Intrasexual Behaviors in the Nonnative Mediterranean House Gecko,
Hemidactylus turcicus

by
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Abstract

Behavioral studies on introduced wildlife can provide valuable insight into its mechanisms of dispersal, habitat use, and the interactions it holds with the native fauna and flora. In this study, intrasexual (same-sex) behaviors of *H. turcicus* geckos were observed through staged interactions using size-matched subjects in a lab arena. Following baseline interaction tests, the same individuals underwent a topical treatment of either testosterone or sesame oil (control) alone. I tested for behavioral differences between the testosterone-treated and sesame oil-treated individuals, and I tested for an effect of testosterone within subjects. Lastly, I tested for a sex difference in the frequency of behaviors observed during the baseline tests. The behaviors recorded included aggressive (e.g. pushups, tail wags, bites), submissive (freezes and retreats), and neutral behaviors (e.g. approaches, licks, vocalizations). Behaviors expressed by testosterone-treated males did not differ from the sesame oil-treated males. The same was true in the female treatment groups, where there was no difference between the treatments, and an overall absence of most aggressive behaviors. Secondly, no significant differences were found between the behaviors of the testosterone-treated subjects and their corresponding baseline results in either sex. Together, these findings suggest that short-term elevations of plasma testosterone do not have a direct influence on the expression of intrasexual behaviors in *H. turcicus*. Longer durations of testosterone treatments (e.g., weeks rather than 3 days) could result in different effects on the behaviors of *H. turcicus* geckos, and castration could also be used in future studies to test if a decrease in circulating plasma testosterone influences the expression of intrasexual behaviors of this House Gecko. Lastly, males were found to be significantly more expressive in both aggressive and

submissive behaviors, than the females in the baseline tests. This is consistent with previous research on *H. turcicus* that reports males as being territorial and engaging in agonistic encounters, but females often participate in communal nesting with members of the same sex.

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Introduction

Introduced species can be very detrimental to their newly colonized habitats. Once an introduced species is known to cause harmful effects on the native fauna or flora, it is said to be 'invasive'. 'Nonnative' is a term often used by biologists in place of the word 'invasive' when researchers cannot determine if the introduced species is truly harmful to the native fauna, flora, or overall ecosystem. Invasive species often provoke a battle toward protecting native fauna and flora from consumption and/or competition with the introduced organisms. Examples can be seen throughout the state of Florida, which now contains more reproducing nonnative lizards than native lizards (discussed by Pernas et al. 2012). In terms of geckos, there are many nonnative species residing in Florida, and several of those same gecko species have been seen in other states of southern North America, such as the geckos in the genus *Hemidactylus*. It should be noted, however, that determining the effects of an introduced species on the colonized environments is not always a simple task, nor is it simple to decide upon an effective method of removal when determined necessary.

Several types of research may be necessary to determine the effects of an introduced species. Both intraspecific and interspecific studies on the introduced species can prove useful in understanding the ecology of the organism (e.g., competition within the introduced species and competition between the introduced species and native species). While data on the introduced species from their native range is valuable to researchers, follow-up studies may also be necessary to better understand the ecology of the organism in its new geographic location. To give an example, the Argentine Black and White Tegu, *Salvator* (formerly *Tupinambis*) *merianae*, has become an invasive species in regions of Florida, and it has been receiving much attention from researchers interested in its behavioral ecology. By studying the behavioral ecology of *S. merianae* within its introduced range, researchers may find reasonable methods toward controlling the species and reducing its threat on the native turtle and crocodilian species (Klug et al. 2015; Mazzotti et al. 2015; Pernas et al. 2012). Studying intraspecific behaviors, such as territorial or reproductive behavior, can provide insight into the social interactions that shape the distribution of a nonnative species.

The Mediterranean House Gecko, *Hemidactylus turcicus*, is an Old World species in the Gekkonidae family that is native to parts of Eurasia and Northern Africa (Briggs 2012). *Hemidactylus turcicus* is a nocturnal gecko with adhesive toe pads, a relatively translucent body covered with bumps, and the capability to vocalize (Briggs 2012; Frankenberg 1982). The species, along with other *Hemidactylus* species, are appropriately referred to as House Geckos as they commonly seek refuge in man-made buildings (Selcer 1986). In the past century *H. turcicus* have far expanded their range outside of the Mediterranean region and now maintain established populations in the Middle East, eastern Africa, the Caribbean, Central America, Mexico, and the southern United States (Briggs 2012; Locey and Stone 2006). The introduced *Hemidactylus turcicus* geckos are very cryptic reptiles, potentially living in and around many of our homes and institutions without our knowledge. As a result, several types of studies on the House Gecko may be needed to understand its potential range, methods of dispersal, breeding behavior, diet, competition, etc. Such research can help biologists better understand the effects of *H. turcicus* on native fauna and how the gecko lives and disperses in the different habitats of the southern states. Whether the introduction of *H. turcicus* to the states should be of much concern to conservationists is largely unknown.

One of the earliest populations of *H. turcicus* observed in the United States was in Key West, Florida by Fowler in 1915 (Meshaka et al. 2006). They have since established populations throughout several states, including Tennessee, and have had several sightings in many others (Locey and Stone 2006; Meshaka et al. 2006; Nordberg et al. 2013). The primary means of introduction of *H. turcicus* to new areas has been documented to be directly related with human transportation, in which populations are established far from other populations because of the intentional or accidental transportation of individuals (Locey and Stone 2008). Eggs of *H. turcicus* have been found in luggage and transported goods, indicating that this type of dispersal is a strong influence on the successful establishment of this species throughout the states (Locey and Stone 2006). It is possible that geckos are also intentionally brought to new regions as a method of pest control, as these geckos hunt and consume a variety of insects. The geckos in this study were sampled from four adjacent buildings on the campus grounds of Middle Tennessee State University (MTSU). It is possible the geckos were brought to the campus with the incidental help of the university's band, which readily travels around the surrounding states, but the true source of the gecko's presence on campus remains uncertain.

While the primary form of dispersal for *H. turcicus* appears to be large-scale movement patterns via transportation by humans, small-scale diffusion dispersion may also occur from within populations of this species (Locey and Stone 2008; Stabler et al. 2011). Diffusion dispersal is described by Locey and Stone (2008) as the movement or dispersion of individuals “from high to low density”. When discussing diffusion dispersal in this study, I am referring to the movement of individual geckos from one area of MTSU to another without the direct influence of humans. Though diffusion rates are presumably low in populations of *H. turcicus*, according to previous studies individual geckos have been seen away from populated buildings, and new inhabitants have been known to arise at buildings adjacent to those with previously established populations (Locey and Stone 2008; Nordberg et al. 2013). It is likely that much of this dispersal within populations is caused by mothers laying their eggs in communal nests slightly farther from the more densely populated areas of buildings (as discussed by Selcer 1986). However, some authors suggest that direct social interactions among the geckos may also contribute to this small-scale population dispersion (Briggs 2012; Locey and Stone 2008). One such interaction seen in *H. turcicus*, as well as several other lizard species, involves agonistic behaviors.

Agonistic behaviors have been observed within *H. turcicus* between adult males and adult males toward juveniles (Briggs 2012). In a lab study *H. turcicus* males were observed to show aggressive behavior “more prevalent[ly]” when another male was overtly near (Briggs 2012). Briggs (2012) also observed some males to become intensely violent toward juveniles. Though these observational tests conducted by Briggs reveal some important insight into the frequency and context of aggressive and territorial behavior in *H. turcicus* males, little has been done to test the underlying physiological mechanisms that drive such tendencies in *H. turcicus*. Likewise, little research has been conducted on female aggression in this species of gecko.

In several vertebrate species, including reptiles, testosterone has been shown to strongly influence the expression of aggressive behavior (reviewed in Adkins-Regan 2005; Archer 1988; DeNardo and Sinervo 1994; Fox et al. 2003; Golinski et al. 2014; Greenberg and Crews 1983; Monaghan and Glickman 1992; Moore 1988; Nelson 2000; Sinervo et al. 2000). Testosterone has become known to many as a male-biased hormone, due to it link to the development of male-typical structures and behavior. Schořálková et al. (2018) recently reported that exogenous treatments of the hormones testosterone and dihydrotestosterone together induced male-typical aggression and the development of the male-copulatory organs (hemipenes) in female *Paroedura picta* geckos. Furthermore, Schořálková et al. (2018) go on to describe how males of *P. picta* would more readily attack females that were treated with the androgens. Cox et al. (2015) found other dimorphic effects of testosterone treatment on the brown anole, *Anolis sagrei*, in which females treated with exogenous testosterone developed larger and brighter dewlaps. These studies strongly suggest that testosterone is responsible for many of the male-typical characteristics seen in reptiles, but there are also numerous studies in which testosterone does not appear to have a direct role in triggering aggressiveness or that reveal other factors involved in its expression (reviewed in Adkins-Regan 2005; Cooper et al. 1987; Fox et al. 2003; Golinski et al. 2014; Kabelik et al. 2004; Wingfield 2005). For example, Moore (1988) discusses testosterone manipulation studies conducted on free-ranging male mountain spiny lizards, *Scleropus jarrovi*. After implanting castrated male *S. jarrovi* with testosterone-implants during the breeding season, Moore (1988) reports that typical breeding season aggressive tendencies were restored, but that when the testosterone implants were given to castrated male *S. jarrovi* during the non-breeding season, the breeding season levels of aggression were not restored. This suggests that other environmental factors are likely contributing to the changes involved in breeding season aggression in *S. jarrovi*. Whether testosterone plays a key role in the agonistic behaviors of *H. turcicus* geckos is unknown.

A common method for testing the effects of testosterone on the expression of aggression is by manipulating circulating levels of the androgen and then observing the changes, if any, in aggressive behavior. Testosterone levels can be manipulated by either experimentally increasing testosterone levels, for example via a testosterone implant, or by lowering circulating testosterone levels via surgical castration (removal of the testes in males). Especially strong evidence for the androgen-dependence of aggressive behavior requires not only a decrease in the expression of aggression upon castration, but also the normal expression of aggressive behavior in castrated individuals given supplemental testosterone (reviewed in Golinski et al. 2014; Moore 1988).

Factors other than testosterone are also known to affect the expression and/or timing of agonistic behaviors in several lizard species (discussed in Fox et al. 2003). Internal factors, such as the secretion of hormones other than testosterone or the amount of energy reserves (e.g., fat), can influence an individual's behavior and the interactions it has with members of its species (Fox et al. 2003; Moore 1988; DeNardo and Sinervo 1994). The surrounding environment, such as changes in temperature or day length, resource availability, home range size, and predation pressure, may also shape the behavioral interactions between members of a species (Fox et al. 2003). The factors that influence the behavioral ecology of *H. turcicus* are mostly unknown.

By examining histological sections of the testes throughout the months of December to August, Rose and Barbour (1968) revealed that spermatogenesis, the process of making sperm cells, commenced in the testes of a southern Louisiana population of *H. turcicus* near the end of fall to the start of winter. By December and into January spermatocytes became much more prevalent and sperm began maturing by spermatogenesis. In April the epididymides were filled with sperm and the epithelium had "reached its maximum height". This suggests that April is the beginning of the reproductive season for males in this population of *H. turcicus* in southern Louisiana. Finally, during July sperm production began to notably decrease and in August the testes atrophied and remained quiescent until the following winter (Rose and Barbour 1968). A study by Selcer (1986) on an *H. turcicus* population in southern Texas also found males to be most reproductively active from April 1 to September 1. In south-central Florida, Meshaka (1995) found local *H. turcicus* breeding cycles to closely match that reported in Louisiana and Texas populations. However, Punzo (2001) studying a population at Tampa University, Florida, stated that male *H. turcicus* were most reproductively active from early June to August 20, which is a smaller frame of reproductive activity than suggested by some of the other claims. These small differences in the periods of reproductive activity among the populations of *H. turcicus* studied in the southern U.S. hint towards some potential variations in the breeding seasons among these populations. Based on these earlier studies, the breeding season of *H. turcicus* in middle TN is estimated to fall between May and August, but studies on the reproductive cycles of *H. turcicus* populations in TN are needed to say this with more certainty.

Hemidactylus turcicus males, like many lizard species, appear to have a prenuptial testosterone cycle in which rising testosterone levels correlate with the maturation of sperm; testosterone levels peak in the spring, and then drop throughout late spring and summer as reproductive activities decrease (Norris and Lopez 2011; Punzo 2001; Rose and Barbour 1968; Selcer 1986). Within the testes, Leydig cells are responsible for producing testosterone (Norris and Lopez 2011). This process of testosterone production is known as steroidogenesis, and it occurs simultaneously with spermatogenesis, the production of sperm cells. The co-production of testosterone and sperm in the testes supports the idea that testosterone may play an important role in the exaggeration of sexual behaviors, such as intrasexual aggression, in seasonal breeders (Golinski et al. 2014; Norris and Lopez 2011). It is therefore expected that circulating testosterone levels for *H. turcicus* males will be highest in spring and influence sexual behaviors, including intrasexual aggression.

In female *H. turcicus* the time of reproductive activity closely matches that of the males (Rose and Barbour 1968; Selcer 1986). Selcer (1986) collected 323 females from a population of *H. turcicus* in southern Texas and tracked their reproductive activities. Selcer (1986) found that females are reproductively active between April 1 and September 1, with most females being active from May 1 to August 15. Communal nesting appears to be common in the females of *H. turcicus*, with nests containing up to twenty eggs (Selcer 1986). Females are reported to lay approximately 2 eggs per clutch and can produce two to three clutches per breeding season (Punzo 2001; Rose and Barbour 1968; Selcer 1986). These nests have been found in closets, boxes, cabinets, and other human belongings (Selcer 1986). Although female *H. turcicus* share a close reproductive cycle with the males, I suspect that female *H. turcicus* normally exhibit very low intrasexual aggression based on the evidence of female communal nesting behaviors. However, because other studies on lizard behaviors have shown testosterone treatments to elevate aggression, including in females (Woodley and Moore 1999; Rhen et al. 1999), I predict that testosterone treatment will increase both male and female aggressive behaviors in *H. turcicus*.

