12-2012

Effects of a chronic increase in plasma corticosterone on the brooding behavior of the prairie skink, *Plestiodon septentrionalis*

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Effects of a chronic increase in plasma corticosterone on the brooding behavior of the prairie skink, *Plestiodon septentrionalis*

A Thesis

Presented to the

Department of Biology

and the

Faculty of the Graduate College

University of Nebraska

In Partial Fulfillment

of the requirements for the Degree

Master of Science

University of Nebraska at Omaha

by

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December 2012

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UMI Number: 1530672

UMI

Dissertation Publishing

UMI 1530672

ProQuest®

Effects of chronic increases in plasma corticosterone on the brooding behavior of the prairie skink, *Plestiodon septentrionalis*

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University of Nebraska, 2012

Advisor: Dr. James D. Fawcett

The effects of chronic corticosterone (CORT) increases on the maternal behavior of the Prairie Skink, *Plestiodon septentrionalis* were studied in the laboratory during the summer of 2012. It was hypothesized that chronic increases in plasma CORT would result in decreased maternal effort and higher egg mortality, with similar results to analogous studies in birds. Plasma CORT was elevated using a non-invasive, exogenous treatment and behaviors were observed using video recordings. CORT treatments significantly reduced the amount of time spent tightly coiled around eggs in experimental individuals, and significantly decreased the amount of time experimental females spend in contact with their eggs in comparison to control females. The treatment also weakly increased the amount of time that experimental females spend outside the nest. No significant difference was found on mortality of eggs, however all eggs that successfully hatched were from control females. These results indicate decreases in maternal effort following increases in CORT similar to those found in avian studies, as well as studies on other vertebrates.

ACKNOWLEDGEMENTS

There are several people without whom the completion of this project would not have been possible. First and foremost, I would like to thank Dr. Fawcett for all of his help. When I settled on my research topic I knew it was something new for him, but he allowed me to pursue it regardless. He was a constant source of support, and always willing to make time for me, even if it was unscheduled, several times per day. He aided me through all of my issues, and read revision after revision of my thesis until we at last reached the final copy. This is a study on the effects of stress, and the irony is not lost on me that it has been a major source of stress for the both of us during my graduate career. For his unwavering patience and support, I am thankful. I owe a large debt of gratitude to my committee as well. Dr. Claudia Rauter was immensely helpful in guiding me through the statistics of the project, and was always willing to make time for me. Without her help, it would have been extremely difficult to interpret my results. Dr. Jeffrey French was also instrumental to the completion of my research, providing valuable advice on how to best record behaviors and helping me to analyze my plasma samples. In addition to my committee, I would like to thank Dr. Davis for supplying me with 100% ethanol, which was necessary to prepare my treatments. I would also like to thank Dr. Tom Bragg for allowing me access to his lab and drying oven. In addition to the faculty who have helped me, I would like to thank the undergraduate students who helped me review my recordings- Adam Blowers, Trace Hardin, and Cassie Hoffman.

This project would also not have been possible without the help of Dr. Tracy Langkilde and her post-doc Dr. Sean Graham at Pennsylvania State University. When it became apparent that we would not be able to analyze plasma samples here at UNO, she

volunteered her services. She also provided me with a wealth of advice in regard to the blood sampling process, treating lizards with hormones, and interpreting the results of plasma samples. I was unsure of what to expect when I reached out to another professor whom I had never met, but she was more than generous, and I am extremely grateful for her help.

I also want to thank my mother Tracy, my father Henry, and loving fiancée Cassie for all of the emotional support they gave me when I was struggling, even riding back and forth to Lake Burchard with me, aiding me in the field. No matter how busy I became, they all reminded me that the end goal was well worth the effort, and that I was surely capable of finishing my work. I would also like to thank the rest of my friends and family who helped me to collect animals- Dan Fogell, Dennis Ferraro, Austin Scarborough, James Russell, and Daniel Noble. Funding was provided by the Nebraska Herpetological Society, the Department of Biology, and the University Committee on Research Activity Center and helped me to minimize the financial impact the project had on me.

This study was approved by the Institutional Animal Care and Use Committee (IACUC #11-037-05-EP).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE   **PAGE**

INTRODUCTION

Corticosterone (CORT) and cortisol are both representatives of the steroid hormone class glucocorticoids. Corticosterone is typically seen as the major stress hormone in reptiles, amphibians and birds while cortisol is more common in mammals, although corticosterone is present in smaller amounts (Romero and Butler, 2007). These stress hormones are released when an individual is exposed to a stressor via a hormonal cascade known as the hypothalamic-pituitary-adrenal axis. The latency of this cascade is the reason that stress responses normally take 3-5 minutes to have a noticeable or measurable effect on an individual (Romero and Butler, 2007). An immediate stressor, such as exposure to a predator would normally evoke a brief, attenuated response. However, other conditions can cause long, extended periods of stress. This occurs when an individual has reached their binding capacity for corticosterone. Additional corticosterone is released into the circulatory system, where it can have effects on behavior. This results in an "allostatic overload" and the engagement of an emergency life-history stage. During this time, the individual will likely engage in self-preservation behaviors rather than what would be normal, such as parental behavior (McEwan and Wingfield, 2003). For example, previous studies have found that Marine Iguanas exposed to El Nino events have increased ACTH responses (Romero and Wikelski, 2001) and that Southern Toads exposed to pollution displayed higher baseline levels of plasma corticosterone than those in pristine conditions (Hopkins *et al*., 1997). When offspring are involved, there is considerable parental-offspring conflict, wherein a parent must maximize their fitness (Trivers, 1985). In this sense, long term increases in corticosterone might serve to cause females to abandon their current reproductive effort in lieu of future

opportunities. In long lived species, it is possible that after the breeding season and during care of eggs that the ACTH response would be more sensitive, in order to maximize fitness over a lifetime. It is important to also note that many other factors can alter the ACTH response, including sex, season, mass and reproductive state (Moore and Jessop 2003). In addition, some species of reptile are capable of down regulation the ACTH response during key times, such as a short breeding period (Moore *et al.*, 2001).

