

THE DEAR ENEMY EFFECT IN MALE BROWN  
ANOLES (*ANOLIS SAGREI*)

By  
ELIZABETH DAWKINS  
Bachelor of Science in Biology  
Loyola Marymount University  
Los Angeles, California  
2016

Submitted to the Faculty of the  
Graduate College of the  
Oklahoma State University  
in partial fulfillment of  
the requirements for  
the Degree of  
MASTER OF SCIENCE  
May 2019

THE DEAR ENEMY EFFECT IN MALE BROWN  
ANOLES (*ANOLIS SAGREI*)

Thesis Approved:

Dr. Matthew Lovern

---

Thesis Adviser

Dr. Barney Luttbeg

---

Dr. Daniel Moen

---

Name: ELIZABETH DAWKINS

Date of Degree: MAY 2019

Title of Study: THE DEAR ENEMY EFFECT IN MALE BROWN ANOLES (*ANOLIS SAGREI*)

Major Field: ZOOLOGY

Abstract:

In the dear enemy effect, territory owners display more aggression towards unfamiliar strangers and less aggression towards familiar neighbors. This biological phenomenon is found in species that have territories that serve a breeding and feeding function. Glucocorticoids, like corticosterone, play an important role in mediating behavioral and physiological responses to stressors, such as increasing aggression in antagonistic encounters. I tested the possibility that corticosterone mediates aggressive behavior associated with the dear enemy effect in male brown anoles with two treatment groups: males with implants containing metyrapone, a glucocorticoid synthesis inhibitor, and males with control blank implants. Focal males in both treatment groups were exposed to stimulus neighbors for four days and subsequently their behavior was measured in trials with a familiar neighbor and an unfamiliar stranger. The aggressive behavior of focal males directed towards neighbors and strangers were recorded and assigned an overall aggression score. Behaviors that were recorded included dewlap extensions, head bob displays, sagittal expansions, dorsal crests, approaches, retreats, and attempted attacks. Overall, male brown anoles displayed more aggression towards strangers than towards neighbors, thus confirming the dear enemy effect. Furthermore, although males given metyrapone implants did not differ from control males in their aggression scores, there was an effect of corticosterone; males with higher plasma corticosterone concentrations exhibited lower aggression scores. These results are discussed within the context of corticosterone and aggression across social contexts.

## TABLE OF CONTENTS

Chapter	Page
I. INTRODUCTION.....	1
The dear enemy effect.....	1
The role of glucocorticoids in stressful encounters .....	3
Study system and research questions.....	5
II. METHODS.....	8
Animal care.....	8
Study males.....	9
Implant procedures.....	9
Trials .....	10
Radioimmunoassay.....	11
Statistical analyses .....	12
III. RESULTS .....	14
Initial and final body condition.....	14
Plasma corticosterone .....	14
Aggression scores and display behavior .....	15
Corticosterone and aggression scores .....	15

Chapter	Page
IV. DISCUSSION.....	17
REFERENCES .....	23
APPENDICES .....	27

## LIST OF TABLES

Table	Page
1. Aggressive score points .....	27

## LIST OF FIGURES

Figure	Page
1. Corticosterone levels of males .....	28
2. Average trial scores.....	29
3. Average stranger-neighbor scores.....	30
4. Corticosterone and aggression score regression .....	31

## CHAPTER I

### INTRODUCTION

#### The dear enemy effect

The “dear enemy effect” is a biological phenomenon displayed by many vertebrate and invertebrate species (Fisher 1954, Tierney et al. 2013). In the dear enemy effect, territory owners display higher levels of aggression towards strangers and lower levels of aggression towards neighbors. One potential cause for this effect is a decreased threat that conspecific neighbors pose compared to that of conspecific strangers with the possibility of strangers attempting to take territory or mates from the resident territory owner (Tierney et al. 2013). The respective threat displayed by neighbors and strangers as well as a territory owner’s familiarity with these threats together most likely influence the degree of aggression shown by a territory owner towards neighbors and strangers (Temeles 1994). The dear enemy effect likely is advantageous because it allows territory owners to minimize the amount of energy lost when fighting conspecifics (Rosell et al. 2008). It is demonstrated by bird, reptile, amphibian, and insect territory owners whose territories serve a breeding and feeding function (Temeles 1994). In several studies that investigated neighbor-stranger discrimination, the dear enemy effect was found in species that had territories that served



a breeding and feeding function but was not found as often in species whose territories only supported one function (Temeles 1994). In more recent years, other studies have investigated the dear enemy effect in group-living species, thereby expanding the focus from single and pair-bonded territory owners. It has been found that there are differences in neighbor-stranger responses in species that live within groups where there are three or more individuals that share a territory (Christensen and Radford 2018).

In the dear enemy effect, signals and different sensory cues are important in differentiating between conspecific neighbors and strangers. For example, Zenuto (2010) examined the dear enemy effect in the subterranean rodent *Ctenomys talarum* (tuco-tuco) and found that the memory of odors played an important role; specifically, that tuco-tucos recognize individual scents. Males that had been familiarized with odors from a conspecific male before interacting with a different male behaved in a similar manner to males that had not been exposed to odors from a conspecific male before contests (Zenuto 2010). In aggressive encounters, natural selection should favor the evolution of signals that increase benefits and decrease costs (Titone et al. 2018). Territoriality is important in the dear enemy effect because it is a characteristic of territory owners that plays a role in the outcomes of aggressive encounters. Territoriality evolves when benefits gained from sole access to restricted resources outweigh the costs of defense (Titone et al. 2018). The benefits of territoriality include access to and familiarity with resources while the costs of territoriality include time, energy, and risk of injury connected to territorial defense (Fox and Baird 1992). The resident effect, where territorial residents generally win conflicts

with intruders, is found in many taxa including lepidopterans, cichlid fish, lungless salamanders, and lizards (Titone et al. 2018). Winning these conflicts often depends upon what signals the animal uses. Many outcomes in aggressive encounters are determined by behavioral displays.