This study was conducted in the spring and summer seasons during the presumed breeding season of *H. turcicus*. I attempt to gain further insight into the social behaviors expressed in adult *H. turcicus* by examining staged intrasexual interactions. I then test testosterone as an underlying influence on aggressive tendencies in adult *H. turcicus* via application of a topical testosterone solution. Therefore, this study has two primary objectives: (1) Do intrasexual aggressive behaviors occur in both males and females of *H. turcicus* and if so, how do the sexes differ in their aggressive tendencies? (2) Does an increase in testosterone intensify these intrasexual aggressive behaviors? I test these questions by staging four behavioral interaction tests: baseline male-male tests, manipulated male-male tests in which one member of each contest has experimentally elevated testosterone, baseline female-female tests, and manipulated female-female tests.

Thesis Statement

The objective of this study is to better understand the intrasexual behaviors of the nonnative Mediterranean House Gecko (*H. turcicus*) and how these behaviors might be affected by changes in circulating testosterone. I expect that males will be significantly more aggressive during intrasexual encounters than females. Furthermore, I predict that treating geckos with topical testosterone will increase the expression of intrasexual aggression in both male and female *H. turcicus*. By studying the intrasexual behavior of *H. turcicus* I hope to increase our understanding of the social behavior of this rapidly spreading nonnative gecko. Additionally, this study may provide insight into the physiological mechanisms that regulate social behaviors in geckos, and how such behaviors might influence the dispersion of this species. Finally, I believe the results of this study will help guide future studies on *H. turcicus*, such as the interactions it may have with other organisms (prey, predators, or competitors) in its introduced range of the southern United States.

Methods and Materials

Capture Efforts

This study took place from April - June of 2018 on the campus of Middle Tennessee State University (Murfreesboro, TN). Thirty-one adult Mediterranean geckos, *Hemidactylus turcicus*, were captured at night by hand (Tennessee Wildlife Resources Agency; permit No. 1483). Capture efforts occurred during April and early May between 20:00-24:00 hrs. Four adjacent buildings on the MTSU campus were found to be inhabited by *H. turcicus* during the capturing efforts: Jones Hall, Wright Music building, Tucker Theater building, and Forrest Hall. Jones Hall, Forrest Hall, and Wright Music were found to contain geckos inhabiting all four faces of the buildings. Tucker Theater appeared to be inhabited by multiple geckos on only one of its four faces, although I did find a single gecko on one of the other three faces on one occasion. The other buildings surrounding these four were only briefly examined for geckos by myself, for which none were found. However, a more careful and thorough survey is necessary to properly determine if other buildings on the MTSU campus are inhabited by *H. turcicus*.

Of the thirty-one adult geckos involved in the behavioral study, nineteen caught were males and the remaining twelve were females. Juvenile and hatchling geckos were intentionally (and successfully) avoided during capture efforts, although many were observed on the buildings. A laser pointer was often used to entice geckos high up on the walls to come down closer for capture (E.J. Nordberg, pers. comm.). Upon capture, geckos were placed individually into a cloth bag and taken to the animal facility within the science building of the MTSU campus. Each gecko had its sex, weight (± 0.1 g), and snout-to-vent length (SVL; ± 0.5 mm) recorded. Sex was determined by examining individuals for the presence of preanal pores. Preanal pores are known to be a male-related characteristic in *H. turcicus*, thus the presence of this characteristic indicated the individual as a male gecko, while the absence of preanal pores indicated a female gecko (Locey and Stone 2006). Individuals with an SVL of 42 mm or greater were classified as adults (Locey and Stone 2006). All captured geckos surpassed this 42 mm rule and were thus determined to be adults.

Housing and Care

Geckos were individually housed in 20.3 x 33.0 x 14.0 cm plastic containers, except for four individuals who were housed in 15.2 x 26.6 x 13.3 cm plastic containers due to limited spacing. The housing and other procedures utilized in this study were approved by the MTSU IACUC committee (permit no. 18-3010). Cage tops were constructed of a flexible metal hardware cloth to prevent geckos from escaping while also providing plentiful airflow into the cage. A coconut husk substrate was used in each cage and 3 to 4 local Magnolia tree leaves were provided as hides for each gecko. Substrate and hides were cleaned or replaced approximately every three weeks or as necessary. Geckos were fed 2-3 crickets *(Acheta domesticus)* dusted in Fluker's Repta Calcium vitamin supplement every Monday, Wednesday, and Friday. Gecko cages were thoroughly misted with water daily. Overhead room lights were preset to activate from 0600 to 1900 to match the day length of Murfreesboro, TN during the summer season. Every four cages shared a heat lamp placed on top of the cages which provided a basking site over the corner of each cage for seven hours a day using timers preset from 1000 to 1700 hrs. The wattages of the heat lamp bulbs ranged from 43W to 75W depending on the sizes of the four cages it was positioned above.

Behavioral Testing and Treatments

To test intrasexual aggressive behaviors between individual *H. turcicus* a 25.4 x 50.8 cm glass aquarium arena was set up with coconut substrate, a cardboard divider, and a cardboard shelter on each side. The morning of each test day, two closely size-matched geckos were placed into opposite ends of the arena and were provided around 10 hours to acclimate to the arena before beginning interaction testing. The cardboard dividers were used to prevent either of the paired geckos from coming into physical contact before beginning the tests. Three arenas were used so that a total of six geckos, in pairs of two, could be examined on each test day. These pairs were observed one at a time. Behavioral trials for the pairs commenced after sunset, between 2000-0111 hrs. One to two hours prior to the start of an interaction each gecko in the arena received water via misting and was fed 1-2 crickets. To minimize the effect of observer interference, I watched from behind a blind set up directly in front of the testing arenas. A dimmable light was used to provide some light for the observer to watch and record the behaviors. To begin an interaction, I removed the cardboard divider, and then took position behind the blind to observe and record the interaction. Behaviors were recorded using a Sony Handycam Vision Hi8 (model: CCD-TRV68). The duration allotted for each trial varied because of two reasons. First, some trials were relatively long because the geckos had a long latency to exhibit any measurable behavior, and second, some trials were ended early to avoid injury to contestants engaged in vigorous fights. On average, trials lasted 41 minutes (Range: 23 – 71 min). Because of these differences in the duration of interactions, I scaled all behavioral data to a time of 30 minutes prior to statistical analyses. This scaling was accomplished by obtaining a correction factor by dividing the real time of each trial by 30 minutes and then using the correction factor value of that trial to divide the behaviors observed by the individuals involved in that trial. Such scaling could result in underestimating the behavioral frequencies of especially aggressive subjects, but was deemed necessary to avoid excessive stress on the subjects. Upon completion of each interaction, geckos were placed back into their home cages. Individuals in each pair were usually identifiable by their distinctive markings, but on occasion individuals were marked with non-toxic paint.

The behaviors recorded were approaches, freezes, retreats, pushups, back arches, tail wagging, biting, fighting, chasing, tongue flicking, licks, and vocalizations. These behaviors have been reported to occur in lizards expressing aggression and/or territorial behavior, including in *H. turcicus* (Briggs 2012; Fox et al. 2003; Norris and Lopez 2011). A pushup display is characterized by the individual bringing their body lower to the ground by bending the limbs, and is followed directly with the individual lifting their body upwards by straightening the limbs. A back arch display occurs when the individual arches the back upwards, which is usually accompanied with stretching of the limbs. Some individuals were noticed to maintain a back arch display for several seconds while in ‘combat’ with its conspecific. Tail wagging is characterized by the individual lifting the tail and swinging it in a side-to-side motion. Even some individuals who had regenerating nubbin tails were observed to wag their tails. Fights were determined when both of the paired individuals participated in the engagement of a mutual state of physical ‘combat’ through a series of exchanged bites and or scratches. Often times a fight would be preceded and followed by vocalizations from one or both of the paired geckos.

However, to help prevent biasing my results I categorized both the multi-click and squeaking vocalizations as neutral behaviors. Methods of identifying and classifying these behaviors were based both on my own observations and previous studies conducted on *H. turcicus*, including Briggs' (2012) study on *H. turcicus* male aggression, Marcellini's (1977) study on gecko acoustic and visual behavior, and Frankenberg's (1982) study on *H. turcicus* vocal behavior.

The observed gecko behaviors were placed into three general categories: aggressive, submissive, or neutral. Aggressive behaviors included back arches, tail wags, pushups, snaps, bites, fights, and chases. Submissive behaviors included freezes and retreats. Freezes were noted when an individual ceased all movement in response to a nearby or approaching conspecific, while retreats were classified as an individual fleeing from a nearby or approaching conspecific. Neutral behaviors included approaches, tongue-flicks, multi-click vocalizations, squeaking vocalizations, and licks. Many of the neutral behaviors could be considered chemical or visual exploratory behaviors, but are listed as neutral for the simplicity of this study.

<u>Male-Male Interactions</u>

I first conducted nine behavioral trials between pairs of non-manipulated males; hereafter called the baseline male-male trials. I tested three pairs a night over the course of three test days spaced by a single day between test days. For the baseline test individuals were placed into the arena at around 0900-1000 and given approximately10 hours to acclimate before undergoing behavioral testing between 2000-0111. The male-male baseline tests occurred from May 14 – 18. Following the completion of these baseline tests and an additional 3 days of rest, I repeated the behavioral tests but this time the plasma testosterone level of one member of each pair was experimentally elevated; hereafter called the testosterone manipulated male-male trials. For this set of tests, the same eighteen males were re-matched into pairs with different opponents of similar size. Plasma testosterone was experimentally elevated by treating half the males, randomly chosen, with a topical suspension of testosterone (1 μg / μl of sesame oil per gram of body weight) on their dorsum immediately caudal to the base of their skull. The suspension was applied using a micropipettor. Control males were similarly treated but with sesame oil alone. Males were treated each morning for three consecutive days, and then observed in the arena the night of the final treatment. Therefore on manipulated interaction test days the male geckos received their final treatment around 0800-0900, were placed into the arena at around 0900-1000, given approximately10 hours to acclimate, and then underwent the behavioral test between 2000-0111. These interactions were observed over the course of three test days, spaced by a single day between the first two test days and two days between the second and last test days. Dr. Matthew Klukowski performed all testosterone and control treatments. All observations were conducted blind to treatment status of animals. One male gecko died for unknown reasons after completion of the baseline male-male trials, but prior to the start of the testosterone manipulated male-male trials. The deceased individual was replaced with a newly captured male. This replacement gecko was given six days to adjust to the animal facility before its involvement in the study. The testosterone manipulated male-male trials occurred from May 23 – 28.

Female-Female Interactions

After completing all the male interaction trials, I conducted six behavioral trials between pairs of non-manipulated females; hereafter called the baseline female-female trials. Just as done with the male baseline tests, females were placed into the arena at around 0900-1000 of test day and given approximately10 hours to acclimate before undergoing the behavioral testing between 2000-0111. The baseline female-female trials took place from May 30 – June 1. The same testosterone and sesame oil solutions as described above were topically applied using the same methodology as was used for the male geckos, prior to conducting a final set of female-female interaction trials; hereafter called the testosterone manipulated female-female trails. As with the males, in this second set of manipulated female trials, the females were paired against different opponents than those they were paired with in the previous tests. Just as conducted with the males, on the mornings of the manipulated interaction test days the female geckos received their final treatment around 0800-0900, were placed into the arena at around 0900-1000, given approximately10 hours to acclimate, and then underwent the behavioral test between 2000-0111. Testosterone manipulated female-female trials occurred from June 4 – 6.

Enzyme-linked Immunosorbant Assay (ELISA)

To ensure that the topically applied testosterone was effective in elevating plasma testosterone concentrations in male and female geckos, an enzyme-linked immunosorbant assay (ELISA) was run on a randomly selected subsample of geckos (n = 8 males and n = 7 females). Blood was collected by making a small incision on the superior surface of the oral cavity adjacent to the orbit of the eye, and then drawing the upwelling blood into a heparinized microcapillary tube. Average bleeding time counted from the moment the gecko was first handled was 180 ± 83.13 sec (Range: 62 - 310 sec). It took less than 5 sec to hand capture each gecko from its container. After centrifugation, the resulting plasma was frozen at -80 C until assayed. A testosterone ELISA kit (Enzo Life Sciences, no. ADI-900-065) was used to conduct the assay on the gecko blood collected. Essentially, this assay utilized an enzyme-linked labeled testosterone to compete for antibody binding sites with the endogenous testosterone from the geckos bled, followed by the use of a standard curve for comparison.

At the time of bleeding, I also measured SVL and mass, as well as total tail length, new tail length (regenerated tail), and rechecked the sex for each of the 15 geckos that were bled. This data was used to confirm that the housing and feeding protocol used was adequate to allow the geckos to at least maintain or even improve their body condition.

Statistical Analyses

The statistics program 'R' was used to conduct the statistical analyses (R Core Team 2018; URL https://www.R-project.org/.) Before conducting the appropriate test, the data was first examined for normality and equal variances with the use of boxplots, histograms, and F-tests.

To test whether the testosterone manipulation was effective in elevating plasma testosterone, the Mann-Whitney U test was used to compare testosterone concentrations of sesame oil (control) and testosterone-treated subjects. These tests were conducted separately for each sex because of the well-established difference in testosterone between the sexes. The Mann-Whitney U test was also used to detect differences in behavior between testosterone-treated and sesame oil-treated geckos within just the testosterone manipulation trials.

Directional Wilcoxon sign-ranked tests were used to test for changes in behavior between the baseline and testosterone manipulation trials within subjects, as well as between body size at initial capture and post-testing.

Finally, I tested for sex differences in behavior with a directional Welch Two Sample t-test that included data from the baseline tests only. The use of directional tests is justified because based on the literature, male lizards often exhibit a greater frequency of the recorded behaviors, especially those associated with territoriality, than females.

