THE EFFECT OF SOCIAL EXPERIENCE ON BEHAVIOR,

STRESS HORMONE, AND REPRODUCTION IN NATIVE

AND INVASIVE *ANOLIS* LIZARDS

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Abstract: Invasive species damage ecosystems, economies, and human health. Understanding the traits that contribute to successful invasion improves our ability to identify and prevent future invasions before damage can occur. *Anolis sagrei* is a small invasive lizard displacing natural populations of its close relative, *Anolis carolinensis*, in the southeastern United States. I staged territorial encounters to compare the behavioral, hormonal, and reproductive consequences of social experience in the invasive and native species. Against conspecific opponents, lizards of both species were more aggressive as residents than as intruders, but only female *A. sagrei* responded to residency with greater plasma corticosterone. In heterospecific trials, males did not differ in behavior or plasma corticosterone based on social experience. Female *A. sagrei* residents and intruders differed in the relationship between reproductive variables and body size, seemingly differing in their investment in current or future reproduction. *A. carolinensis* females did not differ in reproductive activity based on social experience. In long-term experiences, *A. sagrei* females did not vary reproductive investment uniformly but prioritized different reproductive traits based on their social experience. Overall, social experience influences reproductive output in these lizard species, possibly through the mechanism of stress hormones, in ways that likely contribute to *A. sagrei*'s success as an invasive competitor.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

HORMONES, BEHAVIOR, AND INVASION SUCCESS

1.1 Invasive Species

Species that enter a new geographical range, spread rapidly, and have negative effects on other species or the ecosystem are invasive species. Successful invasions can alter ecosystems, harm crops, and harm human health via introduced diseases (Pimentel et al., 2005). Addressing existing invasions is expensive and often ineffective. Understanding the traits that contribute to successful invasions will improve our ability to predict and prevent invasions before potential damage occurs. No single trait consistently predicts invasion success (Chapple et al., 2012), so it is crucial to study the interactions among traits.

1.2 This Research

The main question of this research is: Do hormones and behavior interact to aid the competitive ability of invasive lizards? The two lizard species I work with are excellent models for understanding invasion because one species displaces natural populations of its close relative (Campbell, 2000). The mechanism of displacement is unclear, but both species use complex behaviors (Greenberg & Noble, 1944; Tokarz & Beck, 1987) and have hormonal reactions to social interaction (Tokarz, 1987; Summers et al., 2003); if the species differ in their reactions to social interaction, the invasive species could exploit

those differences during invasion. I predict that interspecific differences in behavior, hormonal activity, and reproduction aid the competitive ability of the invasive lizard. In the chapters that follow, I address the following questions:

1) Does residency in staged territorial conflict affect aggressive behavior and stress hormone activity in males and females of the lizard species *Anolis sagrei* and *Anolis carolinensis*? I hypothesized that, for both species, residents would show more aggressive behaviors and higher plasma concentrations of their main stress hormone than intruders. I found that, in conspecific experiences, lizards of both species showed more aggressive behaviors as residents than as intruders, but only female *A. sagrei* varied stress hormone concentration based on residency. In heterospecific experiences, males did not differ in aggressive behavior or hormone concentration based on residency.

2) Does residency in staged territorial conflict affect relative investment in reproduction and self-maintenance in females of the lizard species *A. sagrei* and *A. carolinensis*? I hypothesized that, for both species, residents would invest more in reproduction and less in self-maintenance than intruders. I found that *A. sagrei* residents and intruders differed in the relationship between reproductive traits and body size, whereas *A. carolinensis* did not vary reproductive traits based on residency.

3) In long-term social experience, are size-matched or size-mismatched pairs at a greater disadvantage? I tested competing hypotheses: (1) Bully hypothesis: mismatched pairs will show greater disruption of reproductive output as the larger lizard harasses the smaller or restricts access to resources; (2) Rivals hypothesis: matched pairs will show greater disruption of reproductive output as lizards devote greater energy to aggression in an uncertain dominance hierarchy. I found that lizards did not uniformly vary all aspects of reproductive output; instead, lizards emphasized different aspects (number of offspring produced, hatching success) based on their size relative to their opponent.

CHAPTER II

BEHAVIORAL AND HORMONAL CONSEQUENCES OF TERRITORIAL CONTESTS

IN *ANOLIS SAGREI* AND *ANOLIS CAROLINENSIS*

2.1 Abstract

Territorial behavior results in differential hormonal reactions based on contest outcome in many reptile species. Here I tested whether residency in staged conflict affects aggressive behavior and stress hormone activity in male and female lizards of two lizard species, the invasive brown anole *Anolis sagrei* and the native green anole *Anolis carolinensis*. These species interact in the field as *A. sagrei* displaces natural populations of *A. carolinensis* by uncertain mechanisms. If the species differ in their behavioral and hormonal responses to territorial conflict, these differences might aid the invader. In conspecific experiences, both species showed more aggressive behaviors as residents than as intruders. Circulating concentrations of stress hormone were higher in intruders than residents for lab-raised female *A. sagrei* but not for lizards of other source, sex, or species, so the relationship between aggressive behavior and hormonal activity is complex. In heterospecific experiences, male *A. sagrei* and male *A. carolinensis* displayed the same aggressive behaviors as in conspecific experiences but at lower frequencies and they did not differ in aggressive behavior or hormonal activity based on residency. I conclude that displacement of *A. carolinensis* populations is likely not due to direct territorial conflict.

2.2 Introduction

Individuals of many species alter their behavior, hormonal activity, and reproductive traits as a result of conflict with others of the same species (Chase & Seitz, 2011). Some species increase their likelihood of winning after experiencing a win (e.g., water skink, *Eulamprus quoyii*, Kar et al., 2016); some species increase their likelihood of losing after experiencing a loss (e.g., copperheads, *Agkistrodon contortrix*, Schuett, 1997; green anoles, *Anolis carolinensis*, Garcia et al., 2014); some species show both effects (e.g., tree lizard, *Urosaurus ornatus*, Zucker & Murray, 1996; White's skink, *Egernia whitii*, McEvoy et al., 2013). Models show that, in many cases, intrinsic attributes such as body size do not fully account for the organization of social groups (although experience likely interacts with intrinsic traits such as the body size of the individuals involved in the conflict (Hsu et al., 2006)), so individuals likely gain some information from the conflict itself (Beacham, 2003). The persistence of the influence of this social experience depends on many factors, which may include expected encounter rates, length of breeding season, or rapidity of population fluctuations (Kasumovic et al., 2010).

Conflict has benefits (e.g., gaining a resource) but also costs (e.g., use of time and energy, risk of injury) (Briffa & Elwood, 2009), so individuals benefit from assessing the likelihood of winning a potential conflict. In anoles, several traits are correlated to fighting ability and thus might help lizards assess their likelihood of winning against an opponent. Dewlap size correlates to maximum bite force, which contributes to fighting success in *A. carolinensis* (Henningsen & Irschick, 2012); dewlap size relative to body size predicts contest outcome in male *A. sagrei* and male *A. carolinensis* (Edwards & Lailvaux, 2013); high rates of head bobbing (rather than head nodding) correlates to success in territory defense in *A. sagrei* males (Simon, 2011); *A. carolinensis* males with black paint over their eyespots (mimicking high serotonin activity) become dominant whereas size-matched males with green paint over their eyespots (mimicking low serotonin activity) become subordinate (Korzan et al., 2002). Larger body mass contributes to dominance in *A. carolinensis* (Evans, 1936); larger male *A. carolinensis* more successfully defend their perches (Tokarz, 1985).

4

However, intrinsic traits such as body mass are not the only predictors of success; residency is also an advantage. Residency can be defined as occupancy over time within an area from which the individual will attempt to drive away others by territorial displays or direct conflict. In the Iberian wall lizard, *Podarcis hispanica*, body size was the first predictor of fight duration, but in cases of small differences between contestants, residency decided contests (Lopez & Martin, 2001). In *A. carolinensis*, smaller males were unlikely to win but residents were more likely to win than intruders (Jenssen et al., 2005); captive *A. carolinensis* males that had resided in a territory for 24 hours won 91% of their conflicts with intruders (Evans, 1936).

Anole lizards are ideal models for determining the influence of conflict on behavior, hormones, and reproduction. *A. carolinensis* use territorial displays to form dominance hierarchies (Greenberg & Noble, 1944). These displays are highly conserved across field and laboratory contexts in *A. carolinensis* males (Lovern et al., 1999), and female *A. carolinensis* use the same signals as males (Jenssen et al., 2000). Females must also be studied in this context because female anoles hold territories (Nunez et al., 1997) and defend their territories with territorial displays (Evans, 1938). When paired under laboratory conditions, female *A. carolinensis* display aggression and form dominant-subordinate relationships in which the dominant lizard displaces the subordinate lizard (Andrews & Summers, 1996).

It is not clear to what extent *A. carolinensis* and *A. sagrei* use information from previous territorial interactions to influence future behavior. In experimental settings, male *A. carolinensis* have been observed to show little effect of contest outcome (Garcia et al., 2012), increase their likelihood of losing after a loss only against familiar opponents (Forster et al., 2005), increase their likelihood of losing after a loss against unfamiliar opponents (Garcia et al., 2014), increase aggression after observing aggression (Yang et al., 2001), or increase aggression after wins and decrease aggression after losses (Garcia et al., 2014).

Heterospecific interactions are especially complex because individuals may misinterpret signals from an individual of a closely related species. *A. sagrei* displaces populations of *A. carolinensis* (Losos & Spiller, 1999), so behavioral interaction is likely. Male *A. carolinensis* perch higher in mixed populations than in *A. carolinensis*-only populations, but in lab conditions male *A. sagrei* show only a slight advantage in occupying preferred perch position; *A. sagrei* females show a much greater advantage (Edwards & Lailvaux, 2013). The behavioral interactions that result in this displacement likely involve the same territorial behaviors that are well-studied in conspecific contexts.

Anoles may show less aggression toward heterospecific competitors than conspecific competitors. In staged field encounters, *Anolis cristatellus* males were more aggressive toward conspecific males than toward *A. gundlachi* males (Hess & Losos, 1991), and in the lab, *A. carolinensis* and *A. sagrei* males were more aggressive toward conspecifics than heterospecifics (Tokarz & Beck, 1987). However, male *Anolis cooki* and *Anolis cristatellus*, competitors for the same microhabitat, are as aggressive toward each other as toward conspecifics (Ortiz & Jenssen, 1982).

It is also possible that interactions would affect the two species unequally. When *A. carolinensis* and *A. sagrei* are introduced to islands without anole populations, *A. carolinensis* population density is limited on islands with *A. sagrei* populations, but *A. sagrei* seem unaffected by the presence of *A. carolinensis* (Losos & Spiller, 1999). When placed in cages with juveniles, *A. sagrei* prefer to eat heterospecific juveniles rather than conspecific juveniles; *A. carolinensis* rarely eat juveniles at all and show no preference when they do (Gerber & Echternacht, 2000). Adult male *A. sagrei* are more likely to eat juveniles than *A. carolinensis* or *A. conspersus* and prefer heterospecific juveniles over conspecific juveniles, whereas *A. carolinensis* and *A. conspersus* show no preference (Gerber, 2000). Some disparity in effect may be driven by differences in territorial behavior; *A. sagrei* display more frequently than *A. carolinensis* in multiple contexts, so it's possible that in the field *A. carolinensis* overestimate *A. sagrei*'s motivation and retreat quickly, whereas *A. sagrei* underestimate *A. carolinensis*'s motivation and do not retreat (Tokarz & Beck, 1987).

Hormonal activity influences behavior in ways that can influence contest outcome, such as increased territorial displays after experimentally elevating testosterone in male *A. sagrei* (Tokarz et al., 2002). The influence of hormonal activity on behavior can vary at the population level, as in eastern fence lizards, *Sceloporus undulatus*, which respond to experimentally elevated corticosterone by decreasing activity in populations free of fire ants but increasing activity in populations invaded by fire ants (Trompeter & Langkilde, 2011).

Contest outcome also influences individuals' hormonal activity. In *A. carolinensis*, testosterone (Hattori, 2009), serotonin (Summers et al., 1997), epinephrine, norepinephrine, and dopamine (Korzan et al., 2002) are influenced by contest outcome. Corticosterone, a glucocorticoid, is of particular interest because it commonly increases in circulation in response to the energetic demands of aggressive behavior in reptiles (Moore & Jessop, 2003) and in turn influences aggressive behavior (Briffa & Sneddon, 2007). Maintaining social status is stressful, and species differ in whether high or low rank correlates to greater stress (Sapolsky, 2005). Glucocorticoids, which function as stress hormones, may be higher in dominants or subordinates, depending on the costs related to maintaining homeostasis (Goymann & Wingfield, 2004). The relationship between glucocorticoids and behavior is influenced by the context of conflict, the intensity, and other physiological and behavioral responses (Summers et al., 2005).

