POSSIBLE SEXUAL SELECTION IN PRE-REPRODUCTIVE HATCHLING COLLARED LIZARDS (*CROTAPHYTUS COLLARIS)*

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POSSIBLE SEXUAL SELECTION IN PRE-REPRODUCTIVE HATCHLING COLLARED LIZARDS (*CROTAPHYTUS COLLARIS)*

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Abstract: We studied the possible adaptive significance of precocial sexual signaling in collared lizard hatchlings. Male hatchlings display intense dorsolateral orange bars at hatching or within the first weeks of life. Hatchling orange bars (HOB) increased in saturation with body size until reaching a threshold, and then faded as adult coloration began to show. In most organisms, sexual dichromatism (usually present only in adults) has been related to androgens and aggressive behavior. It is unknown how androgen levels are related to the expression of the orange bars in male hatchling collared lizards. We tested the hypothesis that male hatchlings with higher levels of androgens would show greater proportion of orange, higher orange saturation (color purity), and more aggressive behavior than those with lower levels of androgens. We first tested this hypothesis by manipulating androgen levels through implants of testosterone (T) and dihydrotestosterone (DHT). Both proportion of orange and orange saturation increased the most with DHT. When DHT was implanted, almost 40% of the variation in aggression was explained by DHT. We then examined relationships of unmanipulated levels of T and DHT with coloration and aggressive behavior in wild-ranging male hatchlings. Although not statistically significant, our results showed a positive trend between orange saturation and DHT. Hatchlings with higher saturated orange bars tended to behave more aggressively than those with less saturated bars. Finally, to determine if HOB are related to fitness in sexually mature males, we measured inferred fitness in yearlings and adults at the same population and related it to the proportion of orange and orange saturation that those yearlings and adults had when hatchlings. Our results suggested that hatchlings with more orange coloration had a higher probability of pair-bonding, displaced less from their natal locations, had larger home range areas (as adults, but not as yearlings), and had higher inferred fitness (as adults, but not as yearlings). Thus, it is suggested that precocial sexual signaling in hatchlings is positively related to estimated fitness in adults as estimated from spatial data, but not so in yearlings when yearling males, unlike adults, likely gain their fitness by sneaking copulations.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure ....Page

CHAPTER I

ONTOGENETIC CHANGES IN COLORATION OF MALE HATCHLING COLLARED LIZARDS (*CROTAPHYTUS COLLARIS*)

Abstract

Adult collared lizards (*Crotaphytus collaris*) are sexually dimorphic and dichromatic, with males having larger body size and displaying bright overall body coloration and intense yellow dewlaps, whereas females are smaller and have dull brown body coloration. They have a polygynous mating system where males are highly territorial and philopatric. Unusually, male hatchling collared lizards also exhibit sexual dichromatism in the form of conspicuous dorsolateral orange bars on the body, which is unexpected because young collared lizards do not reach sexual maturity until the following spring. We studied the ontogeny of the hatchling orange bars in a wild population of collared lizards in north-central Oklahoma. We repeatedly captured male lizards throughout the hatchling season (early August through mid-November). We measured brightness, saturation and hue of the orange bars using a reflectance spectrometer, and using subjective categories in the field. Orange bars were either present at hatching or developed within the first 2-3 weeks of life. Saturation within orange-red wavelengths

(605-700 nm) increased significantly as body size increased until reaching a threshold, and then the amount and intensity of orange began to fade (measured with subjective categories) as adult coloration started to appear.

Introduction

In the animal world it is very common to observe, mostly in males, conspicuous traits like elaborated songs and displays, bright coloration, horns, and other extravagant traits that would seem to reduce survival of their bearers. Darwin (1871) suggested that such traits could evolve through sexual selection, a type of natural selection that deals with characteristics that promote more matings. In most sexually reproducing animals, females tend to be a scarce resource, and they invest more than males in gamete production and offspring care. In contrast, males have the capacity to fertilize many more eggs than females and display greater variation in reproductive success than females (Andersson 1994). This asymmetry in sexual investment means that males are the ones usually competing for females, whereas females are encouraged to look for quality and to choose the best male possible (Krebs and Davies 1993). Thus, sexual selection can operate by favoring traits that offer an advantage when competing against a member of the same sex (usually males) for access to mates, for example fighting (intra-sexual selection), or by favoring traits that make one sex more attractive to the other sex (inter-sexual selection; Krebs and Davies 1993).