Results

<u>Effect of Lab Housing on Gecko Growth</u>

The geckos that were bled exhibited positive growth in both body mass and SVL over the course of laboratory housing. Snout-vent length ($W = 0$, $n = 14$, $p = 0.00053$; Fig. 1) and body mass ($W = 12$, $n = 14$, $p = 0.019$; Fig. 2) were significantly greater upon completion of the behavioral tests than at initial capture.

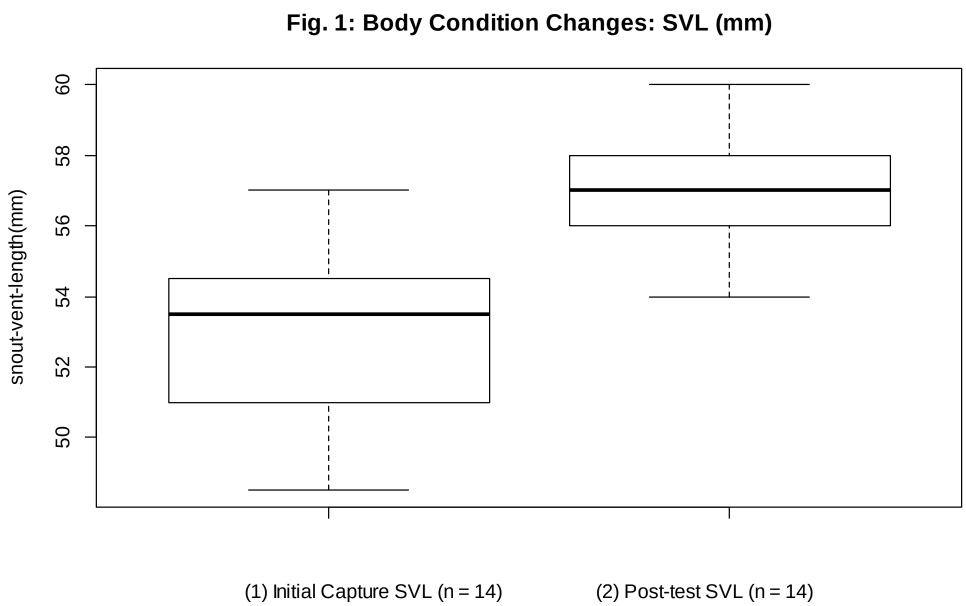

Figure 1: The snout-vent length (SVL) of a subset (i.e., those that were bled, $n = 8$ males, $n = 6$ females) of Mediterranean House Geckos (*Hemidactylus turcicus*) upon initial capture and after completion of the behavioral tests 14 weeks later. The boxplot shows the minimum and maximum values, the median value (darker line), and the first and third quartiles.

Fig. 2: Body Condition Changes: Mass (g)

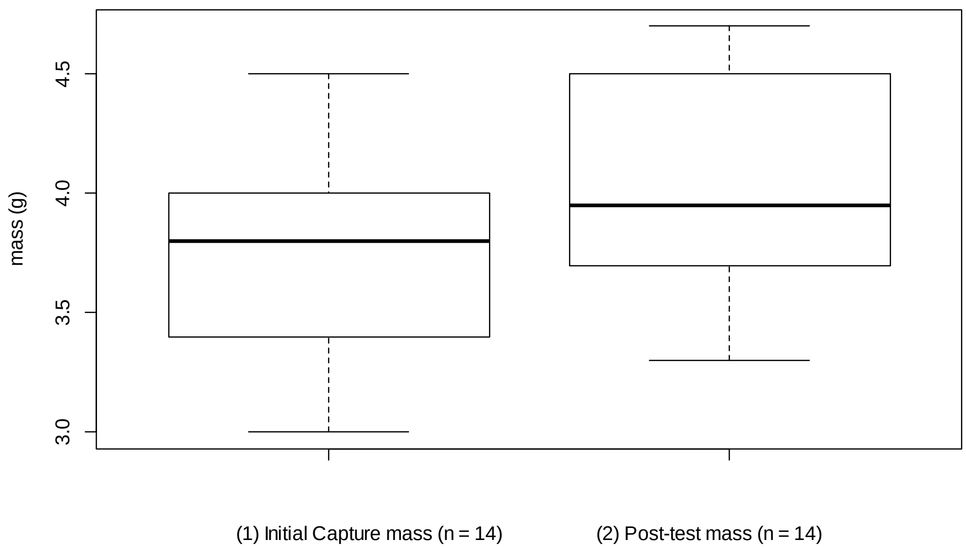

Figure 2: The mass of a subset (i.e., those that were bled, $n = 8$ males, $n = 6$ females) of *H. turcicus* geckos upon initial capture and after completion of the behavioral tests.

Effectiveness of Topically Applied Testosterone

Topically applied testosterone dissolved in sesame oil effectively elevated circulating concentrations of testosterone in male geckos ($U = 16$, $n = 8$, $p = 0.014$; Fig. 3) and in female geckos ($U = 12$, $n = 7$, $p = 0.029$; Fig. 4). Median plasma testosterone concentrations were elevated over 7 fold in male geckos and by 36 fold in female geckos. The lowest concentrations of plasma testosterone were observed in control females (medians: 0.56 vs 2.4 ng/ml in control females vs control males, respectively). However females treated with testosterone had noticeably higher concentrations of plasma testosterone than testosterone-treated males in spite of receiving the same dosage (medians: 20.5 vs 12.0 ng/ml).

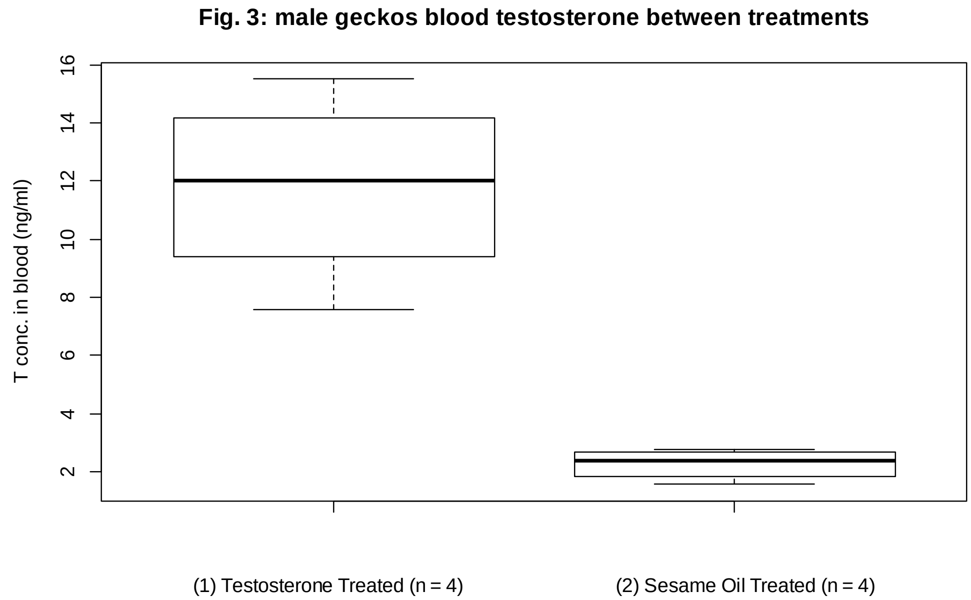

Figure 3: Plasma testosterone levels of a subset of male *H. turcicus* (i.e., those that were bled $n = 8$).

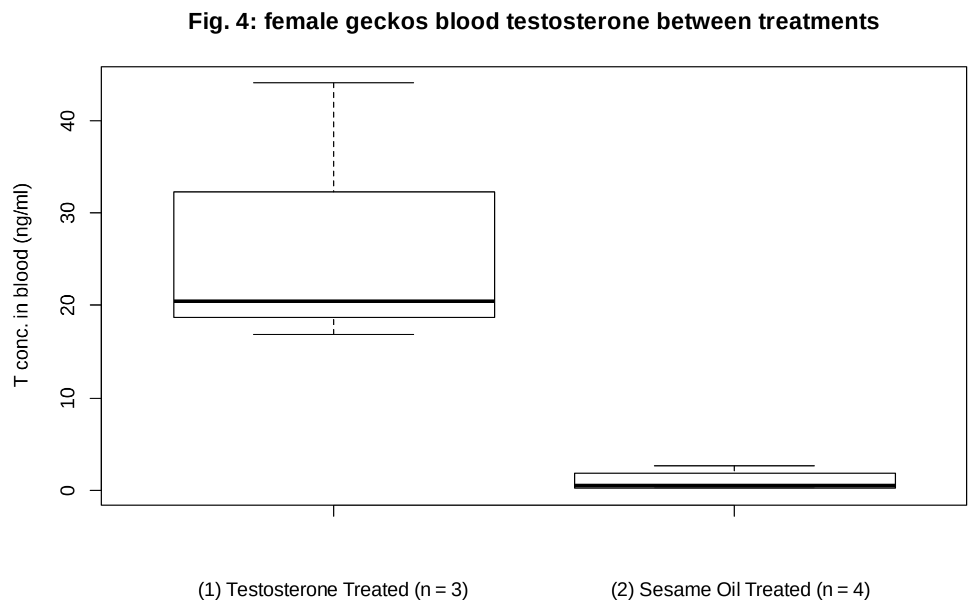

Figure 4: Plasma testosterone levels of a subset of female *H. turcicus* geckos (i.e., those that were bled $n = 7$).

Influence of Testosterone vs Sesame Oil on Intrasexual-Male Behaviors

Male geckos treated with testosterone did not exhibit a greater number of pushups ($U = 41$, $n = 18$, $p = 1$), back arches ($U = 35$, $n = 18$, $p = 0.65$), or tail wags ($U = 32$, $n = 18$, $p = 0.40$) than sesame oil-treated geckos (Fig. 5). Chases and snaps were infrequently observed in both testosterone-treated and sesame oil-treated male geckos. Chases occurred only twice in testosterone-treated and sesame oil-treated males (each from separate males), and did not significantly differ following scaling ($U = 39$, $n = 18$, $p = 0.90$). Snaps were observed on two occasions in the testosterone-treated males and on three occasions in the sesame oil-treated males and thus did not significantly differ between the groups ($U = 37$, $n = 18$, $p = 0.68$). Bites were more common, but also did not occur more frequently in testosterone-treated males than in sesame oil-treated males ($U = 34$, $n = 18$, $p = 0.59$; Fig. 6). Fights were equal between testosterone-treated and control males as I considered a fight to occur when both individuals participated, and each pairs consisted of one sesame oil-treated and one testosterone-treated male. Four fights were observed between testosterone-treated and sesame oil-treated males ($n = 8$), which upon scaling this data to 30-minutes becomes approximately two fights ($n = 4$). Overall, biting incidents and back arch displays were the most frequently observed aggressive behaviors seen in the testosterone manipulated trials.

The frequency of neutral behaviors similarly did not differ between the two male treatment groups. Testosterone-treated and control males performed similar numbers of approaches ($U = 56$, $n = 18$, $p = 0.18$), tongue flicks ($U = 51.5$, $n = 18$, $p = 0.32$), and squeaks ($U = 33$, $n = 18$, $p = 0.43$; Fig. 7). The remaining neutral behaviors, licks and multiple-click vocalizations, were more rarely observed. Licks occurred twice in separate testosterone-treated males and were unseen in sesame oil-treated males, thus the two groups had no significant difference in the occurrence of licks ($U = 33$, $n = 18$, $p = 0.43$). Multiple-click vocalizations occurred twice in sesame-oil treated males and once in testosterone-treated males, and showed no difference in the frequency of multiple-clicking vocalizations between the two groups ($U = 37$, $n = 18$, $p = 0.68$). Approaches and tongue flicks were the most frequently observed neutral behaviors. Lastly, testosterone treatment did not have an effect on the frequency of submissive behaviors. The treatment groups did not differ in the number of freezes ($U = 41.5$, $n = 18$, $p = 0.97$) or retreats ($U = 45.5$, $n = 18$, $p = 0.69$; Fig. 8).

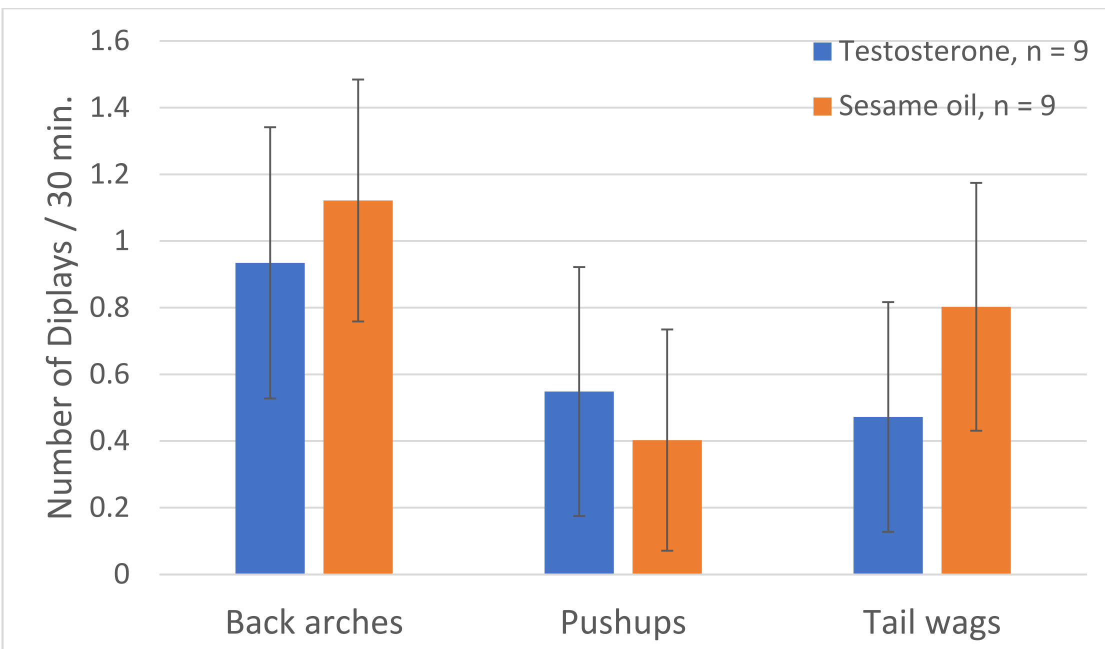

Figure 5: Intrasexual aggression (Mean ± standard error (SE)) in testosterone-treated (n = 9) versus control (sesame oil; n = 9) male *H. turcicus* geckos. Because trials varied in duration, the data were scaled to 30 minutes (see Methods).