### The role of glucocorticoids in stressful encounters

The release of glucocorticoids (e.g., cortisol, corticosterone) and epinephrine, an endogenous stress hormone, are both critical components that enhance an organism's ability to deal with stress (Beylin and Shors 2003, Cahill and Alkire 2003). The glucocorticoid released in some small mammals like mice and voles is corticosterone and in other mammals it is cortisol or a mixture of cortisol and corticosterone (Boonstra 2005). The primary glucocorticoid released for amphibians, reptiles, rodents and birds is corticosterone (Francis et al. 2018). Appropriate learning and behavioral responses are related to the stressor type and level of threat (Thaker et al. 2010). Elevated glucocorticoid levels are critical for modifying behavioral responses to repeated encounters with aversive stimuli and are associated with behavioral changes like increased foraging, aggression, and movement (Thaker et al. 2010). After the occurrence of a stressful event or exposure to a stressor, the HPA (hypothalamic-pituitary-adrenal) axis is activated and glucocorticoids are released within minutes (Thaker et al. 2010). In nature, most stressful encounters like facing predators, are brief and result in a transient increase in plasma glucocorticoid levels (Thaker et al. 2010). Hubbs et al. (2000) investigated the effect of cortisol concentrations in wild-caught female Columbian ground squirrels exposed to predators while maintained in captivity. Their study demonstrated that brief exposure to a predator significantly

increases cortisol levels. Increased glucocorticoid levels are one of the many factors that lead to rapid behavioral and physiological responses. To ensure appropriate behavioral responses in future stressful events, there must be quick activation and retrieval of relevant memories regarding stressors (Thaker et al. 2010). Thaker et al. (2010), tested the role of increased corticosterone in aversive learning of a new stressor by male eastern fence lizards, *Sceloporus undulatus*. They found that blocking corticosterone production with the synthetic compound metyrapone hindered the immediate escape reactions of the male fence lizards that were presented with novel attackers and constrained learning and memory during future confrontations (Thaker et al. 2010).

Corticosterone plays a significant role in memory formation, memory retrieval, and learning and can be affected by stress (Korte 2001). Memory consolidation is also moderated by stress (Yang and Wilczynski 2003). When stress is induced within the consolidation stage after learning, successive memory performance is improved, but when stress is induced within the reconsolidation stage after retrieval, memory performance is disrupted (Yang and Wilczynski 2003). Yang and Wilczynski (2003) found that delayed stress after fear learning selectively boosted the persistence of long-term memory while delayed stress after fear memory reactivation selectively ruined the persistence of long-term memory in work performed with green anole lizards (*Anolis carolinensis*). Yang and Wilczynski (2003) found that stress and corticosterone administration enhanced the late consolidation of memory but disturbed late reconsolidation and that the effects of stress on late consolidation and reconsolidation were dependent on corticosterone synthesis. Their results suggest that the corticosterone system engages in the persistence of late term

memory after acquisition and retrieval (Yang and Wilczynski 2003). Because of the relationship between corticosterone and learning, there is the possibility that corticosterone is involved in the reduction of aggressive behavior as an animal becomes more familiar with social stimuli. When green anole lizards had a recent aggressive encounter, their aggression towards a new challenger increased while their aggression towards a familiar stimulus decreased (Yang and Wilczynski. 2003).

Metyrapone decreases some corticosterone production and release without fully blocking it. Specifically, it decreases rapid, stress-related corticosterone by impeding the 11 $\beta$ -hydroxylation reaction while maintaining basal levels of corticosterone (Baitharu et al 2012, Thaker et al. 2010). Metyrapone decreases cortisol production by impeding cortisol formulation in the adrenal cortex with the inhibition effect lasting over the cycle of cortisol modulation (Antypa et al. 2018, Marin et al. 2011).

#### Study system and research questions

Brown anoles, *Anolis sagrei*, are native to the Bahamas and Cuba and are a trunk-ground ecomorph (Losos 2009). Species of the same ecomorph share similarities in morphology, ecology, and behavior that evolved convergently (Losos 2009). *Anolis sagrei* perch at low heights and on wide surfaces and typically live on and around the base of trees and in other structurally intricate dwellings (Kamath and Losos 2017, Paterson 2002). Brown anoles were introduced to Florida during the 1940s and have since spread throughout the southeast and as far west as Texas (Burger et al. 2004, Lee 1985), where they overlap extensively in range with the native green anole, *Anolis carolinensis*. Brown anoles adapt well to new

environments and have a long breeding season. The reproductive season of brown anoles in Florida populations, for example, ranges from March to October. Around July and August of the reproductive season, female brown anoles are near the peak of egg production and males have enlarged testes, high plasma testosterone levels, and demonstrate high territoriality (Reedy et al. 2018, Paterson 2002). When male and female brown anoles are present within the same area as green anoles, the green anoles display a shift in habitat use and perch higher (Edwards and Lailvaux 2013, Echternacht 1999, Campbell 2000). Brown anoles and green anoles display more readily at conspecifics than heterospecifics, although some heterospecific signaling does occur (Tokarz and Beck 1987). In their study, Tokarz and Beck (1987) tested male-male interactions between brown anoles and green anoles and found that hetero-specifics paired together displayed significantly less aggression towards each other than paired conspecifics. Some signals that come into play during aggressive encounters in brown anole lizards (*Anolis sagrei*) -- the study species in the present work- are visual signals. Male and female brown anoles communicate with head-bobs, push-ups, and dewlap extensions (Reedy et al. 2018). The visual signals that brown anoles use are also critical for maintaining territoriality. When male anoles defend their territory and participate in aggressive encounters with other males, they do a series of displays involving dewlap extensions, head-bobs, and push-ups. Male anoles are territorial and often use visual displays to defend their territories against other males, particularly during the breeding season (Losos 2009). Furthermore, male green anoles display the dear enemy effect with focal males spending more time displaying aggressively towards conspecific strangers than conspecific neighbors (Qualls and Jaeger 1991).

In the present study, I tested if corticosterone mediates aggressive behavior associated with the dear enemy effect in male brown anoles. This addressed the two, related research questions of (1) whether brown anoles exhibit a dear enemy effect and (2) whether corticosterone reduces aggressive output towards familiar individuals. First, I predicted that male brown anoles would respond more aggressively towards unfamiliar males than familiar males, thus demonstrating a dear enemy effect. Second, I further predicted that when corticosterone is pharmacologically blocked using metyrapone, brown anole males would display equal aggression levels to familiar and unfamiliar males because the learning and memory necessary for neighbor-stranger discrimination that is mediated by corticosterone will be reduced. The study that I performed provides more in-depth knowledge about the factors that affect male anole aggression as well as the factors that play a role in the dear enemy effect and the significance of corticosterone's role in the process of learning and memory formation in the context of the physiological stress response.

## CHAPTER II

### METHODS

All work described here was performed as approved by the Oklahoma State University IACUC (protocol # AS-17-4).