Experimentally elevated corticosterone reduces aggression in *A. carolinensis* but increases aggression in *A. sagrei* (Parikh & Lovern, unpublished data). The relationship between contest outcome and corticosterone is similarly complex across species. Post-contest corticosterone is greater in subordinate than dominant individuals in male *A. carolinensis* (Summers et al., 2003) and male copperheads, *Agkistrodon contortrix* (Schuett & Grober, 2000). However, a difference between dominant and subordinate individuals is not always evident in male *A. carolinensis* (Greenberg & Crews, 1990), or may be complicated by the familiarity of the opponent (Ling et al., 2010). The duration of

social interaction may also influence the timing and direction of differences in corticosterone (e.g., tree lizards, *Urosaurus ornatus*, Knapp & Moore, 1995).

In this research I address two primary questions. First, does residency affect behavior in *A. sagrei* and *A. carolinensis*? I hypothesized that residents of both species and both sexes would display more aggressive behaviors than intruders. Second, does residency affect post-experience plasma concentrations of corticosterone? I hypothesized that plasma corticosterone concentration would be greater in residents than intruders for both species and both sexes.

2.3 Methods

2.3.1 Animals

Experiments were conducted in May through August, when lizards are in breeding condition. In 2013 and 2014 I used lab-raised male and female *A. sagrei*. In 2015 and 2016 I used wild-caught male and female *A. carolinensis* and wild-caught male *A. sagrei*. Wild-caught lizards were bought from a commercial supplier (Underground Reptiles, Deerfield Beach, FL). All lizards were adults and had minimum 2 g body mass. All procedures were approved by IACUC as protocol AS1312.

2.3.2 Animal Husbandry

My husbandry protocol was modified from Lovern et al. (2004). Briefly, lizards were housed individually for 14 days prior to each experiment to establish territories and negate previous social experience, as *A. carolinensis* behave as naïve opponents when separated for at least 10 days (Forster et al., 2005). Each lizard was housed in a 38 L glass terrarium with peat moss substrate, a dowel rod for a perch, a water dish, a plastic nest box (9 x 15 x 8 cm) filled with damp peat moss (females only), and a

wire mesh lid. A 60 W bulb (one bulb per two terraria) provided heat and an 18 W Repti-Sun 5.0 UVB bulb (Zoo Med) (one bulb per three terraria) provided UV lighting. Room lights were on from 0700-2100h, UV lights 0800-2000h, and basking lights 0900-1900h daily. Room temperature ranged from 24-38 C and humidity ranged from 50% to 75%. Adjacent terraria were separated by cardboard dividers to prevent visual contact with immediate neighbors. All lizards were offered the same amount of food: mealworms twice per week and crickets twice per week, with one cricket feeding augmented with powdered vitamins (Minerall, Herpetivite). Terraria were misted with water daily. If a nest box was present, it was checked for eggs daily.

2.3.3 Treatment Groups

Lizards were randomly assigned to be residents (intended to gain winning experience) or intruders (intended to gain losing experience). T-tests confirmed that residents and intruders did not differ in mean snout-vent length or mean body mass (all $p > 0.05$) before trials. Lizards were excluded from experiments if their body mass was less than 2 g, if they had a broken or regrown tail, or if they appeared in poor health (abnormally low activity, no observed feeding).

2.3.4 Social Experience Trials

Lizards gained social experience between 1000 and 1400 h (during the lizards' active period). I captured an intruder lizard by hand and released the intruder lizard into the resident lizard's terrarium. Lizards interacted for 10 min while I recorded behaviors from a distance of 8 ft. After this experience trial I captured the intruder by hand to release back into the intruder's home terrarium. To standardize handling stress, I captured and immediately released the resident back into its home terrarium before and after the experience trials.

Species-typical territorial behaviors were observed and recorded: head bobs, dewlap extensions, approaches, retreats, and bites. A head bob was any bout of jerking the head up and down, separated from another bout by at least 3 seconds. A dewlap extension was any individual instance of extension of the throat fan. An approach was movement of at least one body length toward the opponent. A retreat was movement of at least one body length away from the opponent. A bite was any contact between one lizard's mouth and the other lizard.

To estimate investment in self-maintenance, I measured each lizard's snout-vent length and body mass at the beginning and end of the experiment to calculate changes.

2.3.5 Corticosterone

I drew blood from the post-orbital sinus via capillary tube following brief exposure to an inhalant anesthetic agent (isoflurane) 30 min after the staged social experience trial. The order of blood collection was randomized between resident and intruder for each experience trial and total collection time for both lizards did not exceed 4 min. In 2016, baseline corticosterone was sampled 8 d before final experience trials.

Plasma corticosterone concentration was determined by radioimmunoassay. Samples were centrifuged at 4000 rpm for 4 min. Plasma was removed and stored at 4 C. I added 500 µl of ddH$_2$O and 20 µl of tritiated steroid ("tracer") to all sample tubes and standard tubes. I vortexed all tubes, covered them with aluminum foil, and refrigerated them overnight. Steroid was extracted twice with 2 ml diethyl ether and evaporated under nitrogen gas in a water bath for 15 min at 37 ℃. I added 300 µl PBSg to all tubes, vortexed for 5 sec, and refrigerated the tubes overnight. After bringing all tubes to room temperature I vortexed all tubes for 5 sec, added 50 µl of each sample into scintillation vials with 2 ml Ultima Gold scintillation cocktail (Packard), vortexed for 5 sec, and counted radiation using a scintillation counter. I placed 200 µl of each sample into test tubes. To construct a standard curve I

conducted a serial dilution of 250 pg to 1.95 pg. I added 100 µl of antibody and 100 µl of tracer into all

tubes for a final volume of 400 µl per tube. I vortexed for 2 sec, covered tubes with aluminum foil, and

refrigerated them overnight. After bringing all tubes to room temperature I added 500 µl dextran-coated

charcoal in PBS (65% charcoal, 35% PBS) to all tubes to stop the assay and remove unbound tracer. I

vortexed racks manually for 10 sec and, 15 min after adding charcoal solution, centrifuged them at 2200

rpm for 10 min at 4 ℃. I decanted the supernatant into scintillation vials, added 3.5 ml Ultima Gold, and

vortexed. Then I counted on a scintillation counter.

2.3.6 Data Analysis

I used t-tests to detect differences between residents and intruders for each behavior, for

corticosterone concentrations, and for self-maintenance variables. Lizards were assigned to experience 1,

2, or 3 staged experiences; however, as the number of experiences did not significantly affect behavior,

data analysis here includes only the first staged experience for each lizard.

2.4 Results

2.4.1 Behavior: Conspecific Opponents: Anolis sagrei *females*

Female *A. sagrei* residents extended their dewlaps significantly more than intruders (Figure 1).

Residents and intruders did not differ significantly in their change in snout-vent length or body mass ($p > 0.05$ for both variables).

2.4.2 Behavior: Conspecific Opponents: Anolis sagrei *males*

Male *A. sagrei* residents bit more often (Figure 2) and performed significantly more dewlap extensions (Figure 3) than intruders; intruders retreated significantly more often than residents (Figure 2). Residents and intruders did not differ significantly in change in snout-vent length or body mass ($p > 0.05$ for both variables).

2.4.3 Behavior: Conspecific Opponents: Anolis carolinensis *females*

Female *A. carolinensis* residents performed significantly more headbobs than intruders (Figure 4). Residents and intruders did not differ significantly in their changes in snout-vent length or body mass ($p > 0.05$ for both variables).

2.4.4 Behavior: Conspecific Opponents: Anolis carolinensis *males*

Male *A. carolinensis* did not differ significantly in any behavior based on residency (Figure 5). Residents and intruders did not differ significantly in their changes in snout-vent length or body mass ($p > 0.05$ for both variables).

2.4.5 Behavior: Heterospecific Opponents

Neither male *A. sagrei* (Figure 6, Figure 7) nor male *A. carolinensis* (Figure 8) differed significantly in behavior in heterospecific experiences based on residency. In general, behavior did not differ significantly by species (Figure 9). Neither male *A. sagrei* nor male *A. carolinensis* differed significantly in the change in snout-vent length or change in body mass based on residency ($p > 0.05$ for both variables).

12

2.4.6 Corticosterone: Conspecific Opponents: Anolis sagrei

Male *A. sagrei* did not differ significantly in plasma corticosterone based on residency (Figure 10), but female *A. sagrei* had higher plasma corticosterone in intruders than in residents (Figure 10). Within residents and intruders, corticosterone did not vary by sex (Figure 11).

2.4.7 Corticosterone: Conspecific Opponents: Anolis carolinensis

Neither male nor female *A. carolinensis* differed in mean corticosterone by residency (Figure 12). Corticosterone had a greater mean (Figure 13) and greater variance (Figure 14) in *A. carolinensis* females than in males.

2.4.8 Corticosterone: Heterospecific Opponents

Neither male *A. sagrei* (Figure 15) nor male *A. carolinensis* (Figure 16) differed significantly in behavior based on residency against a heterospecific opponent. The two species did not differ significantly at baseline or post-experience corticosterone concentrations (Figure 17). Staged experiences increased mean corticosterone in both *A. sagrei* and *A. carolinensis*, regardless of residency (Figure 18). Variance in corticosterone concentration was significantly greater in *A. carolinensis* than in *A. sagrei* both before and after staged experiences (Figure 19). Variance increased with experience for *A. sagrei* but not *A. carolinensis* (Figure 20).

2.5 Discussion

The first hypothesis, that residents of both species would display more aggressive behaviors than intruders, was supported at the species level, although the specific behaviors affected by residency varied

by species and sex. The second hypothesis, that plasma corticosterone concentration would be greater in residents than intruders for both species and both sexes, was largely unsupported; only for female *A. sagrei* did residency affect corticosterone.

2.5.1 Behavior: Conspecific Opponents: Residency

With the exception of *A. carolinensis* males, all groups were more aggressive as residents than intruders, suggesting the experimental setup successfully simulated territorial conflict, allowing further study of the hormonal and reproductive consequences of such different experiences. Several behaviors that are not significantly different between residents and intruders show trends toward greater aggression in residents, so a larger sample size might increase the number of behaviors that are significantly different based on residency.

2.5.2 Behavior: Conspecific Opponents: Self-Maintenance

Lizards did not differ in their growth and self-maintenance (measured by changes in snout-vent length and body mass) based on residency during social experience trials. This pattern is observed in both species, in lab-raised and wild-caught lizards, and in females and males. Perhaps significant differences would result from the constant interaction of lizards housed together. Future research should explore the effects of a greater number of experiences and of long-term experience on self-maintenance in both female and male lizards.

2.5.3 Behavior: Heterospecific Opponents

Several behaviors in conspecific experiences were similar (headbobs) or identical (dewlap extensions, approaches, retreats, bites) for the two species, so territorial disputes could occur between species in the field. However, in heterospecific experiences with wild-caught *A. sagrei* and *A. carolinensis* males, residency did not affect any behavior for either species. Lizards in heterospecific experiences showed a trend toward fewer behaviors than lizards in conspecific experiences (although this was not statistically tested), suggesting a greater level of caution towards heterospecifics. This result is similar to results found in other anole species: in staged field encounters, *Anolis cristatellus* shows greater aggression toward conspecifics than toward *Anolis gundlachi* (Hess & Losos, 1991). However, some species are quite aggressive toward heterospecifics; *Anolis cooki* and *Anolis cristatellus* compete for the same microhabitat and heterospecific interactions are as aggressive as conspecific interactions for each species (Ortiz & Jenssen, 1982). *A. sagrei* and *A. carolinensis*' caution suggests the displacement of *A. carolinensis* during invasions is not due to aggression between males, but might instead result from resource partitioning (Campbell, 2000; Edwards & Lailvaux, 2012). Growth and self-maintenance traits did not differ based on residency; since both species showed less aggression in heterospecific experiences than in conspecific experiences, the lack of effect of residency is not surprising. Future work should compare the behavioral responses to heterospecific social interactions in lizards from uninvaded and invaded populations.

2.5.4 Corticosterone: Residency

Plasma corticosterone was greater in residents than in intruders for lab-raised female *A. sagrei*; there was no difference based on residents for lab-raised male *A. sagrei*, wild-caught female *A. carolinensis*, or wild-caught male *A. carolinensis*. Behavioral differences based on residency did not always match differences in stress hormones, similar to results in tree lizards, *Urosaurus ornatus*

(Thompson & Moore, 1992). It is possible that the stress of captivity masked any stress due to social interaction for wild-caught lizards. Because the source of study animals differed for the two species, it is not clear whether a species difference exists during conspecific experiences. The advantage of wild-caught lizards is that they likely more closely resemble lizards in the field than lab-raised lizards do, but the disadvantage is that captivity is likely stressful for them, perhaps stressful enough to mask differences in stress hormones based on social experience even when behavior differs between residents and intruders. The advantage of lab-raised lizards is that captivity is familiar to them, so it makes sense that their reaction to social experience would show more subtle differences in stress hormones. Future work should build on this research by conducting similar experiments with wild-caught *A. sagrei* and lab-raised *A. carolinensis*.