As traits are selected for their benefit in male fighting and/or female choice, sexual dimorphism (the morphological differences between males and females of the same species) can be striking. Many studies in different taxa have shown that large male size frequently gives an advantage in dominance contests and fights over females (Andersson 1994). In elephant seals (*Mirounga sp.*), only large males are able to achieve a dominant position and high mating success by winning agonistic encounters against smaller males and chasing them from the breeding group. Thus, small males obtain few or no copulations (Bartholomew 1970; Deutsch et al. 1990; McCann 1981). Large male body size seems then to be strongly favored by sexual contest competition (Bartholomew 1970). Additionally, female northern elephant seals prefer larger males over smaller ones. Females perform evasive movements of the hindquarters to reject being mounted by small adults and through loud calls alert nearby dominant males who chase away the subdominant males (Andersson 1994). Cox (1981) and Cox and Le Boeuf (1977) suggested that this rejection behavior increases the female's chance of mating with the large dominant males. There is also the possibility that females are getting direct benefits by seeking protection from the top-ranking males and mating with them, for example a lower chance of harassment and injuries by sneaker males (Le Boeuf and Mesnick 1991). In another example, the large body size of a male sperm whale is obviously beneficial in contests of strength and body blows during fighting between males (Mann 2000). Conspicuous dimorphism, driven by either male fighting or female choice of mates, is very common in fish as well. In Siamese fighting fish (*Betta splendens*), the sexually dimorphic trait, male standard length, was shown to significantly improve a male's fighting ability (Jaroensutasinee and Jaroensutasinee 2001). It is not just dimorphism in body size that gives males advantages in male-male competition. Males of the Asian stalk-eyed fly (*Cyrtodiopsis whitei*), which have longer stalks than females, display their eye stalks in contests with rivals over females at mating sites. Having long eye stalks appears to increase success in such contests, and female choice also favors long stalks (Burkhardt and de la Motte 1987; Burkhardt and de la Motte 1988).

In reptiles, dominance in contests over females when strength is decisive is also among the factors that can influence the degree of sexual size dimorphism (Andersson 1994). In sand lizards (*Lacerta agilis*), males win contests over smaller males. As size difference increases between males, fight frequency and contest duration decreases (Olsson 1992). In six polygynous species of herbivorous Iguanine lizards, the relative size of the head increased with body size in males but decreased in females (Carothers 1984). As males and females have a similar diet, head size could not be explained by ecological resource partitioning. However, since the jaws are used in male combat, male contest competition was suggested to be the reason why males have larger body and relative head size: Hews (1990) showed that head depth of *Uta palmeri* was favored by sexual selection via male contest competition over territories, which are crucial for attracting females. Hews (1990) also suggested that greater head depth may have been favored because it seemed to help the male maintain its hold on the female during copulation. In a study comparing three populations of collared lizards, large size was found to be under intrasexual selection as larger males in laboratory dyadic encounters had higher agonistic scores in all three populations than smaller males (Baird et al. 1997). Two studies that showed a positive correlation between head size and dominance in lizards suggested that this relation might be due to males with larger heads being able to bite harder (Hews 1990; Perry et al. 2004). Lappin and Husak (2005) tested this hypothesis in male collared lizards and found that bite force, a measure of weapon performance, was a better predictor of fitness than head or body size.

There are numerous examples in the literature regarding intrasexual selection. But the fact that there may be a strong system of male-male competition for females does not imply that females are mating only with males that are good at winning agonistic encounters (Trivers 1976). Instead, systems of male-male competition are expected to evolve also under the influence of female choice, so that males compete among themselves with the purpose of displaying to females that their genes are superior; for example by showing the ability to optimize the trade-off between survival and resource accrual (Trivers 1976). Among the studies that have looked at traits in lizards that help males win mates is a behavioral study on *Anolis garmani*. In this study, Trivers (1976) suggested that female choice for male territory size was a determining factor in shaping male sexual dimorphism. Females seemed to prefer to mate with males whose daughters will have the best chance of both surviving and obtaining resources. Hews (1990), in her study with *Uta palmeri*, also analyzed the importance of female choice on male territory quality. She identified male territory quality to be a direct target of female choice.