#### Animal care

A total of 87 wild-caught adult male brown anoles, *Anolis sagrei*, were purchased from Underground Reptiles (Deerfield Beach, FL) and housed individually in 10-gallon aquaria. Each aquarium had a peat-moss substrate, wooden dowel, and a water dish. Males were fed four times a week with vitamin and mineral supplemented mealworms and crickets (Miner-all, Sticky Tongue Farms, Sun City, CA; and Herptivite, Rep-Cal Labs, Los Gatos, CA). All lizards had access to water ad libitum through daily spraying of cages and in water dishes. The males were exposed to a 14:10 hour light: dark cycle with a combination of UV-emitting fluorescent bulbs and incandescent bulbs which acted as a heat source. Lizard housing and care followed the established protocol from Lovern et al. (2004).

### Study males

Forty males (17 in 2017 and 23 in 2018) were used as focal animals and forty-seven males (18 in 2017 and 29 in 2018) were used as stimulus animals. The snout-vent length (SVL) and mass of all males were measured to the nearest millimeter and 0.01 gram, respectively. Focal and stimulus males were size-matched to minimize potential effects on interactions. The average mass difference between focal and stimulus males was 0.3 g and the average SVL difference was 3 mm. All males were given a week to acclimate to the laboratory environment before receiving implants. Trials between focal and stimulus males took place in July 2017 and June-July 2018, well within the reproductive season both years.

### Implant procedures

A total of 18 males (10 in 2017 and 8 in 2018) received metyrapone implants (1  $\mu\text{g}$  in 2 mm silicone pellets, implanted subcutaneously and dorsolaterally) and 20 (7 in 2017 and 13 in 2018) received blank implants (2 mm silicone pellets implanted subcutaneously and dorsolaterally). Metyrapone was purchased from Sigma (M296; St. Louis, MO) and silicone type A medical adhesive was purchased from Dow Corning (Midland, MI). All surgical instruments were sterilized with a bead sterilizer (Simon Keller AG, Fine Science Tools, Foster City, CA) at 250°C for three seconds. Before being injected subcutaneously into the anoles, implants were sterilized with Sporox and dried. Lizards were anesthetized with isoflurane and their left sides were wiped with antiseptic (chlorohexidine) before injection of the implant via pit tagger (Biomark, Boise, ID). After the implant was injected,



the site was sealed with Vetbond (3M, St. Paul, MN) and males were placed in individual plastic containers to completely recover from the anesthesia before being placed back into their individual tanks (recovery for all animals was under 20 min).

### Trials

For the trial set up, there were three tanks aligned in a row with a single anole in each tank. The anole located in the center was the focal male and anoles in adjacent tanks were stimulus males. Six days after focal males received implants and were set up in the lab, dividers were removed between the tanks of focal males and one of their immediately adjacent neighbors so that they could fully see each other in their respective tanks. Focal males were housed like this for four days, after which dividers were put back into place between the tanks. Each focal male participated in one neighbor and one stranger trial. On the morning of the fifth day dividers were removed and aggression scores were calculated for focal males towards stimulus males (either the neighbor or the stranger) by measuring behavior for ten minutes following divider removal; whether the neighbor or the stranger trial was first was chosen arbitrarily. Other studies have demonstrated individual recognition occurs rather than place recognition, although that was not tested (e.g. Lovern unpublished, Husak and Fox 2003). Aggression scores were calculated based on total points of behaviors exhibited by the focal male in the trials; behaviors included were head bob displays, dewlap extensions, sagittal expansions, dorsal crests, approaches, retreats, and attempted attacks (following Lovern & Jessen 2003). Points were assigned to behaviors based on increasing aggressive intent (Table 1) (modified from Lovern et al.

2001). On the sixth day, the remaining trial was conducted using the other stimulus male (neighbor or stranger). On the seventh day, final SVL and mass measurements were taken, and blood was collected from each focal male after euthanization with isoflurane and rapid decapitation with a scalpel. After blood samples were centrifuged, plasma was collected and then frozen at -20 °C for later radioimmunoassay.

### Radioimmunoassay

Radioimmunoassay procedures determined plasma concentrations of corticosterone (Wingfield and Farner 1975, Lovern and Adams 2008). Briefly, plasma samples were thawed, vortexed, and measured to the nearest  $\mu\text{L}$  (10-61  $\mu\text{L}$  range), after which 500  $\mu\text{L}$  of ddH<sub>2</sub>O was added to each sample to provide sufficient volume for extraction. Tritiated corticosterone from Perkin Elmer (NET-399, Boston, MA) also was added to each sample, samples were vortexed, and refrigerated overnight at 4 °C.

On the second day of the radioimmunoassay, samples were extracted twice with two mL of diethyl ether and dried off with nitrogen gas in a water bath at 37 °C. After all samples were dry, 300  $\mu\text{L}$  of PBSg (phosphate buffer solution with gelatin) was added to all the samples and each sample was vortexed for several seconds before being refrigerated overnight at 4 °C.

On the third day of the radioimmunoassay, after all samples reached room temperature, they were vortexed and 50  $\mu$ L of each sample were aliquoted into scintillation vials for recovery determination. Two mL of Ultima Gold (Perkin Elmer) were then added and each sample was vortexed again. After each sample was vortexed, 200  $\mu$ L were aliquoted into test tubes for the radioimmunoassay along with tritiated corticosterone and corticosterone antibody (B3-163; Endocrine Sciences, Calabasas, CA). All the samples were then vortexed gently for a few seconds and refrigerated.

On the fourth day, the radioimmunoassay was stopped with dextran-coated charcoal solution, samples and standards were centrifuged at 2200 rpm for 10 min at 4 °C, and the supernatant was decanted into scintillation vials. Ultima Gold was added, samples were vortexed, and then they were read on a scintillation counter for sample quantification. The standard curve, from 500 – 1.95 pg, was run in duplicate and samples were run singly, corrected for individual recovery and sample volume to arrive at final concentrations. Average recovery across both the 2017 and 2018 assays was 88%, intra-assay coefficients of variation (CVs) were 4% (2017) and 6% (2018), and the inter-assay CV, calculated from the two runs of this assay, was 15%.

### Statistical analyses

All statistical analyses were performed in SigmaPlot (v. 11.0). Corticosterone values were log-transformed prior to analyses to normalize their distribution. Overall aggression scores were calculated for individual focal males as the aggression scores from the neighbor trial

subtracted from the aggression score from the stranger trial. A positive aggressive score signifies more aggression displayed towards strangers than neighbors and a negative aggressive score signifies less aggression displayed towards strangers than neighbors. Unless specified otherwise, test statistics and p-values are from general linear model ANOVAs.