Figure 6: Physical bites (Mean ± SE) during aggressive intrasexual encounters in testosterone-treated (n = 9) versus control (sesame oil; n = 9) male *H. turcicus* geckos. Because trials varied in duration, the data were scaled to 30 minutes.

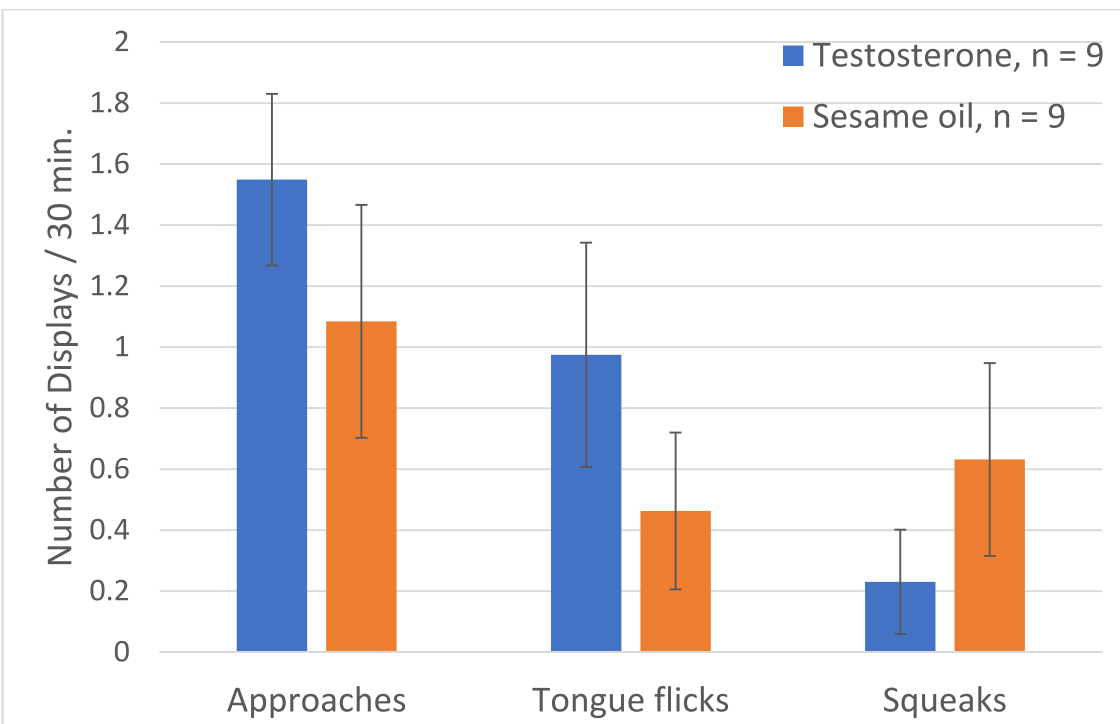

Figure 7: Intrasexual neutral behaviors (Mean ± SE), approaches, tongue flicks, and squeaks, in testosterone-treated (n = 9) versus control (sesame oil; n = 9) male *H. turcicus* geckos. Because trials varied in duration, the data were scaled to 30 minutes.

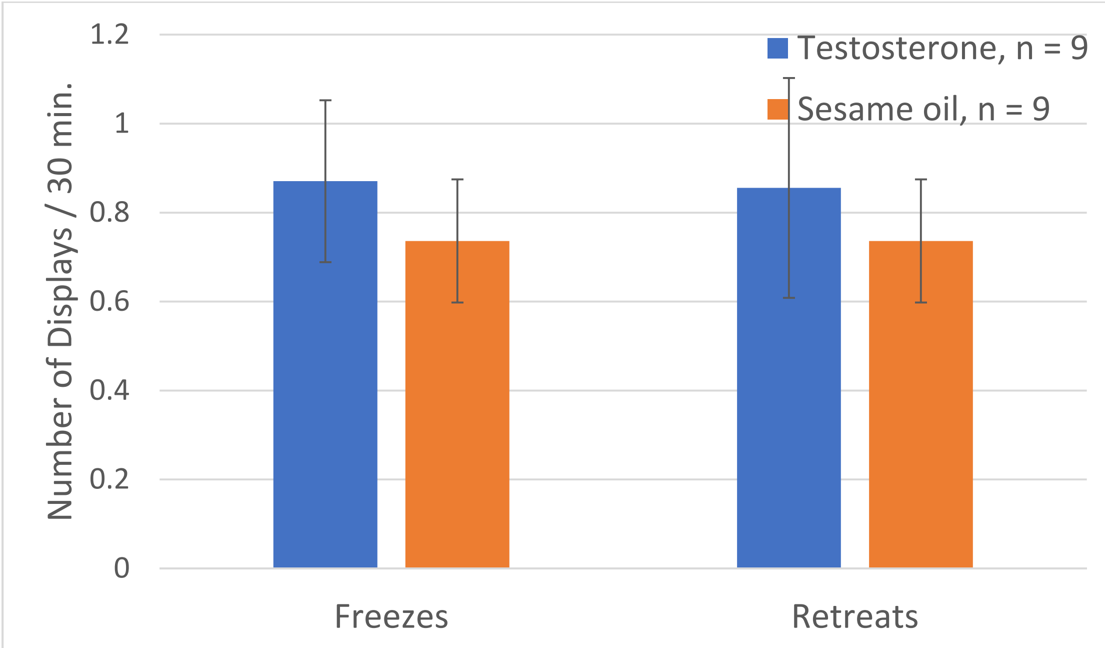

Figure 8: Intrasexual submissive behaviors (Mean ± SE), freezes and retreats, in testosterone-treated (sesame oil: *n* = 9) versus control (*n* = 9) male *H. turcicus* geckos. Because trials varied in duration, the data were scaled to 30 minutes.

<u>Intrasexual Male Behaviors Before and after Testosterone Treatment</u>

Male geckos did not perform more pushup displays after they were treated with testosterone than they did during the baseline male-male tests ($W = 0$, $n = 9$, $p = 0.37$; Fig. 9). No male geckos performed pushups pre-testosterone treatment (baseline tests), but two of the nine male geckos performed pushups post-testosterone treatment. Similarly, back arches ($W = 6$, $n = 9$, $p = 0.21$; Fig. 10) and tail wags ($W = 3$, $n = 9$, $p = 1$; Fig. 11) did not significantly differ in male geckos pre- and post-testosterone treatment, but five of the nine male geckos did perform more back arches post-testosterone treatment than observed during their baseline interactions. Snaps ($W = 3$, $n = 9$, $p = 1$; Fig. 12), bites ($W = 4$, $n = 9$, $p = 0.21$; Fig. 13), fights ($W = 8$, $n = 9$, $p = 0.68$; Fig. 14), and chases ($W = 1$, $n = 9$, $p = 1$), were also all found to be unaffected by testosterone manipulation, but five of the nine males performed more bites after testosterone treatment.

Similar to the aggressive behaviors, the neutral behaviors showed no significant changes after testosterone manipulation. Male geckos performed similar numbers of clicking vocalizations ($W = 1$ $n = 9$, $p = 1$), tongue flicks ($W = 16$, $n = 9$, $p = 0.83$), squeaks ($W = 8$, $n = 9$, $p = 0.36$), and licks ($W = 6$, $n = 9$, $p = 0.86$) after they were treated with testosterone as they did in the baseline tests. Male geckos did not perform more approaches after treatment, but the difference approached significance ($W = 6$, $n = 9$, $p = 0.055$; Fig. 15), and seven of nine males approached more following treatment of testosterone. Lastly, submissive behaviors were unaffected by testosterone treatment. Males treated with testosterone performed a similar number of retreats ($W = 10$, $n = 9$, $p = 0.55$) and freezes ($W = 17$, $n = 9$, $p = 0.57$) as they had in the baseline tests.

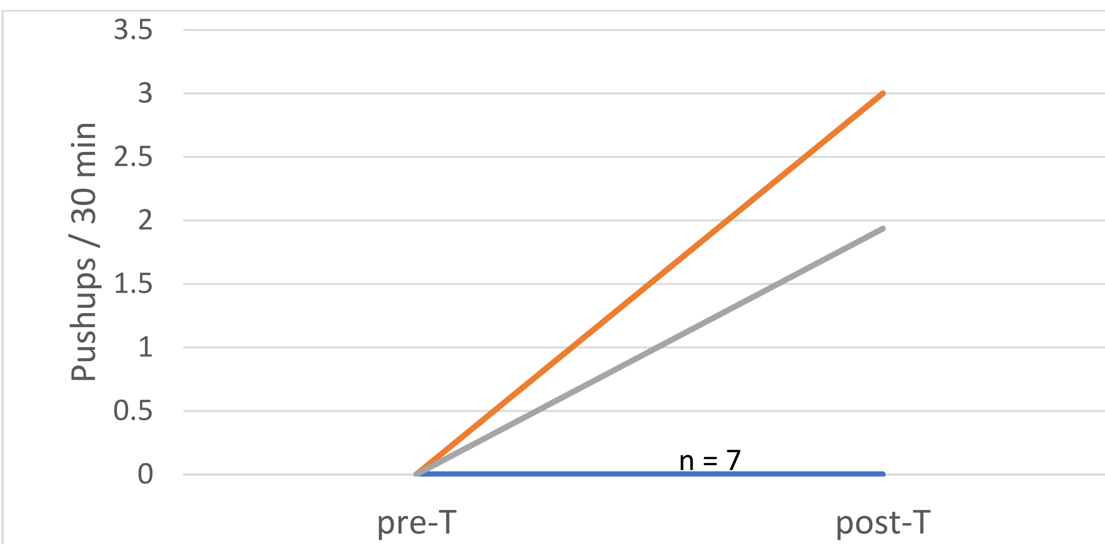

Figure 9: The number of times a male *H. turcicus* gecko exhibited pushup displays toward his opponent prior to testosterone treatment (e.g., in the baseline tests) and after testosterone treatment. Individual lines represent individual geckos ($n = 9$). Seven individuals exhibited zero pushup displays both pre- and post-testosterone treatment. Note that geckos faced different, size-matched conspecifics pre-and post-testosterone.

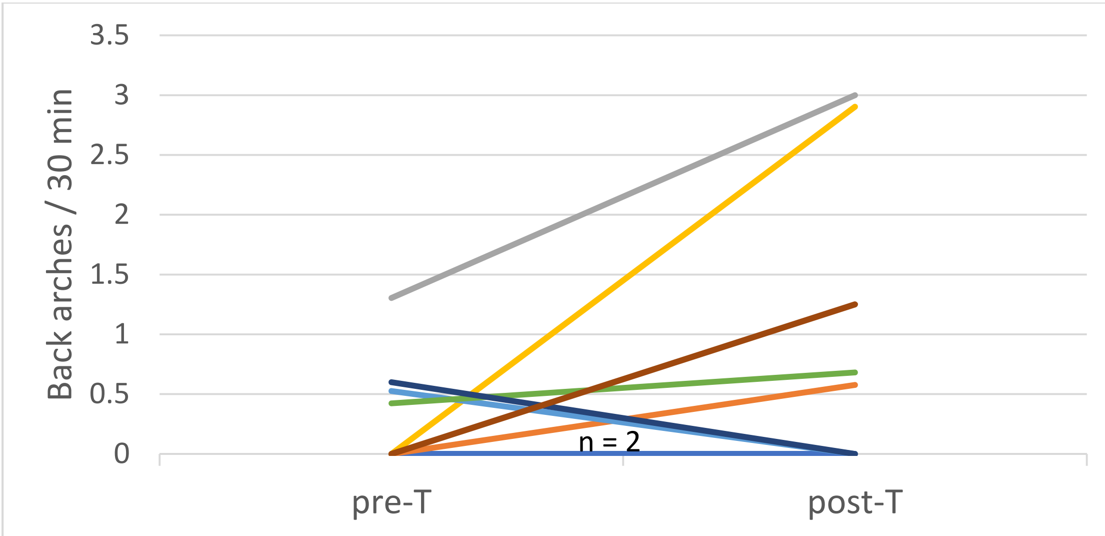

Figure 10: The number of times a male *H. turcicus* gecko exhibited back arch displays toward his opponent prior to testosterone treatment (baseline tests) and after testosterone treatment.