Sexual dimorphism in coloration, or sexual dichromatism, is also much in evidence among animals. The extravagant colorful tail of the peacock is one of the most well-known examples. Courting males of the three-spine stickleback (*Gasterosteus aculeatus*) develop an intense blue eye coloration, which has been shown to be important in intersexual selection since males with this secondary sexual characteristic are the most attractive to females (McLennan and McPhail 1989). In general, females typically prefer conspicuously colored males as mates (Hill 1990) and such males often achieve greater social dominance or greater access to resources (e.g., females) or both (Zamudio and Sinervo 2000). This differential mating advantage of males with conspicuous coloration results in sexual dichromatism via sexual selection. For example, intrasexual selection has been suggested in the evolution of the red head coloration in male skinks of *Plestiodon laticeps* (Vitt and Cooper 1985). During the breeding season, males develop a bright red head coloration that is absent in females. The dramatic coloration of their heads likely helps males win agonistic contests with other males. In a study comparing three populations of collared lizards, results both between and within populations showed supporting evidence for female preference of males with brighter coloration via intrasexual selection in at least one population (Baird et al. 1997).

In all animals, the reproductive benefits that conspicuous coloration yields have been studied only in adults (sexually mature individuals), and because secondary sexual characteristics are thought to be expressed only when animals are able to reproduce, there is no apparent reason for the presence of these sexual traits in sexually immature organisms. Nevertheless, sexual dichromatism has been observed in a few cases. In juveniles of Bearded Tits, the plumage remains the same between the sexes but males develop a bright orange beak about a week after fledging (Marin et al. 1994). In European Bee-eaters, their juvenile plumage seems to be a significant indicator of sex (Kapun et al. 2011), males having a yellowish-brown back and females green back feathers.

The eastern collared lizard (*Crotaphytus collaris*) is found from the Ozarks of southern Missouri and Arkansas, westward through Kansas, Oklahoma, Texas, New Mexico, Arizona, and south into northern Mexico (Stebbins 2003). This diurnal species prefers rocky habitats with sparse vegetation. It feeds on a variety of prey including spiders, beetles, grasshoppers and other insects (Ivanyi 2009). Individuals are highly territorial and adults exhibit strong sexual dichromatism, with males being bright bluish-green dorsally with a pale to near-fluorescent yellow throat. Adult females are mostly brown to dull olive (McCoy et al. 1997). Adults can reach a maximum SVL of 110 mm, and males are larger than females. Every season, yearling and adult collared lizards emerge from hibernation in late March to mid-April. Mating behavior reaches its peak in early summer and females lay one or two clutches every season with 8-14 eggs per clutch (Trauth et al. 2004); some females will lay up to three clutches in some years (Baird 2004). Incubation lasts about 60 days; the first hatchlings of the season begin to appear in late July and continue to hatch until early October (Trauth et al. 2004). Adults begin to retreat underground in late summer and are rarely observed after late August. Interestingly, during the reproductive cycle, adult females develop orange dorsolateral bars that presumably cycle in intensity with ovarian steroid hormones (Cooper and Ferguson 1972). Many authors have suggested that the orange bars of female *C. collaris* inhibit male aggression (e.g., Carpenter 1967; Cooper Jr. and Greenberg 1992; Cooper and Ferguson 1972). Baird (2004) directly tested this courtship rejection hypothesis by painting orange bars on some females and obscuring the natural orange bars of other females, expecting that females lacking the orange bars would be courted more frequently and more intensely by males than females with orange bars. However, he found that males, instead of being repelled, were instead attracted to females with orange bars. Females with orange bars painted on were courted longer and received more courtship displays and contacts by males than brown females.

Something more unusual is that sexual dichromatism is also seen in juvenile *C. collaris*. Males either hatch with, or develop within two weeks, conspicuous dorsolateral orange bars (Fig 1.1). These orange bars fade after a couple of months as the maturing males begin to attain their typical adult green and yellow coloration, although they are still seen in some small yearlings at the beginning of the next year (Husak et al. 2004). Husak et al. (2004) tested the widespread hypothesis that orange bars in small male yearlings served as a form of female mimicry, reducing aggression from adult males, but they found no support for this function of the bars. Their result is not surprising since we now know that the orange bars in adult females do not serve the purpose of dissuading adult males (Baird 2004). Also, at the time of the year when juveniles develop the most intense orange bars (end of August and into October), most adults (both sexes) have already retreated underground for the winter (Trauth et al. 2004; Crosby and Fox, pers. comm.). Thus, the orange bars in hatchlings appear to be a visual signal directed toward other hatchlings, not adults (Fox et al., in prep). In fact, Fox et al. propose that the hatchling orange bars are used as a sexual signal to distinguish males from females and then the sexes are treated differently. Hatchling males are more aggressive to hatchlings with orange bars than without them, driving rival males away from their developing territories, while they accept the close presence of hatchling females without orange bars, perhaps to begin to form pair bonds with them, a possible expression of precocial sexual selection.