## CHAPTER III

### RESULTS

#### Initial and final body condition

Male body condition for all individuals across years was calculated as the residuals of the regression of mass onto SVL. Focal males in 2018 had a significantly higher initial body condition ( $F(1,35)=6.984, p=0.013$ ) and final body condition ( $F(1,35)=70.212, p=0.001$ ) than focal males in 2017. There were no treatment effects on either initial ( $F(1,35)=1.267, p=0.269$ ) or final ( $F(1,35)=0.532, p=0.471$ ) body condition, and there were no significant interactions between year and treatment for initial ( $F(1,35)=1.521, p=0.226$ ) or final ( $F(1,35)=1.711, p=0.200$ ) body condition.

#### Plasma corticosterone

There were both year and treatment effects on plasma corticosterone concentrations in focal males. Corticosterone was significantly higher for males in 2017 than for males in 2018 ( $F(1,35)=20.900, p=0.001$ ). And, control males had significantly higher

corticosterone concentrations than did males treated with metyrapone ( $F(1,35)=4.507$ ,  $p=0.042$ ) (Fig. 1). There was no significant interaction between year and treatment ( $F(1,35)=0.0372$ ,  $p=0.848$ ).

### Aggression scores and display behavior

Across year and treatment, focal males were more aggressive towards stranger males than towards neighbor males (paired t-test:  $t=1.938$ ,  $p=0.031$ ) (Fig. 2). There were no significant year ( $F(1,35)=0.679$ ,  $p=0.416$ ) nor treatment ( $F(1,35)=0.614$ ,  $p=0.439$ ) effects on difference in aggression scores, however, nor was there a significant interaction ( $F(1,35)=0.115$ ,  $p=0.737$ ) (Fig. 3). Finally, display behavior was unaffected by year and treatment as well. Neither the frequency of dewlap extensions nor head bobbing displays expressed during aggression trials differed across year and treatment (general linear model ANOVA; all  $p>0.05$ ). Although a part of the aggressive score, these behaviors were the most frequently demonstrated by focal males and were looked at to evaluate whether they were the drivers of the aggressive scores.

### Corticosterone and aggression scores

Although there was no treatment effect on overall aggression scores, there was an effect of measured corticosterone on aggression levels. Specifically, as concentrations of plasma corticosterone levels increased, the difference in aggression scores decreased revealing a significant negative relationship (simple linear regression, SLR:  $F(1,35)=4.238$ ,  $p=0.047$ ).

As corticosterone levels increased aggression levels towards neighbors did not change (SLR:  $F(1,35)=0.11, p=0.740$ ), but aggression levels towards strangers decreased (SLR:  $F(1,35)=5.86, p=0.021$ ). Males with higher corticosterone concentrations were less likely to discriminate between neighbor and stranger males via differences in directed aggressive intent. Furthermore, this negative relationship between corticosterone and overall aggression score did not seem to be driven by specific display behavior, as there was no relationship between corticosterone and stranger-neighbor dewlap extensions (SLR:  $F(1,35)=1.635, p=0.210$ ) nor between corticosterone and stranger-neighbor head bobs (SLR:  $F(1,35)=3.213, p=0.082$ ).

## CHAPTER IV

### DISCUSSION

This study examined whether male brown anoles would demonstrate more aggression towards unfamiliar males than towards familiar males and whether there was a relationship between corticosterone and displayed aggression. Overall, male brown anoles displayed more aggression towards unfamiliar males than towards familiar males since the difference in aggressive scores with strangers were significantly higher than with neighbors. This supports my first prediction that male brown anoles would show the dear enemy effect. My study also found that there is no specific treatment effect of metyrapone on aggression scores; males given metyrapone did not show a difference in aggression between neighbors and strangers compared to control males. This fails to support my second prediction that males given metyrapone would show differences in aggression when compared to control males. Males given metyrapone did not show diminished discrimination between neighbors and strangers. However, my study did find that there is a relationship between plasma corticosterone and overall aggression in male brown anoles. Specifically, opposite to what I predicted, I found that as corticosterone levels increased the total aggressive



scores (stranger-neighbor score) decreased revealing a significant negative relationship. At higher corticosterone levels, there was a smaller difference in stranger-neighbor scores. Overall, the results of this study demonstrate that males can discriminate between strangers and neighbors in a territorial context and that corticosterone influences the aggression shown.

High corticosterone levels in vertebrates have been affiliated with a range of swift behavioral changes such as increases in foraging, aggression, and locomotion (Thaker et al. 2010). The effects of elevated corticosterone can vary across and within species. In one population of Eastern fence lizards that lived with fire ants, elevated corticosterone increased activity while in another population that did not live with fire ants, elevated corticosterone decreased activity (Wack et al. 2013, Trompeter and Langkilde 2011). One reason that there may be differences in behavioral responses to elevated corticosterone is that corticosterone mediates species-specific and context-specific interactions (Wack et al. 2013, Orchinik et al. 2002). The length of glucocorticoid exposure is important in establishing the effect of glucocorticoids on aggression (Soma et al. 2018). Thaker et al. (2010) found that suppressing acute corticosterone elevations in male fence lizards hinders aversive responses during the first encounter with a novel attacker as well as subsequent learning and recollection. In adult rats, acute injections of corticosterone result in increased aggression while blocking corticosterone with metyrapone result in decreased aggression (Soma et al. 2008). There are some instances where increased corticosterone can decrease aggression, like when aggression is reduced in wild song sparrows that experience chronic exposure to high corticosterone (Soma et al. 2008, Wingfield and Silverin 1986).

A study that highlighted the importance of corticosterone on aggressive behavior in green anoles (*Anolis carolinensis*) was done by Yang and Wilczynski (2003). They examined the relationship between behavioral habituation towards social or non-social stimuli and corticosterone (Yang and Wilczynski 2003). They examined this relationship by measuring plasma corticosterone after periods of exposure to social or non-social stimuli and for a short time blocking corticosterone secretion that occurs with the exposure to a social or non-social stimulus. They found that males exposed to another male for the aggressive encounters showed higher plasma corticosterone levels than males exposed to a non-aggressive encounter, a non-social video and that this higher corticosterone level was maintained through the fifth day. They also found that control males exposed to the aggressive video showed a slow increase followed by a decrease in aggression while males treated with metyrapone exposed to the aggressive video showed high aggression that did not decrease (Yang and Wilczynski 2003). Their results suggest that corticosterone has a moderating effect in behavioral habituation (Yang and Wilczynski 2003). They did not find any significant correlations between corticosterone levels and behavior levels in either experiment conducted (Yang and Wilczynski 2003). In the present study, I found a significant negative relationship between corticosterone and aggression levels towards strangers with higher corticosterone being associated with less aggression. Overall, both studies reveal a relationship between corticosterone and aggression in anoles although the contexts of the studies may explain the different results. Whereas Yang and Wilczynski (2003) examined aggression in social vs. non-social settings over the course of repeated

exposures, I examined aggression in a territorial context in which the variable was a neighbor or stranger male.