Stress hormones such as cortisol and corticosterone can have dramatic effects on individual animal behaviors, particularly during the mating season. An individual at this point in their life is likely to have an increased amount of corticosterone, and in this sense it may be considered to be adaptive because corticosterone is inducing behaviors which increase fitness. For example, male frogs have increased plasma corticosterone during reproductive behaviors such as mate calling (Moore and Jessop, 2003). However, further increases in plasma corticosterone may be caused by factors other than the season, such as a lack of resources or overcrowding. In these situations, increased plasma corticosterone could have detrimental effects on both the individual and their offspring such as a decrease in immune function (Comendant *et al.*, 2003) or a reduction of reproductive behaviors, possibly including parental care. The way that a vertebrate reacts to increased stress hormones allows them to behave in ways that are most adaptive for their overall fitness, (Moore and Jessop, 2003).

To understand parental care, it is important to have a clear definition of what it is. In 1972, Trivers coined the term "parental investment", which includes "any investment by the parent in an individual offspring that increases the offspring's chance of surviving at the cost of the parent's ability to invest in other offspring." In 1991, Clutton-Brock

further refined this idea into three more precise definitions- parental care, parental

expenditure, and parental investment. Under this definition, parental care describes any

form of parental behavior that is likely to increase the fitness of offspring. Perhaps most

importantly, parental investment is the extent to which parental care of individuals

reduces the parent's residual reproductive value, which a parent would use to determine

how much of an investment to make. Clutton-Brock's definition for forms of parental

care also includes care of eggs in ectotherms, where species likely must account for

variation in abiotic conditions, similar to the behavior of *P. septentrionalis*. The term

brooding will be used to describe the behavior of *P. septentrionalis,* and is a more narrow

description of the parental care observed in this species. This term "describes behaviors

of the parent while closely attending its nest and progeny" and does not include defense

of territory outside the nest (Somma, 2003).

Because there have been very few papers outlining the effects of corticosterone on

reptilian behaviors, especially parental, it is important to examine related phylogenetic

taxa for background information. The parental behaviors of birds have been widely

studied, and there have been several studies on the effects of increased corticosterone on

avian parental behavior. Artificially increased corticosterone has a variety of effects. In

some cases, an increase in corticosterone yields decreased parental effort, or in extreme

cases complete abandonment of a nest (Chastel *et al.*, 2005; Groscolas *et al.*, 2008;

Angelier *et al.*, 2009). King Penguins abandoning their nests were found to have two-

four- fold increases in plasma corticosterone (Groscolas *et al.,* 2008). Black-Legged

kittiwakes that received corticosterone implants decreased parental effort, visiting their

nests less often (Angelier *et al.,* 2009). In both of these cases, the results indicated that

individuals became more interested in their own welfare as opposed to that of their offspring. Kittiwake chicks that had received corticosterone implants begged more than normal, and parents that had received corticosterone implants spent significantly more time away from the nest foraging (Kitaysky *et al.,* 2001). These trips away from the nest left chicks alone more often and potentially more susceptible to predators. In some cases however, plasma corticosterone may be seen as beneficial. Mourning Doves given a corticosterone implant reared nestlings with significantly more weight than individuals with lower corticosterone levels. Following the acute stressor of capture, however, parental effort decreased (Miller *et al.,* 2009). The results of these studies indicate that corticosterone may function to reduce reproductive and parental behaviors in birds, possibly by regulating other hormones such as prolactin (Angelier et al. 2009). This reaction to increased corticosterone is expected in individuals where the value of future reproductions outweighs the value of the current reproductive effort. This idea is supported by previous research (Bokony *et al.*, 2009).

The parental behaviors of reptiles have been studied to a much smaller extent. However, there have been several studies on how corticosterone affects other behaviors, and it is important to review them in order to hypothesize how this hormone might affect parental care. It is well known that stress can have significant negative effects on reptile health, such as decreased immune responses (Warwick *et al.,* 1995). High plasma corticosterone has been shown to alter an individual's social behaviors, as well as their normal routine. For example, male *Uta stansburia* lizards given corticosterone implants show significantly smaller home ranges (DeNardo and Sinervo, 1994). Furthermore, if females have long periods of high plasma corticosterone during the development of eggs

or while carrying offspring, offspring can be born with similarly high levels of plasma

corticosterone. This can affect a variety of offspring behaviors. *Zootoca vivipara* lizard

offspring whose mothers were subjected to increased corticosterone took significantly

longer to emerge from shelters after simulated predator attacks (Uller and Olsson*, 2006*).

In some cases, seasonal plasma corticosterone can vary between sexes and may interact

with androgens to regulate reproductive behaviors (Grassman and Hess, 1992). Each of

these examples highlights the fact that when faced with an increased amount of

corticosterone, normal conditions or behaviors were altered to benefit survival or fitness.

While these interactions have been well studied, there has been no attention given to the

role of corticosterone in reptilian parental behaviors.

Parental behavior in lepidosaurian reptiles is a rare phenomenon. The only taxon

of reptiles which show universal parental care are the crocodilians. Parental care, while

rare in reptiles, appears to be widespread in the genus *Eumeces* or *Plestiodon*. For

example, several related species such as *Plestiodon laticeps*, *P. fasciatus,* and *P.

obsoletus* have been shown to display egg brooding behavior (Noble and Mason 1933;

Evans, 1959). Other genera of skink have also evolved forms of parental care. *Eutropis

longicaudata* in Taiwan has been shown to exhibit nest defense from predators, as well as

the ability to home in on their nest after being removed from it (Huang and Pike, 2011a;

Huang and Pike, 2011b). It is important to note that egg defense is very different from

brooding, which will be outlined below.

