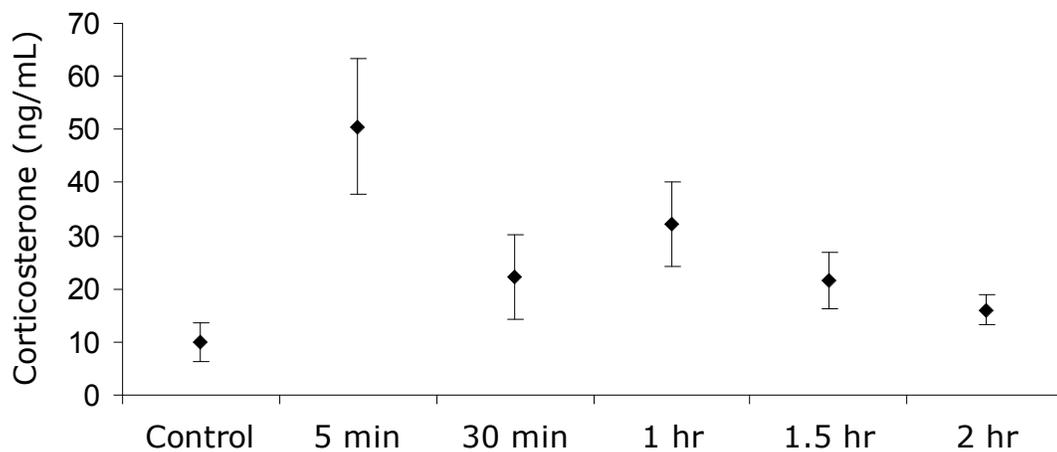
Figure 1. Changes in circulating plasma CORT levels across time following a chasing stressor. Values represent means ± 1 SE.
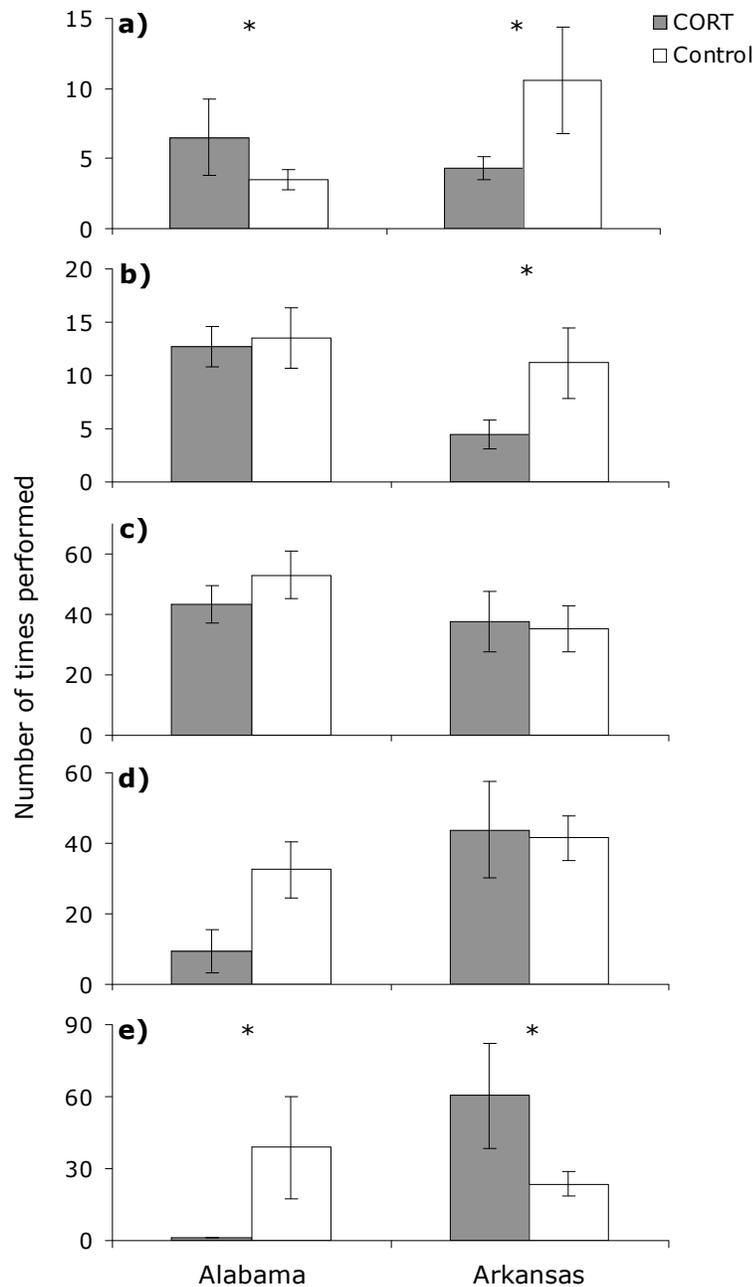
Figure 2. Frequency of A) moving, B) basking, C) eyes closed, D) vigilance, and E)