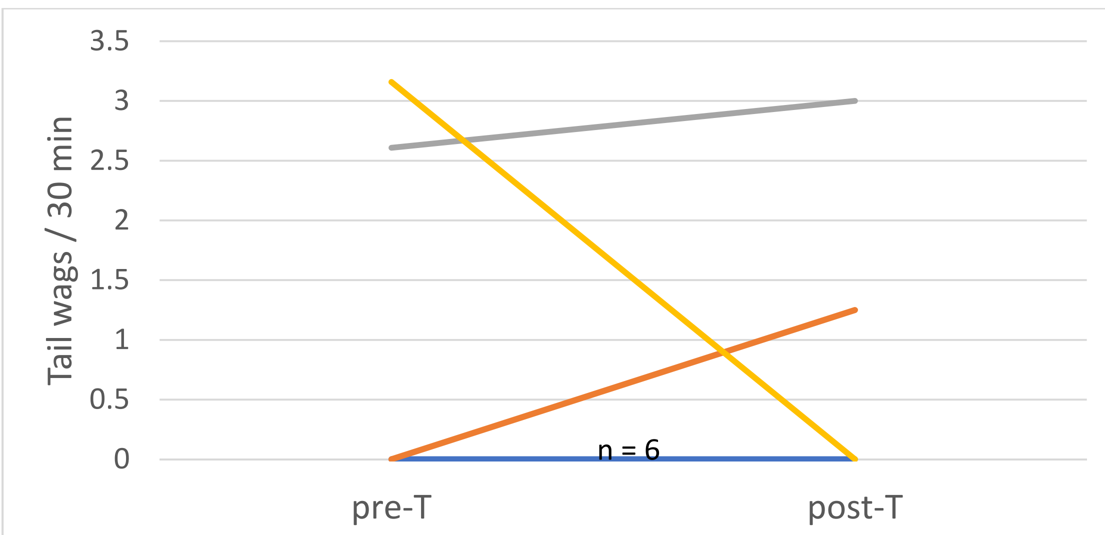

Figure 11: The number of times a male *H. turcicus* gecko exhibited tail wag displays toward his opponent prior to testosterone treatment (baseline tests) and after testosterone treatment.

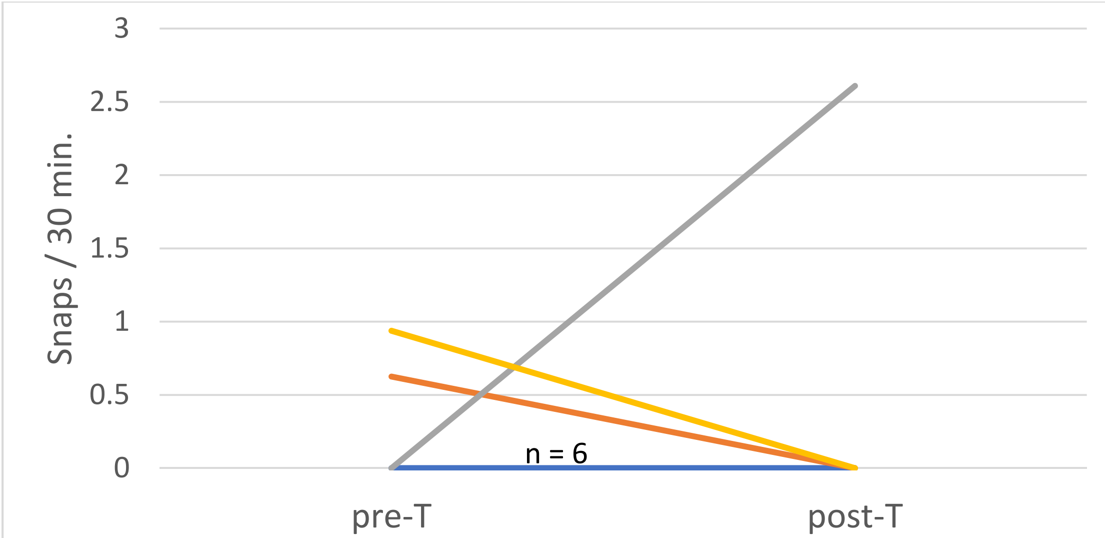

Figure 12: The number of times a male *H. turcicus* gecko exhibited snaps toward his opponent prior to testosterone treatment (e.g., in the baseline tests) and after testosterone treatment.

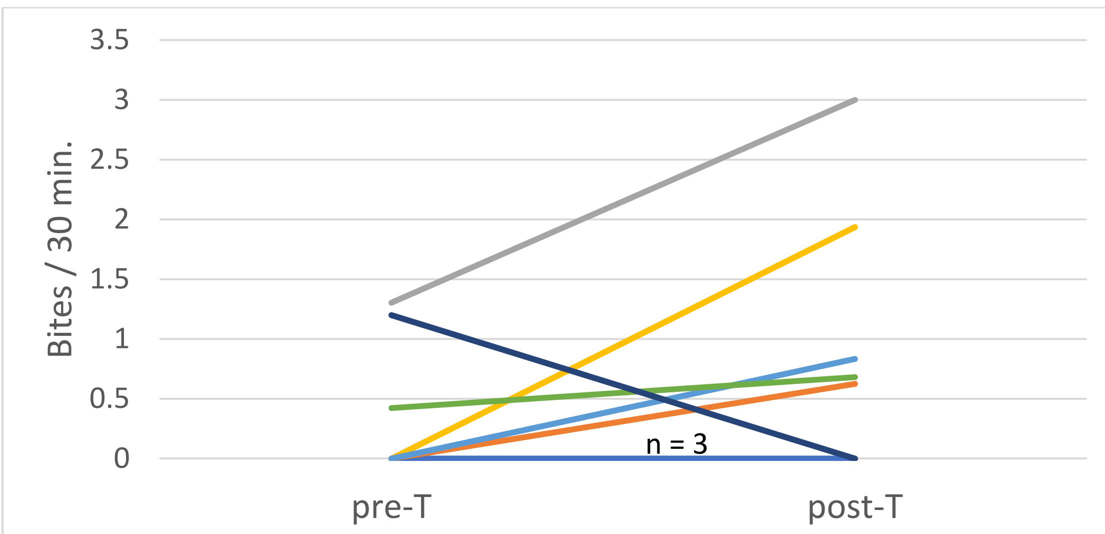

Figure 13: The number of times a male *H. turcicus* gecko bit his opponent prior to testosterone treatment (baseline tests) and after testosterone treatment.

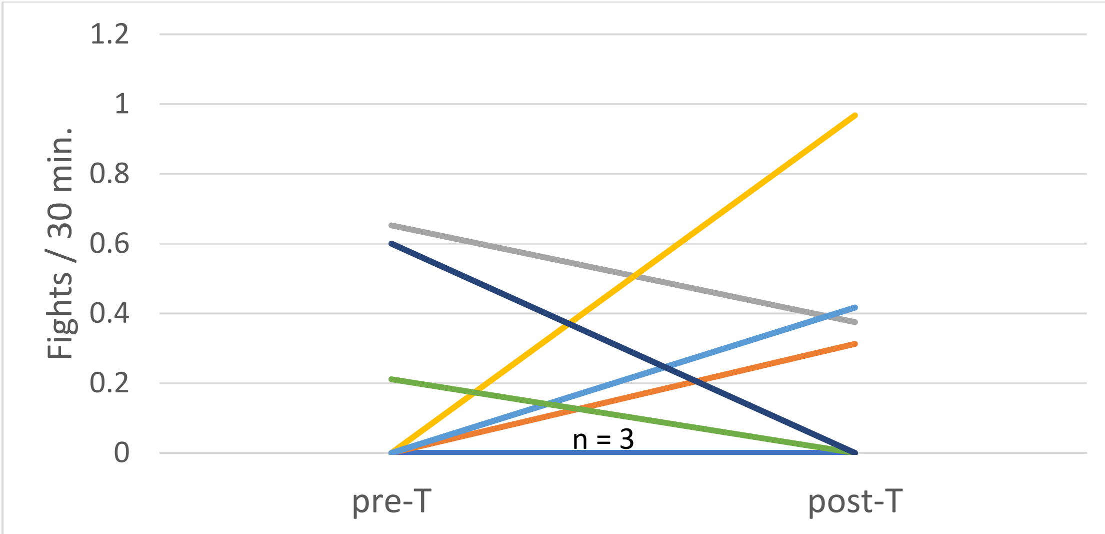

Figure 14: The number of times a male *H. turcicus* gecko fought with his opponent prior to testosterone treatment (baseline tests) and after testosterone treatment.

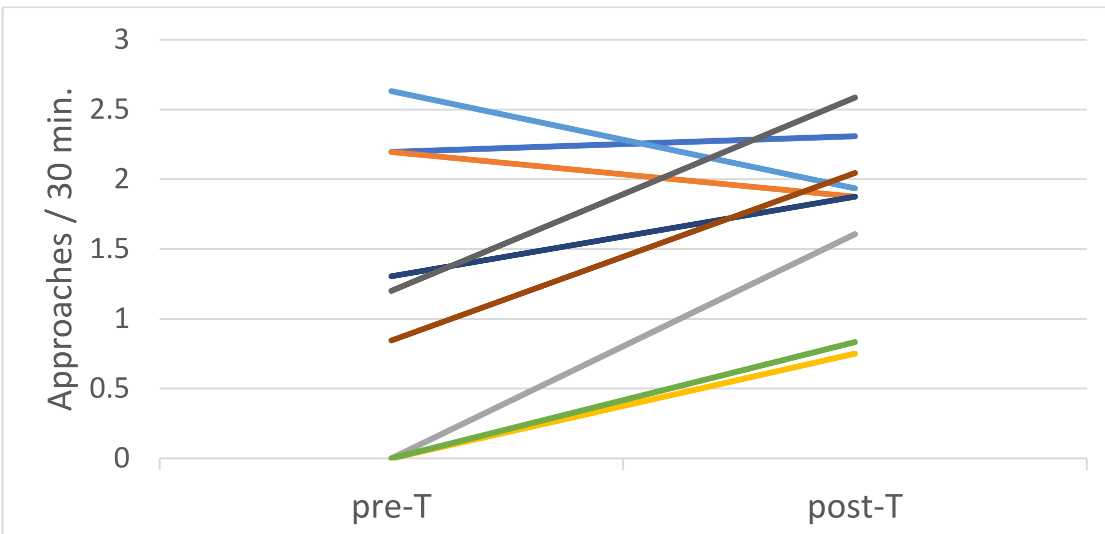

Figure 15: The number of times a male *H. turcicus* gecko approached his opponent prior to testosterone treatment (baseline tests) and after testosterone treatment.

<u>Influence of Testosterone vs Sesame Oil on Intrasexual-Female Behaviors</u>

Most aggressive displays, including pushups, back arches, bites, fights, and chases, did not occur in any of the females treated with testosterone during the contests with sesame oil-treated females. There was only one tail wag incident, performed by a sesame oil-treated female, and one snap incident, performed by a different sesame oil-treated female. Behaviors were too infrequent to perform statistical tests.

Neutral behaviors were also infrequent among the testosterone-treated and sesame oil-treated females. No vocalizations (multi-clicks and squeaks) were heard during these manipulated female tests. Tongue flicks ($U = 18.5$, $n = 6$, $p = 1$) and licks ($U = 21.5$, $n = 6$, $p = 0.53$; Fig. 16) did not vary significantly between the treatments in the female geckos. Finally, freezes ($U = 15.5$, $n = 6$, $p = 0.67$) and retreats ($U = 15.5$, $n = 6$, $p = 0.67$; Fig. 17) did not differ between sesame oil-treated and testosterone-treated females. Interestingly enough, the three females that exhibited freezes were not the same three females that exhibited retreats, meaning the submissive behaviors were expressed by different individuals at different times. This differs from the results seen in the testosterone manipulated male trials, where 11 out of the 17 males that exhibited either freezes or retreats also exhibited the other during that same trial.

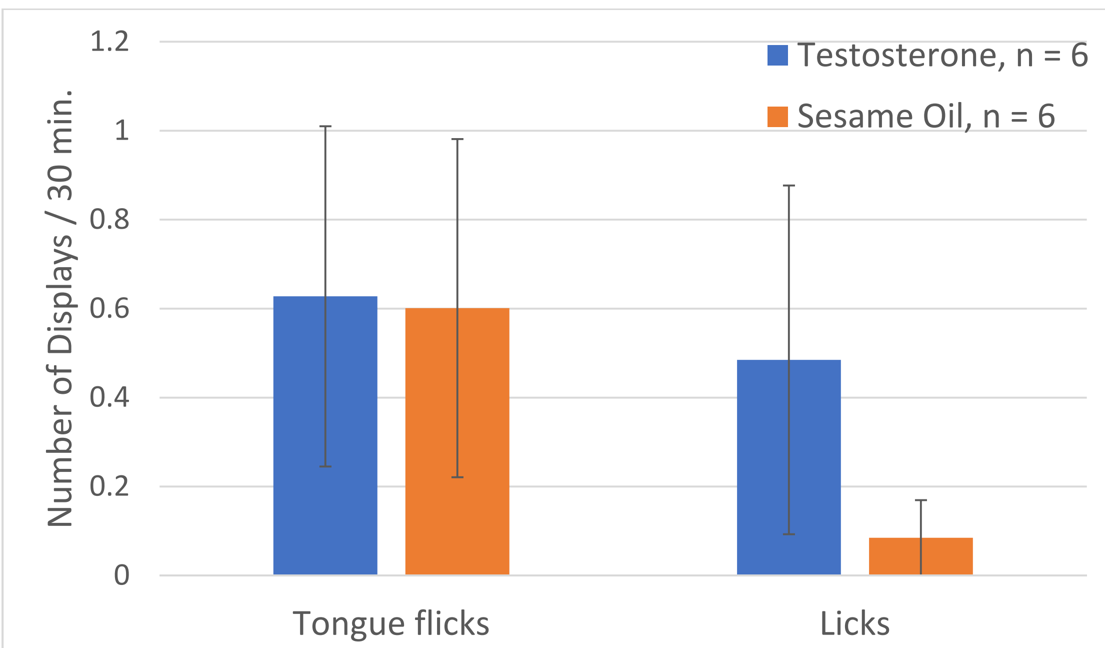

Fig. 16: Intrasexual neutral behaviors (Mean ± SE) in testosterone (*n* = 6) vs control (sesame oil; *n* = 6) female *H. turcicus* geckos (*n* = 6). Because trials varied in duration, the data were scaled to 30 minutes.