The relationship between stress hormone activity and fitness is complex. The relationship between stress hormone activity and variables used to estimate fitness may be positive, negative, or nonexistent (Bonier et al., 2009). Future work should investigate the effects of social interaction on short-term and long-term hormone activity and the relationship between stress hormone activity and measures of fitness in these species.

2.5.5 Corticosterone: Females vs. Males

It is interesting that lab-raised female *A. sagrei* showed differences based on residency but males did not; perhaps dominance hierarchies are more stable for females than for males, so a losing experience corresponds to a longer-term reduction in access to resources. Perhaps the difference between sexes relates to differences in reproductive investment; nearly every female is expected to reproduce in the field, but only the most dominant males reproduce, leading to a greater range of reproductive success among males (Gerber, 2000).

Lab-raised *A. sagrei* showed no sex differences in plasma corticosterone, but corticosterone was greater in female wild-caught *A. carolinensis* than in males. It is unclear whether this species difference is actually due to species or animal source. Perhaps differences in females' status more greatly affect behavior towards males than toward other females; other work with female *A. carolinensis* shows that social status does not affect perch site selection, body color, or prey capture, but does affect frequency of signals to males (Andrews & Summers, 1996).

2.5.6 Corticosterone: Heterospecific Opponents

Neither wild-caught *A. sagrei* males nor wild-caught *A. carolinensis* males differed in plasma corticosterone based on residency in heterospecific experiences. This result is consistent with the lack of effect of residency on behavior in heterospecific experiences. Perhaps both species are so cautious that neither recognizes residency or threats to residency. The lack of behavioral or hormonal differences based on residency supports the conclusion that displacement of *A. carolinensis* during invasion is not caused by direct conflict between males (Edwards & Lailvaux, 2013).

2.5.7 Limitations

In this study, lizard interactions were standardized at 10 minutes to aid statistical analysis, whereas interactions in the field vary in duration as individuals abandon contests they are unlikely to win.

These experiments tested lizards in pairs only. Social interactions in the field often involve only two individuals, but more individuals may be involved directly or may observe the social interactions of others. Studies have found that aggression in pairs may show low correlation to aggression in groups (Chase et al., 2003) or high correlation (Dugatkin & Druen, 2004). Future work should focus on larger

groups and observers, as models predict observation of others interacting could contribute to social