hiding behavior performed by lizards following experimental elevation of CORT levels.

Bars represent means ± 1 SE, weighted for total number of behaviors performed during a

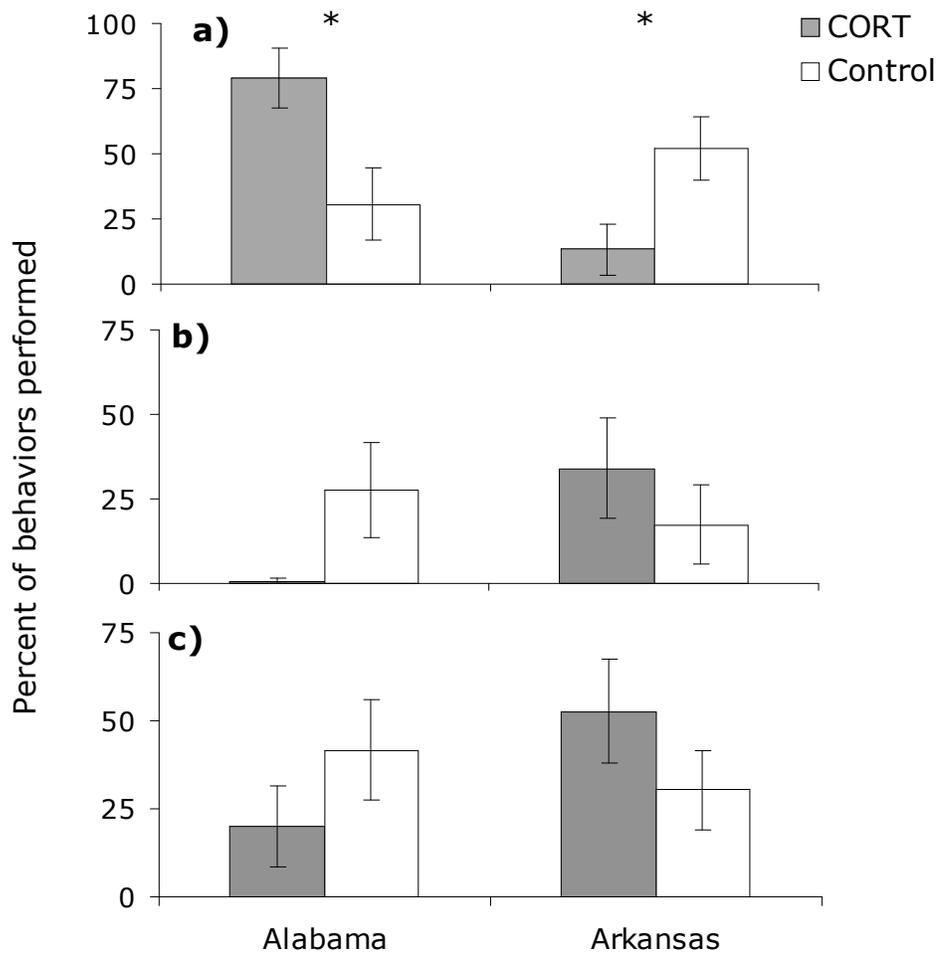30-minute trial. * indicates statistically significant differences.

Figure 3. The percentage of time lizards spent A) on the basking log, B) under the
basking log, and C) on substrate of the experimental enclosure following experimental
elevation of CORT levels (grey bars) versus control lizards (white bars). Bars represent
means ± 1 SE. * indicates statistically significant differences.