Plestiodon septentrionalis, the Northern Prairie Skink is native to Nebraska and

known to exhibit parental care in the form of egg brooding (Noble and Mason, 1933;

Breckenridge, 1943; Ballinger *et al.*, 2010; Fogell, 2010). During egg brooding, after

depositing eggs, the female remains with them until hatching, exhibiting near constant nest attendance (Breckenridge, 1943). During this time, she may move the eggs around the nest, retrieve displaced eggs, or coil around the eggs. The female may even consume eggs which obtain fungal infections, in order to prevent the spread of fungus (Somma, 1989). The presence of the female may help deter some small predators from the nest; however the small size of the individuals makes it unlikely that they would be capable of true nest defense. A previous study indicated that the hydric environment of the nest has a significant effect on the health and mortality of eggs (Somma and Fawcett, 1989). When the brooding female is removed from the nest, significantly higher egg mortality was exhibited in moist conditions. Egg brooding behavior and the manipulation of eggs is likely a method of maintaining the best possible hydric environment for developing eggs. Indeed, recent research on Children's Pythons has shown that brooding allows them to manipulate the rate of water loss in eggs (Stahlschmidt and DeNardo, 2010). In a previous study of the skink *Eumeces okadae*, researchers removed brooding females from their nests in the field. In nests where the females were removed, of 133 eggs 86 had disappeared, and 41 remained but did not hatch. It is possible that unfavorable nesting environment could be responsible for these eggs not hatching (Hasegawa, 1985). This egg brooding behavior, in addition to their small size and availability makes *P. septentrionalis* an ideal subject to study the effect of corticosterone on parental behaviors of reptiles.

Study Objectives

The primary goal of this study was to determine if a chronic, long term increase in plasma corticosterone has a significant effect on the brooding behavior of *P.*

septentrionalis. Since this field is largely unexplored, any understanding of how hormones might interact with or direct these behaviors will help to broaden our understanding of not only reptilian maternal behavior, but also of how chronic increases of corticosterone in a reptile during parental care compare to similar interactions in other vertebrates . The only study that could be located tested the effect of ovariectomy on brooding behavior in a closely related species, *P. fasciatus*. Ovariectomy had no effect on the brooding behavior of these individuals. Even after undergoing surgery, females returned to their nests and brooded eggs (Stewart and Duvall, 1985). Many studies that have examined the effects of long term increases of corticosterone in birds have suggested the possibility that these high levels depress prolactin. If the results of this study parallel those found in birds, it might provide evidence that prolactin is the hormone responsible for maintain parental care in reptilian species and incentivize new research.

MATERIALS AND METHODS

Pilot Study

In the summer of 2011, a pilot study was performed to ensure that the proposed corticosterone treatments would be successful in *P. septentrionalis*. During the last week of May and first week of June, five female and five male lizards were collected from Burchard and Douglas Counties, Nebraska and returned to room 209 Allwine Hall at the University of Nebraska at Omaha. Lizards were allowed to acclimate to captive conditions for four weeks to ensure they reached baseline corticosterone levels. Previous research has indicated that reptiles experience a spike in plasma corticosterone following collection and placement in captivity, but that this returns to lower levels after a week (Langkilde and Shine, 2006).

Success of treatments was determined by analyzing plasma samples. I attempted to draw blood from the caudal vein, however no blood could be recovered using this method due to the small size of *P. septentrionalis*. Next, blood was sampled using the post-orbital method. A small capillary tube was inserted behind the eye ball and rotated gently. This ruptured the postorbital sinus, and blood was collected within the capillary tube. This technique was first shown to be successful by (Maclean *et al.*, 1973), and has since become the standard blood collection technique used on small lizards (Dr. Tracy Langkilde, Pennsylvania State University (PSU), personal communication, 2011). This method also allowed for rapid sampling. Previous research has shown that if a sample is not obtained within two minutes of disturbance, the corticosterone values will reflect the stress of sampling rather than baseline values (Langkilde and Shine, 2006). The use of

anesthesia was considered, but was determined to be too dangerous for use with *P. septentrionalis* due to their small size.

To account for any potential effects of the treatment process, all individuals were given a4.5 µl treatment consisting of only sesame oil. This treatment was pipetted onto the interscapular area of the lizards at 0830. At 1430, 20 µl blood samples were taken from the post-orbital sinus to determine baseline plasma corticosterone values. Blood samples were then placed in a centrifuge and spun at 3,000XG for 5 minutes to separate plasma. Plasma was then transferred from the collection tubes using a pipette (Fisherbrand Finnipipette 0-2.5 µl) to a second, clean collection tube. Plasma samples were quickly moved to a -20C freezer for storage.

After the initial blood sampling, individuals were allowed to recover for one week. This was necessary due to their small size and invasive nature of sampling methods (Dr. Tracy Langkilde PSU, personal communication 2011). Following the recovery period, a 4.5 µl corticosterone treatment was administered to all individuals. Ten mg of corticosterone (Sigma C2505) were diluted in commercial, organic sesame oil to a volume of 4.5 µl. Because reptilian skin is highly lipophilic, steroid molecules such as corticosterone are easily absorbed (Knapp and Moore, 1996). Hence, treatments were applied exogenously to the interscapular region using a pipette (Fisherbrand Finnipipette, 0.5-10 µl). This method was first introduced by (Knapp and Moore, 1996), and is commonly used to increase circulating steroid hormone levels in reptiles. After 6 hours, 20 µl blood samples were taken from each individual. Plasma was separated as previously described, and stored in a -20C freezer. Due to the small amounts of corticosterone present in *P. septentrionalis* plasma, samples were unable to be analyzed at UNO. In November 2011,

all samples were placed on dry ice and shipped to Dr. Tracy Langkilde at PSU, who offered to analyze the samples. Under her supervision, all samples were analyzed using the enzyme-linked immunoabsorbent assay method (ELISA) and a commercially prepared assay kit (Enzo Life Sciences ADI-900-097). The results of the analysis indicated that the treatments significantly increased circulating plasma corticosterone six hours after treatment ($p= 0.054$). Following Dr. Langkilde's advice, treatments were applied twice per day to reflect highly stressful environments. This method was chosen because it is more practical than using dermal patches, which would have to be reapplied each day.