organization in similar ways to direct involvement (Dugatkin & Earley, 2003).
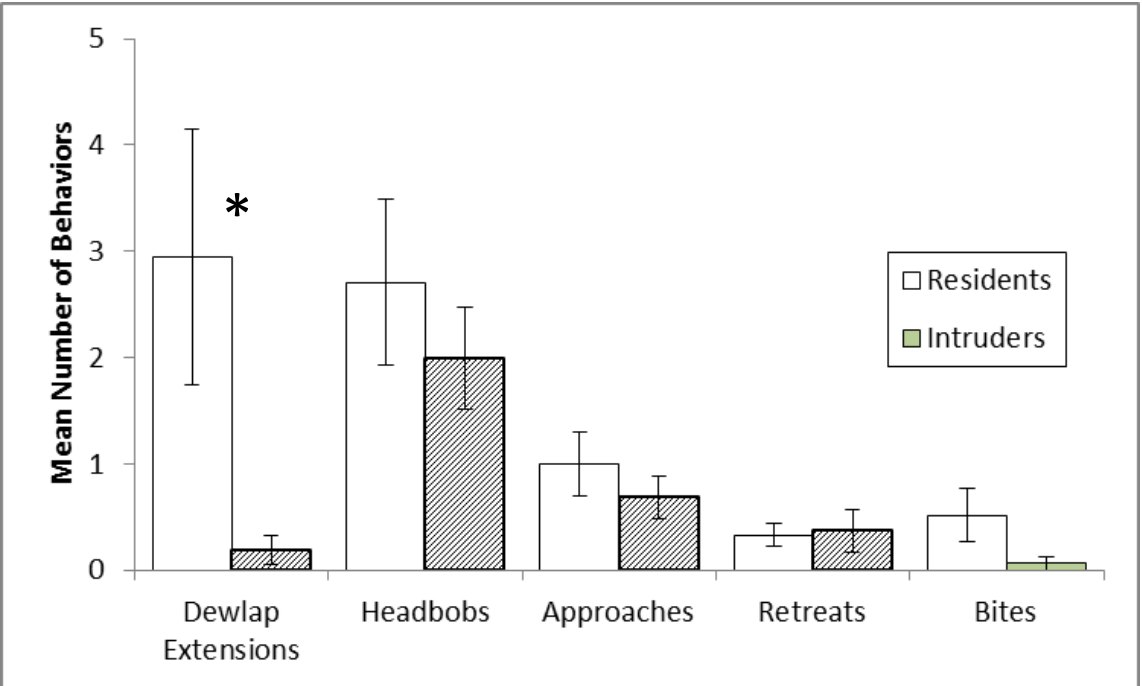
Figure 1. Behavior by residency in female *A. sagrei* (n = 21 resident trials, 16 intruder trials; error bars represent one standard error). T = -2.37, p = 0.028 for dewlap extensions and p > 0.05 for all other behaviors. An asterisk indicates a significant difference.
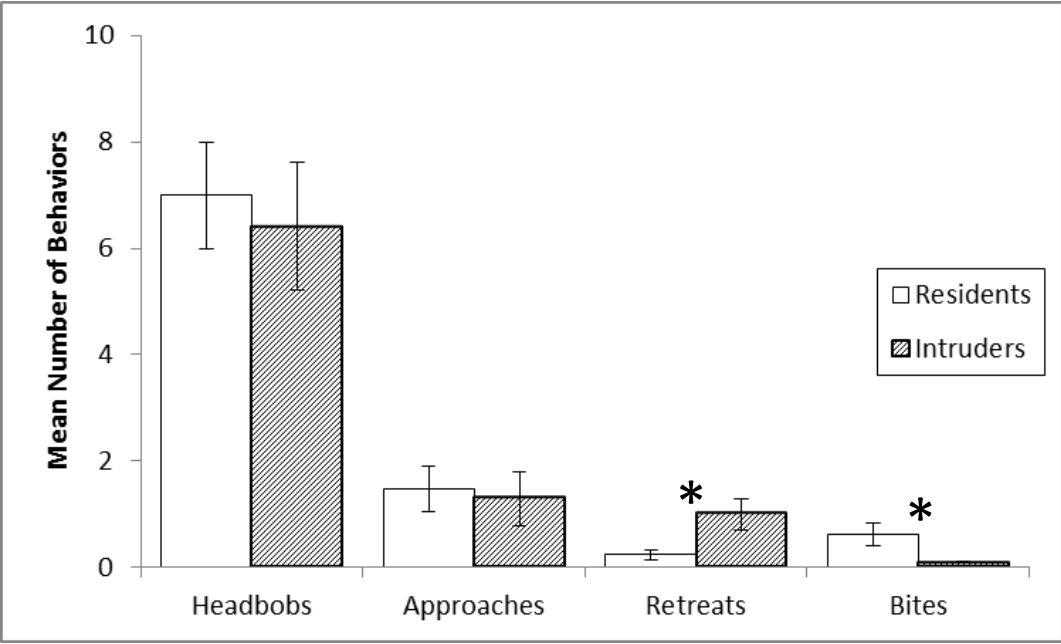
Figure 2. Behavior by residency in male *A. sagrei* (n = 21 resident trials, 17 intruder trials; error bars represent one standard error). T = 2.44, p = 0.025 for retreats, t = -2.43, p = 0.024 for bites, and p > 0.05 for all other behaviors. An asterisk indicates a significant difference.
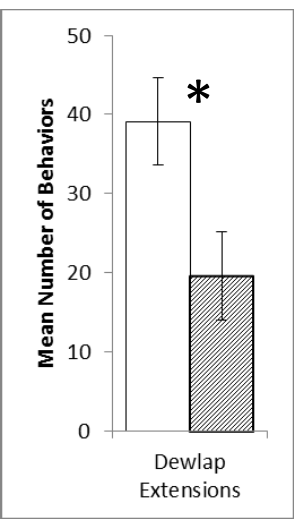
Figure 3. Dewlap extensions by residency in male *A. sagrei* (n = 21 resident trials, 17 intruder trials; error bars represent one standard error). T = -2.50, p = 0.017. An asterisk indicates a significant difference.
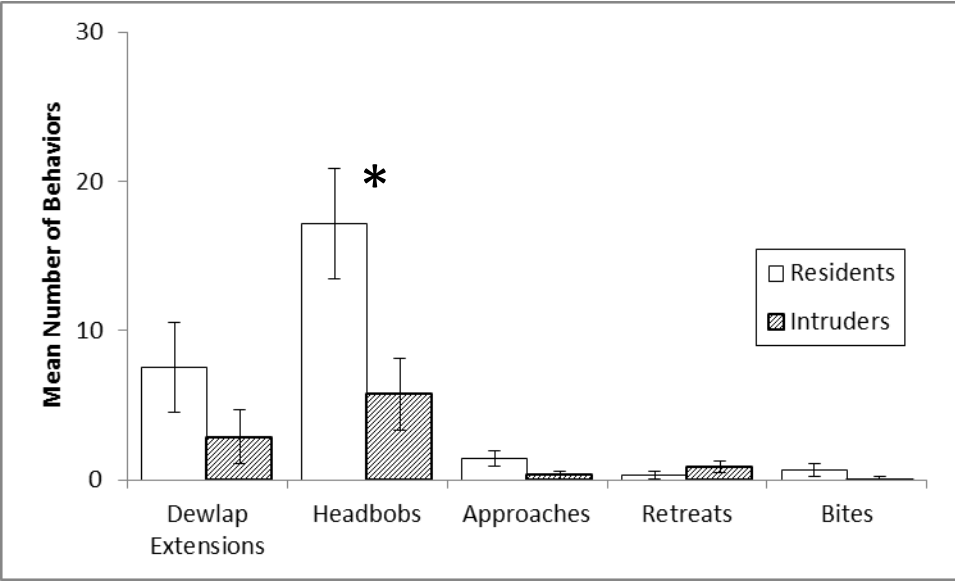
Figure 4. Behavior by residency in female *A. carolinensis* (n = 9 resident trials, 9 intruder trials; error bars represent one standard error). T = -2.56, p = 0.024 for headbobs and p > 0.05 for all other behaviors. An asterisk indicates a significant difference.
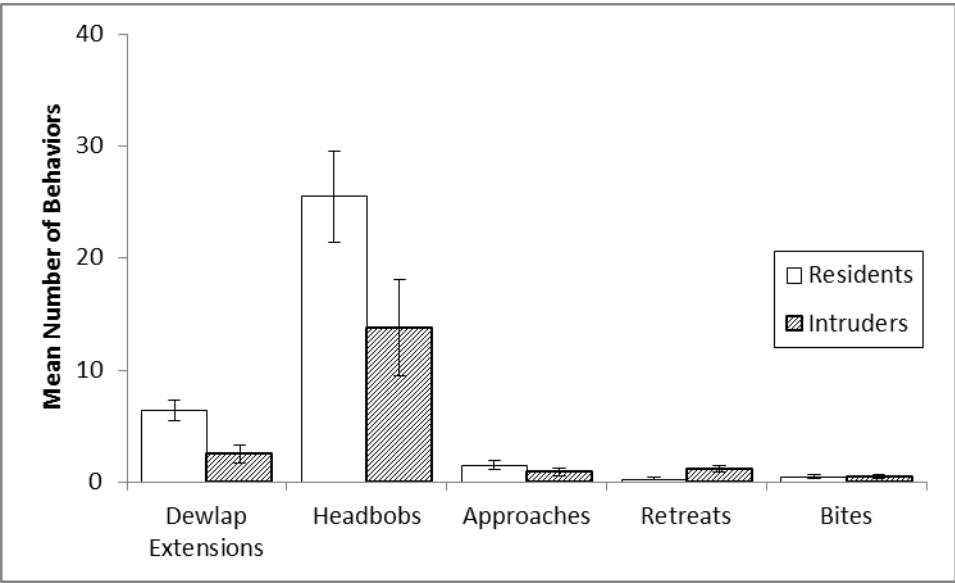
Figure 5. Behavior by residency in male *A. carolinensis* (n = 11 resident trials, 11 intruder trials; error bars represent one standard error). P > 0.05 for all behaviors. An asterisk indicates a significant difference.
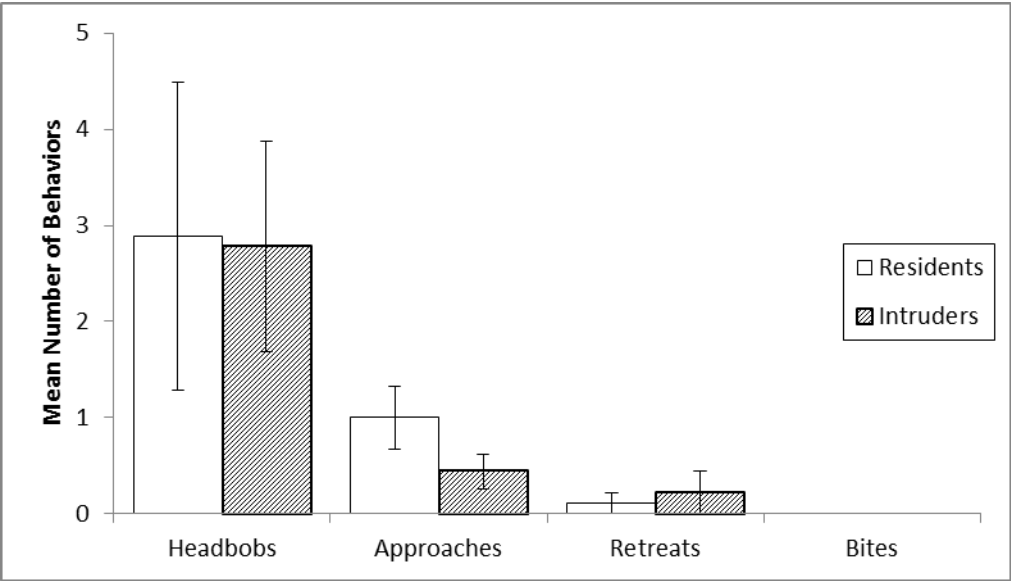
Figure 6. Behavior by residency in male *A. sagrei* in heterospecific experiences (n = 9 resident trials, 9 intruder trials; error bars represent one standard error). P > 0.05 for all behaviors. An asterisk indicates a significant difference.
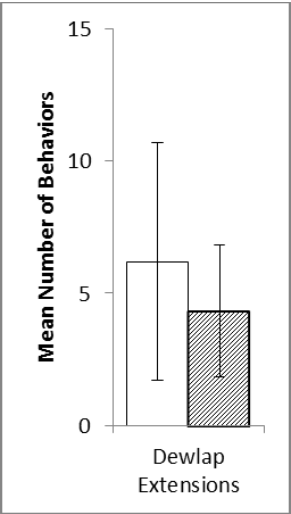
Figure 7. Dewlap extensions by residency in male *A. sagrei* in heterospecific experiences (n = 9 resident trials, 9 intruder trials; error bars represent one standard error). P > 0.05. An asterisk indicates a significant difference.
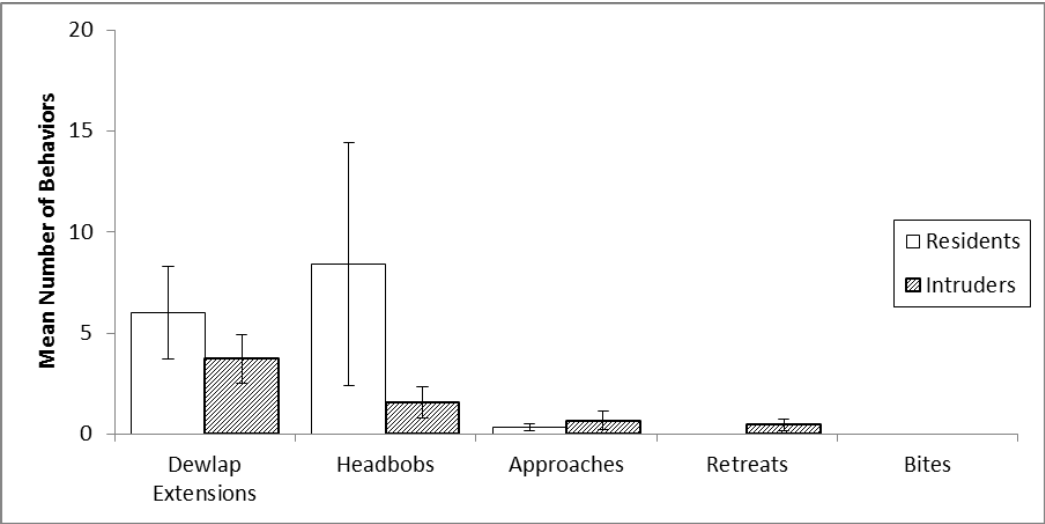
Figure 8. Behavior by residency in male *A. carolinensis* in heterospecific experiences (n = 9 resident trials, 9 intruder trials; error bars represent one standard error). P > 0.05 for all behaviors. An asterisk indicates a significant difference.
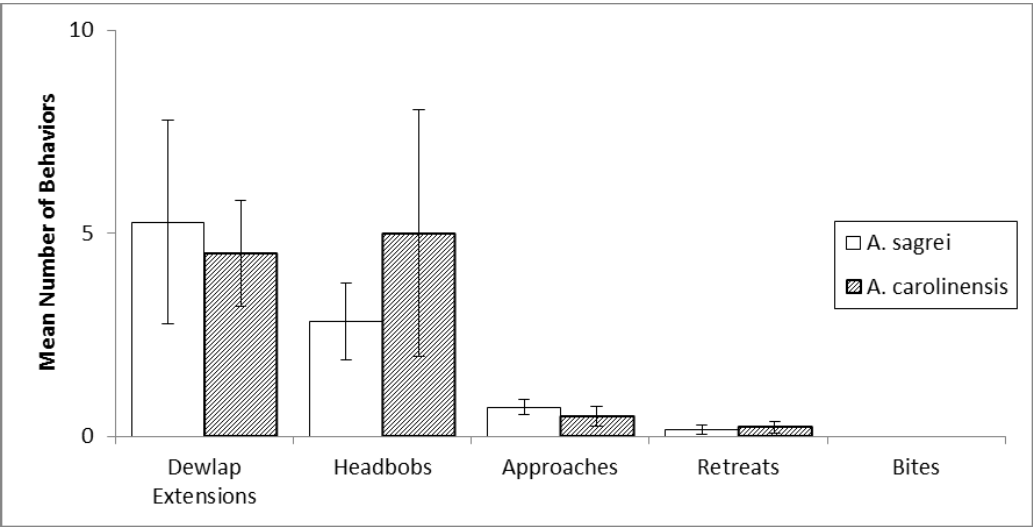
Figure 9. Behavior by species in male *A. sagrei* and *A. carolinensis* in heterospecific experiences (n = 18 *A. sagrei* trials, 18 *A. carolinensis* trials; error bars represent one standard error). P > 0.05 for all behaviors. An asterisk indicates a significant difference.
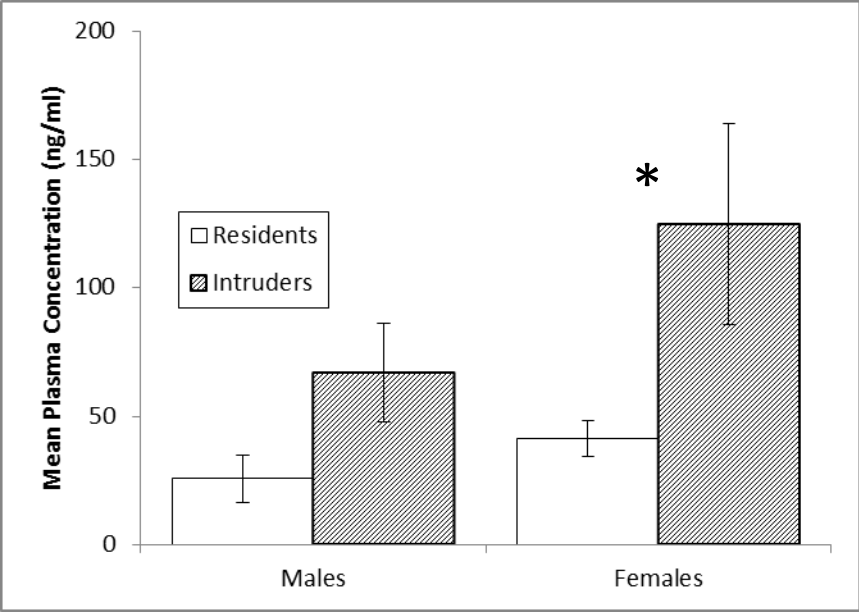
Figure 10. Corticosterone by residency within sex in *A. sagrei* (n = 10 male residents, 8 male intruders, 12 female residents, 11 female intruders; error bars represent one standard error). P > 0.05 for males, T = -2.2, p = 0.040 for females. An asterisk indicates a significant difference.
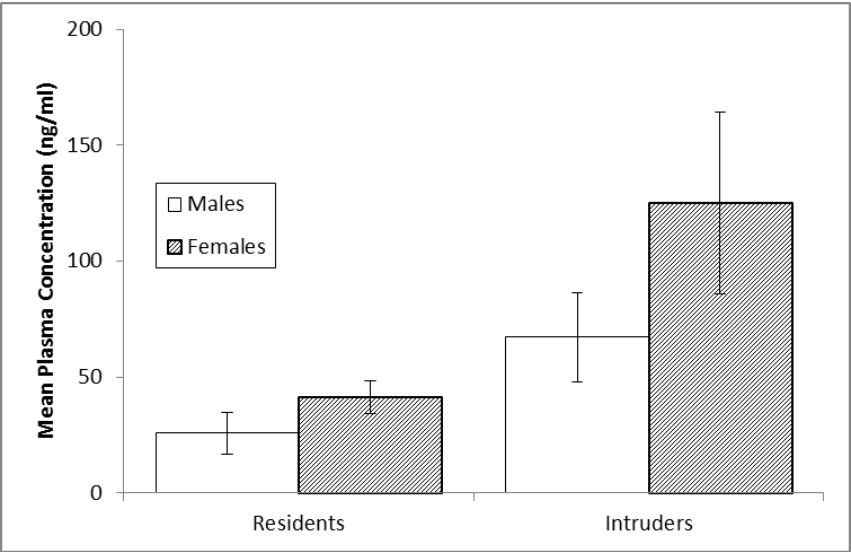
Figure 11. Corticosterone by sex within residency in *A. sagrei* (n = 10 resident males, 12 resident females, 8 intruder males, 11 intruder females; error bars represent one standard error). P > 0.05 for residents and intruders. An asterisk indicates a significant difference.
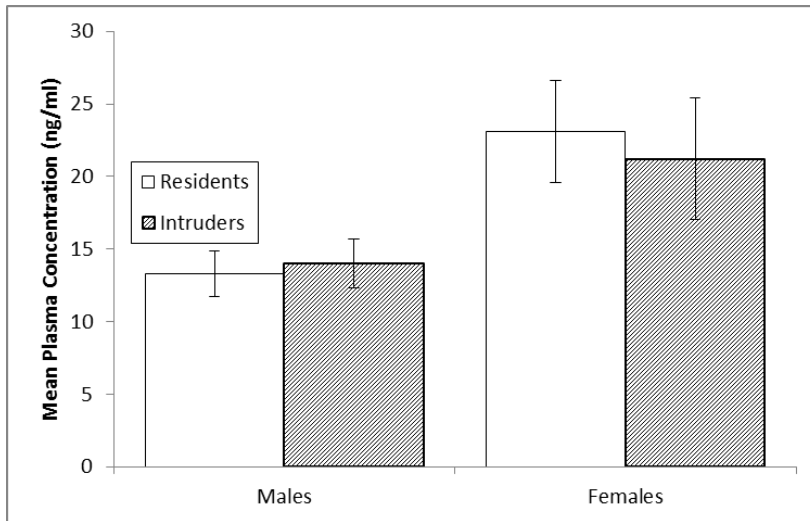
Figure 12. Corticosterone by residency within sex in *A. carolinensis* (n = 11 male residents, 11 male intruders, 9 female residents, 8 female intruders; error bars represent one standard error). P > 0.05 for males and females. An asterisk indicates a significant difference.
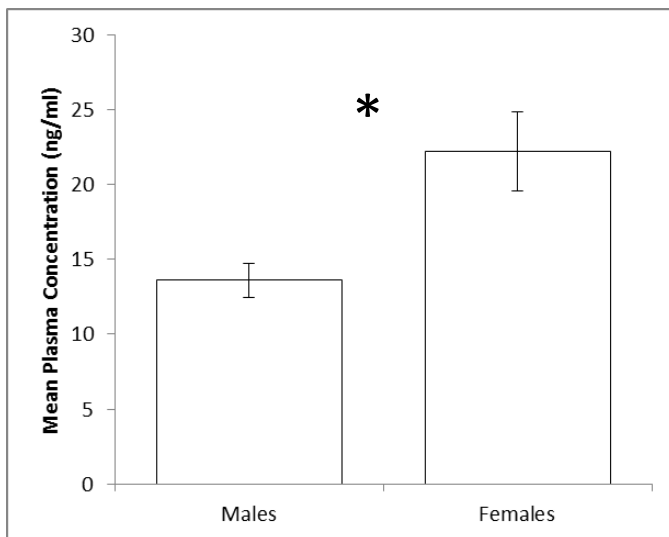
Figure 13. Corticosterone by sex in *A. carolinensis* (n = 22 males, 17 females; error bars represent one standard error). T = 3.0, p = 0.007. An asterisk indicates a significant difference.