McMann and Paterson (2012) assessed whether wild resident male brown anoles exhibit similar display differences between a close confrontation with a neighbor versus a stranger. They were also interested in the relative frequencies of nodding and bobbing displays. They exposed a resident male brown anole to either a neighbor or stranger in a cage that was one meter away and documented display behavior of the territory resident for thirty minutes (McMann and Paterson 2012). They recorded the number of head bob displays, the type of head bob display (nodding or bobbing), the presence or absence of crest erection during each head bob display, and the number of dewlap extensions (McMann and Paterson 2012). They found that while confrontation type did not differ in number of nodding displays, proportion of head bob displays with crest erections, or number of dewlap extensions, resident male brown anoles demonstrated fewer bobbing displays towards neighbors than towards strangers. In a previous study conducted, Paterson and McMann found that focal males showed more bobbing displays towards strangers than towards neighbors in close encounters in a neutral arena (Paterson and McMann 2004). The results of this study suggest that the reaction of a resident male brown anole to a neighbor male is different and less aggressive than the reaction to a stranger male (McMann and Paterson 2012).

Similarly to the behaviors that McMann and Paterson (2012) recorded, in this study the number of dewlap extensions, head bob displays, and crest erections were recorded along with additional behaviors which included attacks, retreats, sagittal expansions, and approaches. Equivalently to what they found regarding dewlap extensions, I also found no significant difference between the dewlap extensions that were directed towards neighbors and strangers by control focal males and focal males treated with metyrapone. I did not find a significant difference between head bob displays directed towards neighbors and strangers by focal control males and focal males treated with metyrapone. However, in this study, head bob displays were treated as one behavior and were not categorized into bobbing or nodding displays as they were in McMann and Paterson's study (2012). The results obtained from this study demonstrate an overall dear enemy effect as do the results from McMann and Paterson's (2012) since more aggression was shown towards strangers than neighbors.

Another factor to consider in the dear enemy effect is the identity of neighbors and strangers and the process of identifying them. Husak and Fox (2003) investigated whether male territorial collared lizards could recognize neighbors and would increase aggression towards displaced neighbors. They found that territorial collared lizards increased displayed aggression towards displaced neighbors but showed more aggression towards strangers, suggesting that resident males can recognize individuals outside of location (Husak and Fox 2003). In a study by Forster et al. (2005), they found that green anoles seem to learn information that is distinct to their opponent during an initial aggressive encounter and that the relevance of this information decreases the more time passes

between encounters. Both studies highlight the significance of distinguishing the difference between individuals in aggressive encounters. Future studies investigating the dear enemy effect in male brown anoles could specifically investigate cues that are important in identifying neighbors and strangers.

In future studies investigating the dear enemy effect in male brown anoles, in addition to examining aggression towards neighbors and strangers, androgen levels could also be measured since they also play a critical role in male aggression. It would also be worthwhile to investigate this phenomenon in other anole species and to study same dear enemy effect in female anoles. Studying the dear enemy effect in female anoles is critical in expanding the field because female aggression is not as widely examined as male aggression. Were there to be differences in the females as well, there would be very interesting implications for our understanding of social system, territoriality, and the endocrine underpinnings that have evolved as mediators of these behaviors.

## REFERENCES

- Antypa, D., Vuilleumier, P., & Rimmele, U. (2018). Suppressing cortisol at encoding reduces the emotional enhancement in subjective sense of recollection. *Neurobiology of Learning and Memory*, *155*, 86-91.
- Beylin, A. V., & Shors, T. J. (2003). Glucocorticoids are necessary for enhancing the acquisition of associative memories after acute stressful experience. *Hormones and Behavior*, *43*(1), 124-131.
- Baitharu, I., Deep, S. N., Jain, V., Barhwal, K., Malhotra, A. S., Hota, S. K., ... & Ilavazhagan, G. (2012). Corticosterone synthesis inhibitor metyrapone ameliorates chronic hypobaric hypoxia induced memory impairment in rat. *Behavioural Brain Research*, *228*(1), 53-65.
- Boonstra, R. (2005). Equipped for life: the adaptive role of the stress axis in male mammals. *Journal of Mammalogy*, *86*(2), 236-247.
- Burger, J., Campbell, K. R., & Campbell, T. S. (2004). Gender and spatial patterns in metal concentrations in brown anoles (*Anolis sagrei*) in southern Florida, USA. *Environmental Toxicology and Chemistry*, *23*(3), 712-718.
- Cahill, L., & Alkire, M. T. (2003). Epinephrine enhancement of human memory consolidation: interaction with arousal at encoding. *Neurobiology of Learning and Memory*, *79*(2), 194-198.
- Campbell TS. (2000). Analysis of the effects of an exotic lizard (*Anolis sagrei*) on a native lizard (*Anolis carolinensis*) in Florida, using islands as experimental units. PhD Thesis. Univ. of Tennessee, Tennessee, USA.
- Christensen, C., & Radford, A. N. (2018). Dear enemies or nasty neighbors? Causes and consequences of variation in the responses of group-living species to territorial intrusions. *Behavioral Ecology*, *29*(5), 1004-1013.
- Echternacht, A. C. (1999). Possible causes for the rapid decline in population density of green anoles, *Anolis carolinensis* (Sauria: Polychrotidae) following invasion by the