Main Study

Acquisition of Study Animals

Individuals were collected from the end of April through the beginning of June, 2012. Two female lizards were collected from Douglas County, six females and all males were collected from Burchard County. Males were identified by the presence of orange breeding coloration on the throat and jaw (Figure 1). Females were recognized by their lack of male breeding colors and relatively stout abdomens. All individuals were collected either by hand beneath natural or artificial debris, or in pitfall traps (Figure 2).

Figure 1: Male *Plestiodon septentrionalis* with orange breeding coloration along

the jaw. This trait was used to determine sex of individuals.

Figure 2: Typical habitat of *Plestiodon septentrionalis*. Pitfall traps are placed beneath boards.

Experimental Design

Eight females were systematically arranged into experimental or control groups. Individuals in the experimental group received bi-daily exogenous corticosterone treatments designed to significantly increase plasma corticosterone levels for up to six hours. Individuals in the control group received bi-daily treatments consisting of sesame oil only. For both groups, the soil moisture level of the nest was raised to 20-25%, and was kept within this range for the duration of the experiment.

Each nest was an experimental unit. At the end of the experiment, mortality of eggs from each group was noted. Eggs were considered dead based on gross appearance or absence from the nest, in which case it was assumed to be oophagous behavior by the female.

Animal Maintenance

Field-caught animals were brought to the lab and housed in 37.85 liter aquariums. Each aquarium was filled with 2,000 grams of commercial topsoil and 500 grams of commercial sand. This substrate was recommended by Daniel Fogell (Southeast Community College) and has been previously used to house and allow females to successfully care for eggs. It also controlled for the differences in soil composition that existed at different collection sites. Above the enclosures 48" R-Zilla UVA/UVB light strips were hung 1.5 meters above the floor of the enclosures in order to simulate a 14L:10D cycle and provide crucial UV rays which are necessary for reptile health. Each enclosure also had a 50 watt heat lamp on one side of the cage, which allowed individuals to thermoregulate. Translucent, 15x15.5 cm red acrylic plates were placed at the warm end of each enclosure to provide shelter under which females could lay eggs.

Initially, males were housed with females for captive breeding. Mating was witnessed in

several enclosures (Figure 3), and was assumed to have taken place in all enclosures.

Every three days, the males were placed with another female in order to ensure that

mating would occur. After three weeks, all females exhibited signs of being gravid.

Specifically, females had stout abdomens, with abdominal swelling along their sides due

to the presence of large oviductal eggs. At this point, all males were removed from the

enclosures, and females remained solitary for the remainder of the experiment.

Figure 3: Male and female individual copulating in the laboratory. Note: Female has regenerating tail.

Feeding and Water

Females were offered food ad-libitum daily. The domestic crickets or domestic mealworms were dusted with Repti-vite calcium powder in order to ensure adequate calcium intake. Water dishes were refilled each day, and the entire enclosure was hand-misted using an aspirator.

Maintenance of Nest Moisture and Temperature

Because of the size of the enclosures, it was not possible to maintain the entire substrate at the same moisture content. In order to properly maintain nest moisture, the following method was used (Somma and Fawcett, 1989). Each morning at 0830, a small soil sample was taken from the edge of each nest. These samples were weighed, and this mass was recorded as a wet weight. Samples were then placed in drying ovens for three hours, and weighed again to obtain a dry weight. This dry weight was subtracted from the wet weight, and then this number was divided by the original wet weight. This process yielded the percent moisture of each sample. To maintain the nests at a moisture of 20-25%, samples below this threshold were misted with water to bring them within this range. Water was applied using an aspirator around the edges of the nest.

Air temperature was checked to ensure the nests had an appropriate temperature. A wet/dry thermometer was used to determine air temperature. Using this in conjunction with a RYOBI laser temperature gun, it was determined that when the air temperature was at 29.4C, nest cavity temperature was 26.6-27.7C. This temperature matches that of previous research on *P. septentrionalis* (Breckenridge, 1964). Air temperature was used to determine nest temperature in order to minimize disturbance to females.

Hormone Application

Results of the pilot study, as well as results from previous literature have found that 45 μg of corticosterone is sufficient to increase circulation CORT levels for roughly six hours (Knapp and Moore, 1996; Cote *et al.*, 2006). Hence, bi-daily treatments were applied twice per day, first at 0830 and again at 1430. This ensured that stress levels were kept high throughout the day. During treatments, 4.5 μl of CORT solution were applied to the interscapular region of experimental females. This amount was chosen to allow quick absorption of the corticosterone. Control females received 4.5 μl treatments consisting of sesame oil only. If the female was under the cover of the acrylic plate, the plate was lifted briefly to allow application of the treatment.

Behavioral Observation

Behavior observations were accomplished using two Sony Handycam camcorders. Cameras were chosen because when observed over the course of a long period by an observer, the behavior of the lizards was altered (Personal Observation). Cameras also allowed for more consistent and longer data-recording periods. Beginning at 0930, cameras were set up facing an enclosure, and the view of the camera was focused on the nest (Figure 4). The cameras had a night vision mode (Sony Nightshot) which allowed for high visibility through the red acrylic plates. Individuals were recorded for one hour, after which the cameras were moved to two new individuals. Each morning, taping began with) random individuals to account for any effect time of day may have on behavior. Following personal observation of individuals and the previous work of Lou Somma (1985), two patterns of behaviors were outlined: positional and active (Tables 1 and 2).