Figure 14. Variance in corticosterone in *A. carolinensis* in conspecific experiences (n = 22 males, 17 females). F = 4.2, p = 0.001. An asterisk indicates a significant difference.
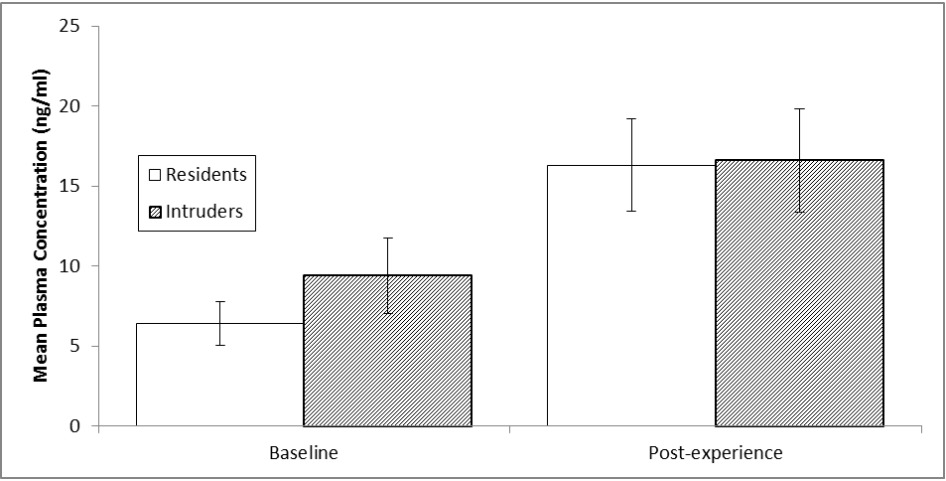
Figure 15. Corticosterone in male *A. sagrei* in heterospecific experiences (n = 8 residents, 8 intruders; error bars represent one standard error). P > 0.05 for baseline and post-experience concentrations. An asterisk indicates a significant difference.
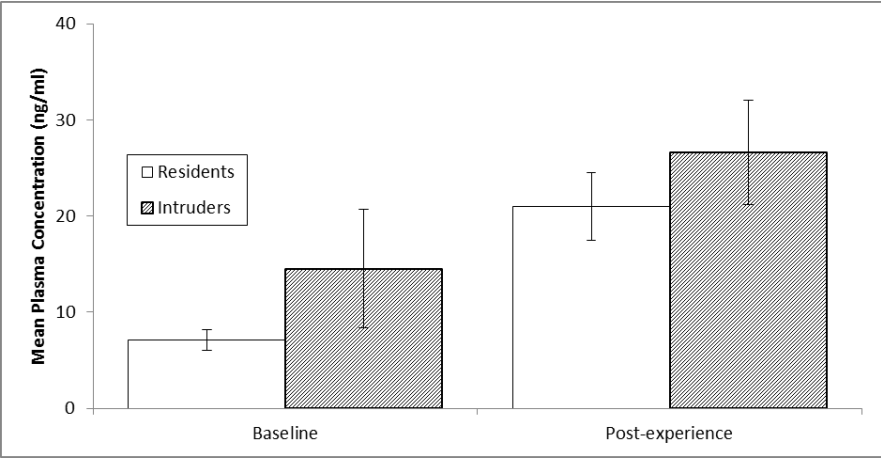
Figure 16. Corticosterone in male *A. carolinensis* in heterospecific experiences (n = 9 residents, 9 intruders; error bars represent one standard error). P > 0.05 for baseline and post-experience concentrations. An asterisk indicates a significant difference.
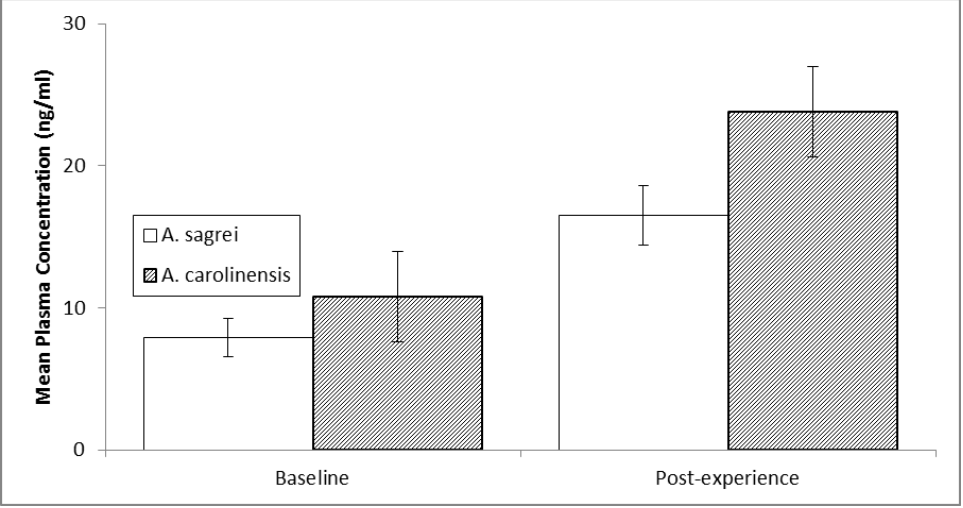
Figure 17. Corticosterone by species in male *A. sagrei* and *A. carolinensis* in heterospecific experiences (n = 16 *A. sagrei*, 18 *A. carolinensis*; error bars represent one standard error). P > 0.05 for both baseline and post-experience concentrations. An asterisk indicates a significant difference.
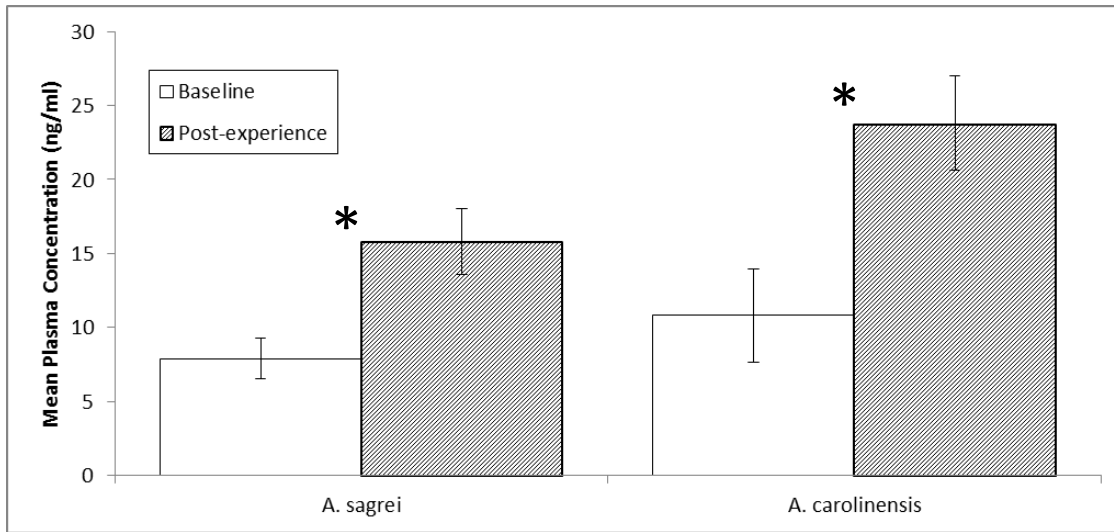
Figure 18. Corticosterone in male *A. sagrei* and *A. carolinensis* in heterospecific experiences (n = 16 *A. sagrei*, 18 *A. carolinensis*; error bars represent one standard error). T = -3.5, p = 0.003 for *A. sagrei* and t = -3.5, p = 0.003 for *A. carolinensis*. An asterisk indicates a significant difference.
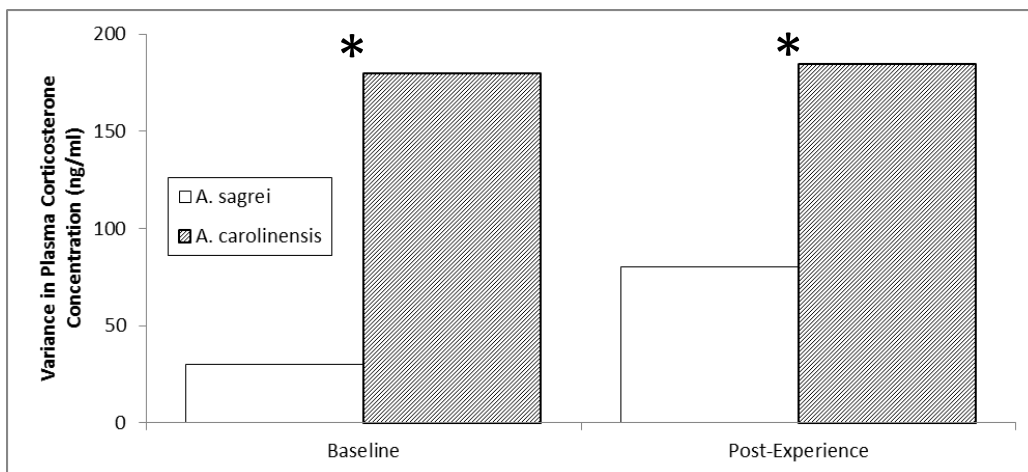
Figure 19. Variance in corticosterone in male *A. sagrei* and *A. carolinensis* in heterospecific experiences (n = 16 *A. sagrei*, 18 *A. carolinensis*). F = 0.2, p = 0.001 for baseline concentrations and f = 0.4, p = 0.048 for post-experience concentrations. An asterisk indicates a significant difference.
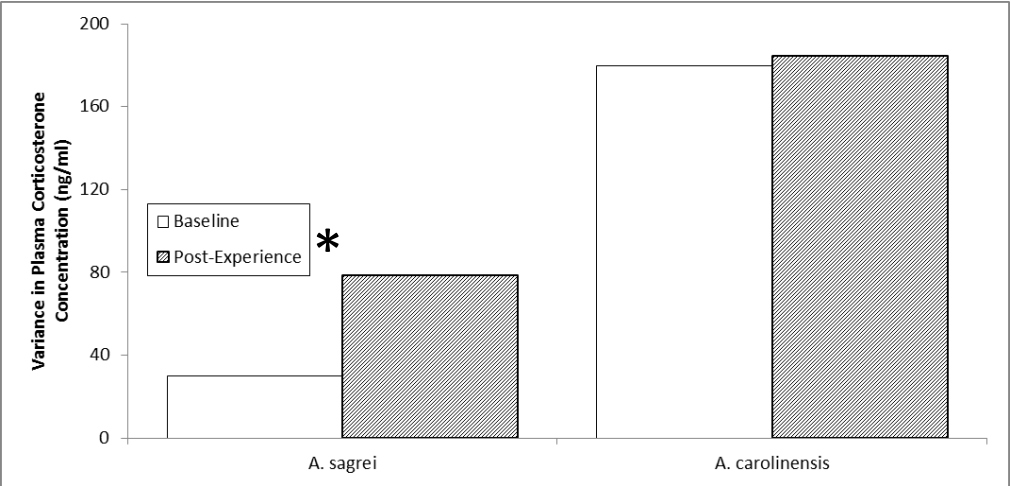
Figure 20. Variance in corticosterone in male *A. sagrei* and *A. carolinensis* in heterospecific experiences before and after experiences (n = 16 *A. sagrei*, 18 *A. carolinensis*). F = 0.4, p = 0.030 for *A. sagrei* and p > 0.05 for *A. carolinensis*. An asterisk indicates a significant difference.

CHAPTER III

TERRITORIAL CONTESTS AFFECT REPRODUCTION

IN *ANOLIS SAGREI* AND *ANOLIS CAROLINENSIS* FEMALES

3.1 Abstract

Territorial species compete over resources such as food and shelter, resulting in unequal access to resources that are useful in producing offspring. Inappropriate behavior during a territorial dispute is costly because an individual who is too bold wastes energy on an unwinnable fight and an individual who is too cautious abandons useful resources. Some species avoid inappropriate behavior by using information from previous territorial disputes to alter territorial behavior, becoming more or less aggressive based on previous successes or failures. This study investigates the effects of territorial competitions on females' investment in the competing interests of reproduction and self-maintenance. Female *Anolis sagrei* and *Anolis carolinensis* were exposed to brief conspecific interactions as residents or intruders. Lab-raised *A. sagrei* residents and intruders differed in the relationship between reproductive variables and body size. Wild-caught *A. carolinensis* did not differ in reproductive or self-maintenance traits based on residency. Social experience influences reproduction in lab-raised *A. sagrei*. Social experience may influence reproduction in wild-caught *A. carolinensis* in ways that are masked by the stress of captivity.

3.2 Introduction

Female anoles hold territories (Nunez et al., 1997) and use territorial displays to defend them (Evans, 1938); these displays are the same as those displayed by territorial males, although at lower frequencies (Jenssen et al., 2000). In the lab, paired female *A. carolinensis* form dominant-subordinate relationships that affect behavior, although social status does not seem to affect prey capture (Andrews & Summers, 1996). Even in the absence of overt territorial behaviors, territorial interactions affect hormonal activity (Davis & Marler, 2003).