- brown anole, *Anolis sagrei*, in the southeastern United States. *Anolis Newsletter*, 22, 27.
- Edwards, J. R., & Lailvaux, S. P. (2013). Do interspecific interactions between females drive shifts in habitat use? A test using the lizards *Anolis carolinensis* and *A. sagrei*. *Biological Journal of the Linnean Society*, 110(4), 843-851.
- Fisher, J. (1954). Evolution and bird sociality. *Evolution as a Process*, 71, 83.
- Forster, G. L., Watt, M. J., Korzan, W. J., Renner, K. J., & Summers, C. H. (2005). Opponent recognition in male green anoles, *Anolis carolinensis*. *Animal Behaviour*, 69(3), 733-740.
- Fox, S. F., & Baird, T. A. (1992). The dear enemy phenomenon in the collared lizard, *Crotaphytus collaris*, with a cautionary note on experimental methodology. *Animal Behaviour*.
- Francis, C. D., Donald, J. W., Fuxjager, M. J., Goymann, W., Hau, M., Husak, J. F., ... & Miller, E. T. (2018). Metabolic scaling of stress hormones in vertebrates. *Integrative and Comparative Biology*, 58(4), 729-738.
- Hubbs, A. H., Millar, J. S., & Wiebe, J. P. (2000). Effect of brief exposure to a potential predator on cortisol concentrations in female Columbian ground squirrels (*Spermophilus columbianus*). *Canadian Journal of Zoology*, 78(4), 578-587.
- Husak, J. F., & Fox, S. F. (2003). Adult male collared lizards, *Crotaphytus collaris*, increase aggression towards displaced neighbours. *Animal Behaviour*, 65(2), 391-396.
- Kamath, A., & Losos, J. B. (2017). Does ecological specialization transcend scale? Habitat partitioning among individuals and species of *Anolis* lizards. *Evolution*, 71(3), 541-549.
- Korte, S. M. (2001). Corticosteroids in relation to fear, anxiety and psychopathology. *Neuroscience & Biobehavioral Reviews*, 25(2), 117-142.
- Lee, J. C. (1985). *Anolis sagrei* in Florida: phenetics of a colonizing species I. Meristic characters. *Copeia*, 182-194.
- Losos, J. B. (2009). *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles* (Vol. 10). Univ of California Press.
- Lovern, M. B., McNabb, F. A., & Jenssen, T. A. (2001). Developmental effects of testosterone on behavior in male and female green anoles (*Anolis carolinensis*). *Hormones and Behavior*, 39(2), 131-143.
- Lovern, M. B., & Jenssen, T. A. (2003). Form emergence and fixation of head bobbing displays in the green anole lizard (*Anolis carolinensis*): a reptilian model of signal ontogeny. *Journal of Comparative Psychology*, 117(2), 133.

- Lovern, M. B., Holmes, M. M., & Wade, J. (2004). The green anole (*Anolis carolinensis*): a reptilian model for laboratory studies of reproductive morphology and behavior. *Ilar Journal*, 45(1), 54-64.
- Lovern, M. B., & Adams, A. L. (2008). The effects of diet on plasma and yolk steroids in lizards (*Anolis carolinensis*). *Integrative and Comparative Biology*, 48(3), 428-436.
- Marin, M. F., Hupbach, A., Maheu, F. S., Nader, K., & Lupien, S. J. (2011). Metyrapone administration reduces the strength of an emotional memory trace in a long-lasting manner. *The Journal of Clinical Endocrinology & Metabolism*, 96(8), E1221-E1227.
- McMann, S., & Paterson, A. (2012). Display Behavior Of Resident Brown Anoles (*Anolis Sagrei*) During Close Encounters With Neighbors And Non. *Herpetological Conservation and Biology*, 7(1), 27-37.
- Orchinik, M., Gasser, P., & Breuner, C. (2002). Rapid corticosteroid actions on behavior: cellular mechanisms and organismal consequences. In *Hormones, Brain and Behavior* (pp. 567-600). Academic Press.
- Paterson, A. V. (2002). Effects of an individual's removal on space use and behavior in territorial neighborhoods of brown anoles (*Anolis sagrei*). *Herpetologica*, 58(3), 382-393.
- Paterson, A. V., & McMann, S. (2004). Differential headbob displays toward neighbors and nonneighbors in the territorial lizard *Anolis sagrei*. *Journal of Herpetology*, 38(2), 288-292.
- Qualls, C. P., & Jaeger, R. G. (1991). Dear enemy recognition in *Anolis carolinensis*. *Journal of Herpetology*, 25(3), 361-363.
- Reedy, A. M., Pope, B. D., Kiriazis, N. M., Giordano, C. L., Sams, C. L., Warner, D. A., & Cox, R. M. (2017). Female anoles display less but attack more quickly than males in response to territorial intrusions. *Behavioral Ecology*, 28(5), 1323-1328.
- Rosell, F., Gundersen, G., & Le Galliard, J. F. (2008). Territory ownership and familiarity status affect how much male root voles (*Microtus oeconomus*) invest in territory defence. *Behavioral Ecology and Sociobiology*, 62(10), 1559-1568.
- Soma, K. K., Scotti, M. A. L., Newman, A. E., Charlier, T. D., & Demas, G. E. (2008). Novel mechanisms for neuroendocrine regulation of aggression. *Frontiers In Neuroendocrinology*, 29(4), 476-489.
- Temeles, E. J. (1994). The role of neighbours in territorial systems: when are they'dear enemies'?. *Animal Behaviour*, 47(2), 339-350.



- Thaker, M., Vanak, A. T., Lima, S. L., & Hews, D. K. (2009). Stress and aversive learning in a wild vertebrate: the role of corticosterone in mediating escape from a novel stressor. *The American Naturalist*, *175*(1), 50-60.
- Tierney, A. J., Andrews, K., Happer, K. R., & White, M. K. M. (2013). Dear enemies and nasty neighbors in crayfish: Effects of social status and sex on responses to familiar and unfamiliar conspecifics. *Behavioural Processes*, *99*, 47-51.
- Titone, V., Marsiglia, F., Mangiacotti, M., Sacchi, R., Scali, S., & Zuffi, M. A. L. (2018). Better to be resident, larger or coloured? Experimental analysis on intraspecific aggression in the ruin lizard. *Journal of Zoology*, *304*(4), 260-267.
- Tokarz, R. R., & Beck Jr, J. W. (1987). Behaviour of the suspected lizard competitors *Anolis sagrei* and *Anolis carolinensis*: an experimental test for behavioural interference. *Animal Behaviour*, *35*(3), 722-734.
- Trompeter, W. P., & Langkilde, T. (2011). Invader danger: lizards faced with novel predators exhibit an altered behavioral response to stress. *Hormones and Behavior*, *60*(2), 152-158.
- Wack, C. L., Ratay, M. K., & Woodley, S. K. (2013). Effects of corticosterone on locomotory activity in red-legged salamanders. *Herpetologica*, *69*(2), 118-126.
- Wingfield, J. C., & Farner, D. S. (1975). The determination of five steroids in avian plasma by radioimmunoassay and competitive protein-binding. *Steroids*, *26*(3), 311-327.
- Wingfield, J. C., & Silverin, B. (1986). Effects of corticosterone on territorial behavior of free-living male song sparrows *Melospiza melodia*. *Hormones and Behavior*, *20*(4), 405-417.
- Yang, E. J., & Wilczynski, W. (2003). Interaction effects of corticosterone and experience on aggressive behavior in the green anole lizard. *Hormones and Behavior*, *44*(3), 281-292.
- Zenuto, R. R. (2010). Dear enemy relationships in the subterranean rodent *Ctenomys talarum*: the role of memory of familiar odours. *Animal Behaviour*, *79*(6), 1247-1255.