Figure 4: Video cameras positioned to record individuals for behavioral observation.

Table 1: Descriptions of Positional Behaviors (From Somma 1985).

Positional Behavior	**Description**
Coiling	Female tightly coils around eggs.
Alongside	Female lays alongside eggs, contacting many.
Between	Female positions herself between the eggs, separating them.
Over	Female positions herself over top of eggs.
No Contact	Female remains under the shelter, but provides no contact to eggs.
Not Present	Female is absent from the shelter.

Table 2: Descriptions of Active Behaviors (From Somma 1985).

Active Behavior	Description
Pushing	Female changes the position of an egg without overturning it.
Rolling	Female changes the position of an egg by overturning it.
Nudging	Female nudges an egg, but does not change its position.
Biting	Female bites an egg.
Eating	Female consumes an egg.
Tongue Flick	Female contacts an egg with her tongue.
Digging	Female excavates the nest or area around it using her forelimbs.

Positional behaviors describe the position of a female in relation to her nest. For positional behaviors, instantaneous sampling was used at thirty second intervals. Behavior totals were weighted against total observations to obtain a percentage of time spent in each position. For active behaviors, one-zero sampling was used for each behavior at 30 second intervals. Active behavior totals were weighted against the total number of observation intervals to obtain frequency of occurrences.

Data Analyses

Recordings of individuals resulted in many hours of recording, with 20 hours of recording minimum per each individual. In order to account for differing lengths in brooding periods for positional behaviors, all observations were weighted against the total recorded to provide a percentage of observations for each behavior. For active behaviors, observation totals were weighted against the number of observations in which the individual was found within the nest. For egg mortality, the total number of hatching eggs was weighted against the total number of eggs to obtain a percentage of mortality. The Ryan-Joiner test was used to test for normality, and the majority of the recorded data was found to be not normally distributed. Because of the lack of normality and small sample size, the Mann-Whitney test for non-parametric data was used to determine whether significant differences between treatments existed in each behavior. Pearson's correlation test was used to determine if any relationships existed between behaviors. Female snout-vent length was also included in this analysis to determine if size of females had any effect on treatment. All statistical tests were performed using the Minitab statistical software package. For all tests, a standard alpha level of 0.05 was used to determine significance. Due to small sample sizes, it was not possible to determine effect sizes.

RESULTS

Pilot Study Results

Blood samples were assayed for baseline and treatment induced plasma corticosterone values. Treatment increased average plasma corticosterone from 39.10 ± 6.21 ng/mL to 67.45 ± 18.5 ng/mL. Using a repeated measures ANOVA, a significant increase was found between the two samples (p=0.054).

Female SVL and Egg Number

Female SVL and number of eggs were measured at the beginning of the experiment. Average SVL for the control group was 66.75 cm, and average SVL for the experimental group was 70.325. Average number of eggs per female in the control group was 11, and the average number of eggs per female in the experimental group was 10.25. Using a simple t-test, no significant differences for SVL or egg number were detected (Table 3).

Table 3: Two sample t-test results for comparison of snout-vent lengths and egg number between control and experimental females. Significant results are indicated by bold text.