Anolis lizards store sperm from multiple males for months (Calsbeek et al., 2007), choosing from among the sperm of several males post-mating to maximize offspring quality (Calsbeek & Bonneaud, 2008). *Anolis* lizards lay one-egg clutches with no parental care (Tokarz & Jones, 1979). However, reproduction is still costly and there are trade-offs between reproduction and survival. When the ovaries of *A. sagrei* were surgically removed to prevent reproduction, females increased survival to the end of the breeding season by 56%, to the end of the winter by 96%, and to the next year by 200% (Cox & Calsbeek, 2009). The trade-off could be due to intrinsic factors such as reduced resources available to self-maintenance while reproducing or to extrinsic factors such as increased predation risk while reproducing (Cox & Calsbeek, 2009). *A. sagrei* females experience the best growth, body condition, fat, and survival through the breeding season with a bilateral ovariectomy, intermediate performance for all variables with unilateral ovariectomy, and the worst performance with sham surgery (Cox et al., 2014).

Reproductive activity also affects stress hormones. Female tree lizards, *Urosauraus ornatus*, differ in the intensity of their stress responses, as measured by increases in plasma corticosterone, based on their reproductive stage (Woodley & Moore, 2002). Female Texas horned lizards, *Phrynosoma cornutum*, have higher corticosterone during egg-laying (Wack et al., 2008).

Territorial behavior can influence reproductive investment. At high conspecific population densities, when territorial behavior is common, *A. carolinensis* and *A. sagrei* females reduced the number of eggs produced (but not egg size or lipid content); at high heterospecific population densities, *A. carolinensis* but not *A. sagrei* females reduced the number of eggs produced (Vincent, 2002).

Traits that are useful in territorial defense are also useful in reproduction. Larger *A. sagrei* females produce heavier eggs (Warner et al., 2013) and are more likely to survive the breeding season, although they have no survival advantage over the winter (Delaney & Warner, 2016). Territorial behavior of parents may affect indirectly their offspring through differential access to food resources and subsequent investment in offspring. *A. carolinensis* females with greater diet quality laid more but not heavier eggs (Lovern & Adams, 2008); *A. sagrei* females with greater diet quality had heavier eggs, heavier offspring, faster offspring growth, and higher offspring survival, apparently by greater yolk investment (Warner & Lovern, 2014).

Maternal hormonal activity can affect offspring. Lizards transfer corticosterone to developing embryos before laying the eggs (Uller et al., 2009). Maternal corticosterone can adversely affect reptile embryos' physical development (Uller et al., 2009) and hatching success (Meylan et al., 2010). *A. carolinensis* reproductive behavior is affected by the stress associated with dominance hierarchies (Greenberg, 2002). High stocking densities in captive American alligators (*Alligator mississippiensis*) raised plasma corticosterone and reduced nesting success (Elsey et al., 1990). Elevated corticosterone in the eggs of tree lizards, *Urosauraus ornatus*, decreased time to hatching, which limits time to develop before hatching, which can decrease hatchling survival (Weiss et al., 2007). Elevated corticosterone adversely affects lizards' offspring body size (Uller et al., 2009) and body condition (Cadby et al., 2010), although not all species show this effect (e.g., common lizard, *Lacerta vivipara*, Uller & Olsson, 2006). The effect of corticosterone on offspring can differ by species; elevated corticosterone levels during embryonic development increase the growth rate of the Eastern three-lined skink, *Bassiana duperreyi* but decrease growth rate of the jacky dragon, *Amphibolurus muricatus* (Warner et al., 2009).

Exposure to maternal corticosterone during embryonic development also affects offspring later in life. Elevated corticosterone during embryonic development in common lizards, *Lacerta vivipara*, decreases juvenile sprint speed and tendency to flee (Meylan & Clobert, 2004). Experimentally elevated corticosterone in eggs increased juveniles' time to emerge from shelter after a simulated attack, indicating greater caution (Uller & Olsson, 2006). Sometimes effects are complex, as in *Lacerta vivipara*; experimentally elevated corticosterone in mothers reduced juvenile body size and body condition but increased juveniles' physiological performance (Meylan et al., 2010). Corticosterone exposure at different developmental stages and of different durations has different effects on juveniles' dispersal strategies (Vercken et al., 2007).

Females should alter the sex ratio of their offspring to increase their fitness (Sheldon & West, 2002). Theoretically, females in good condition should produce more sons to maximize their reproductive output (Trivers & Willard, 1973); "good condition" may include "high status" and high status contributes to good condition through greater access to food and shelter resources. When body condition is good, males tend to have greater reproductive success than females, and when body condition is poor, females tend to have greater reproductive success than males; mammalian data support this (Trivers & Willard, 1973).

Sex ratios can be influenced by social status, as in female Barbary macaques, *Macaca sylvanus*, which produce more sons when they have high status and more daughters when they have low status (Paul et al., 1992). The hormones associated with different social statuses may be the mechanism of adjusted sex ratios. Elevated corticosterone in female common lizards, *Lacerta vivipara*, increases survival of their male offspring (Meylan & Clobert, 2005), biases offspring toward females in the Jacky dragon, *Amphibolurus muricatus* (Warner et al., 2009), and biases offspring toward males in the Eastern three-lined skink, *Bassiana duperreyi* (Warner et al., 2009).

I hypothesized that residents would invest more in reproduction than intruders by producing either greater quantity or quality of offspring, with offspring sex ratios biased toward males in residents and toward females in intruders, representing a greater investment in current reproduction in response current success in territorial conflict. I also hypothesized that intruders would invest more in self-maintenance than residents by losing less body mass during the experiment, representing a greater investment in future reproduction by investing in current survival in response to current failure in territorial conflict.

3.3 Methods

3.3.1 Animals

Experiments involved 19 lab-raised *Anolis sagrei* females (in 2013 and 2014) and 12 wild-caught *Anolis carolinensis* females (in 2015) purchased from Underground Reptiles (Deerfield, FL). All lizards were adults and had minimum 2 g body mass. Experiments took place during the breeding season (May through August) when *A. sagrei* (e.g., Cox & Calsbeek, 2015) and *A. carolinensis* (e.g., Husak et al., 2007) defend territories. All procedures were approved by IACUC as protocol AS1312.

3.3.2 Animal Husbandry

My husbandry protocol was modified from Lovern et al., 2004. Briefly, lizards were housed in 110 L glass terraria in mixed-sex groups to gain mating experience prior to experiments, then housed individually for 14 days prior to each experiment to establish territories and negate previous social experience, as *A. carolinensis* behave as naïve opponents when separated for at least 10 days (Forster et al., 2005). Each lizard was housed in a 38 L glass terrarium with peat moss substrate, a dowel rod for a perch, a water dish, a plastic nest box (9x15x8 cm) filled with damp peat moss, and a wire mesh lid. A

60 W bulb (one bulb per two terraria) provided heat and an 18 W Repti-Sun 5.0 UVB bulb (Zoo Med) (one bulb per three terraria) provided UV lighting. Room lights were on from 0700-2100h, UV lights 0800-2000h, and basking lights 0900-1900h daily. Room temperature ranged from 24-38 C and humidity was maintained between 50% and 75%. Adjacent terraria were separated by cardboard dividers to prevent visual contact with neighbors. All lizards were offered the same amount of food: mealworms twice per week, crickets twice per week; once per week, crickets were dusted with vitamins (Minerall, Herpetivite). Terraria were misted with water daily.

Nest boxes were checked daily for eggs. Eggs were removed, weighed, and placed in a small glass jar of vermiculite (10 g vermiculite, 10 mL distilled water); moisture was retained by a square of plastic wrap fixed over the mouth of the jar by a rubber band. Eggs were incubated at 28 C and checked daily for hatchlings.

3.3.3 Treatment Groups

Treatment groups were identical to those described in chapter 1. Lizards were randomly assigned to be residents (intended to gain winning experience) or intruders (intended to gain losing experience). T-tests confirmed that residents and intruders did not differ significantly in mean snout-vent length or mean body mass ($p > 0.05$) before trials. Lizards were excluded from experiments if their body mass was less than 2 g, if they had a broken or regrown tail, or if they appeared in poor health (abnormally low activity, no observed feeding).

3.3.4 Social Experience Trials

Lizards gained social experience between 1000 and 1400 h (during the lizards' active period). I captured an intruder lizard by hand and released the intruder lizard into the resident lizard's terrarium.

Lizards interacted for 10 min while I recorded behaviors from a distance of 8 feet. After this experience I captured the intruder by hand to release back into the intruder's home terrarium. To standardize handling stress, I captured and immediately released the resident back into its home terrarium before and after the experience.

To test whether staged experiences simulated successful and unsuccessful terrirorial conflict, species-typical territorial behaviors were observed and recorded: head bobs, dewlap extensions, approaches, retreats, and bites. A head bob was any bout of jerking the head up and down, separated from another bout by at least 3 seconds. A dewlap extension was any individual instance of extension of the throat fan. An approach was movement of at least one body length toward the opponent. A retreat was movement of at least one body length away from the opponent. A bite was any contact between one lizard's mouth and the other lizard.

3.3.5 Variables

To estimate investment in reproduction, I measured reproductive traits for each lizard throughout the rest of the experiment: the number of eggs laid, the number of eggs hatched, mean egg mass, mean days to hatch, mean hatchling mass, mean hatchling snout-vent length, and offspring sex ratio. To estimate investment in self-maintenance, I measured snout-vent length and body mass at the beginning and end of the experiment to determine change in each variable.

3.3.6 Data Analysis

I used t-tests to detect behavioral differences between residents and intruders. T-tests were used to compare each reproductive and self-maintenance variable in resident and intruder lizards. To address the pseudoreplication of siblings, I calculated the mean value for all offspring for each lizard for

statistical analysis. I ran simple regressions to compare maternal snout-vent length and reproductive variables among all lizards, among winners, and among losers.

3.4 Results

3.4.1 A. sagrei

Residents and intruders did not differ significantly in any reproductive or self-maintenance variable ($p > 0.05$ for all variables). Residents and intruders did differ in the relationship between maternal snout-vent length and reproductive variables; the mean number of eggs laid, mean egg mass, mean days to hatch, and mean hatchling mass were positively related to maternal snout-vent length in residents, whereas in intruders mean days to hatch was negatively related to maternal snout-vent length (Table 1).

3.4.2 A. carolinensis

Residents and intruders did not differ significantly in reproductive or self-maintenance variables ($p > 0.05$ for all variables). No reproductive variable was significantly related to maternal snout-vent length ($p > 0.05$ for all variables).

3.5 Discussion

The first hypothesis, that residents would invest more in reproduction than intruders, was not supported in *A. sagrei* or *A. carolinensis*, although *A. sagrei* residents and intruders differed significantly in the relationship between reproductive variables and maternal body size. The second hypothesis, that

37

intruders would invest more in self-maintenance than residents, was not supported, as residents and intruders did not differ significantly in self-maintenance variables

3.5.1 A. sagrei

Lab-raised *A. sagrei* females did not show the expected differences in reproductive variables based on residency. Since *Anolis* lizards are estimated to live several years in the field (Toda et al., 2010), it would be advantageous to the losing lizard (with limited food and shelter resources) to maximize her chances of survival to the next breeding season to try reproducing again. Winners have resources now, so should invest in maximizing their reproduction now. Winners can prioritize current reproduction whereas losers, although still reproducing now, should invest more than winners in future reproduction. Perhaps a greater number of experiences would result in differential reproductive investment.

The relationship between reproductive variables and maternal body size (measured by snout-vent length) differed between residents and intruders. Body size seems more important to lizards who have successfully defended a territory than those who have not, even when food resources are equal. It is possible that, although smaller residents would benefit from investing as much in each individual offspring as larger residents, some morphological or physiological constraint prevents them from doing so. Another possibility is that lizards of different ages prioritize current and future reproduction differently, as described by the cost-of-reproduction hypothesis (Jasienska, 2009). Smaller lizards are younger, and although they are successfully defending a territory now, they may not be successful in the future. With potential breeding seasons in their future, they might invest less in each individual offspring now if they can spend those energetic resources on increasing their chances to survive to the next breeding season to reproduce again. Larger, older lizards are closer to the end of their lives, so investing

maximally in each offspring now represents a lesser cost to future reproductive opportunities than for younger lizards.

3.5.2 A. carolinensis

Wild-caught *A. carolinensis* females did not significantly differ in reproductive variables based on residency, nor was any measured reproductive variable related to maternal body size, although egg production was quite low (a total of 14 eggs), so a larger sample size might yield statistical differences based on residency. It is also possible that the stress of captivity masked any differences in reproduction and self-maintenance based on residency. The complex relationship between stress and reproduction involves hormones not measured in this study (e.g., elevated levels of epinephrine suppress oviposition in *A. carolinensis* (Jones et al., 1983)), so future work should take multiple hormones into account.

3.5.3 Limitations

It is difficult to draw conclusions about species differences because the *A. sagrei* in this study were lab-raised and the *A. carolinensis* were wild-caught. Attempts at studying lab-raised *A. carolinensis* yielded too little data to analyze. Future work should study lizards from similar sources to make direct comparison more robust, especially in long-term interactions and heterospecific interactions. Future work should also compare the effects of maternal status on offspring traits as juveniles and adults, as the associated stress hormones may influence traits such as behavior in offspring (Vercken et al., 2007).

