8-2013

Effects of Habitat Enrichment on The Stress Level of The Sit-and-Wait Predator Sand Boa (Gongylophis Colubrinus)

Jose Manuel Cantu Martinez
University of Texas-Pan American

EFFECTS OF HABITAT ENRICHMENT ON THE STRESS LEVEL OF THE SIT-AND-WAIT-PREDATOR SAND BOA (*GONGYLOPHIS COLUBRINUS*)

A Thesis

by

JOSE MANUEL CANTU MARTINEZ

Submitted to Graduate School of the
University of Texas-Pan American
In partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE

August 2013

Major Subject: Biology

EFFECTS OF HABITAT ENRICHMENT ON THE STRESS LEVEL OF THE SIT-AND-WAIT-PREDATOR SAND BOA (*GONGYLOPHIS COLUBRINUS*)

A Thesis
by
JOSE MANUEL CANTU MARTINEZ

COMMITTEE MEMBERS

Dr. Frederic Zaidan III
Chair of Committee

Dr. Rober Edwards
Committee member

Dr. Teresa Feria
Committee Member

August 2013

ABSTRACT

Cantu Martinez, Jose Manuel, *Effects of habitat enrichment on the stress levels of the sit-and-wait predator sand boa (*Gongylophis colubrinus*)*, Master in Science, August, 2013, 35 pages, 5 figures, 3 tables, references, 39 titles

Conditions in which the animals are housed vary according to animal use. Conditions unfamiliar or unsuited to organisms usually induce stress. Stress is mirrored by a hormone cascade resulting in glucocorticoids. Of the glucocorticoids corticosterone is regarded as the most important. The corticosterone levels of sand boas (*Gongylophis colubrinus*) were measured under enriched and barren soil and cage features. Enriched soil consisted of enough dirt to allow borrowing. Enriched cage featured consisted of a wooded ball. Barren soil consisted of newspaper as substrate. Barren cage features consisted of plane newspaper. Snakes were subject to treatment for two weeks. No significant difference was found between treatments of both soil and features using non-parametric Wilcoxon signed ranks test (P=.285, Z=-1.069,Fig 2). A low sample size prevented possible further analysis. However, data was suggestive of an increase in stress with cage features and a decrease in stress with enriched substrate.

DEDICATION

This Thesis is dedicated to my family. My father and mother, Jose Manuel Cantu Ramos and Gabriela Martinez Santos, which supported me in every sense of the word throughout this endeavor. My brother and sister, Alberto Cantu Martinez and Gabriela Cantu Martinez, and my wife Flor Sandoval. Without your support and affection this would not be possible

ACKNOWLEDGMENTS

I want to thank my committee, Dr. Frederic Zaidan, Dr. Robert Edwards, and Dr. Teresa Feria, for helping and supporting me throughout my scholar endeavor. Thanks to Dr. Michael Persans and Mr. Tom Eubanks for technical support. Thanks to Dr. Kristine Lowe and Dr. Frank Drrigil for advice and support throughout. I would also like to thanks Mr. Gilbert Martinez and Mr. Edwin Quintero for help during the different phases of this research. I would like to finally thank my wife Flor Sandoval for being at my side at every step of the way.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The raising and caring of animals has become a necessity to humans. Different species of livestock and fishes are reared in captivity to provide food for human populations (Brown et al. 2003). Endangered species are reared in zoological and botanical parks and government institutions to ensure their survival despite conditions of wild populations. Universities and scientific institutions keep species for research purposes (Kulpa-Eddy et al. 2005). Regardless of why species are kept in captivity, their well-being is not always prioritized.

Environmental conditions have long been recognized as important factors in the overall health of organisms (Benaroya-Milshtein et al. 2004). The degree to which the best conditions are upheld in captivity depends on the "use" of the animal. The "animal use" affects the conditions and environment the animals are held (Newberry 1995). In commercial rearing sites, the main objective is to get more product (e.g., eggs, meat, etc.) per unit capital. Federal laws prohibit animal mistreatment (Langkilde and Shine 2005). But even with that protection, the conditions in which farm animals are raised are sometimes deplorable. As long as the objective is achieved. the stress and well-being of an organisms is a secondary priority. Enrichment, as a part of the animal well-being, is often forgotten. Habitat enrichment is usually done to increase output of product or to improve the public image of the product (Curtis 1993).

On the conservation side the short- and long-term well-being of animals before and after release is of utmost importance. Zoological institutions and their workers have long acknowledged that enrichment and human care plays a key role in the well-being of animals (Mellen and MacPhee 2001). In zoos, a maximization of habitat enrichment to exposure is often achieved (Akers and Schildkraut 1985). However, monetary constrains limit the ability of any zoological institution to grant every animal the full spectrum of enrichment.

For animals held in a laboratory, the motivations for enrichment yet again change. Animal health and well-being is required by animal care and use committees and federal legislation such as the Federal Animal Welfare Act (FAWA) (Kulpa-Eddy et. al. 2005, Newberry 1995, Lutz and Novak 2005). Most of these regulations impact higher vertebrates, especially mammals. Dogs are required to exercise and non-human primates are required to have "toys" (Kulpa-Eddy et. al. 2005). For reptiles, amphibians, and fishes, the mandatory conditions to keep an animal do not go beyond food, water, and enough space to exercise. Outside of mammals and avian reptiles, any form of enrichment beyond basic need is voluntary (Kulpa-Eddy et. al. 2005). Outside of legal reasons, funding and space limit the ability of researchers to accommodate and upkeep organisms in complexly enriched habitats. Well-being, by itself, is a strong motivator for high-quality captive conditions. Furthermore, data obtained from animals held in substandard conditions might be tainted by added stress or abnormal behavior (Newberry 1995, Lutz and Novak 2005, Langkilde and Shine 2006, Rosier and Langkilde 2011).

Stress and Stress Sources

The ultimate goal of any organism is to pass its genes to the next generation. In order to do so, an organism has to survive and cope with environmental dangers. Response to external stimuli can occur on the behavior or in the physiology of the organisms (Manzo et. al. 1994, Moore and Jessop 2003). Stress is mirrored in the activation of the hypothalamic-pituitary-adrenocortical complex and the sympathetic-adrenomedullar complex (Marashi et al. 2003, Moore et al. 2000, Moore and Jessop 2003). An increase in glucocorticoids is a classic marker of these complexes being activated in vertebrates (Belliure et al. 2004, Langkilde and Shine 2006). Of the glucocorticoids, corticosterone is usually the most important in reptiles, amphibians, birds, and other vertebrates (Langkilde and Shine 2006, Romero 2004). The concentration of corticosterone is measured in the fluid or tissue of the animal and it's correlated with stress levels (Marashi et al. 2003, Jones and Bell 2004). External and internal factors such as environment and physiology can affect the activity of these complexes (Manzo et. al. 1994)

The causes of stress are often not simple. The behavioral, physiological, and environmental context has to be taken into account when considering a source of stress (Moore and Jessop 2003, Jones and Bell 2004). On the behavioral context, it is important to consider a species life history. Intra- and inter-specific competition, as well as prey interactions, can cause stress levels to fluctuate (Smith and John-Alder 1999). Species that show intraspecific competition will show an increase in corticosteroids when exposed to a member of another species (Langkilde and Shine 2006). Such an increase in corticosterone might not be present in social species. Smith and John-Alder (1999) found that eastern fence lizard (*Sceloporus undualtus*) in the breeding season (high testosterone) showed aggression toward conspecific lizards. Such aggression was not found outside of the breeding season. Time of peak hormone

secretion is correlated with the organism's life history (Chan and Callard 1972). In diurnal mammals. hormone secretions are at their peak in early morning and decline throughout the day with a low at midnight (Chan and Callard 1972, Zolovick et. al. 1966). Chuckwallas (*Diposaurus dorsalis*) were found to have a peak hormone secretion at 4 p.m. with a low at 4 a.m. (Chan and Callard 1972).