	t	P
SVL	-0.97	0.386
Egg Number	0.47	0.66

Positional Behaviors

Significant differences were found between the experimental and control groups for two positional pattern behaviors (Figures 5 and 6). For all other positional behaviors, no significant effect of treatment was found (Table 5).
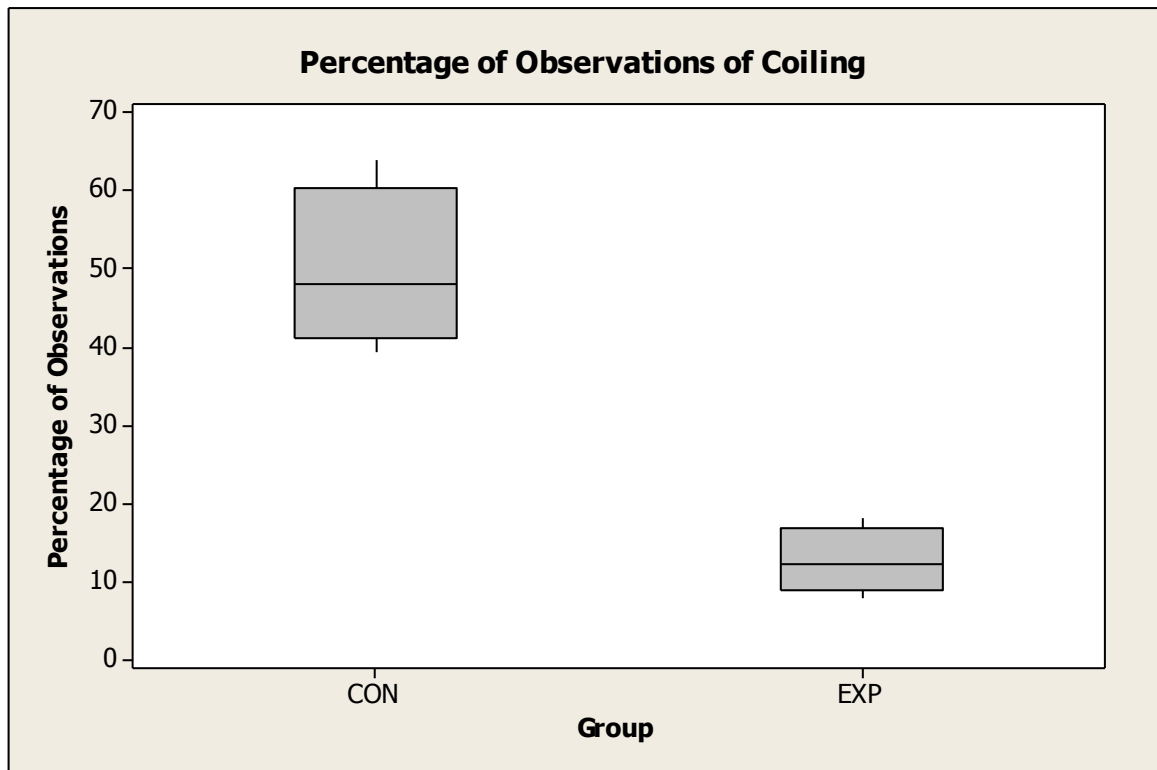
Figure 5: Percentage of observations of coiling behavior for control vs. experimental
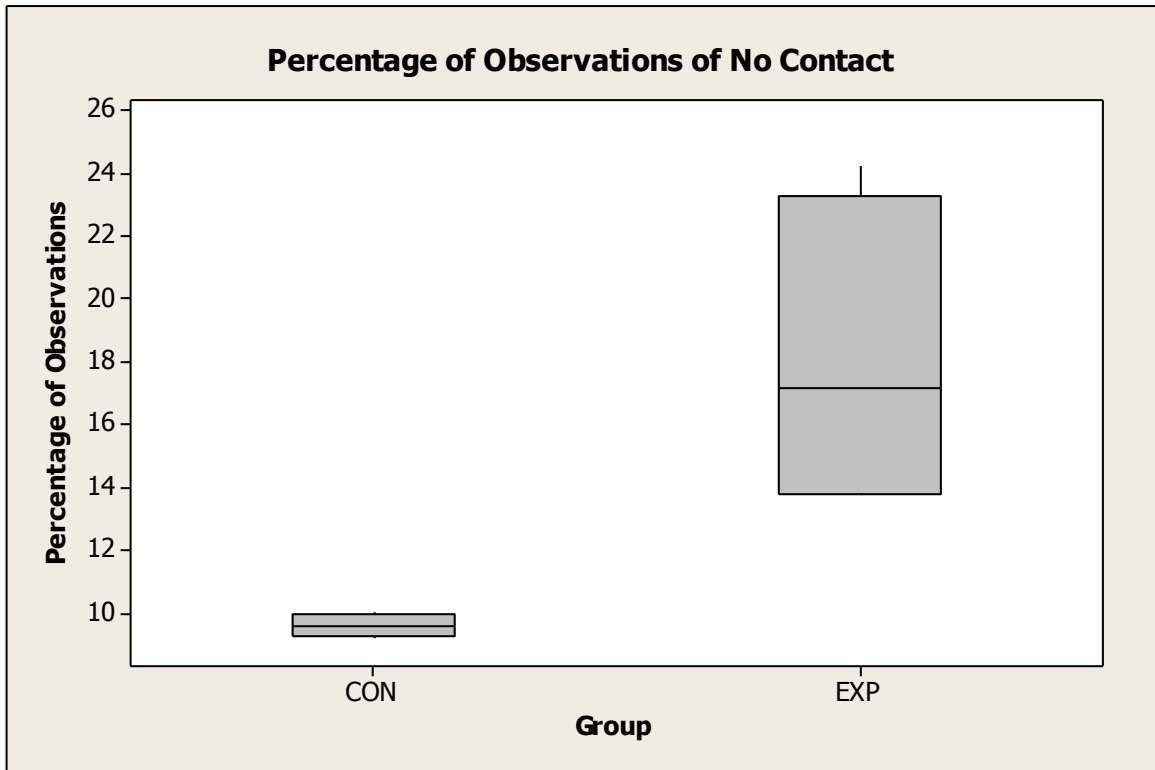
females.

Figure 6: Percentage of observations of no contact for control vs. experimental females.

Table 5

Table 5: Mann-Whitney test results for the effect of treatment on positional behaviors. Significant results are indicated by bold text.

	Z	**P**
Coiling	0.36	**0.03**
Alongside	0.03	0.88
Over	-0.02	0.47
Between	-0.07	0.19
No Contact	-0.08	**0.03**
Not Present	-0.16	0.06

Active Behaviors

No significant effects of treatment were found between control and experimental groups for any of the seven active behaviors (Table 4). Eating behavior occurred only twice in all observations, and as a result was not analyzed.

Table 6: Mann-Whitney test results for the effect of treatment on active behaviors. Significant results are indicated by bold text.

	Z	P
Nudging	0.01	0.67
Biting	-0.00	0.67
Rolling	-0.01	0.31
Digging	-0.01	0.66
Pushing	-0.01	0.89
Tongue Flicking	0.00	1.0

Oviposition and Egg Clutches

Females oviposited from May 29th to June 18th, 2012. Brooding periods ranged from 22 to 38 days. Egg clutches for the control group ranged from 6 to 16, with a median value of 11. Egg clutches for the experimental group ranged from 7 to 14, with a median value of 9.5.