Table 1. Relationship between maternal snout-vent length and offspring variables in lab-raised female *A. sagrei* with conspecific opponents (n = 20 resident lizards, 18 intruder lizards).

Variable	Residents			Intruders		
	R^2	Coefficient	P value	R^2	Coefficient	P value
Mean Number of Eggs Laid	**31.3%**	**0.87**	**0.016**	19.1%	0.47	0.054
Mean Egg Mass	**40.2%**	**26.44**	**0.006**	0.1%	1.59	0.889
Mean Days to Hatch	**32.4%**	**0.54**	**0.017**	**26.6%**	**-0.56**	**0.028**
Mean Hatchling Mass	**39.7%**	**24.69**	**0.007**	0.1%	1.58	0.882
Mean Percent Female Offspring	1.1%	0.02	0.683	3.1%	-0.03	0.501

CHAPTER IV

THE REPRODUCTIVE EFFECTS OF LONG-TERM SOCIAL EXPERIENCE

IN AN INVASIVE LIZARD

4.1 Abstract

The formation of dominance hierarchies reduces the costs of competition for resources. Different dominance rankings may lead to different physiological and behavioral traits. Previous work demonstrated that female lizards (*Anolis sagrei*) that achieved different rankings in brief (10 min) interactions invested differently in reproduction. This study investigates the effect of long-term interactions between female pairs which were either size-matched or size-mismatched in both conspecific and heterospecific pairs. I tested competing hypotheses: (1) Bully hypothesis: mismatched pairs will show greater disruption of reproductive output as the larger lizard harasses the smaller or restricts access to resources; (2) Rivals hypothesis: matched pairs will show greater disruption of reproductive output as lizards devote more energy to aggression in an uncertain dominance hierarchy. In conspecific pairs, mismatched pairs laid more eggs than matched pairs, supporting the rivals hypothesis. In heterospecific pairs, mismatched pairs produced offspring with greater hatchling mass, supporting the rivals hypothesis, but matched pairs had greater hatching success, supporting the bully hypothesis. Lizards may not alter all aspects of reproductive output together but rather emphasize different traits to maximize fitness in different social circumstances.

4.2 Introduction

Individuals in a population compete for finite resources such as food, space, or mates. Competition is costly as competitors risk injury and spend energy and time. Some species minimize the costs of competition by forming dominance hierarchies (Hsu et al. 2006).

Dominance hierarchies are formed as individuals compete. Some individuals win these competitions and gain greater access to resources; others lose and have reduced access. Differential access to resources results in a social structure with high-status and low-status individuals: a dominance hierarchy. Dominance hierarchies reduce the costs of competition for low-status individuals if they are aware of their low status as they may avoid competitions they are unlikely to win and they may prioritize other tasks (Hsu et al. 2006).

High-status individuals and low-status individuals may vary greatly in physiology and behavior (Sapolsky 2005). *Anolis* is a genus of lizard species commonly used to study dominance hierarchy formation as they establish and defend small territories when space is limited, as under laboratory conditions (Andrews and Summers 1996). Previous work in our lab demonstrated that female *Anolis sagrei* alter reproductive investment based on the outcome of brief interactions; after a 10 min interaction using residency to control outcome (the resident lizard would "win" and the intruder would "lose"), hatchlings of winners were significantly heavier than the hatchlings of losers, despite equal food resources for all lizards (Magaña, chapter 3 of this dissertation).

These results demonstrate differential reproductive investment based on the outcome of brief interactions, but the similarity of these experimental conditions to natural populations is unclear. If potential territories are limited, interactions in the field may be prolonged and residency may be uncertain. Tree lizards, *Urosaurus ornatus*, experience different stress hormone activity in short-term and long-term interactions (Knapp & Moore, 1995). If hormonal activity differs similarly in *Anolis*

species, the reproductive consequences of social interaction may also differ based on the duration of social interaction.

The species of an individual's opponent could also matter. Since *A. carolinensis* and *A. sagrei* occupy similar microhabitats that overlap at the trunk and ground (Edwards & Lailvaux, 2012) and eat similar arthropod prey (Campbell, 2000), perhaps the presence of the other species increases corticosterone in ways that affect reproduction. A greater effect on one species than in the other would be an advantage to the less-affected species.

Further, individuals may respond differently to long-term interaction with opponents of similar and dissimilar size. Large body size contributes to success in territory defense (e.g., male *A. sagrei*, Tokarz 1985), although body size is sometimes unrelated to contest outcome (e.g., male *Egerni whitii*, McEvoy et al. 2013). Like males, *Anolis* females defend territories (Nunez et al. 1997) and under laboratory conditions paired females assume dominant and submissive statuses characterized by differences in aggressive behaviors (Andrews and Summers 1996).

In short-term (10 min) interactions with lizards of the same species, both *A. carolinensis* and *A. sagrei* were more aggressive as residents than as intruders (Magaña, chapter 1 of this dissertation). However, in interactions with lizards of a different species, males did not show this residency effect (Magaña, chapter 1 of this dissertation). In this laboratory study I tested whether the impact of social interaction on reproductive output would be greater in female lizards of similar size (matched pairs) or dissimilar size (mismatched pairs). I tested competing hypotheses: (1) **Bully hypothesis:** mismatched female pairs will show greater disruption of reproductive output as the larger lizard harasses the smaller lizard or restricts access to resources; (2) **Rivals hypothesis:** matched female pairs will show greater disruption of reproductive output as the lizards devote greater energy to aggressive behaviors in an uncertain dominance hierarchy. Disruption is defined here as change relative to baseline in any reproductive trait that may reduce fitness, e.g., producing fewer eggs or smaller hatchlings. I conducted

two experiments, one with *A. sagrei* females only, and one with *A. sagrei* paired with *A. carolinensis*, to test the hypothesis that *A. carolinensis* would experience greater disruption of reproduction than *A. sagrei* in mixed-species pairs, contributing to the success of *A. sagrei* as an invader.

4.3 Methods

4.3.1 Animals

Experiments involved 16 lab-raised female *A. sagrei* in conspecific pairs (2015) and 16 wild-caught *A. sagrei* and 16 wild-caught *A. carolinensis* in heterospecific pairs (2016) purchased from Underground Reptiles (Deerfield, FL). Experiments took place during the breeding season (May through August) when *A. sagrei* and *A. carolinensis* defend territories (e.g., *A. carolinensis* Husak et al., 2007; *A. sagrei* Cox & Calsbeek, 2015). All procedures are approved by IACUC as protocol AS1312.

4.3.2 Animal Husbandry

Prior to conspecific experiences, laboratory-hatched and raised *A. sagrei* were housed in mixed-sex groups in 110 L glass terraria from hatching through their first winter prior to their first breeding season. Prior to heterospecific experiences, wild-caught *A. sagrei* and *A. carolinensis* were weighed and their snout-vent lengths were measured upon arrival at the lab. Lizards were housed in conspecific groups of 8 in 110 L glass terraria for 18 days prior to the experiment; one conspecific male was introduced to each terrarium for 2 days to allow mating opportunities.

My husbandry protocol was modified from Lovern et al., 2004. Prior to experiments, each 110 L terrarium was equipped with one 60-W heat lamp and pairs of terraria shared one Repti-Sun 5.0 UV lamp. For experiments, lizards were transferred to 38 L glass terraria in pairs according to their treatment

group. Female *A. sagrei* store sperm throughout the breeding season, so females could continue to produce eggs without access to males (Tokarz 1998). Rows of 38 L terraria shared one 60-W heat lamp per two terraria and one Repti-Sun 5.0 UV lamp per three terraria. Cardboard dividers between terraria prevented lizards from seeing their neighbors. Room lights were turned on 0800-2000 h, UV lights turned on 0900-1900 h, and heat lamps turned on 1000-1800 h. Room temperature ranged from 24-38 C and humidity was maintained between 50% and 75%. Each terrarium contained one dowel rod perch, a water dish, and a lidded plastic container (9 x 15 x 8 cm) half-filled with damp peat moss in which lizards laid their eggs. Drinking water was provided *ad libitum* via water dishes and daily misting of terraria. Lizards were offered mealworms two days per week and crickets twice per week, once per week enriched by coating with powdered Miner-all (Sticky Tongue Farms) and powdered Herpetivite (Rep-Cal) prior to offering to lizards.

Nest boxes were checked daily and eggs were removed, weighed, and incubated in vermiculite (10 ml water added to 10 g vermiculite) at 28 C. Eggs were checked daily for hatchlings; on the day of hatching I weighed and measured the snout-vent length of each hatchling.

4.3.3 Treatment Groups

For each experiment (conspecific experiences and heterospecific experiences), lizards were ranked by body mass measured to the nearest 0.01 g. Lizards in the greater half of each sample were categorized as "big" and lizards in the lesser half categorized as "little." T-tests were used to compare "big" and "little" lizards: in the conspecific experiment, "big" lizards and "little" lizards differed significantly in mean body mass (the "big" lizards' mean was 2.8g, the "little" lizards' mean was 2.2g, t = 5.75, p < 0.001) but not snout-vent length (the "big" lizards' mean was 42.9mm, the "little" lizards' mean was 41.3mm, t = 0.98, p = 0.344); in the heterospecific experiment, "big" lizards and "little" lizards differed significantly in mean body mass (the "big" lizards' mean was 2.1g, the "little" lizards'

mean was 1.6g, t = 7.04, p < 0.001) and snout-vent length (the "big" lizards' mean was 44.6mm, the "little" lizards' mean was 40.8mm, t = 4.19, p < 0.001). Matched pairs consisted of two "big" lizards or two "little" lizards whereas mismatched pairs consisted of one "big" and one "little" lizard. Experiment start date and duration were constant for all conspecific pairs and for all heterospecific pairs.

4.3.4 Variables

I measured variables estimating investment in reproduction: the number of eggs laid, the number of eggs hatched, mean egg mass, mean days to hatch, mean hatchling mass, mean hatchling snout-vent length, mean hatchling body condition (snout-vent length divided by body mass), and offspring sex ratio. Reproductive variables were measured for each conspecific pair (maternity could not be determined) and for each lizard in heterospecific pairs (maternity was determined by egg characteristics; *A. sagrei* lay smaller eggs than *A. carolinensis* (*A. carolinensis* eggs are approximately 340 mg, *A. sagrei* eggs approximately 165 mg, Magaña, unpublished data)). I also measured variables estimating investment in growth and self-maintenance: change in snout-vent length, change in body mass, and change in body condition (body mass divided by snout-vent length) during the experiment (7 weeks for the conspecific pairs, 4 weeks for the heterospecific pairs).

4.3.5 Data Analysis

To compare matched and mismatched pairs, I used t-tests to compare means for the reproductive variables of each pair and the self-maintenance variables of each lizard. To address the pseudoreplication of siblings, each pair's mean value for each reproductive variable was used in statistical analysis.

4.4 Results

4.4.1 Conspecific Opponents: Anolis sagrei

Mismatched pairs produced significantly more eggs than matched pairs (Figure 21), although it is unclear whether both lizards in a pair produced equally. Relatedly, mismatched pairs had a significantly smaller inter-egg interval than matched pairs (Figure 22). Mismatched and matched pairs did not differ significantly in any other reproductive variable (p > 0.05 for all variables). Neither "big" lizards nor "little" lizards differed significantly in self-maintenance variables based on whether they lived in matched or mismatched pairs (p > 0.05 for all variables).

4.4.2 Heterospecific Opponents: Anolis carolinensis

No *Anolis carolinensis* female laid eggs.

4.4.3 Heterospecific Opponents: Anolis sagrei

"Big" lizards in matched and mismatched pairs did not differ significantly in reproductive variables (p > 0.05 for all variables). Among "little" lizards, matched lizards had a significantly higher percentage of their eggs survive to hatching than mismatched lizards (Figure 23). Neither "big" lizards nor "little" lizards differed significantly in self-maintenance variables based on whether they lived in matched or mismatched pairs (p > 0.05 for all variables).

4.4.4 Heterospecific Opponents: Anolis sagrei *and* Anolis carolinensis

A. sagrei and *A. carolinensis* did not differ significantly in changes in snout-vent length or body mass over the course of the experiment; significant differences did not exist for "big" lizards in matched pairs or mismatched pairs, nor for "little" lizards in matched pairs or mismatched pairs (p > 0.05 for all variables).

Regarding general behavior, *A. carolinensis* lizards typically approached food more quickly than *A. sagrei*, although lizards seemed to eat an approximately equal amount of food. No fighting was observed, and lizards often shared the perch under the heat lamp.