Physiological context applies to all organisms regardless of life history. The effect of stress can be easily seen on the behavior and physiology of an organism. Reproductive behaviors are most times reliant on hormone levels (Zaidan et al. 2003). A change in hormone levels can alter the reproductive patterns of an organism or stop them altogether (Moore et al. 2003). Furthermore, stress can alter energy allocation patterns and escape behaviors, potentially decreasing an organism chances for survival (Wingfield et al. 1998, Moore et al. 2003). Physiological stress can be related to the increase or decrease of corticosterone and testosterone (Moore et al. 2003). The correlation between these two hormones is species-specific and dependent on environmental factors.

It is in the environmental context that humans can play a big role. The baseline level of stress depends on the conditions the animal is held in and other variables to which the animal is exposed. Recent research has shown that seemingly harmless practices put a lot of stress on captive organisms. Moore et al. (1991) found that both short-term collecting and long-term housing on foreign enclosures increased corticosterone and decreased testosterone. Langkilde and Shine (2006) found that common practices like microchip implantation increased stress hormones more than less used methods like toe clipping for marking lizards. Furthermore, the exposure to an unfamiliar environment showed similar corticosterone responses, as a seemingly traumatic event. like a tail being broken or a blood sample taken in lizards (Langkilde and Shine

2006). Other factors, like reproductive behavior and immune responses, are also related to stress physiology (Jones and Bell 2004)

Most experiments measure either physiological outcome (e.g. change in corticosterone levels) or behavioral outcome (e.g. change in time performing an action). On the experiments researching corticosterone levels, a variety of methods have been used. Renee and Lankilde (2011) took a blood sample from the postorbital sinus of *Sceloporus undulatus*. Langkilde and Shine (2011) follow a similar procedure on *Eulamprus heatwolei*. Corticosterone was later assessed by levels in blood plasma. Case et al. (2005) assessed the corticosterone levels of eastern box turtles (*Terrapene ornata*) from feces.

It is widely accepted that stress causes an increase in glucocorticoid hormones like corticosterone in reptiles (Moore et al. 1991, Moore et al. 2000, Jones and Bell 2004). Sources of reptile stress have been documented previously (e.g., . Moore et al. 1991, Langkilde and Shine 2006, Case et al. 2005). Most of these studied research interactions of enrichment and stress without sharing a basic habitat structure. Individual sources of stress and their interactions still remain poorly understood in reptiles.

History of habitat enrichment

Starting on the early 1900s Robert Yerkes proposed an approach for well-being of captive animals based on research with non-human primates. He stated that primates need objects to play and work to promote well-being (Kulpa-Eddy et. al. 2005, cited in Shepherdson 1998). This introduced the concept of enrichment to an otherwise barren, minimalistic environment.

Hedinger was another of the pioneers on habitat enrichment. Hedinger pointed out the significance of enrichment, maintenance, and diet regimes on animal well-being (Hediger 1950, 1969). These notions drastically changed the views of animal husbandry at the time. Having a large number of animals in close quarters was no longer acceptable without proper care. Proper species-specific care was a novel idea at the time. The behavioral psychologist, Allan Neuringer, showed that when given the opportunity, animals will prefer food supplied in a non-constant regime over food constantly and predicatively supplied (Neuringer 1969). This showed that animal husbandry is more complex than simply providing food and water to an animal and that animal relationships and interactions are a complex phenomenon. Both Hedinger and Neuringer gave the new area of animal well-being a foundation.

Debra Forthman-Quick discussed the different ideas about animal husbandry and care. Forthman-Quick (1984) suggested that technology should be used in unison with passive techniques of enrichment to an animal's environment. At that time, animal enrichment began a transformation from mainly a zoo practice to a widely used science. In 1985, two new amendments to the (FAWA) reshaped enrichment: exercise for dogs and enhanced environments that promote psychological well-being for non-human primates (Kulpa-Eddy et al. 2005). Shepherdson (1998) defined environmental richness as the giving of stimuli necessary for the animal to reach optimal psychological and physiological well-being. Lutz and Novak (2005) presented another possible enrichment factor: social interactions. A solitary species might not behave normal when introduced with conspecifics. Short and long term stress, as well as violence, and lack of appetite might be triggered by this. On the other hand, social species might benefit from social interaction, exhibiting more normal behavior.

A natural environment alongside proper social environment could help animals exhibit more natural behaviors (Poole 1992, Lutz and Novak 2005), reduce stress (Case et al. 2005), and promote overall well-being and health (Case et al. 2005, Rosier and Langkilde 2011). On all definitions, enrichment falls close to the idea of mimicking the organism's natural environment and natural history to provide physical and psychological well-being (Case et al. 2005, Newberry 1995, Lutz and Novak 2005).

Psychological and physical well-being, ideas presented by Sheperdosn (1998), are normally attributed to different animal groups. Psychological well-being and behavior have been well studied in animals with high mental functions like mammals and birds (Almli and Burghardt 2006). Federal mandates in FAWA order for enriched environments in birds, aquatic mammals, and non-human primates (Kulpa-Eddy 2005). Physiological well-being is usually studied in animals with lower brain function such as reptiles and fishes. Overall it has been shown that habitat enrichment increases memory, adaptive and behavioral plasticity, long-term survivorship, overall health, and lowers stress indicators (Brown et al. 2003, Benaroya-Milshtein et al. 2004, Lutz and Novak 2005, Almli and Burhardt 2006, Case et al. 2005).

Enrichment in reptiles

It is a common belief that the captive requirements of reptiles are minimal. These ideas come from the notion that reptiles are highly adaptable and tolerant to a range of condition (Case et al 2005). With these notions in mind, most of the practices and care provided to reptiles are based on intuition (Langkilde and Shine 2006). It is usual in the scientific community to keep reptiles in conditions without enrichment. Newspaper as substrate and cover and a water bowl are commonly accepted conditions for reptiles. Similar conditions are used in herpetoculture.

Housing a large number of reptiles with enriched habitats can be problematic for monetary and for upkeeping reasons. Furthermore no federal mandate for reptile enrichment is in place (Kulpa-Eddy et. al. 2005). Corticosterone has been found to severely affect sexual performance and normal hormone control in several taxa (Manzo et. al. 1994). Thus avoiding such stress would promote overall well-being.