Ontogenetic color changes among lizards have received little attention (Cooper Jr. and Greenberg 1992), and few studies exist that detail the adaptive significance of juvenile coloration (e.g., Clark and Hall 1970; Cooper and Vitt 1985; Huey and Pianka 1977). Eastern Collared Lizards represent a good model to examine ontogenetic changes in coloration, especially those related to sexual selection. The first objective of this study was to determine the ontogenetic pattern of development of the orange bars in male hatchlings of collared lizards. We hypothesize that orange bars increase in intensity as lizards grow, and once hatchlings reach a certain size, the orange bars will start to fade while the adult coloration begins to appear.

Methods

This study was conducted using a population of collared lizards from Sooner Lake dam in Pawnee County, Oklahoma. We followed the first male hatchlings appearing in the season of 2013 by catching them as soon as possible after hatching (early-August) and re-captured them repeatedly throughout the season until they went underground for the winter (late-October). Every time a hatchling was caught, we recorded date and location and measured its snout-vent length (SVL) with a ruler and mass with a Pesola spring scale. To identify each individual lizard permanently, we marked animals with a unique combination of toe clips. We removed only the very distal end of three toe tips per lizard, and no more than one toe tip per foot. To be able to identify animals from a distance without being disturbed, we painted each animal with a unique color code combination (Fox 1978). Each lizard received 4 small dots of non-toxic acrylic paint on its back. As with any marks placed on the outside of the lizard, these paint dots were lost when the animal molted its skin; thus, paint dots were reapplied when necessary.

To measure the subjective intensity of the orange bars in the field, we assigned four categories: zero being complete absence of the orange bars and three being the most intense orange. Subsequently, hatchlings were brought to the lab and spectral reflectance of orange bars was quantified using an Ocean Optics USB4000 spectrometer and deuterium tungsten halogen DH-2000-BAL light source connected to a fiber optic probe. The probe was mounted within a probe holder that ensured readings were taken from areas 3 mm in diameter at a constant 7-mm distance from the surface with both illumination and reflectance measurement at a 90° angle to the surface. Spectral reflectance was measured at 320–700 nm as this represents the broadest range of wavelengths known to be visible to lizards (Loew et al. 2002). Measurements were taken when lizards reached an optimal body temperature of 35–40 ºC after holding them in individual glass terraria with an overhead 60-w incandescent light for an hour. We measured reflectance of three separate orange bars at three places per bar. These nine measurements were averaged to produce one orange bar reflectance value for each lizard on that date. Reflectance measurements were then converted into the three most frequently reported color variables: hue (H), saturation (S) and brightness (B) using CLR: COLOUR ANALYSIS PROGRAMS v1.05 (Montgomerie et al. 2008). This software package was downloaded from http://post.queensu.ca/~mont/color/.

For each of the chosen color variables (H, S, and B), one can calculate a large number of indices (Montgomerie et al. 2008). We selected H_3 for hue, S_1R for saturation and B_2 for brightness. H_3 (wavelength at the reflectance midpoint between minimal and maximal reflectance, or λ_{Rmid}) is considered the most useful index for hue because it depends on more than one reflectance estimate. Reflectance at each wavelength can have random fluctuations, which can unduly bias the single value of λ_{Rmax} or λ_{Rmin}, making those indices less reliable indicators of hue (Hill and McGraw 2006). Furthermore, λ_{Rmid} explained 78% of the variation perceived in hue by the human eye as measured in the CIELAB color space (Pryke et al. 2001). S_1, the most utilized index for saturation

(Andersson et al. 1998; Ornborg et al. 2002; Siefferman and Hill 2005; Smiseth et al. 2001), is calculated by dividing the total reflectance in the wavelength region of interest (i.e., color) by the total reflectance across the visible spectrum. The region of interest in this case corresponded to the orange-red coloration of the orange bars, or 605-700 nm. S_1R was the index used for this region of the light spectrum (λ 605-λ 700). B_2 refers to the mean brightness, an index of total reflectance across all wavelengths that can be compared among individuals and species (Hill and McGraw 2006).