4.5 Discussion

4.5.1 Conspecific Opponents: Anolis sagrei

Differences in reproductive output in mismatched and matched pairs offer more support for the rivals hypothesis than the bully hypothesis, although that support is limited to a single aspect of reproduction: the number of offspring produced. Mismatched pairs laid more eggs than matched pairs (Figure 21), which supports the rivals hypothesis. This result is expected if matched lizards devote more energetic resources to aggressive behavior in an uncertain dominance hierarchy. Although it is possible that the greater egg production in mismatched pairs is solely due to the larger lizard, which would offer more support for the bully hypothesis, the low mean inter-egg interval for both pairing types (3.2 d for mismatched pairs, 4.4 d for matched pairs) suggests both lizards in a pair laid eggs, as individuals of the closely-related *A. carolinensis* lizard lays eggs at intervals of approximately 7-10 d (Jenssen & Nunez, 1998). It is possible that a larger sample size would yield results that more strongly support one or both hypotheses.

4.5.2 Heterospecific Opponents: Anolis carolinensis

A. carolinensis's failure to reproduce is puzzling, as this protocol has been successful in the past. The lizards ate consistently, maintained healthy body mass, and had access to males prior to the experiment. Another study found that *A. carolinensis* females reduced the number of eggs produced at high population densities of *A. sagrei* (Vincent, 2002); however, in this study, the lack of reproduction in unpaired control lizards (not discussed in this dissertation) suggests that the lack of reproduction was not a response to the presence of *A. sagrei*.

4.5.3 Heterospecific Opponents: Anolis sagrei

Matched "little" lizards had greater hatchling success than mismatched "little" lizards (Figure 23), possibly supporting the bully hypothesis, if little lizards in mismatched pairs experienced greater harassment or resource restrictions than lizards in matched pairs. The lack of effect in "big" lizards is unexpected; perhaps a larger body size allows compensation for the effects of social experience in some way. A larger sample size would increase the power of the statistical tests and could reveal significant differences in reproductive variables based on pairing type.

4.5.4 Limitations

The classification of lizards as "big" or "little" is relevant only to this sample; it is not clear that this artificial classification scheme reflects real differences in wild populations. The different sources of lizards complicate direct comparison between types of social interaction, as lizards in conspecific experiences were lab-raised and lizards in heterospecific experiences were wild-caught. The inability to determine maternity in conspecific experiences also complicates comparison. It is not clear whether *A. sagrei* females would alter investment in reproduction if their heterospecific opponents also reproduced.

It is possible that neither the bully hypothesis nor the rivals hypothesis is strongly supported in this experiment because they both accurately describe social interactions, producing offsetting disadvantages. It could be that mismatched pairs are at a disadvantage as the bigger lizard harasses the smaller lizard and restricts her access to resources (bully hypothesis) whereas matched pairs are *also* at a disadvantage as the similarly-sized lizards spend energy in territorial conflict (rivals hypothesis). If both occur to similar extents, the resulting disadvantages could be approximately equivalent.

4.5.5 Future Research

Future work would benefit from a protocol that determines the maternity of offspring of conspecific pairs to detect differences in the responses of large and small lizards to conspecific social experience.

Increased range of lizards' ages and body sizes would make experimental social experiences more closely resemble natural social experiences. All lab-raised lizards in conspecific experiences hatched in the same season, so their age range was approximately three months. In a natural population, lizards that survive to their full life span may interact with lizards one or even two seasons younger and thus much smaller (e.g., Fitch, 1972). It is possible that for some reproductive traits in this study matched and mismatched pairs showed no significant differences because mismatched pairs were not mismatched enough. An increased size differential in mismatched pairs might trigger further changes in reproductive output. Sample sizes were also small, thus limiting the power of the tests.

Future work should also investigate stress hormones as a potential mechanism of differences in reproductive output between matched and mismatched pairs. Social status affects stress hormones (Sapolsky, 2005), and maternal stress hormones during fetal development affect juvenile traits (Vercken et al., 2007), so stress hormones may be the mechanism of the differences seen in this study. Sampling circulating corticosterone at multiple time points throughout the long-term interaction would allow

50

detection of the concentration and fluctuations of stress hormones in lizards in different social

circumstances.
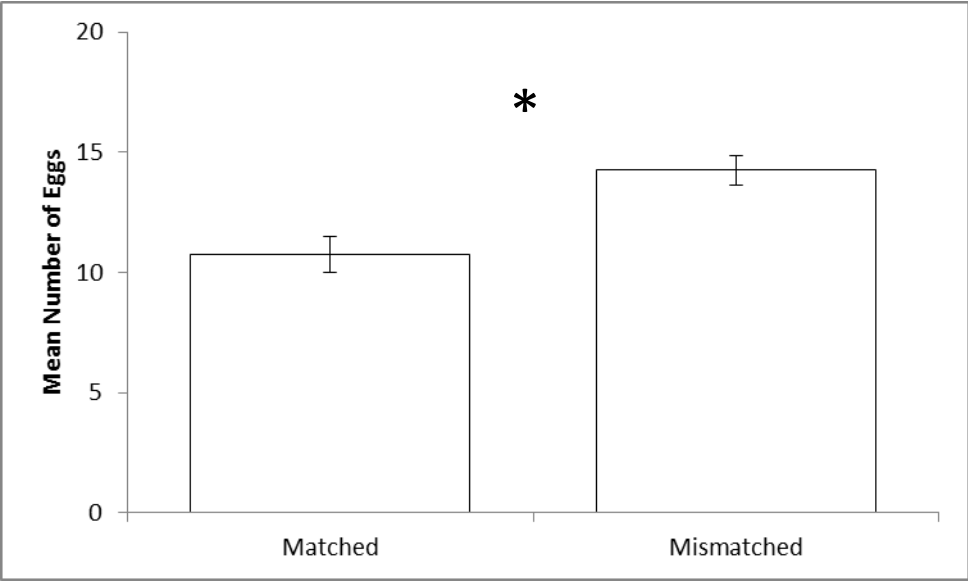
Figure 21. Mean number of eggs produced by pairing type in female *A. sagrei* in conspecific pairs (n = 4 matched pairs, 4 mismatched pairs; error bars represent one standard error). T = -3.58, p = 0.016. An asterisk indicates a significant difference.

Figure 22. Mean inter-egg interval by pairing type in female *A. sagrei* in conspecific pairs (n = 4 matched pairs, 4 mismatched pairs; error bars represent one standard error). T = -3.96, p = 0.007. An asterisk indicates a significant difference.
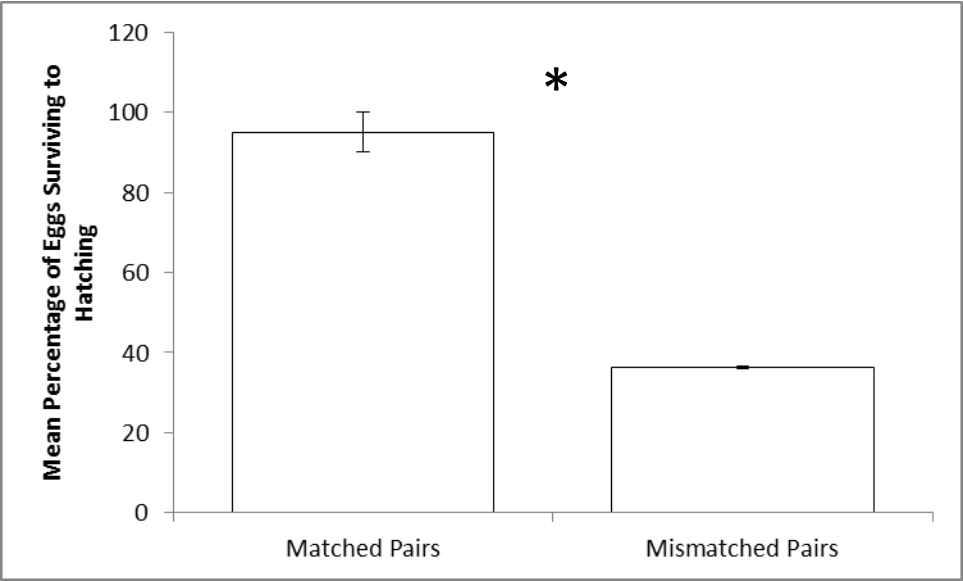
Figure 23. Mean percentage of eggs that survived to hatching in "little" female *A. sagrei* in heterospecific pairs (n = 4 matched lizards, 4 mismatched lizards; error bars represent one standard error). T = 3.6, p = 0.012. An asterisk indicates a significant difference.
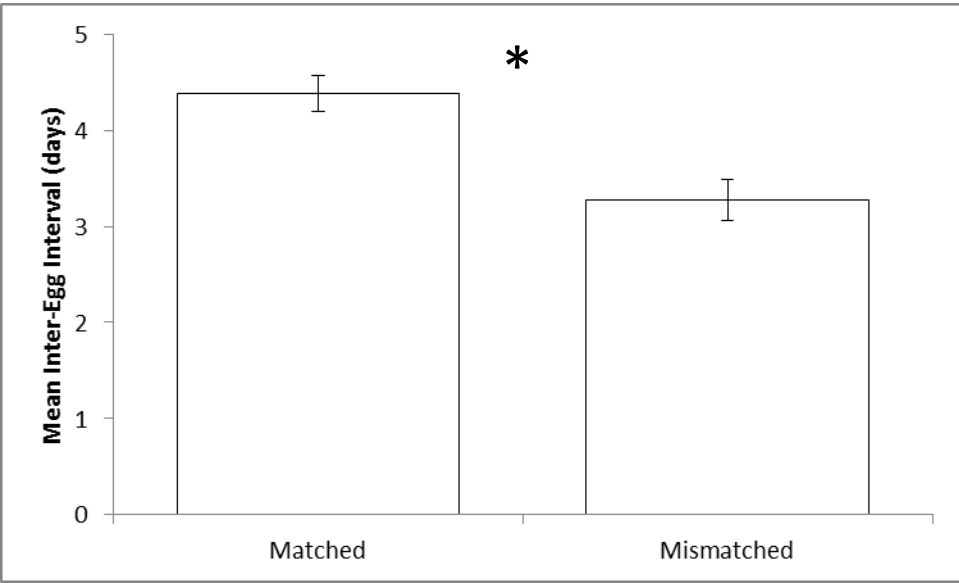
CHAPTER V

OVERALL CONCLUSIONS

Social experience influences reproductive output, possibly through stress hormone mechanisms, in a lizard. The interaction between social experience and reproduction likely contributes to the invasive species' success as a competitor.

5.1 Chapter 2: Behavior and Corticosterone and Short-term Social Experience

In conspecific experiences, residents behaved more aggressively than intruders, consistent with residents winning and intruders losing. Residency had little to no effect on self-maintenance at this scale. In heterospecific experiences, residency did not affect the aggressive behaviors of males. Lizards were cautious against heterospecifics, even though lizards of these species likely interact in the field as they compete for microhabitats (Edwards & Lailvaux, 2012) and prey (Campbell, 2000).

Plasma corticosterone concentration differed significantly based on residency in lab-raised female *A. sagrei* and in no other group, despite the effect of residency on aggressive behavior. It is possible that females' hormonal response to residency is greater due to more stable dominance hierarchies for females than for males or because average reproductive investment is greater for females than for males (Gerber, 2000). Because the source of study animals differed for the two species, it is not clear whether a species difference exists during conspecific experiences. Future work should investigate

corticosterone concentration differences during heterospecific experiences. Other hormones such as epinephrine should also be investigated, as epinephrine suppresses oviposition in *A. carolinensis* (Jones et al., 1983).

Heterospecific opponents seem to negate the effect of residency on plasma corticosterone as well as on aggressive behavior. Although these species share similar microhabitats, it is unclear whether lizards from single-species populations would recognize an opponent of the other species as a direct threat. Future work should focus on lizards from mixed-species populations and on females in both short-term and long-term interactions.

5.2 Chapter 3: Reproduction and Short-term Social Experience

This sample of lab-raised *A. sagrei* did not significantly vary reproductive investment based on residency, although the relationship between reproductive variables and maternal body size did differ significantly based on residency. Future work should focus on a broader range of body sizes (and ages) of lizards to more closely resemble field conditions.

This sample of wild-caught *A. carolinensis* females also did not significantly differ in reproductive variables based on residency. Future work should investigate the effects of long-term interactions. Future work should also incorporate *A. sagrei* and *A. carolinensis* from the same source to allow for direct comparison.

5.3 Chapter 4: Reproduction and Long-term Social Experience

In lab-raised *A. sagrei* in long-term experience against a conspecific opponent, mismatched pairs laid significantly more eggs than matched pairs, seemingly supporting the rivals hypothesis. Future work

should determine the maternity of each offspring, involve a greater range of lizard sizes and ages, and track stress hormone activity throughout the experience.

In wild-caught *A. sagrei* in long-term experience against a heterospecific opponent, matched pairs had significantly greater hatching success than mismatched lizards, supporting the bully hypothesis, among "little" lizards only. It is not clear whether conspecific and heterospecific social experience actually supports different hypotheses or if this result is the product of different sources of lizards and small sample size. It is also not clear why *A. carolinensis* failed to reproduce in heterospecific experiences. Future work should investigate reproductively active *A. carolinensis* and track stress hormone activity throughout the experience.

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