Intuition becomes unreliable when dealing with organisms phylogenetically distant to humans (Langkilde and Shine 2006). In recent years, several authors have started to research the validity of common practices (e.g., Langkilde and Shine 2006, Almli and Burghardt 2006). The results from these authors have proven that reptiles might not be as flexible and resilient with their environmental condition as it was believed.

Case et al. (2005) studied the effect of enrichment on behavior and stress hormones of eastern box turtles (*Terrapene carolina*). It was found that *T. carolina* housed in enriched environments had a significantly lower stress level than turtles housed on barren environments. Furthermore, the percentage of time spent on escape behavior was also significantly decreased with an enriched behavior. Almli and Burghardt (2006) tested the ability of a group of rat snakes (*Elaphe obsoleta*) to complete cognitive tasks while previously exposed to barren and enriched environments. Snakes exposed to an enriched environment were faster to finish the objectives, grew faster, and gained more mass than snakes exposed to a barren environment (Almli and Burghardt 2006). Langkilde and Shine (2006) studied the effect of commonplace laboratory practices on the corticosterone level of southern water skinks (*Eulamprus heatwolei*). The study found that placing a lizard in a foreign enclosure raises the corticosterone level more than twofold compared with control lizards. Sex, as a variable, showed a great importance in hormone levels. Other studies have corroborated that sex can drastically change the hormonal change

given a stressor (Moore et al. 1991). Female lizards showed twice as much corticosterone regardless of treatment when compared with male lizards (Moore et. al. 1991). These studies helped shed a light on commonplace practices on reptiles and their validity in regards to animal well-being.

Jones and Bell (2004) found that the corticosterone levels of white skinks (*Egernia whitii*) did not significantly change over a four week captive period. Skinks were held on pelleted paper substrate to allow for burrowing. A terracotta base was also provided for basking (Jones and Bell 2004). Manzo et al. (1994) investigated corticosterone and testosterone levels after long term capture in Italian wall lizard (*Podarcis sicula*). It was found that corticosterone had a peak after 24 hours of capture and followed with a decline ending 7 days later. After 8 days of treatment, *P. sicula* corticosterone levels came back close to levels at capture. Housing substrate or habitat features were not specified on this study (Manzo et. al. 1994).

The little information available on environmental enrichment on reptiles points to the fact that the environment has more to do with well-being than common intuition dictates. Few experiments have looked at the effect of enrichment with regard to corticosterone. The few papers that do look at it, focus on an overall effect of enrichment (i.e., enriched vs. barren). The effect of each particular variable on enrichment (i.e., soil, features in the environment, hiding space, etc.) has not been properly assessed.

Previous research on enrichment has been done mainly on organisms with an active strategy (e.g., *Elaphe* and *Sceloporus*). Foraging mode (the continuum between sit-and-wait and active) drastically changes the time and energy budget of organisms (Anderson and Karasov 1981). Sit-and-wait predators forage less than active, spending less energy and time interacting

with the environment. Both strategies have advantages and disadvantages. Active predators have a much higher metabolic rate that demands more food (Anderson and Kasarov 1981). More food would imply more time foraging. More time foraging increases interaction with features in the environment. A sit-and-wait predator would have much lower metabolic demands. Soil/floor conditions are of utmost importance given that sit-and-wait species rely on camouflage and burrowing. Interaction with environmental features is usually not as important as with active predators. Thus each strategy brings an attachment to either environmental features or floor/soil conditions. Such conditions could bring along psychological attachments to the environment that when broken promote stress. Overall the effect of enrichment on a sit-and-wait predator has not been determined.

Sand boas (*Gongylophis colubrinus*, formerly *Eryx*) are medium sized fossorial snakes inhabiting North-East Africa from Egypt to Tanzania (Tokar 1995, 1996). *Gongylophis colubrinus* follows a sit-and-wait predatory strategy. It burrows into the sand and waits for a prey item to walk close to them and then proceeds to bite, constrict, and consume their prey. The natural habitat of *G. colubrinus* includes arid coast plains, foothills, and deserts (Tokar 1996). The common name sand boas come from their preference of sand and sandy soils. Being a natural burrower, *G. colubrinus* has developed adaptations to living in such environments. Klein and Deuschle (2010) found that the outer scale layer of *G. colubrinus* show a significantly higher thickness compared with more inner layers of scales. Adaptations, such as this, have been found in other sand swimming organisms like the sand skink (*Scincus scincus*) (Rechenberg and El Khyari 2004). Such adaptations allow the reptiles to withstand the friction from ‘swimming’ in the sand (Klein and Deuschle 2010). A question follows from having such a strong physical

adaptation to their environment: does this physical adaptation have psychological attachments? And if they do: do deviations from natural conditions induce stress on the organisms?

The goal of this research is to assess whether commonplace practices used in reptiles impose unnecessary stress on the organisms and whether enrichment lowers such stress. In order to do so, the effects of soil and object enrichment on the stress hormone corticosterone were studied in *G. colubrinus*.

I hypothesized that *G. colubrinus* exposed to enriched soil will show a significantly lower level of corticosterone compared to barren housed snakes. This because a deep soil in which to borrow would mimic *G. colubrinus* natural habitat. I also hypothesized that object enrichment will not decrease basal levels of corticosterone. It is known that interest in foreign objects wanes with time (e.g., Lutz and Novak 2005). Thus, the long-term exposure to a foreign object will theoretically not lower stress indicators.

CHAPTER II

MATERIALS AND METHODS

Pre-treatment

Gongylophis colubrinus were used as a model organism. *G. colubrinus* are easily kept in captivity and follow a sit-and-wait predatory strategy. Tweleve *G colubrinus* were used as the model organism (3 males, 9 females). Snakes were housed individually in plastic containers measuring 40x27x13cm. A temperature of 24-25 °C was held through the experiment. A natural photoperiod for Hidalgo County, Texas, at the time of the experiment, was used (11L:13D). All snakes were in a post-digestive state at the beginning of the experiment. Snakes were kept on a rack separated from each other. Snakes were kept at the same position in the racks to prevent variation in results due to positioning factors. Snakes were divided randomly for treatments by flipping a coin. During treatments no cage was washed to avoid extra stress from new odors. Urates and feces were removed between changes of substrate when found.