Survival of Eggs

No significant effect of corticosterone was found between the survival of eggs belonging to control females and experimental females (Figure 17). Two control females had at least one egg hatch. No eggs belonging to experimental females hatched. Each egg clutch was converted to a percent hatching for comparison.
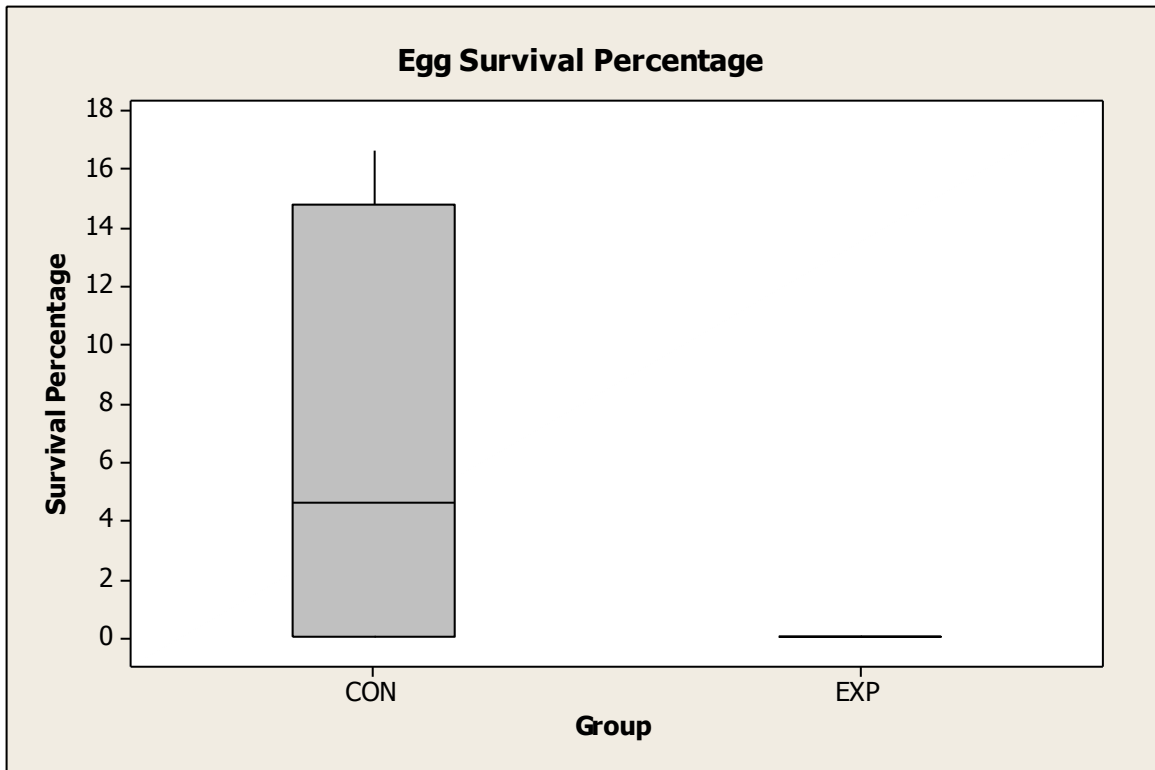
Figure 17: Egg survival percentage for control vs. experimental females.

Correlations

Pearson correlation tests were used to determine if there were any relationships between positional behaviors, active behaviors, and snout-vent length (SVL) of females from both control and experimental groups (Tables 3 and 4). Significant correlations were found between rolling and biting in control females, SVL and not present in experimental females, and tongue flicking and biting in experimental females.

Table 6- Pearson correlation values and p-values for control female positional behaviors and SVL. Pearson correlation values are listed above p-values. P-values in bold text indicate significant results.

	Coiling	Alongside	Over	Between	No Contact	Not Present
Alongside	-0.774					
	0.226					
Over	-0.066	0.268				
	0.934	.732				
Between	-0.701.	0.486	0.679			
	0.299	0.514	0.321			
No Contact	0.002	-0.628	-0.478	-0.018		
	0.998	0.372	0.522	0.982		
Not Present	0.296	-0.689	-0.850	-0.018	0.811	
	0.704	0.311	0.150	0.419	0.189	
SVL	0.779	-0.716	0.451	-0.105	0.089	0.089
	0.221	0.284	0.549	0.895	0.911	0.987

Table 7- Pearson correlation values and p-values for control female active behaviors and SVL. Pearson correlation values are listed above p-values. P-values in bold text indicate significant results.

	Nudging	Biting	Rolling	Digging	Pushing	Tongue Flicking
Biting	0.423					
	0.577					
Rolling	0.444	0.999				
	0.556	**0.001**				
Digging	0.275	0.794	0.744			
	0.725	0.206	0.226			
Pushing	-0.505	0.473	0.466	0.177		
	0.495	0.527	0.534	0.823		
Tongue Flicking	0.250	0.817	0.830	0.313	0.703	
	0.750	0.183	0.170	0.687	0.297	
SVL	0.876	-0.046	-0.018	-0.189	-0.060	-0.060
	0.124	0.954	0.982	0.811	0.269	0.940

Table 8- Pearson correlation and p-values for experimental female positional behaviors and SVL. Pearson correlation values are listed above p-values. P-values in bold text indicate significant results.

	Coiling	Alongside	Over	Between	No Contact	Not Present
Alongside	0.617					
	0.383					
Over	-0.623	0.048				
	0.377	0.952				
Between	0.943	0.660	-0.710			
	0.057	0.340	0.290			
No Contact	-0.409	-0.654	0.491	-0.757		
	0.493	0.346	0.509	0.243		
Not Present	-0.466	-0.787	-0.457	-0.260	0.049	
	0.591	0.213	0.543	0.740	0.951	
SVL	-0.466	-0.650	-0.383	-0.245	-0.127	0.964
	0.534	0.350	0.617	0.755	0.873	**0.036**

Table 9- Pearson correlation and p-values for experimental female active behaviors and SVL. Pearson correlation values are listed above p-values. P-values in bold text indicate significant results.