The effect of corticosterone on thermoregulation and cloacal temperature in

Sceloporus undulatus from two sites in The United States

Abstract

Animals respond to stressors by secreting stress hormones, such as corticosterone (CORT). This facilitates the survival of animals by redirecting resources towards escape. CORT affects a variety of physiological and behavioral parameters. Recent research has found that elevated CORT levels affect basking behavior of lizards, including the fence lizard, *Sceloporus undulatus*. However, it's unclear whether this is due to changes in temperature regulation itself or the result of other behavioral changes, such as the use of shelter (and basking platforms) to escape a threat. I tested how elevated CORT affects thermoregulation by directly measuring the affect of CORT on the selected body temperature of fence lizards. CORT levels did not affect lizard selected body temperatures. Rather than affecting temperature regulation itself, it appears that CORT affects the lizards use of shelters sites. This highlights the need to carefully assess the mechanisms driving changes caused by elevated stress levels if we are to understand the adaptive basis of this physiological stress response.

Introduction

Corticosterone (CORT) is the major glucocorticoid stress hormone in reptiles, amphibians, birds, and many rodents (Romero 2004), secreted when the hypothalamic-pituitary-adrenal (HPA) axis is activated by a stressor (Sapolsky et al. 2000). Therefore,

circulating glucocorticoid levels in the blood can provide a useful index of stress and can be used to monitor population health (Kort et al. 2005; Creel et al. 2002). CORT has been shown to improve survival through its suppressive, stimulating, and preparative effects on physiology and behavior, including metabolism, cardiovascular function, appetite, memory formation, and reproductive function when elevated short-term (Astheimer et al. 1994; Sapolsky et al. 2000).

Since CORT can induce alternate behavioral strategies (Astheimer et al. 1992; Gray et al. 1990; Ramenofsky et al. 1992), it is important to understand the physiological aspect driving changes in behavior. The relationship between thermoregulatory behavior and thermal physiology has been a huge focus for evolutionary physiologists (see reviews by Huey 1982; Angilletta et al. 2002). Shuttling heliothermic animals, such as lizards, behaviorally regulate their body temperature by moving in and out of the sun rather than physiologically regulating their body temperature (Bartholomew 1982; Huey 1982; Stevenson 1985). Thermoregulatory behavior is important many important processes such as locomotor ability, foraging, and digestion (Harwood 1979; Bennet 1980; Avery et al. 1982;). CORT has been found to alter the amount of time lizards spent basking, (Preest and Cree 2008; Belliure and Clobert 2004; Trompeter unpubl. data). These observed behavioral changes could be driven by changes in thermoregulation, such as shifts in preferred body temperature as a result of CORT levels. Corticosteroids at high circulating levels mobilize lipids from fat stores (Guillette et al. 1995). This could facilitate lizard thermoregulation and enable them to reach preferred body temperatures more quickly, devoting less time to regulating their temperature (Belliure and Clobert 2004). Thermoregulatory behavior, like CORT, influences locomotion and foraging

(Bennet 1980; Avery et al. 1982). CORT-enhanced locomotor activity was associated with changes in thermoregulatory behavior and CORT was suggested to have an important role in thermoregulatory activity (Belliure and Clobert 2004). Alternatively, CORT-induced changes in basking behavior could be the result of some other behavioral shift that is unrelated to temperature regulation, such as the use of elevated basking platforms. To distinguish these, it is necessary to quantify the relationship between CORT levels and body temperature. We test this by experimentally altering CORT levels of the fence lizard, *Sceloporus undulatus*, and quantifying the impact on selected body temperatures in the lab.

Methods

The lizards used in this study were hatched from eggs obtained from females collected from two sites: the Solon Dixon Forestry Education Center, Escambia county, Alabama (31.15°N, 86.71°W) and St Francis National Forest, Lee county, Arkansas (34.73°N, 90.70°W). Lizards were raised under common conditions: housed in same sex groups of 4 or 5 in plastic terrariums 33 × 33 × 48 cm (L × W × H), with a paper towel substrate lining the enclosure. Enclosures were furnished with a water dish and a plastic log was provided for basking and as shelter. The log was positioned directly beneath a 60-watt light bulb, which provided heat for 8 hours per day, allowing the lizards to thermoregulate naturally. Fluorescent lights illuminated the room and were set on a

10:14 light:dark schedule. Lizards were 19-20 months old (10.28 ± 0.40 SE g) at the

time of this study.