Blood samples were taken using a 1ml syringe (25Gx1 (0.5 mm x 25 mm). The syringe was inserted below the 12^{th} post-cloacal scale to prevent hemipenal/hemiclitoral damage. The blood vessel from which the blood samples were acquired is the caudal artery or vein. A second and third puncture were done one scale down anterior from the previous puncture wound if no blood was present on the previous puncture. In the instance where no blood was collected on the

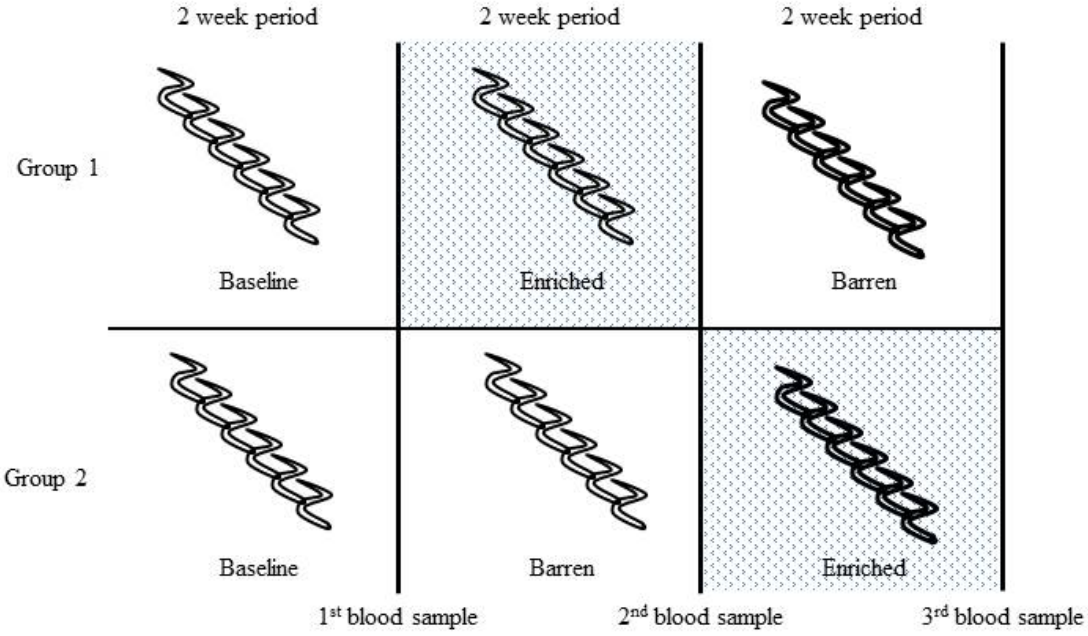
syringe, a 70 μL heparinized micro capillary tube was used to collect blood and fluids from around the puncture wound. Samples were collected between 1000 and 1400 CST. Blood samples were centrifuged and then stored at -80 °C prior to assay.

Soil enrichment

Phase I researched the effects of soil enrichment in the stress hormone corticosterone. Snakes were randomly selected to groups. One group was exposed to enriched conditions and the other to barren conditions simultaneously for a period of two weeks. Barren condition cages consisted of folded newspaper as substrate and a corrugated piece of newspaper as overhead cover. Enriched environments consisted of 4-6 cm of loose dirt as substrate. On enriched conditions, snakes had enough dirt to borrow completely into it. These settings were chosen to emulate common laboratory settings. Barren conditions were chosen to emulate common reptile housing conditions. No other objects were present in the cages during the experiment (Fig 1). All snakes were set on barren conditions for a 2-week period prior to the beginning of the experiment. A blood sample was taken after the 2-week period and before the first exposure to a treatment to measure basal corticosterone levels. Half of the snakes (n=6) were exposed to enriched conditions and the other half remained under barren conditions. Snakes were exposed to a treatment for a period of 2 weeks. The 14 day period was chosen to ensure an acclimation to conditions without short term stress tainting the data. This ensured a true reading of corticosterone under these conditions. After the first period, a second blood sample was taken from the snakes. The snakes were immediately switched from enriched to poor conditions and vice versa. The second exposure also had a duration of 2 weeks. At the end of the second exposure a final blood sample was taken.

All the blood samples were taken within a time frame of 5 minutes starting with needle insertion. This was done in an attempt to minimize stress added from handling the animal. Substrate on barren cages was switched for a new newspaper between every treatment. The substrate change allowed to account for the stress of being handled and placed in a foreign soil between treatments. Phase I ran from November 25 to December 23, 2012.

Figure 1.- Experimental design showing a caricature of snakes. Dotted squares represent enriched conditions. Blank squares represent barren conditions. Each square represent a 2-week period.



Toy enrichemnt

Phase II researched the effects of object enrichment in the stress hormone corticosterone. Barren conditions consisted of folded newspaper as substrate and a corrugated newspaper as overhead cover. Enriched conditions consisted of folded newspaper as substrate, corrugated newspaper as overhead cover, and a wooden ball (circumference = 14.5 cm, 35.4 g). Wooden balls were previously submerged and covered in paraffin to avoid any residual odors. Blood sampling followed the same protocol used in Phase I. Phase II ran from February 11 to March 25, 2013

Sample analysis

Blood samples were sorted and thawed. To calculate the total amount of sample to be used, the amount of plasma on the microcentrifuge capillary tube was measured to the nearest millimeter. Knowing the total capacity of the microcentrifuge capillary tubes (70 μL) and the total length of the tubes, a simple mathematical equation was used. Blood plasma length (mm) was multiplied by the total volume of the tube and then divided by the total length to get the sample volume. Blood plasma was pushed out of the microcentrifuge capillary tube using a plunger and poured into a 1.5ml microcentrifuge tube. Samples were diluted 10-fold when possible. Some samples had to be further diluted 5-fold to have the necessary volume for the hormone essays The hormone concentration of samples were essayed using a Corticosterone EIA Kit (Cat. No. 500655, Lot No. 0429055). The protocol provided by Cayman was used to run the essay.

Validation

The validity of using the Cayman corticosterone kit to measure blood plasma corticosterone on *G. colubrinus* was tested. Parallelism was tested by replicating the serial dilution standards protocol using a sample from the studied species. Quantitative recovery was tested in two steps. First, a sample's concentration of hormone for a sample was tested. Second, an known amount of hormone was added to the previous sample and subsequently tested. The difference in hormone should be equal to the additional hormone added. A high concentration of corticosterone obscured analysis. Thus, light absorbance units from the plate reading were using to analyze the validation. An inverse correlation existed between absorbance units and corticosterone levels. The cottonmouth (*Agkistrodon piscivorus),* another sit-and-wait predator, was also analyzed for comparison.

Cayman kit standards showed a linear correlation of 0.946 Light Absorption Units (LAU) (Fig. 2). *G. colubrinus* showed a linear correlation of 0.0888 LAU. *Agkistrodon piscivorus* showed a linear correlation of 0.6018 LAU. The very poor positive correlation of *G. colubrinus* indicates that something in the sample was preventing the kit to work properly. A correlation like *A. piscivorus'* was expected but not obtained. Further research is needed to corroborate the validity of the data on this paper with this validation results. Furthermore, explanation on what causes such obscurity of results could prove very useful.

Gongylophis colubrinus showed 0.623 LAU on the quantitative recovery (Fig. 3). After spiking, the sample read 0.611 LAU. *A. piscivorus* showed 0.287 LAU on the original sample and 0.299 LAU on the spiked sample. The desired results are not found. *G. colubrinus* showed a higher LAU on the spiked sample, indicating a lower hormone concentration. These results showed the

expected pattern. *G. colubrinus* showed a decrease in LAU after the spiking, which mirrored an increase in hormone concentration. In contrast *A. piscivorus* showed a small decrease in hormone concentration. A similar pattern was also seen in the diamondback waternskae (*Nerodia rhombifer*)(unpublished data). *Gongylophis. colubrinus* data fitted the desired pattern. However, further validation would clarify whether or not something in its blood obscures the proper functioning of the kit.