## APPENDICES

Table 1- Points assigned to different aggressive behaviors displayed by focal males. Aggression scores were calculated by summing the total of points of all behaviors exhibited during the ten-minute trials.

Behavior	Points Assigned
Dewlap Extension	1 point
Head bob display	2 points
Sagittal expansion	3 points
Dorsal crest	3 points
Approach	4 points
Retreat	-4 points
Attack	5 points

Figure 1. This graph shows the average (+SE) corticosterone levels of control and treatment males in 2017 and 2018. There were significant year ( $F(1,35) = 20.900, p = 0.001$ ) and treatment ( $F(1,35) = 4.507, p = 0.042$ ) effects.

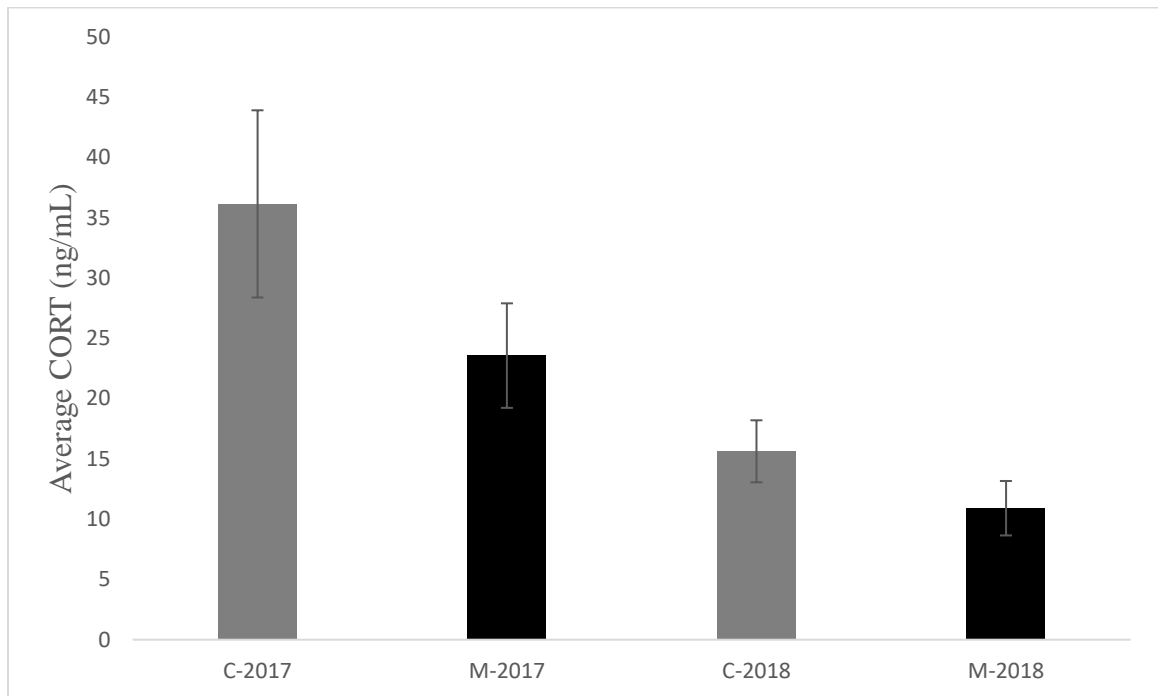


Figure 2. This graph shows the average neighbor trial score and average stranger trial score of all control and treatment males. Males displayed significantly more towards strangers than neighbors (one sample t-test:  $t=1.938$ ,  $p=0.031$ ).

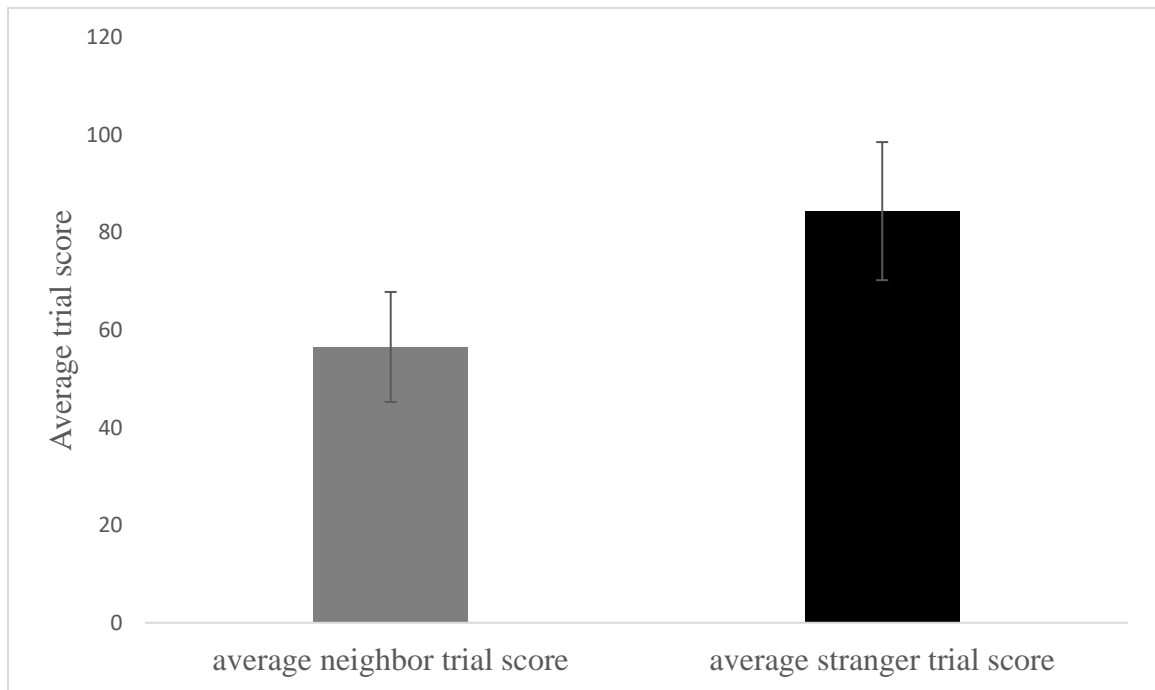


Figure 3. This graph shows the average (+SE) stranger-neighbor aggression score of control and treatment males in 2017 and 2018. There were no significant year or treatment effects.