To see how closely subjective orange categories correlated with individual color variables, we tested for significant correlations using linear regression and assessed normality of the residuals by visual inspection of a normal Q-Q plot. Violation of the normality assumption is important only for statistical significance testing, and does not affect parameter estimates (Berry and Feldman 1985). We tested for normality of color variables (H, S and B) using the Shapiro–Wilkes normality test. Based on results of the test, we log transformed B prior to regression analysis. To examine the relationship between coloration and growth, we applied linear mixed-effects models using SVL as the fixed effect and H, S, and ln(B) as the dependent variables. We specified a random effect of each individual lizard so the models account for the non-independence of repeated measurements on the same lizard.

Since one of the goals of this chapter was to correlate the presence of the orange bars with the growth of the hatchlings, we stopped taking spectral measurements in larger juveniles when the orange bars started to fade and the yearling coloration (green limbs/dorsum and yellow throat) began to appear. Therefore, we used the subjective orange categories, which we continued to record, to show the relationship between orange coloration and body size from the time of appearance of the hatchlings to when they went underground for the winter. To test whether colors increased and then faded as the lizards grew, we used a linear mixed-effects quadratic model with a fixed effect of SVL (and a random effect of each individual lizard to account for the repeated measurements on the same lizard) with the equation $y = a + \beta_1 * SVL + \beta_2 * SVL^2$. Degrees of freedom and p-values for regression coefficients were calculated based on Satterthwaite's approximations. Mixed-effects modeling was performed using package lmerTest in program R version 3.1.2.

Results

Beginning on August 6, 2013, we followed a total of 15 male hatchling collared lizards through their first season. Each lizard was caught and measured 2 to 4 times during the 2013 season, collecting 41 measurements in total. Males grew at a mean rate of 0.80 mm/day (SD = 0.14). Orange coloration was observed in all 15 males from the first capture on. From data collected on other juveniles raised from hatching in captivity the previous year (Andrea Crosby, unpublished data) and from Trauth et al. (2004), juvenile size at hatching is 32-36 mm SVL. Thus, the three smallest male hatchlings that were caught for the first time at 42, 44 and 45 mm SVL had an estimated age of 10, 12 and 14 days, respectively. Subjective orange categories assigned in the field were not significantly correlated with color variables measured using the spectrophotometer. Subjective orange categories did not show a significant regression with brightness ($r^2 = 0.001$ $p = 0.854$, $n = 41$), saturation ($r^2 = 0.036$, $p = 0.234$, $n = 41$), or hue ($r^2 = 0.037$, $p = 0.231$, $n = 41$) (Figure 1.2). Visual inspection of normal Q-Q plots indicated that residuals were not normally distributed for any of the regressions.

We found a statistically significant relationship between the fixed effects of SVL on S_1R ($p < 0.001$, $df = 3$) but not between SVL and B_2 ($p = 0.231$, $df = 15.5$) or H_3 ($p = 0.857$, $df = 23.9$) (Figure 1.3 and Table 1.1). The quadratic regression suggested that orange coloration was low at smaller body sizes (40-55 mm SVL), increased to a peak at intermediate sizes (55-65 mm SVL), then fell at larger sizes (65-84 mm SVL). When male hatchlings were first caught, we observed all 4 subjective orange categories in different individuals (Figure 1.4). The fixed effect of SVL was marginally significant at $\alpha = 0.1$ ($p = 0.103$, $df = 9$) and was significant for SVL^2 ($p = 0.085$, $df = 8.8$; Table 1.2). Examination of the Q-Q plot showed the residuals were not normally distributed. Although this should not affect parameter estimates, p-values should be interpreted with caution.