Figure 2.- Linear correlation of the light absorbance of the serial dilution. Rhomboids represent Cayman standards. Squares represent *Gongylophis colubrinus*. Triangles represent *Agkistrodon piscivorus*.

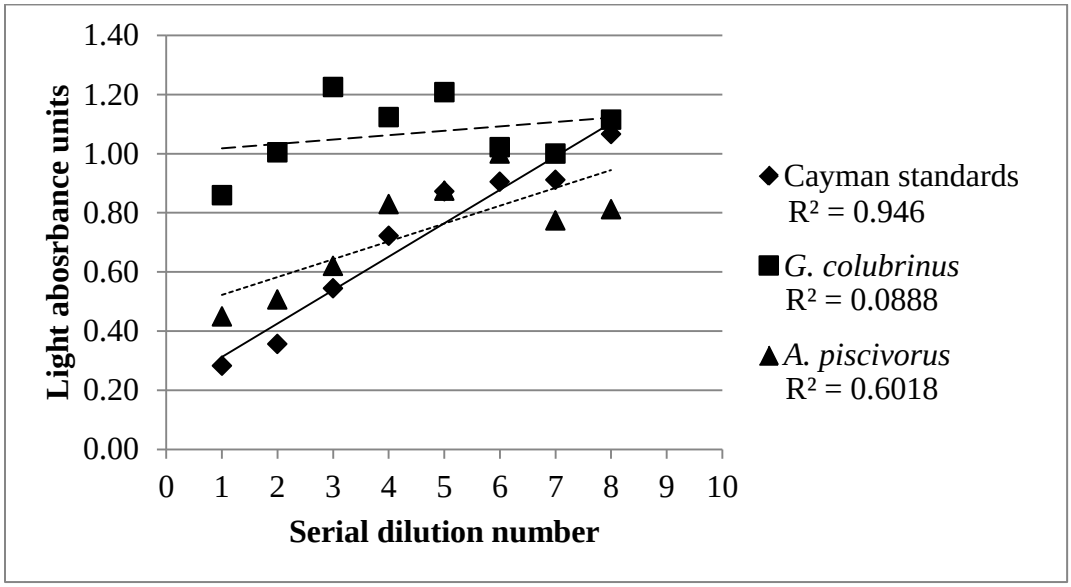
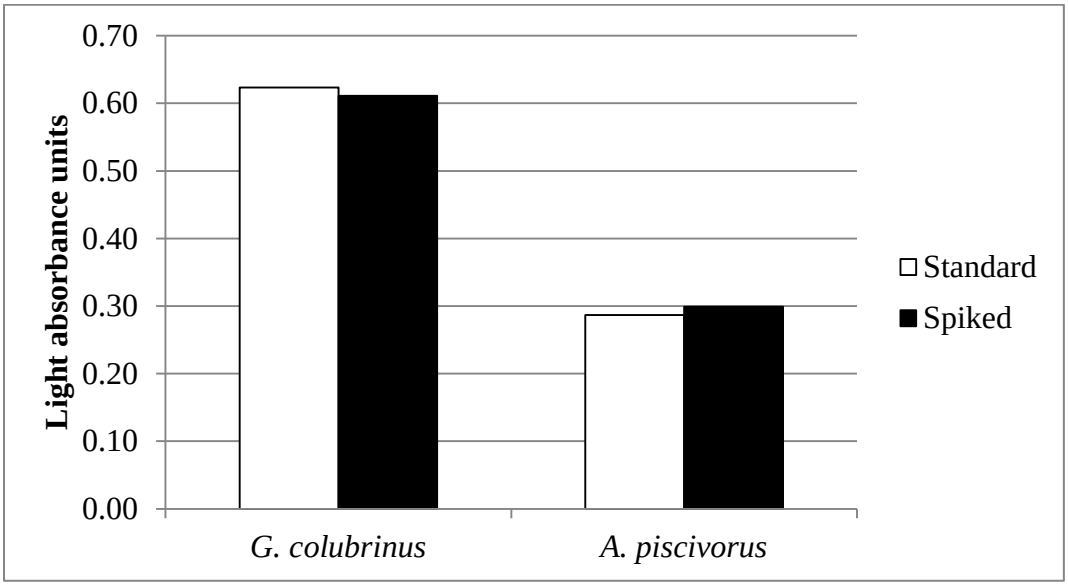


Figure 3.- Quantitative recovery of sample. White bars represent standard unaltered samples. Black bars represent samples that were spiked with extra corticosterone.



CHAPTER III

RESULTS

Soil enrichemnt

No samples were obtained from one snake. Four snakes had only one sample point and were excluded from the final analysis. A total of 9 snakes had viable samples and were used for calculations (3 males, 6 females). Males had an average .3668 ng/ml of corticosterone at the beginning of the experiment. Males showed an average 4.608 ng/mL of corticosterone when exposed to an enriched environment (n=3) and 11.422 ng/ml of corticosterone on a barren environment (n=2, Table 1). Females had an average baseline corticosterone ratio of 3.457 ng/ml (n=6). After exposure to an enriched environment females had an average 4.896 ng/ml (n=4). When exposed to a barren environment females showed an average 10.771 ng/ml (n=4, Table 1). Due to large difference in corticosterone levels between snakes, the percent change between treatments and baseline was used for calculations (Table 2). Due to the small sample sizes, non-parametric statistics were used. No significant difference was found between treatments using a Wilcoxon signed ranks test (P=.285, Z=-1.069, Fig. 4).

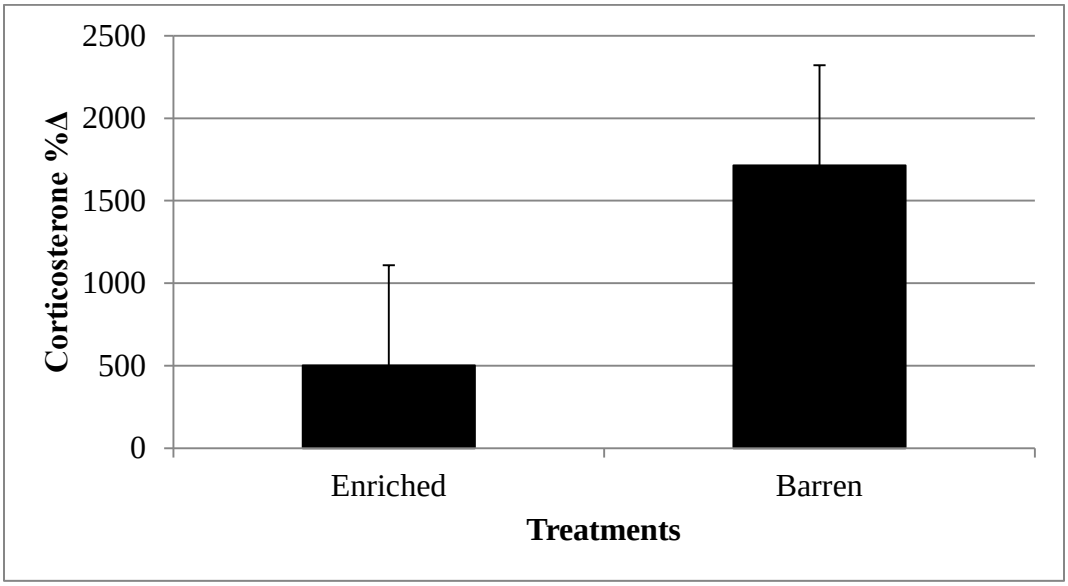
Table 1.- Average corticosterone levels for snakes subject to enhance and barren substrates.