Testing the effect of CORT on thermoregulation

Lizards were weighed and placed individually into experimental enclosures 89 ×

43 × 33 cm (L × W × H) for one week to allow them to acclimate to these new conditions

and for their CORT levels to return to baseline levels (Langkilde and Shine 2006,

Langkilde unpubl. data). The enclosures were furnished with a sand substrate, a piece of

corrugated plastic to act as a shelter and basking site, and a water dish. A 60-watt

incandescent light bulb provided heat for 8 hours per day, allowing the lizards to

thermoregulate naturally. A 250-watt heat lamp was positioned at a 45° angle from the

center of the terraria to the bottom of one corner to provide additional heat during the

trials,

Twenty lizards were assigned to each of two treatment groups, CORT application

and control, with sex and site equally represented in each. I manipulated circulating

levels of corticosterone using a non-invasive method modified from Meylan et al. (2003).

I experimentally elevated the CORT levels of lizards assigned to the CORT treatment by

transdermally delivering CORT (minimum 92%; Sigma-Aldrich, Inc., St. Louis, MO) in

sesame oil (1.6 mg corticosterone / 1 mL sesame oil) to the lizards using a pipettor.

Lizards assigned to the control treatment were handled in the same way, but only sesame

oil was applied.

Prior to the start of each trial the vials containing control oil and oil plus CORT

were vortexed for 5 seconds to ensure they were thoroughly mixed. The lizard was

removed from the experimental enclosure and 0.5 µL/g body mass of sesame oil or oil plus CORT was applied to its dorsal surface. The lizard was returned to the experimental enclosure, the 60-watt lamp was turned off and the basking shelter was removed in order to eliminate lizards preferentially using that specific location in the gradient. The lizard was left undisturbed for 15 minutes to allow the sesame oil (or sesame oil plus CORT) to be absorbed into the bloodstream (Langkilde unpubl. data). After 15 minutes, the 250-watt heat lamp was turned on, creating a thermal gradient of 22.5 to 42.2°C. The lizard was then left undisturbed for 30 minutes to allow it time to explore the thermal gradient and regulate its body temperature, after which I captured the lizard and immediately measured its internal cloacal temperature using a thermocouple thermometer (Fluke Corporation, Evertt, WA). I also measured the temperature of the sand where the lizard had been sitting immediately before capture using an infrared thermometer (Raytek Corporation, Santa Cruz, CA).

Statistical analyses

Body temperature data were non-normal (one-sample Kolmogorov-Smirnov Test: $Z = 1.655$, $p = 0.008$). We therefore analyzed these data using non-parametric Mann-Whitney U tests in SPSS (version 17.0) (SPSS Inc., Chicago, IL).

Results

CORT levels did not significantly affect lizard selected body temperatures ($U =$ 168.50, p = 0.40; Fig 1a). Males had higher selected body temperatures than did females ($U = 127.00$, p = 0.048; Fig. 1b). Lizards from both sites had similar selected body temperatures ($U = 148.50$, p = 0.12; Fig. 1c).

There was a strong correlation between the temperature of the sand where a lizard chose to thermoregulate and internal lizard body temperature (CORT: $R^2 = 0.552$, Control: $R^2 = 0.643$) (Fig. 3).

Discussion

CORT levels affect a variety of physiological and behavioral factors, facilitating survival of animals faced with a threat. Previous research on fence lizards has shown that CORT affects basking behavior (Trompter unpubl. data). Our results suggest that this change is basking behavior is not driven by changes in thermoregulation as a result of CORT levels, as lizard selected body temperature was not affected by experimentally elevated CORT levels.

The body temperatures recorded for males and females in this study fall within the range of those previously reported this species (Angilletta et al. 2002). CORT affects basking behavior of *S. undulatus* (Trompter Part 1 this thesis). The results of this study suggest that this behavioral shift has more to do with the use of the basking sites than it

has to do with changes in preferred body temperature. In the previous study, basking was recorded as lizards being up off the ground and on top of their basking log, which as positioned under the heat lamp. Although changes in the use of this log likely affected the lizards body temperature, this study suggests that these lizards do not alter their preferred body temperature in response to elevated CORT levels. In addition to serving as a basking platform, the provided logs also served as shelters. Fence lizards will seek shelter in refuge sites, and escape up trees to avoid predators (reference from the previous chapter about escaping up trees). CORT causes changes in behavior that allow animals to survive in the face of threats: initiating the fight to flight response. Therefore, in light of the results of this study, the documented shift in basking behavior likely reflects a behavioral escape response to CORT rather than a thermoregulatory shift.