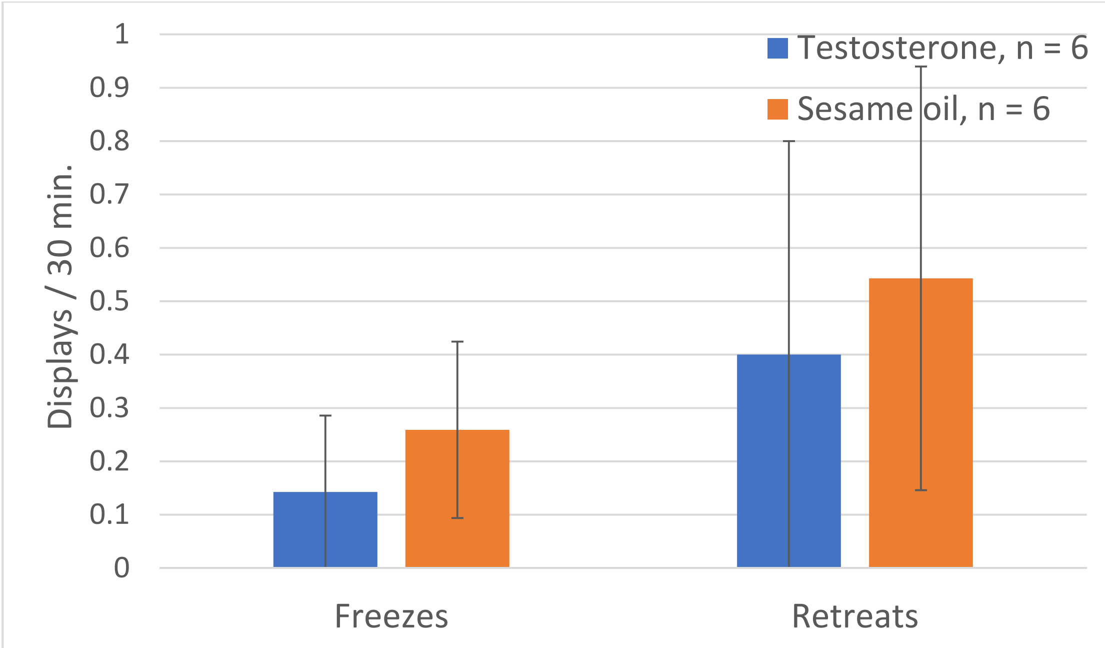

Fig. 17: Intrasexual submissive behaviors (Mean ± SE) in testosterone (n = 6) vs control (sesame oil; n = 6) female *H. turcicus* geckos. Because trials varied in duration, the data were scaled to 30 minutes.

Intrasexual Female Behaviors Before and after Testosterone Treatment

In both the baseline and the testosterone treatment trials of female-female interactions there were no occurrences of pushups, back arches, bites, fights, or chases and thus there were no apparent influences of testosterone on these behaviors. A single tail wag was expressed by a female during the baseline tests, but no tail wags were observed from this individual after receiving the testosterone treatment, nor was it observed from any of the other females treated with testosterone. Similarly, freezes (W = 3, n = 6, p = 1) and retreats (W = 1, n = 6, p = 1) did not vary with testosterone treatment. Finally, neutral behaviors such as approaches (W = 12, n = 6, p = 0.28), tongue flicks (W = 3, n = 6, p = 0.58), squeaks (W = 1, n = 6, p =1) and licks (W = 0, n = 6, p = 0.37) also did not differ between females when in the baseline trials and after testosterone treatment (Fig. 18).

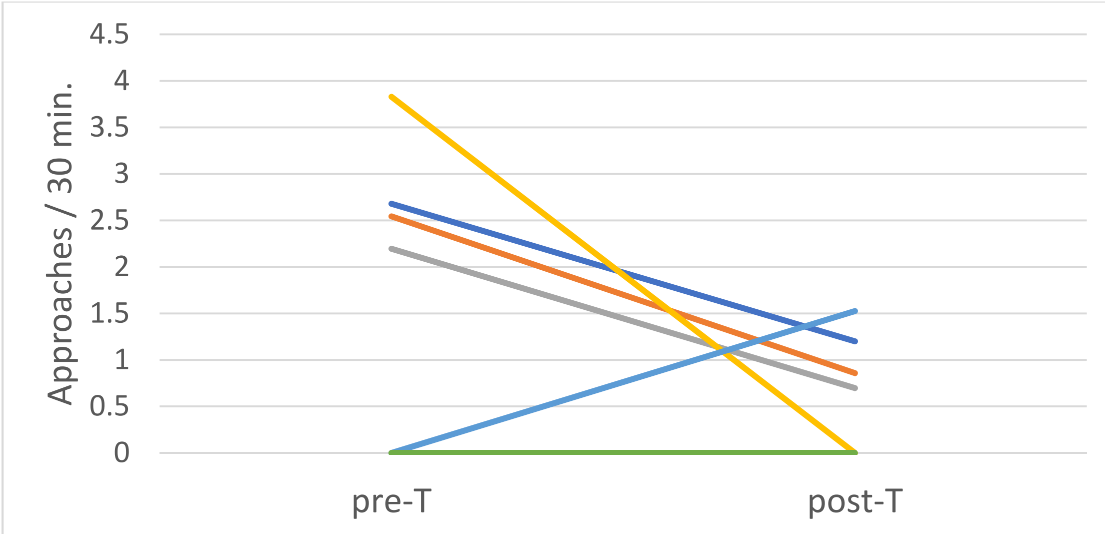

Figure 18: The number of times a female *H. turcicus* gecko approached her opponent prior to testosterone treatment (baseline tests) and after testosterone treatment. Individual lines represent individual geckos ($n = 6$). Note that geckos faced different, size-matched conspecifics pre-and post-testosterone.

Sex Differences in Intrasexual Behaviors during the Baseline Test

Males participating in the male-male baseline tests performed significantly more pushups ($T = 1.77$, $df = 28$, $p = 0.048$), back arches ($T = 3.0$, $df = 28$, $p = 0.004$), and tail wags ($T = 2.65$, $df = 28$, $p = 0.0079$) than females did in the female-female baseline tests (Fig. 19). Additionally, snaps ($T = 1.75$, $df = 28$, $p = 0.049$), bites ($T = 2.73$, $df = 28$, $p = 0.0058$), and fights ($T = 3.87$, $df = 28$, $p = 0.00062$), were all observed significantly more often in males than females (Fig. 20). Chases ($T = 1.37$, $df = 28$, $p = 0.094$) were the only aggressive behavior that did not occur significantly more in males than females. Multiple-click vocalizations ($T = 1$, $df = 28$, $p = 0.17$) and licks ($T = 1.53$, $df = 28$, $p = 0.072$) were infrequently observed in both male and female intrasexual baseline tests, and neither of these behaviors had a significant difference in occurrence between the sexes. Though squeaks and approaches were more commonly observed, they did not occur more often in the baseline males than females (squeaks: $T = 1.72$, $df = 28$, $p = 0.051$ and approaches: $T = -0.14$, $df = 28$, $p = 0.55$). However, tongue flicks were observed significantly more often in the males ($T = 2.04$, $df = 28$, $p = 0.027$; Fig. 21). For submissive behaviors, freezes ($T = 0.55$, $df = 28$, $p = 0.30$) and retreats ($T = 1.67$, $df = 28$, $p = 0.054$) were not significantly greater in males (Fig. 22).

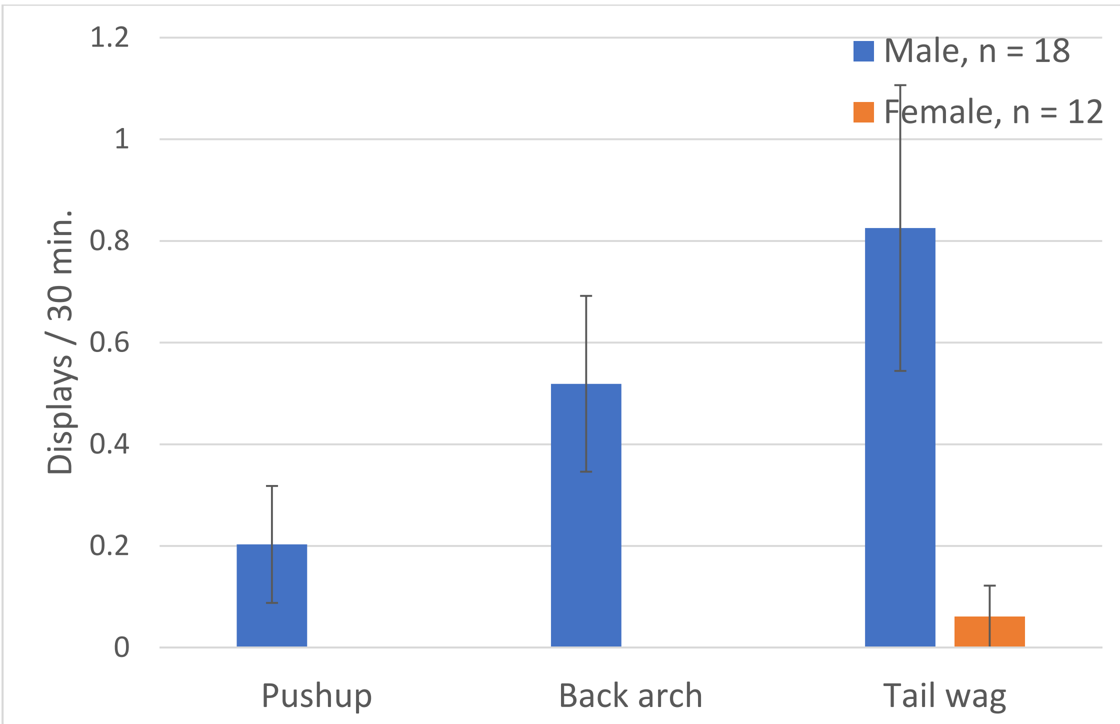

Figure 19: Intrasexual aggression (Mean ± SE) in baseline male ($n = 18$) vs baseline female ($n = 12$) *H. turcicus* geckos. Because trials varied in duration, the data were scaled to 30 minutes.

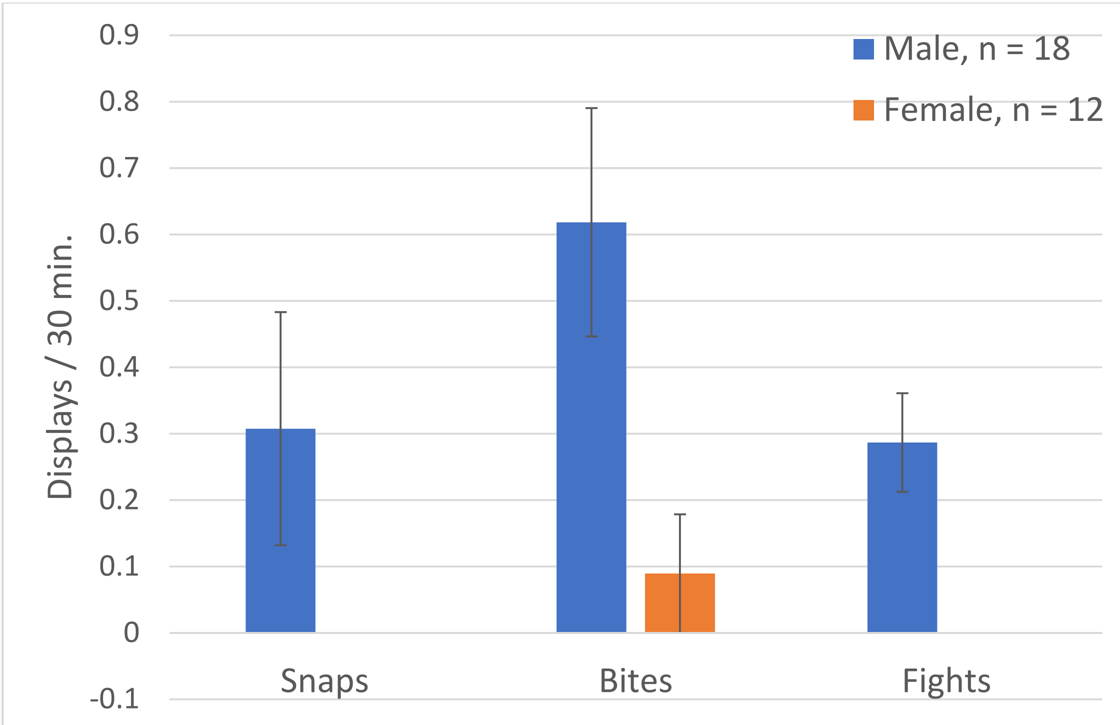

Figure 20: Intrasexual overt aggression (Mean ± SE) in baseline male vs baseline female *H. turcicus* geckos.

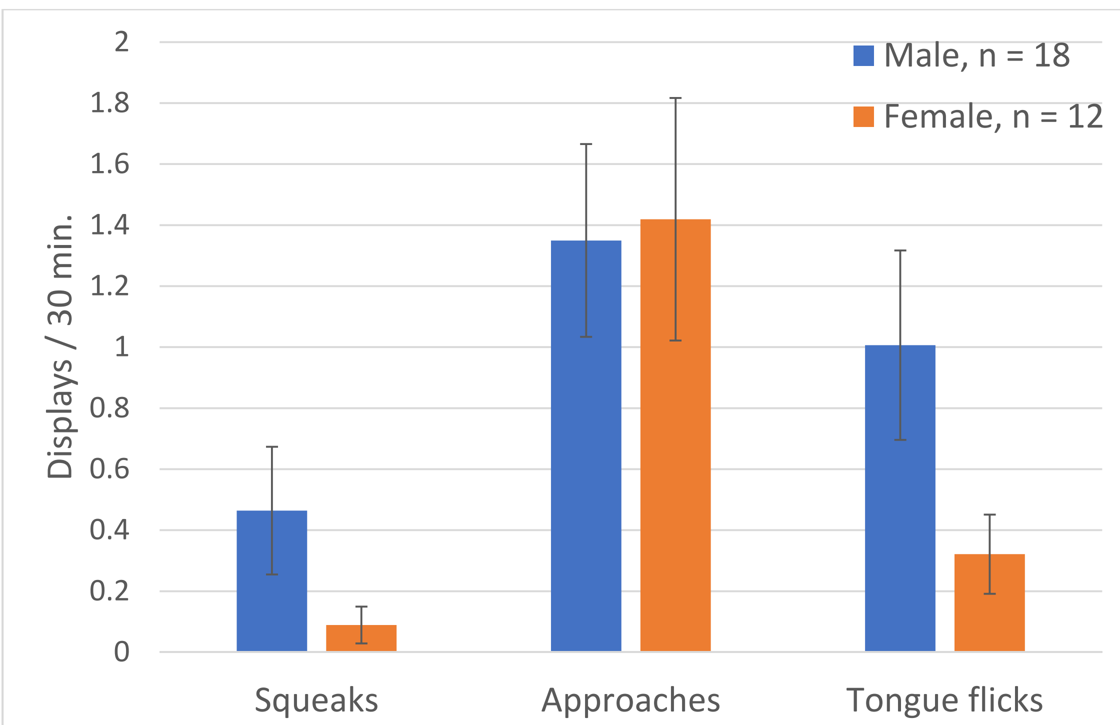

Figure 21: Intrasexual neutral behaviors (Mean ± SE) in baseline male versus baseline female *H. turcicus* geckos.