	Corticosterone (ng/ml)		
	Base	**Enriched**	**Barren**
Males	.3668	4.608	11.422
Females	3.457	4.896	10.771

Table 2.- Percent change in corticosterone from baseline levels to treatment in Phase I (substrate).

Snake #	**Sex(M1, F2)**	**Enriched %Δ from base**	**Barren %Δ from base**
2	Male	626.9	338.7
5	Male	2201.9	
1	Female	-32.6	
6	Female	319.0	-32.5
8	Female		709.0
9	Female	-62.1	
12	Female	-40.4	40.0
16	Female		7517.9

Figure 4.- Average change in percentage from baseline corticosterone between treatments and in Phase I (Substrate).



Toy enrichment

No samples were obtained from one snake. Five snakes had only one sample point and were excluded from final analysis. One snake had no base level of corticosterone so it was not used for calculations. A total of 7 snakes had viable samples and were used for calculations (1 males, 6 females). Male corticosterone baseline was. 957 ng/ml (n=1). After exposure to an enriched environment males showed an average corticosterone of 4.314 (n=2). After exposure to a barren environment, one male showed a corticosterone level of 1.750 ng/ml (n=1, Table 3). Females showed an average corticosterone level of 3.657 ng/ml (n=6). After exposure to an enriched environment female corticosterone averaged 5.516 ng/ml (n=6). Females exposed to a barren environment showed an average of 1.863 ng/ml (n=4, Table 3). Due to large differences in corticosterone levels between snakes, the percent change between treatments and baseline was used for calculations (Table 4). No significant difference was found between treatments using a Wilcoxon signed ranks test (P=.715, Z=-.365, Fig. 5).

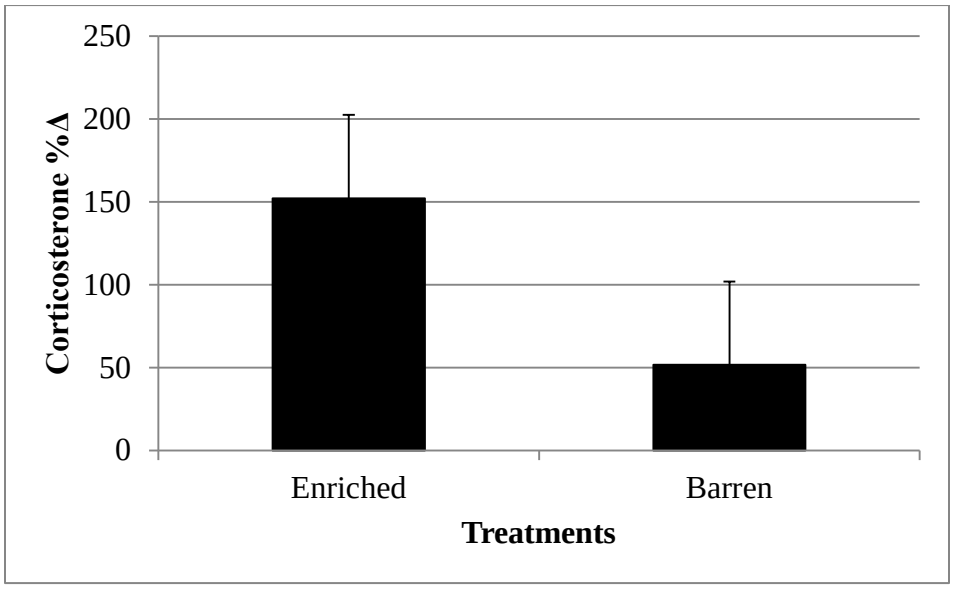
Hypothesis testing

Based on the results, I reject my first hypothesis. Environmental enrichment did not significantly decrease corticosterone levels compared with barren settings. I failed to reject my second hypothesis, finding that toy enrichment did not significantly decrease corticosterone levels compared to barren setting

Table 3.- Percent change in corticosterone from baseline levels to treatment in toy enrichemnt

Snake #	Sex(M1, F2)	Enriched %Δ from base	Barren %Δ from base
7	Male	302.7	
1	Female	123.7	
3	Female	-7.6	
8	Female	-19.3	10.3
12	Female	90.6	118.3
13	Female	423.2	-60.2
15	Female	-27.2	138.3

Fig. 5.- Average percent change in corticosterone between treatments and baseline corticosterone levels in toy enrichemnt.



CHAPTER IV

DISCUSSION

Experimental design

Blood samples were more difficult to obtain than previously predicted. The strong constrictor muscles on the tail of the *G. colubrinus* prevented blood flow to the tail area. It was noted that when snakes relaxed their tails, blood rushed to the puncture sites on several occasions. The sampling success rate was 59.53% including all attempted samples.

The odor of conspecific individuals is something to consider in future research. Although visual contact was impossible, olfactory contact could have occurred. This might have added stress to the snakes. For the purpose of this research, this variable was ignored. Since the baseline mark for corticosterone was taken with the same experimental design as the blood samples from treatments, the effect of olfactory contact was constant throughout the experiment.

Langkilde and Shine (2006) found that *E. heatwolei* exposed to predator scent increased their blood corticosterone levels. In a laboratory setting with multiple species present, this physiological response could be a factor.

My research was focused on the long-term well-being of the animals. Langkilde and Shine (2006) found that after two weeks from the time a stressor was given to their organisms,

stress levels were highest. After 2 weeks, corticosterone levels started to fall. The effect of short-term stressors was best sampled at 1 hour after a stressor (2006). The two week exposure to treatments used in this study goes in parallel with the findings of Langkilde and Shine (2006)

Several papers have shown a change in corticosterone with temporal variation (e.g., Chan and Callard 1972, Langkilde and Shine 2006). Jones and Bell (2004) found that variations within the active period of the skink, *Egernia whitii*, did not significantly change corticosterone levels. *Gongylophis colubrinus* were observed exploring their enclosure at morning hours (pers. obs.). All samples were conducted at the same time throughout the research. Thus it is assumed that sampling time had no major effect on the results of this experiment.