Although this study found males and females had different selected body temperature, previous research on these same lizards (Trompeter Part 1 this thesis) found no difference in basking behavior of males and females. This apparent mis-match of results may be due to the timing of these studies. Both studies were prior to the lizards breeding season, from January to March, so these differences are likely not driven by seasonal changes in activity. They could, however, to be due to changes in lizard maturation. These studies were conducted exactly one year apart. Males begin to have more testosterone than females at 11 months of age (Cox et al. 2005). As males enter sexual maturity, testosterone influences daily activity period and movement (Marler and Moore 1989, 1991; DeNardo and Sinervo 1994; Klukowski et al. 2004). Cox et al. (2005) found that exogenous testosterone (within the natural physiological range) increased daily activity period and movement. I did not test behavior during the thermoregulation trials

so it is possible that males could have been more active, increasing their body temperature (Belliure and Clobert 2004).

CORT plays an important role in facilitating survival, and has been found to affect a range of behavioral and physiological parameters. This study reveals that previous findings suggesting an affect of CORT on thermoregulatory behavior are actually driven by escape, rather than temperature regulation behavior. This highlights the need for researchers to carefully examine the mechanisms driving observed changes. Such studies will provide insight into the adaptive importance of animals' physiological stress responses.

Acknowledgments

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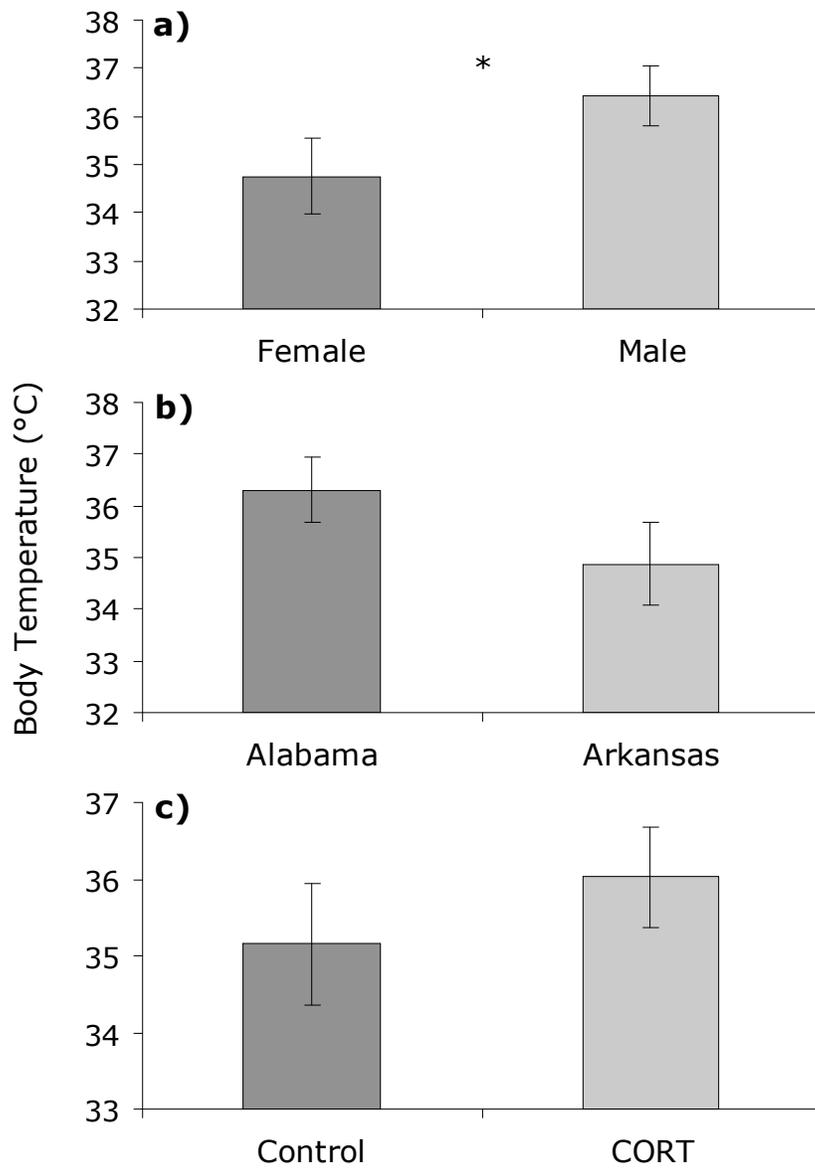77, 1037, 1042.