	Nudging	Biting	Rolling	Digging	Pushing	Tongue Flicking
Biting	0.235					
	0.765					
Rolling	0.407	0.275				
	0.593	0.725				
Digging	0.879	-0.229	0.421			
	0.121	0.771	0.579			
Pushing	0.463	0.915	0.061	-0.015		
	0.537	0.085	0.939	0.985		
Tongue Flicking	0.236	0.973	0.475	-0.178	0.820	
	0.764	**0.027**	0.525	0.822	0.180	
SVL	-0.783	-0.283	-0.886	-0.743	-0.255	0.578
	0.217	0.717	0.114	0.257	0.745	-0.422

DISCUSSION

Despite advances in knowledge of reptilian parental behavior, studies on the hormonal control of the phenomenon are nearly non-existent to this day. This study has shown that chronic increases in corticosterone can cause *P. septentrionalis* to alter its maternal behavior. Specifically, individuals with chronic increases in corticosterone spend five-fold less time coiled around eggs, and spent significantly less time in contact with eggs. Although the difference of time spent in the nest was not significantly different, it was very near so. It is also interesting that the treatment caused significant differences in some behaviors, but not in others. The difference in frequency for active behaviors were not significant for any individual action, but was extremely small in all cases. It is possible that these behaviors occur naturally in low frequencies, and to observe a significant difference would be extremely difficult. There are very few significant correlations between behaviors, indicating that each behavior is independent. Again, however, the small sample size must be taken into consideration with these results, as some behaviors neared correlation. Interestingly, a positive correlation was found for experimental females SVL and absence from the nest. This is interesting because larger females would be expected to better attenuate their stress response and spend more time in the nest similar to control females, despite treatment. There is strong evidence to support the idea that long term stress can alter the behavior of individuals (McEwen and Wingfield, 2003). This study was designed to simulate an allostatic overload, and the results reflect the response of *P. septentrionalis* to such an overload.

Brooding Behavior

As previously stated, there have been several studies on the effect of corticosterone on the parental effort of birds. It can be somewhat difficult, however, to compare the parental effort of avian species to those of reptiles. Many birds are capable of true nest defense, and provide warmth to eggs by placing themselves over the nest. Studies on birds often consider parental effort to include nest attendance, defense, and thermoregulation of eggs. Brooding behavior for reptiles is markedly different. Venomous and large bodied snakes are likely capable of nest defense, and many pythons are capable of shivering thermogenesis, which allows them to thermoregulate eggs (Somma, 2003). Prairie skinks, however, are unlikely to be able to provide either of these benefits to developing eggs. Previous research has suggested that skinks might brood eggs in an effort to control the hydric environment (Somma and Fawcett, 1989). The results of this study do not suggest otherwise. However, in order to compare the results of this study to those performed on avian species, maternal effort of female skinks must be defined. In order to compare the results of this study to those in avian species, I consider time spent in contact with eggs to be maternal effort.

Comparison to Previous Studies

There have been nearly no studies on the effects that corticosterone might have on the maternal care of reptiles. However, it has been shown that female alligators kept in high stocking densities had higher plasma corticosterone, and exhibited lower nesting success (Elsey *et al.*, 1990). Although it is a viviparous lizard, research on the skink *Pseudemoia entrecasteauxii* has shown that corticosterone treatments lead to the female provisioning

more energy to her own body condition at the cost of her offspring (Itonaga *et al.*, 2012), an interesting viviparous analog to egg brooding species. As previously mentioned, this interaction has been studied mostly in birds. When definitions for parental effort are set for both *P. septentrionalis* and various birds, the results of this study show marked similarity to many studies on avian species (Angelier *et al.*, 2009; Groscolas *et al.*, 2008; Spee *et al.*, 2010; Chastel and Lormee, 2002; Silverin, 1998; D'Alba *et al.*, 2011; Ouyang *et al.*, 2012). In each of these cases, increases in corticosterone and decreases in prolactin led to decreased parental effort. In more extreme cases, nest abandonment occurred. Similarly to this study, (Angelier *et al.*, 2009 and Kitaysky *et al.*, 2001) found that black legged kittiwakes decrease nest attendance after receiving corticosterone implants. Female *Ficedula hypoleuca*, the pied flycatcher also respond to experimental increases in stress with a decrease in nest attendance and parental effort, provisioning less food to their offspring. Individuals with CORT implant raised fewer successful offspring than did control individuals (Silverin, 1986). The results of my research indicate significant decreases in some maternal behaviors, specifically coiling behavior and contacting eggs. These behaviors help to maintain a proper hydric environment, and so a decline in these behaviors in comparison to control individuals likely has negative effects on eggs in the same way that that the absence of a brooding bird would. Although there was no significant difference in the percent mortality of eggs, it is of interest to note that of the eggs that did hatch, all were from control females. The effects of corticosterone on maternal care in mammals have been studied as well, with similar results. Female mice treated with long term CORT increases show significant decreases in maternal care (Brummelte and Galea, 2010). Other vertebrates such as fish have similar responses to

stress treatment as well. Bass have not been shown to reduce parental effort in response to the stress hormone cortisol, but after prolonged increases abandon their nest similarly to birds (Dey *et al.*, 2010). In comparison to the large breadth of previous research, my study fits in well and supports the hypothesis that long term increases in CORT cause a reduction in maternal behavior of *P. septentrionalis*. The role of chronic CORT or cortisol increases in affecting parental and maternal behavior appears to be similarly conserved throughout vertebrate evolution.

Possible Limitations and Future Research

There were potential limitations with the project that may have affected the final results. Roughly two thirds of the way into the organism collection period, heavy construction occurred at the most populated collection site, Lake Burchard. This affected my ability to collect the amount of individuals desired, and resulted in a low sample size. It is likely that a significant effect may have been found for some behaviors with a larger sample size. In particular, the behavior of not being present within the nest was extremely close to significance. In addition, each individual was provided with a single shelter, where eggs were laid. It is possible that some individuals spent time in the nest due to the fact that it was the only shelter available. Further research could provide a second source of cover in order to alleviate this potential problem. Furthermore, response to long term CORT increases can vary based on the age and experience of individuals (Love *et al.,* 2004). It is not possible to know the ages of individual *P. septentrionalis* captured, so this may have had an effect.

Conclusions

Significant effects of corticosterone treatment were found on coiling and no contact behaviors. No significant effect of corticosterone was found on active pattern behaviors. I conclude that this research, while somewhat limited, provides information to help fill in the knowledge gap regarding the effects of long term CORT increases on maternal behavior of reptiles. *P. septentrionalis* exhibits a reduction of maternal behavior when faced with long term corticosterone increases, similar to other vertebrate taxa.

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