Effect of enrichment on corticosterone

This research findings did not show that enrichment helps lower the stress of animals in captivity. It is important to consider the sit-and-wait strategy used by *G. colubrinus*. Given that *G. colubrinus* is not only a sit-and-wait predator, but also a burrower, the non-effect of soil enrichment is surprising. Data is suggestive of an effect of soil enrichment (Fig. 4). A larger sample size might prove soil enrichment to be significant.

The lack effect of toy enrichment is not surprising given that its sit-and-wait strategy guarantees a minimal interaction with the habitat's features. Nevertheless, the low sample size grants room for further investigation. More natural habitat features could prove to significantly affect corticosterone, in contrast with man-made objects. The problem with this approach would be the consistency of such features (e.g., equal size, weight, etc). Data was suggestive of an increase in corticosterone levels with enrichment. A larger sample size could prove this to be significant.

A small sample size prevented an analysis separated by sexes or sex effects. The results of this research could not validate Rosier and Langkilde (2011) due to non-significance due to the small sample size. Rosier and Langkilde (2011) found no significant effect of enrichment on *S. undualtus* survival, behavior, or basal corticosterone levels.

Ramifications of this research

It should be considered that an increase in stress indicators is not a phenomenon unique to laboratories. Organisms are subject to several stressors in their natural habitats from such factors as predators or drought. Thus stress suffered under captive conditions might not be as bad for organisms as thought. Nevertheless, the scientific objective should always be to provide the clearest data possible with the least suffering from the organism.

Although suggestive to the contrary, enriched soil failed to significantly lower corticosterone levels compared to barren soil. These results suggest that current housing practices in herpetoculture and on the scientific community are valid for sit-and-wait predators. A barren, easy to maintain, habitat did not promote an increase in corticosterone and stress in sit-and-wait predators. Given the low sample size and uniqueness of *G. congylopus'* lifestyle, more research is needed on this subject to further understand this phenomena. A species specific approach should always be taken when considering housing conditions for an animal.

Although not significant, enrichment in the form of toys, suggested an increase in corticosterone in *G. colubrinus*. Given the spherical shape of the object, snakes were able to move the toy while moving themselves. The movable object inside their enclosures might have increased the corticosterone in this case.

Other studies studying effects of long term capture on corticosterone have been done before. These studies did not focus on the housing conditions but rather on the overall effect of long term capture. Jones and Bell (2004) housed *E. whitii* in pelleted paper with a basking platform. They found found that after a month, corticosterone levels were back to normal baseline levels. Based on their description of their environmental set up, Jones and Bell (2004) followed my definition of enriched environment. These results would go alongside the results of this research. Although non-significant, the difference between enriched and barren treatments is suggestive of a pattern. Other papers, like Manzo et al. (1994), did not specifiy captive conditions, thus comparisons are not possible.

No water or food was provided during the experiment. This might have added stressors to the experimental settings obscuring the results. Other researchers (e.g., Manzo et.al. 1994, Jones and Bell 2004) feed their research animals during their studies. In retrospect, this approach might be the best. Langkilde and Shine (2006) saw an increase in corticosterone for a period of an hour after a stimulus was applied. Two hours after the stimuli, corticosterone levels were back to normal. If this pattern holds for feeding, the feeding of animals while the research is going on should not obscure the results. Further, the obscuring of the data as a result of the dehydration might outweigh any benefits.

Physiological and hormonally oriented research must account for the physical state of the animal prior and during treatment. Avoiding any variable outside of the scope of the experiment is a priority. Some species adapt well to captivity, while others do not (Jones and Bell 2004). Cree et. al. (2000) found no significant difference in the corticosterone levels of eastern bearded dragons (*Pogona barbata*) after 3.5 or 24 hours. Most other research suggest a significant increase in corticosterone levels (i.e. Manzano et. al. 1994, Langkilde and Shine 2006). It is important to find a species that shows little to no stress response to common stressors. Eventually such species could become model organisms for the field of herpetology.

Future research

A larger and more comprehensive study of soil and toy enrichment should be done. *Gongylopus colubrinus* is not a good candidate for similar future studies. The difficulty to obtain samples overshadows any benefit of the organism. Other more abundant sit-and-wait predators like *Agkistrodon piscivorus* and *Agkistrodon controrix* seem to be better candidates. These species are abundant in the field and readily collectable on a larger scale. Furthermore previous studies have shown that blood samples from *Agkistrodon* species are readily taken (Zaidan et. al. 2003).

A complete assay of stressors on an organism would further clarify the role of enrichment. Knowing all the stress sources (i.e., dehydratiation, temperature change, etc.) could help model future legislation regarding mandatory enrichment on reptiles. On the research perspective, knowing what source of stress is most important could help scientists maximize their resources when it comes to housing animals. If a complete assay of stressors is desired, olfactory contact should also be taken into account.

Due to the limited sample size sex as a variable could not be assessed. Langkilde and Shine (2006) found that female *E. heatwolei* showed an increase in corticosterone at least threefold compared to males. This is a significant difference and should not be neglected in future research. A larger sample size with a good ratio of males to females should allow for clarification of this phenomena when it comes to soils and toy enrichment.

The effect of soil and toy enrichment on an organism with an active strategy should be assessed. Given that an active strategy implies a higher interaction with the environment, the effects of enrichment on organisms with this strategy could further clarify the use of enrichment and its role.

An active forager like *Pituophis* of *Thamnophis* could have other advantages from the research perspective. Less invasive means of measuring stress response like breathing rates have been found to correlate with blood corticosterone levels (Langkilde and Shine 2006). A large active organism would allow for easily observed respirations per minute. A comparison of traditional glucocorticoid levels with respiratory rate could change the standard way stress is measured in snakes (Langkilde and Shine 2006).

LITERATURE CITED

Akers J.S. and Schildkraut D.S. 1985. Regurgitation/reingestion and coprophagy in captive gorillas. Zoo. Bio. Vol 4, Pp 99-109

Almli L.M. and Burghardt G.M. 2006. Environmental Enrichment Alters the Behavioral Profile of Ratsnakes (*Elaphe*). Journal of Applied Animal Welfare. Vol 9, No 2, Pp 85-109

Anderson R.A. and Karasov W.H. 1981. Contrast in Energy Intake and Expenditure in Sit-And Wait and Widely Foraging Lizards. Oecologica. Vol 49, No 1, Pp 67-72Belliure J.,

Benaroya-Milshtein N., Hollander N., Kukulansky T., Raz N., Wilf A., Yaniv I., and Pick C.G. 2004. Environmental enrichment in mice decreases anxiety, attenuates stress responses and enhances natural killer cell activity. European Journal of Neuroscience. Vol 20, Pp 1341-1347.

Brown C., Davidson T., Laland K. 2003. Environmental enrichment and prior experience of live prey improve foraging behavior in hatchery-reared Atlantic salmon. Journal of Fish Biology. Vol 63, Pp 189-196.