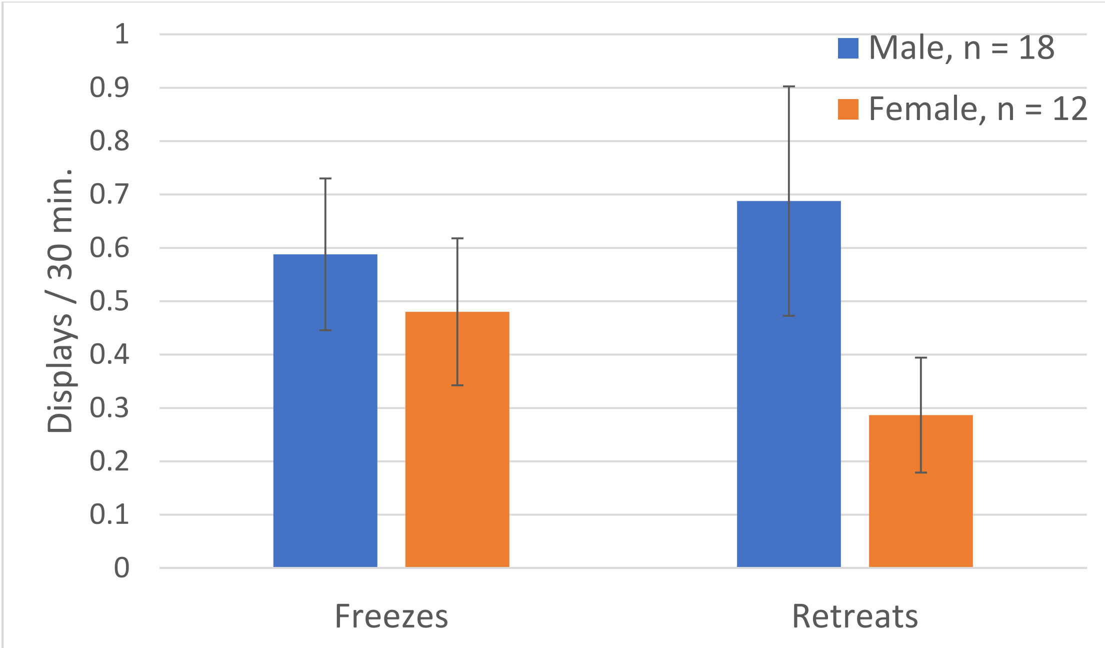

Figure 22: Intrasexual submissive behaviors (Mean ± SE) in baseline male versus baseline female *H. turcicus* geckos.

Discussion

Changes in Body Size over the Course of the Study

The geckos that were measured for snout-vent length (SVL) and body mass during pre- and post-test points of this study revealed a significant growth in both SVL and mass. This indicates that the housing conditions and frequency of cricket feedings used in this study were suitable for *Hemidactylus turcicus* geckos living in a lab setting for at least a duration of 3 months. Mediterranean House Geckos thus appear to acclimate reasonably well to captivity. This is also supported by the finding that the geckos exhibited many of their natural behaviors in the artificial testing conditions. Overall, *H. turcicus* has the potential to be a productive model species for elucidating the proximate mechanisms underlying stereotyped lizard behaviors.

Influence of Testosterone on Male Intrasexual Behaviors

Intrasexual behaviors did not differ between testosterone-treated males and control males, nor were they found to differ between the testosterone-treated males and their corresponding baseline results. Furthermore, based on the subset of subjects that were bled, the topical application of testosterone was highly effective in raising plasma testosterone concentrations, at least in the short term. Taken together, these results suggest that there was no influence of testosterone on any of the recorded behaviors in male Mediterranean House Geckos. In other words, treating *H. turcicus* with testosterone topically for three consecutive days will increase their circulating plasma testosterone, but will likely have little effect on their behavior during a same-sex encounter, at least when tested in a similar context.

The results of this study discredit my hypothesis that an increase in testosterone via topical application will increase the male-male aggression expressed by *H. turcicus*. This does not mean, however, that testosterone is not an important regulator in male-typical aggression of this gecko, but that other physiological factors influence the effects of testosterone, as found in several previous studies on lizard behavior (e.g. DeNardo and Sinervo 1994; Golinski et al. 2010; Moore 1988; Norris and Lopez 2011). Additionally, a greater sample size could provide more insight on small effects of testosterone on *H. turcicus* behavior that were not observed during this study.

While it currently appears that male Mediterranean House Gecko behavior is unaffected by experimentally elevated plasma testosterone concentrations, there are alternative explanations for the lack of an effect. For example, males in this study may have been relatively slow to approach and investigate one another. Gollinski et al. (2014) reported that in their hormonal study on the Madagascar ground gecko, *Paroedura picta*, geckos with experimentally increased testosterone often did not investigate the intruder, but aggression often followed in the geckos that did encounter the intruder. In future studies on House Gecko behavior, one could utilize a smaller testing arena which might facilitate closer contact between the males. Secondly, this study only observed behaviors of *H. turcicus* during the presumed breeding months, when endogenous testosterone is already believed to be at its highest in males, and thus fails to test the influences of testosterone on male-male encounters outside of the breeding season. Besides changing the timing of the tests, I could have also castrated males to achieve greater control over their testosterone levels. Thirdly, by reusing the same males before and after treatments, I have allowed males that may have underwent a recent agonistic encounter to return into the arena where the results of that recent encounter might influence their behavioral outcomes (Wingfield 2005). In addition to the potential shortcomings mentioned, tests were always conducted in an arena after removing males from their home cages. Future studies may choose to test males in their established home cages by introducing conspecifics. This may reduce the potential behavioral effects of handling the individuals.

<u>Influence of Testosterone on Female Intrasexual Behaviors</u>

Many aggressive displays were completely absent during the female-female *H. turcicus* interaction trials both before and after testosterone treatment, suggesting that females of this species are typically nonaggressive toward adult female conspecifics. Additionally, the displays that were expressed by females did not differ between the testosterone-treated females and the sesame oil-treated females. Despite the evidence that topical testosterone significantly raised circulating levels of testosterone in the blood of the females treated, it did not significantly influence any of their behaviors expressed. This suggests that testosterone does not cause an increase in female intrasexual behaviors in *H. turcicus*, at least not under my testing conditions (e.g., time of year, testing arena size and context).

Future studies of *H. turcicus* females could examine how changes in hormones influence intersexual (female - male) behaviors, such as receptivity. For example, an increase in testosterone or estradiol might induce a change in female *H. turcicus* mating behavior. Future studies could also test for behavioral effects of different dosages and durations of hormone treatment. For example, Rhen et al. (1999) conducted a study of intersexual behaviors in the female leopard gecko, *Eublepharis macularius*, and tested across three different concentrations of testosterone over two different durations: low (1 ng/ml), medium (100 ng/ml), or high (200 ng/ml) over short (8 days) or long (35 days) durations of delivering the testosterone. Rhen et al. (1999) reported that female leopard geckos became less appealing to male mates following the medium and high treatments of testosterone over both short and long durations of treatment when compared to the females treated with low testosterone. Additionally, female leopard geckos treated with the medium and high levels of testosterone for a long duration were unreceptive and significantly more aggressive towards males compared to the females treated for the short duration. This suggests that female behavior of some gecko species may have a complicated physiological relationship to testosterone. Because I only exposed Mediterranean House Geckos of both sexes to testosterone for a duration of 3 consecutive days, I am unaware of the differences that long-term treatments could induce in males and females of the species. Whether hormonal treatments delivered on *H. turcicus* females as hatchlings could cause different effects than treatments delivered to adult females is another question left to be answered.

Sex Differences in Baseline Intrasexual Behaviors

Through this study and others, it is apparent that *H. turcicus* geckos use different behaviors and methods of communication to interact with members of their own sex, with variations in their expression occurring between the two sexes. Males appeared to utilize vocalizations and aggressive displays substantially more than females, while females were readily tolerant towards members of their own sex and rarely engaged in agonistic behaviors. As expected, untreated male *H. turcicus* were found to express most aggressive behaviors significantly more often than the untreated females of the species, with the exception of chases in this study. This is consistent with the findings of previous studies involving *H. turcicus* behavior, in which males will display territorial aggression and females will participate in communal nesting with other females (Selcer 1986). Future studies on intersexual behaviors of *H. turcicus* adults, as well as the behaviors that occur between adults and young, may provide further explanations on the ecology of this non-native, possibly invasive, gecko.

Many of the behaviors observed in *H. turcicus* during this study are also known to occur in other lizard species, but the contexts for their usage, as well as the physiology behind their expression, appear to have great variability among and within species (DeNardo and Sinervo 1994; Fox et al. 2003; Golinski et al. 2010; Norris and Lopez 2011; Sinervo et al. 2000). As previously stated, female *H. turcicus* may be less aggressive toward same-sex individuals than males due to their tendency to share spatial nesting sites, a behavior known as communal nesting, in which females lay their eggs with other females (Selcer 1986). Males on the other hand may act aggressive toward conspecific males to maintain their territory and/or control of females, as it may increase their chance of successfully passing on their own gametes. Aggression is a common behavioral trait observed in many male vertebrates (Adkins-Regan 2005; Archer 1988; DeNardo and Sinervo 1994; Monaghan and Glickman 1992; Nelson 2000).

Most of the neutral behaviors recorded in this study were fairly common in both sexes of *H. turcicus*. Some of these behaviors, like tongue flicks and approaches, likely contribute to the initial investigation of a conspecific. These behaviors may occur prior to aggressive or submissive behaviors, as the individual attempts to identify the sex and status of their nearby conspecific. Vocalizations, however, appeared to be slightly more common in the males, who produced squeaks significantly more than females. I suspect that these squeaks were most likely produced submissively in response to an aggressive conspecific male, and because females were rarely aggressive, submissive behavior in females was less common. Multi-clicks were not heard from any females, before or after receiving testosterone treatment. The multi-clicks were rarely exhibited in the male pairs, but eight multi-click calls did occur across both the manipulated and baseline male tests. However, four of these eight multi-click calls were produced by a single male (three during the baseline tests and one after receiving testosterone), meaning that only five of the eighteen males produced multi-click calls throughout the study. As stated previously, testing males in smaller arenas or in their home cages might elicit a greater frequency of vocalizations.

Observations on Vocalizations

Overall vocalizations were poorly monitored in this study (e.g., I did not utilize sensitive audiorecording equipment), but I did attempt to quantify the more obvious vocalizations. I noticed two general types of vocalizations in *H. turcicus*, multi-clicking and squeaks. It appeared to me that the low-pitch, multi-clicking vocalizations accompanied aggressive behavior while the more drawn-out, high-pitched squeaks accompanied submissive behavior, but further investigation is required to say this with more certainty. Frankenberg (1982) found *H. turcicus* males to produce multi-click and squeak calls toward both males and females, but even these multi-click calls tended to differ in their repertoire when made towards other males than when made towards females. These findings persuaded Frankenberg (1982) to describe the multiple-click calls collectively as “advertisement calls”, due to variations in their context, from territoriality to mate attraction. Frankenberg (1982) describes some squeaks of *H.*

turcicus as “release calls”, when the gecko producing them is being chased or attacked by another. Frankenberg (1982) also relates some multi-clicks to males preparing to approach or attack a conspecific. Jono and Inui (2012) also found male and female Japanese House Geckos, *Gekko japonicas*, to exhibit calls during aggressive intrasexual interactions. Furthermore, they found the calls of *G. japonicas* to vary in their chirp duration based on whether it was being produced during an intrasexual or intersexual encounter. Despite the suggestive literature of multiple-click calls being used aggressively in intrasexual encounters of Gekkonidae lizards, I decided to avoid including these multi-click vocalizations in the aggressive behaviors because of the complexity in the context of such calls, in addition to the poor monitoring of the vocalizations during this study. However, I feel it should be noted that I believe most of the multi-clicking vocalizations observed in this study were used in either an aggressive or territorial circumstance. Similarly, I exclude squeaks from submissive behaviors due to the same challenges in clarifying their behavioral context, but I believe their occurrence in this study typically accompanied submissive acts, such as retreats. As previously stated, I categorized both the multi-click and squeaking vocalizations as neutral behaviors to prevent biasing my results. Future studies may choose to monitor the context of these vocalizations more carefully to gain better insight on the purpose of their usage by *H. turcicus* geckos.

<u>Conclusion</u>

Although topical application of testosterone was successful at raising the blood testosterone concentration in the geckos treated, the observed behaviors after the treatments did not significantly differ in frequency from the baseline trials in either males or females. This suggests that either testosterone does not influence these behaviors, or that the hormone has a much more complicated influence on the behaviors that may not express itself after a topical testosterone treatment of merely three consecutive days. Further studies on *H. turcicus* are needed to understand better the physiological influences that androgens have on the expression of these behaviors.