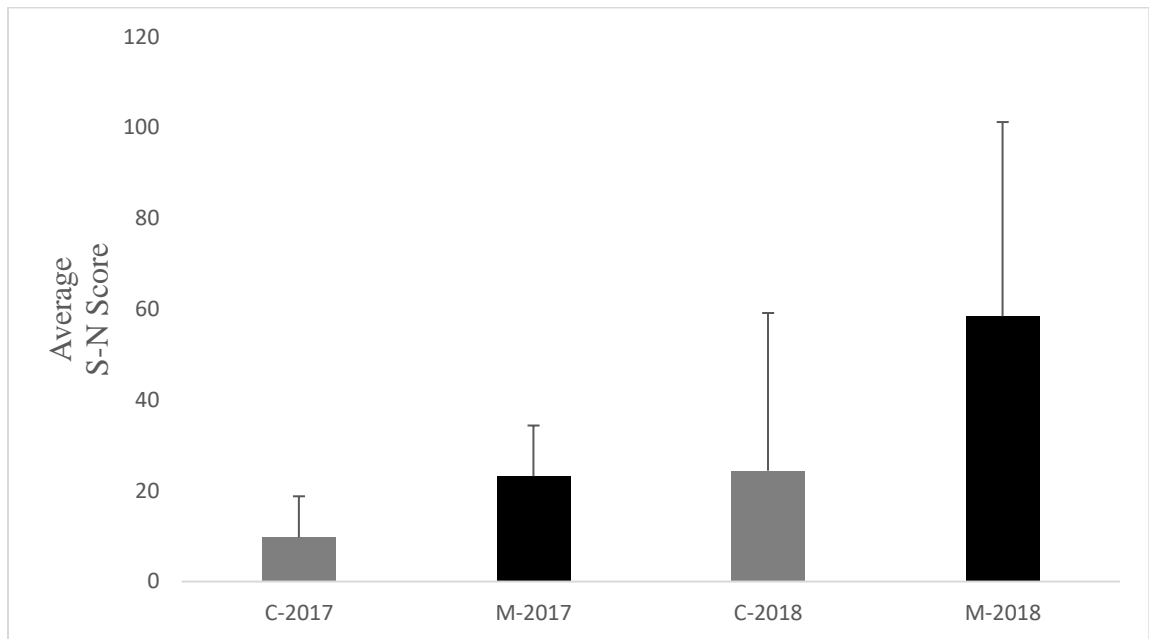
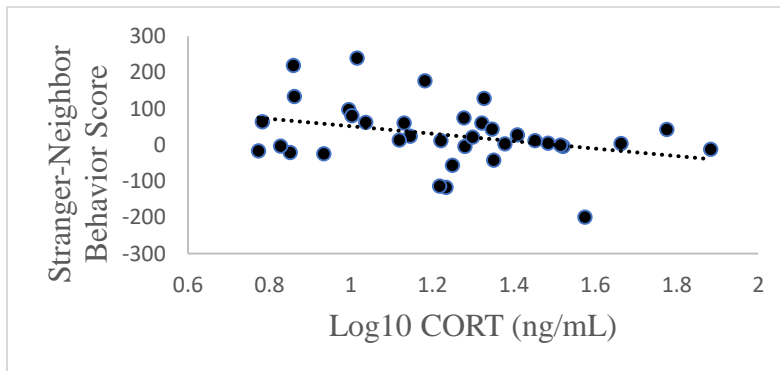
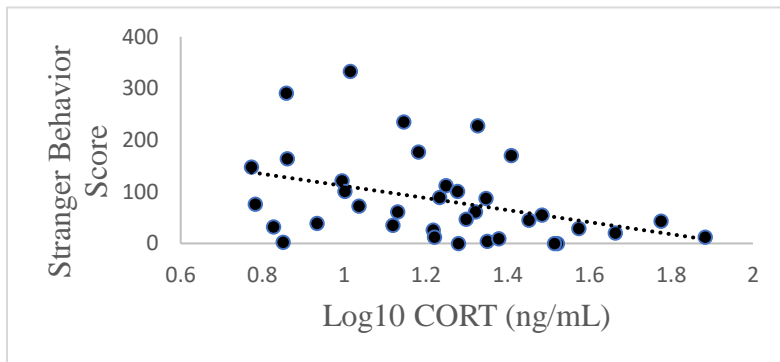
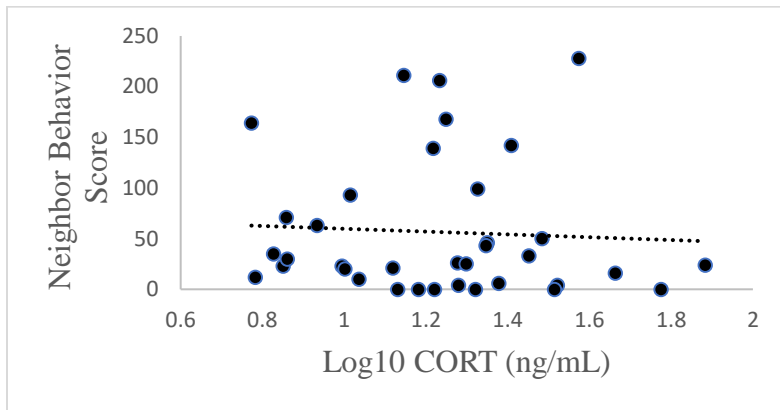


Figure 4. As corticosterone levels increased the difference in levels of aggression shown towards neighbors did not change (a) ( $F(1,35) = 0.11, p = 0.740$ ), strangers decreased (b) ( $F(1,35) = 5.86, p = 0.021$ ), and , and stranger-neighbor decreased (c) ( $F(1,35) = 4.238, p = 0.047$ ). This graph shows the corticosterone levels of control and treatment males used in 2017 and 2018.



VITA

Elizabeth Silva Dawkins

Candidate for the Degree of

Master of Science

Thesis: THE DEAR ENEMY EFFECT IN MALE BROWN ANOLES (*ANOLIS SAGREI*)

Major Field: Zoology

Biographical:

Education:

Completed the requirements for the Master of Science in Zoology at Oklahoma State University, Stillwater, Oklahoma in May 2019.

Completed the requirements for the Bachelor of Science in Biology at Loyola Marymount University, Los Angeles, CA 2016.

Experience:

**Graduate Teaching Assistant, Fall 2016 to present**

Biology 1114 Introductory Lab: Oklahoma State University.

- Facilitated labs, reviewed student manuscripts, and participated in preparatory meetings
- Fed animals daily, cleaned animal enclosures, and prepared animal diets