Case B.C., Lewbart G.A., and Doerr P.D. 2005. The physiological and behavioral impacts of and preference for an enriched environment in the eastern box turtle (*Terrapene Carolina Carolina*). Applied animal behavioral science. Vol 92, Pp 352-365

Chan S. and Callard I.P. 1972. Circadian rhythm in the secretion of corticosterone by the desert iguana *Diposaurus dorsalis*. Gen. Comp. Endocrinol. Vol 18, pp 565-568

Cree A., Amey A.P., and Whittier J.M. 2000. Lack of consistent hormonal responses to capture during the breeding season of the bearded dragon, *Pogona barbata*. Comp. Biochem. And Physiol. Part A. Vol 126, No 2, Pp 275-285

Curtis S.E. 1993. The physical environment and swine growth. In: G.R. Hollis (Editor), Growth of a pig. CAB International, Wallingford, Uk. Pp 93-105

Forthman-Quick D. 1984. An integrated approach to environmental engineering in zoos. Zoo Biol. Vol 3, Pp 65-78

Hediger H. 1950. Wild animals in captivity. London: Butterworths. P 207

Hediger H. 1969. Man and animal in the zoo. London: Routledge and Kaegan Paul. P 303.

Jones S.M. and Bell K. 2004. Plasma corticosterone concentrations in male of the skink E*gernia whitii* during acute and chronic confinement, and over a diel period. Comaprative Biochemistry and Physiology. Vol 137, Pp 105-113.

Klein M.G. and Deuschle J.K. 2010. Material properties of the skin of the Kenyan sand boa *Gongylophis colubrinus* (Squamata, Boidae). J. Comp. Physiol. Vol 196, Pp 659-668

Kulpa-Eddy J.A., Taylor S., and Adams K.M. 2005. USDA Perspective on Environmental Enrichment for Animals. ILAR journal. Vol 46, No 2, Pp 83-94

Langkilde T. and Shine R. 2005. Competition for crevices: interspecific conflict influences retreat-site selection in montane lizards. Oecologia. Vol 140, Pp 684-691

Langkilde T. and Shine R. 2005. How do water skinks avoid shelters already occupied by other lizards?Behaviour. Vol 142, Pp 203-216

Langkilde T. and Shine R. 2006. How much stress do researchers inflict on their study animals? A case study using a scincid lizard, *Eulamprus heatwolei*. Journal of Experimental Biology. Vol 209, Pp 1035-1043

Lutz C.K., and Novak M.A. 2005. Environmental Enrichment for Nonhuman Primates: Theory and Application. ILAR journal. Vol 42, No 2.

Manzo C., Zerani M., Gobbetti A., Maddalena D.F., and Angelini F. 1994. Is Corticosterone involved in the reproductive Processes of the Male Lizard, *Podacris sicula sicula*? Hormones and Behavior. Vol 28, Pp 117-129

Marashi V., Barnekow A., Ossendorf E., and Sachser N. 2003. Effects of different forms of environmental enrichness on behavioral, endocrinological, and immunological parameters in male mice. Horm. Beha. Vol 43, Pp 281-292

Mellen J., and MacPhee S.M. 2001. Philosophy of Environmental Enrichment: Past, Present, and Future. Zoo Biology. Vol 20, Pp 211-226

Meylan S., and Clobert J., 2004. Prenatal and postnatal effects of corticosterone on behavior in juvenile of the common lizards, *Lacerta vivipara*. J. Exp. Zool. Vol 301, Pp 401-410

Moore M.C., Thompson C.W., and Marler C.A. 1991. Reciprocal Changes in Corticosterone and Testosterone Levels following Acute and Chronic Handling Stress in Tree Lizard, *Urosaurus ornatus*. General and Comparative embryology. Vol 81, Pp 217-226

Moore I.T., Lemaster P., Mason R.T. 2000. Behavioral and hormonal response to capture stress in the male red-sided garter snake, *Thamnopis sirtalis parietalis*. Animal Behaviour. Vol 59, Pp 529-534

Moore I.T. and Jessop T.S. 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. Hormones and behavior. Vol 43, Pp 39-47

Neuringer A. 1969. Animals respond to food in the presence of free food. Science. Vol 166, Pp 399-401

Newberry R.C. 1995. Environmental enrichment: Increasing the biological relevance of captive environments. Applied Animal Behavior Science. Vol 44, Pp 229-243

Poole T.,B., 1992. The natural evolution of behavioral needs in mammals. Animal Welfare. Vol 1, Pp 203-220

Rechemberg I and El Khyari A.R. 2004. Der Sandskink der Sahara—Vorbild fur Reibungs- und VerschleiBminderung. Http://www.bionik.tuberlin.de/institute/festo04.pdf, Berlin.

Romero M.L. 2004. Physiological stress in ecology: lessons from biomedical research. Trends Ecol. Evol. Vol 19, Pp 249-255

Rosier R.,L., and Langkilde T. 2011. Does environmental enrichment really matters? A case study using the eastern fence lizard, *Sceloporus undualtus*. Applied Animal behavioral Science. Vol 131, Pp 71-76

Shepherdson D.J. 1998. Tracing a path of environmental enrichment in zoos. In: Shepherdson

D.J., Mellen J.D., Huchins M eds. Second Nature: Environmental enrichment for Captive Animals. Washington DC: Smithsonian Institution Press. Pp 1-12

Smith L.C. and John-Alder H. 1999. Seasonal Specificity of Hormanal, Behavioutal, and Coloration Responses to Within- and Between-Sex Encounters in Male Lizards. Hormones and Behaviour. Vol 36, Pp 39-52

Tokar A.A. 1995. Taxonomic revision of the genus *Gongylophis* Wagler 1830: *G. conicus* (Schneider 1801) and *G. muelleri* Boulenger 1892 (Serpentes Boidae). Tropical Zoology. Vol 8, No 2, Pp 347-360

Tokar A.A. 1996. Taxonomic revision of the genus *Gongylophis* Wagler 1830: *G. colubrinus* (L. 1758) (Serpentes Boidae). Tropical Zoology. Vol 9, No 1, Pp 1-17

Wingfield J.C., Maney D.L., Breuner C.W., Jacobs J.D., Lynn S., Ramenofsky M., and Richardson R.D. 1998. Ecological bases of hormone-behaviour interactions: the "emergency life history stage". Am. Zool. Vol 38, Pp 191-206

Zaidan F. III., Kreider D.L., and Beaupre S.J. 2003. Testosterone cycles and reproductive energetics: Implications for Northern Range Limits of the Cottonmouth (Agkistrodon piscivorous leucostoma). Copeia. Vol 2003, No 2, Pp 231-240

Zolovick A., Upson D.W., and Eleftheriou B.E. 1966. Dirunal variation in plasma glucocorticoids levels in the horse (*Equus caballus*). J. Endocrinol. Vol 35, Pp 249-253

BIOGRAPHICAL SKETCH

Jose Manuel Cantu Martinez graduated from the University of Texas-Pan American on Summer 2013, with a Master in Science. He obtained his Biology/Business Management double major at the University of Texas-Pan American on fall 2010. He conducted research under Dr. Frederic Zaidan III between Spring 2009 and Summer 2013. His mailing address is 1104 N 3rd ave, Edinburg, Texas 78541