Studying intrasexual behaviors is only a piece in understanding this rapidly spreading non-native gecko. Many additional behavioral and ecological studies should be conducted to appreciate how *H. turcicus* is using its newly colonized range in the U.S., and whether we should be concerned for the native organisms it interacts with. Finally, there is still much potential for the use of *H. turcicus* as a study organism of gecko behaviors and their mechanisms of communication through visual, auditory, and chemical signals.

Literature Cited

Adkins-Regan, E. (2005). Mating, Fighting, Parenting, and Signaling. In: Hormones and Animal Social Behavior. Adkins-Regan, ed. Princeton University Press, Princeton and Oxford, pp. 49-52.

Archer, J. (1988). The Behavioral Biology of Aggression. Cambridge University Press.

Briggs, V. S. (2012). Agonistic behavior of the mediterranean gecko *Hemidactylus turcicus*. *Zoological Studies* **51**: 956-965.

Cooper, W. E., Mendonca, M., Vitt, L. (1987). Induction of orange head coloration and activation of courtship and aggression by testosterone in the male broad-headed skink (*Eumeces laticeps*). *Journal of Herpetology* **21**: 96-101.

Cox, C. L, Hanninen, A. F., Reedy, A. M., Cox, R. M. (2015). Female anoles retain responsiveness to testosterone despite the evolution of androgen-mediated sexual dimorphism. *Functional Ecology* **29**: 758-767.

DeNardo, D., Sinervo, B. (1994). Effects of steroid hormone interaction on activity and home-range size in male lizards. *Hormones and Behavior* **28**: 273-287.

Fox, S., McCoy, J. K., Baird, T. (2003). Lizard Social Behavior. The Johns Hopkins University Press.

Frankenberg, E. (1982). Vocal behavior of the mediterranean house gecko, *Hemidactylus turcicus*. *Copeia* **1982**: 770-775.

Frankenberg, E. (1984). Interactions between two species of colonizing house geckos, *Hemidactylus turcicus* and *Hemidactylus garnotii*. *Journal of Herpetology* **18**: 1-7.

Golinski, A., Kubička, L., John-Alder, H., Kratochvíl, L. (2014). Elevated testosterone is required for male copulatory behavior and aggression in Madagascar ground geckos (*Paroedura picta*). *General and Comparative Endocrinology* **205**: 133-141.

Golinski, A., John-Alder, H., Kratochvíl, L. (2010) Male sexual behavior does not require elevated testosterone in a lizard (*Coleonyx elegans, Eublepharidae*). *Hormones and Behavior* **59**: 144-150.

Greenberg N., Crews, D. (1983) Physiological Ethology of Aggression in Amphibians and Reptiles. In: Hormones and Aggressive Behavior. Svare, ed. Plenum Press, New York, pp. 479-498.

Kabelik, D., Weiss, S. L., Moore, M. C. (2004). Testosterone, aggression, and amygdala morphology in the tree lizard*, Urosaurus ornatus*. *Integrative and Comparative Biology* **44**: 580.

Klug, P.E., Reed, R. N., Mazzotti, F. J., Collier, M. (2015). The influence of disturbed habitat on the spatial ecology of Argentine black and white tegu (*Tupinambis merianae*), a recent invader in the Everglades ecosystem (Florida, USA) *Biological Invasion* **17**: 1785-1797

Jono, T., Inui, Y. (2012). Secret calls from under the eaves: acoustic behavior of the Japanese house gecko, *Gekko japonicus*. *Copeia* **2012**: 145-149.

Locey, K., Stone, P. (2006). Factors affecting range expansion in the introduced Mediterranean gecko, *Hemidactylus turcicus*. *Journal of Herpetology* **40**: 526-530.

Locey, K., Stone, P. (2008). Ontogenetic factors affecting diffusion dispersal in the introduced Mediterranean gecko, *Hemidactylus turcicus*. *Journal of Herpetology* **42**: 593-599.

Marcellini, D. (1977). Acoustic and visual display behavior of Gekkonid lizards. *American Zoology* **17**: 251-260.

Mazzotti, F. J., McEachern, M., Rochford, M., Reed, R. N., Eckles, J. K., Vinci, J., Edwards, J., Wasilewski, J. (2015). *Tupinambis merianae* as nest predators of crocodilians and turtles in Florida, USA *Biological Invasions* **17**: 47-50.

Meshaka, W. (1995). Reproductive cycle and colonization ability of the Mediterranean gecko (*Hemidactylus tucicus*) in South-central Florida. *Florida Scientist* **58**:10-15.

Meshaka, W., Marshall, S., Boundy, J., Williams, A. (2006). Status and geographic expansion of the Mediterranean gecko, *Hemidactylus turcicus*, in Louisiana: implications for the Southeastern United States. *Herpetological Conservation and Biology* **1**: 45-50.

Monaghan, E. P., Glickman, S. E. (1992). Hormones and Aggressive Behavior. In: Behavioral Endocrinology. Becker J. B., Breedlove, S. M., Crews, D., ed. The MIT Press, Cambridge, Massachusetts, pp. 271-279.

Moore, M.C. (1988) Testosterone control of territorial behavior: tonic-release implants fully restore seasonal and short-term aggressive responses in free-living castrated lizards. *General and Comparative Anatomy* **70**: 450-459.

Nelson, R. (2000). Affiliative and Aggressive Behavior. In: An Introduction to Behavioral Endocrinology. 2nd ed. Nelson, ed. Sinauer Associates, Inc., Sunderland, Massachusetts, pp. 395-445.

Nordberg, E., A. Blanchard, T., Cobb, V., Scott, F., Howard, S. R. (2013). Distribution of a non-native gecko (*Hemidactylus turcicus*) in Tennessee. *Journal of the Tennessee Academy of Science* **88**: 64-66.

Norris, D. O., Lopez, K. H. (2011). Hormones and Reproduction of Vertebrates. Vol. 3. Reptiles. Academic Press, Elsevier Inc.

Pernas, T., Giardina, D. J., McKinley, A., Parns, A., Mazzotti, F. J. (2012). First observations of nesting by the Argentine black and white tegu, *Tupinambis merianae*, in South Florida **11**: 765-770.

Punzo, F. (2001). The Mediterranean gecko, *Hemidactylus turcicus*: life in an urban landscape. *Florida Scientist* **64**: 56-66.

Rhen, T., Ross, J., Crews, D. (1999). Effects of testosterone on sexual behavior and morphology in adult female leopard geckos, *Eublepharis macularius*. *Hormones and Behavior* **36**: 119-128.

Rose, F. L., Barbour, C. D. (1968). Ecology and reproductive cycles of the introduced gecko, *Hemidactylus turcicus*, in the Southern United States. *The American Midland Naturalist* **79**: 159-168.

Schořálková T, Kratochvíl L, Kubička L. (2018). To fight or mate? Hormonal control of sex recognition, male sexual behavior and aggression in the gecko lizard. *Hormones and Behavior* **97**: 18-24.

Selcer, K. (1986). Life history of a successful colonizer: the Mediterranean gecko, *Hemidactylus turcicus*, in Southern Texas. *Copeia* **1986**: 956-962.

Sinervo, B., Miles, D., Frankino, A., DeNardo, D., Klukowski, M. (2000). Testosterone, endurance, and Darwinian fitness: natural and sexual selection on the physiological bases of alternative male behaviors in side-blotched lizards. *Hormones and Behavior* **38**: 222-233.

Stabler, L. B., Johnson, W. L., Locey, K. J., Stone, P. A. (2011). A comparison of Mediterranean Gecko (*Hemidactylus turcicus*) populations in two temperate zone urban habitats. *Urban Ecosystems* **15**:653-666.

Wingfield, J. C. (2005). A continuing saga: The role of testosterone in aggression. *Hormones and Behavior* **48**: 253-255.

Woodley, S. K., Moore, M. C. (1999). Female territorial aggression and steroid hormones in mountain spiny lizards. *Animal Behaviour*. **57**: 1083-1089.

APPENDICES

IACUC

INSTITUTIONAL ANIMAL CARE and USE COMMITEE
Office of Research Compliance,
010A Sam Ingram Building,
2269 Middle Tennessee Blvd
Murfreesboro, TN 37129

MIDDLE TENNESSEE STATE UNIVERSITY

IACUCN006: FCR PROTOCOL APPROVAL NOTICE

Friday, March 30, 2018

Principal Investigator	**Matthew Klukowski**
Co-Investigator(s):	Clinton Warren
Investigator Email(s):	*matthew.klukowski@mtsu.edu; crw6u@mtmail.mtsu.edu*
Department/Unit:	BIOLOGY

Protocol ID:	**18-3010**
Protocol Title:	***The effects of testosterone on aggressive behavior in male Mediterranean House Geckos***

Dear Investigator(s),

The MTSU Institutional Animal Care and Use Committee has reviewed the animal use proposal identified above under the ***Full Committee Review (FCR) mechanism***. The IACUC met on 3/23/2018 to determine if your proposal meets the requirements for approval. The Committee determined through a unanimous vote that this protocol meets the guidelines for approval in accordance with PHS policy and has approved this protocol pending to modifications. The IACUC also voted to allow Designated Member Review (DMR) to inspect your revised protocol. In the light of the revisions, the DMR has determined that your protocol now eligible for the proposed animal use. A summary of the IACUC action(s) and other particulars of this this protocol are tabulated below:

IACUC Action	**APPROVED for one year from the date of this notification**
Date of Expiration	**3/31/2019**

Number of Animals	35 (THIRTY FIVE)	
Approved Species	**Hemidactylus turcicus (wild caught)**	
Category	☐ **Teaching**	☒ **Research**
Subclassifications	☐ Classroom ☐ Laboratory	☒ Laboratory ☒ Field Research ☐ Field Study ☒ Handling/Manipulation ☐ Observation
	Comment: Field Research licence expires in March, 2019	
Approved Site(s)	Field and SCI1170I	
Restrictions	**Must comply with all FCR requirements and Vivarium policy.**	
Comments	NONE	

This approval is effective for three (3) years from the date of this notice. This protocol **expires on 3/31/2021** The investigator(s) MUST file a Progress Report annually regarding the status of this study. Refer to the schedule for Continuing Review shown below; NO REMINDERS WILL BE SENT. A continuation request (progress report) must be approved by the IACUC prior to

3/31/2019 for this protocol to be active for its full term. Once a protocol has expired, it cannot be continued and the investigators must request a fresh protocol.

Continuing Review Schedule:

Reporting Period	Requisition Deadline	IACUC Comments
First year report	2/28/2019	Yet to be completed
Second year report	2/28/2020	Yet to be completed
Final report	2/28/2021	Yet to be completed

Post-approval Amendments:

Date	***Amendment***	***IACUC Notes***
NONE	NONE	NONE

MTSU Policy defines an investigator as someone who has contact with live or dead animals for research or teaching purposes. Anyone meeting this definition must be listed on your protocol and must complete appropriate training through the CITI program. Addition of investigators requires submission of an Addendum request to the Office of Research Compliance.

The IACUC must be notified of any proposed protocol changes prior to their implementation. Unanticipated harms to subjects or adverse events must be reported within 48 hours to the Office of Compliance at (615) 494-8918 and by email – compliance@mtsu.edu.

All records pertaining to the animal care be retained by the MTSU faculty in charge for at least three (3) years AFTER the study is completed. Be advised that all IACUC approved protocols are subject to audit at any time and all animal facilities are subject to inspections at least biannually. Furthermore, IACUC reserves the right to change, revoke or modify this approval without prior notice.

Sincerely,

Compliance Office

(On behalf of IACUC)

Middle Tennessee State University

Tel: 615 494 8918

Email: iacuc_information@mtsu.edu (for questions) and

Iacuc_submissions@mtsu.edu (for sending documents)

TENNESSEE WILDLIFE RESOURCES AGENCY

ELLINGTON AGRICULTURAL CENTER
P. O. BOX 40747
NASHVILLE, TENNESSEE 37204

Scientific Collection Permit : 1483

Issue_date: 3/27/2018

Expiration_date: 3/27/2019

Pursuant to authority of T.C.A. 70-2-213: Matthew Klukowski

and the following additional permittees: Clinton Warren

Are granted permission to take the following species: 40 Sceloporus undulatus (Eastern fence lizard), 10 Plestiodon inexpectatus (Southeastern Five-lined skink), 10 Anolis carolinensis (Green anole), Unlimited Hemidactylus turcicus (Mediterranean House gecko), 20 Nerodia sipedon (Common Water snake), 10 Regina septemvitatta (Queen snake), 10 Nerodia rhombifer (Diamondback Water snake), 10 Nerodia erythrogaster (Plainbelly Water snake)

Restricted to the following locations: Rutherford, Wilson, Bedford, Cannon, and Coffee counties in middle TN; Reelfoot Lake (Lake and Obion counties); Chattanooga Chickamauga creek and tributaries (Hamilton county).

Restricted to the following collection methods: Lizards are captured by noosing and by hand. Snakes are captured by hand.

Subject to the following rules: Wildlife may not be held longer than 24 hours without prior approval. All containers and equipment utilized in the collection of amphibians and reptiles shall be decontaminated and disinfected for ranavirus and other pathogens. This permit is invalid unless accompanied by all applicable federal permits.
No species listed by TW RA as endangered, threatened, in need of management, or of greatest conservation need may be taken without approval; release these species immediately. Report the occurance of endangered or threatened species to TW RA within five days.
Prior to collecting in the field, you are required to notify the TWRA Regional Dispatcher with the name(s) of person(s) doing the collecting, where, when and what species you will be collecting. Contact information is attached.

Ed Carter

Executive Director, Tennessee W ildlife Resources Agency

3/27/2018

Date

The State of Tennessee - *AN EQUAL OPPORTUNITY EMPLOYER*