Figure 1. Average cloacal temperature for A) sex, B) site, and C) treatment. * indicates statistically significant differences.
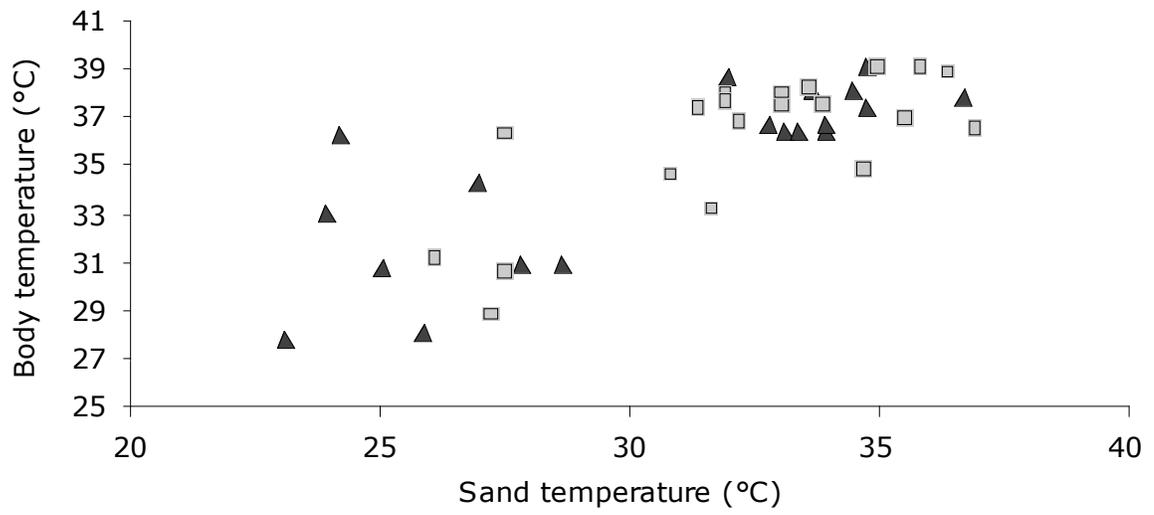
Figure 2. Sand Temperature of chosen thermoregulation spot along gradient versus

cloacal temperature.

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EDUCATION

The Pennsylvania State University 2006-2009
Major/Biology
Member of Schreyer Honors College
Fall 2006/2007/2008/2009 and Spring 2007/2008/2009 Dean's List

WORK EXPERIENCE

Langkilde Laboratory, Undergraduate Researcher: September 2008-Present
Participating in the Center for Emergency Medicine EMT-Basic Course with Clinical Internship in Pittsburgh: Summer 2009
Intern at The London Clinic of Dermatology in St. Johns Wood, London: Summer 2008
Carnegie Pediatrics, Secretary in Pediatric Office: January 2006-February 2006, August 2008
Squirrel Hill Movie Theatre, Concessions: November 2005-February 2006, March 2009
Emma Kaufmann Camp, Counselor: Summer 2006 and 2007.
Emma Kaufmann Camp Counselor in Training: Summer 2004

AWARDS AND RECOGNITION

Recipient of Phi Kappa Phi Outstanding Junior Award in Research: 2009
Phi Eta Sigma National Honors Fraternity 2007-Present
Recipient of Eberly College of Science Undergraduate Research Grant: 2008
Recipient of Lynne Robins Memorial Endowment Fund Israel Experience Scholarship: May 2005

STUDY ABROAD

Arcadia Education Summer London Internship Program 2008
Passport to Israel Participant - Participated in an Israeli Army Camp and Spent time on a Kibbutz: Summer 2005

SCHOOL ACTIVITIES

Panhellenic Dance Marathon (THON) Morale Committee: 2006-2007, 2007-2008
Service sorority Member of Gamma Sigma Sigma Spring: 2007-present
Membership Vice President of Gamma Sigma Sigma: Spring 2010
Member of THON Family Relations Committee for Gamma Sigma Sigma: Spring 2009
Business Division of *The Daily Collegian* Trainee: Spring 2007

COMMUNITY SERVICE

Completed at least 35 volunteer hours for various organizations each academic year
Volunteered at Beth Shalom Sunday School, tutoring elementary children in Hebrew-100 hours
Participated in Medallion Ball for St. Lucy's Auxiliary Fund. November 2005