Discussion

We found that hatchlings' orange bars follows a general pattern where saturation (measured spectrally) increases with body size until reaching a threshold, and then decreases in intensity (as measured by subjective categories) as hatchlings continue to grow in body size. A few studies examining the role of juvenile coloration in lizards have been documented in the literature. Adults of *Eremias lugubris* have a cryptic coloration that blends with their semi-desert habitat. However, the juveniles have a very conspicuous jet-black body and walk with a distinctive, even, stiff-arched gait. When juveniles reach the adult stage both coloration and arch-walking behavior disappears (Huey and Pianka 1977). This appears to be a case of Batesian mimicry, where juvenile lizards are mimicking the abundant, sympatric and noxious "oogpister" beetle (*Anthia* spp). Cases of conspicuous coloration of tails in juvenile lizards that fade when reaching adulthood are fairly common (Hawlena et al. 2006). The bright blue tail of the five-lined skink (*Plestiodon fasciatus*), which fades away when juveniles reach their second year, has been suggested to have an intraspecific function by inhibiting attacks from aggressive adult males (Clark and Hall 1970). The red coloration in juvenile spiny-footed lizards (*Acanthodactylus erythrurus*), is another example of juvenile coloration serving to reduce adult aggression (Fresnillo et al. 2015). All of these cases relate the ontogenetic coloration with escape behavior, foraging mode, or anti-predator defense mechanisms (Hawlena et al. 2006) that diminish with age and/or size. Thus, once juvenile lizards get too big to be effective mimics or to assuage aggression of conspecific adult males, the juvenile coloration is lost. The hatchlings' orange bars observed in male collared lizards do not seem, however, to be an example of anti-predator coloration. Instead, it is more likely that this coloration actually makes the hatchlings more conspicuous to potential predators, as is the case with brighter coloration in adults (Husak et al. 2006).

We did not find any significant relationship between the spectrometer variables and our subjective orange categories. With the subjective categories, we were attempting to measure the degree of conspicuousness of the orange coloration, and the subjective categories encompassed the amount of orange as well as its hue and intensity (saturation), so the two measures are really not the same. Additionally, since more than one observer assigned orange categories, it was hard to have a standardized subjective measurement, even though every observer was trained in the scale and his/her categories were cross-checked for consistency with other observers at the start of the field season. We expected saturation to be positively correlated with the orange categories because subjective categories were expected to increase as the orange turns from less intense pigmentation (lower subjective category) to more pure or saturated orange pigmentation (higher subjective category) through ontogeny. Saturation, also known as “spectral purity,” is a measure of the degree to which a color appears to be pure (Hill and McGraw 2006), and thus can be thought of as a measure of “orangeness.” Brightness was not expected to correlate with the subjective categories since the latter addresses “orangeness,” not brightness. Brightness describes the amount of black in a color, making the color in question appear darker or lighter (Hill 2010). Therefore, among the three color variables, H, S, and B, we considered saturation (S_1R) to be the most biologically relevant for describing the development of the orange bars. Our subjective categories could have actually been measuring a combination of all three of these color variables, and therefore no one color variable by itself related strongly to them.

Male hatchling collared lizards showed sexual dichromatism (orange bars) as soon as 10 days after hatching, this being the earliest age at which we were able to catch them in the wild. However, the orange coloration can be present from the moment the males hatch as it has been observed in previous studies in the laboratory (Crosby and Fox, pers. observ.). In the blunt-nosed leopard lizard (*Gambelia sila*), the juveniles of both sexes develop different amounts of yellow coloration under the tail and under their hind limbs (Montanucci 1965).

As we hypothesized, saturation of the orange bars increased significantly as the lizards grew. The maximum size reached by a hatchling before saturation started to decrease was 84 mm SVL. This result is consistent with Trauth et al. (2004), who report that the orange bars are lost at around 82 mm SVL. In a previous year, a yearling collected at the same study site was mistaken for a hatchling due to the presence of orange bars. However, the time of capture (early May), did not match the usual hatching time that begins in early August, i.e., this was a small yearling not a hatchling. The body size of this particular yearling, 86 mm SVL, is relatively close to the maximum body size (84 mm SVL) found in hatchlings still showing the orange coloration. Taken together, these data suggest that the expression of the orange coloration is related to the lizard's growth and appears to make the hatchlings more conspicuous as they grow in body size. This increased conspicuousness could actually be beneficial if the hatchling's orange bars are used in hatchling-hatchling communication, repelling rival mates and attracting potential future mates (i.e., precocial sexual selection).

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Table 1.1. Fixed effects of body size (Slope Estimate), measured as snout-vent length (mm), on color variables (Dependent Variable), estimated with linear mixed-effects models to account for repeated measurements on individual lizards. Degrees of freedom and P-values were estimated using Satterthwaite's approximation.

Dependent Variable	**Slope estimate**	**Std. Error**	**df**	**P-value**
Log(Brightness)	0.0036	0.0026	15	0.189
Saturation	0.0017	0.00036	3	<0.001
Hue	-0.0003	0.00016	24	0.857

Table 1.2. Quadratic model of fixed effects of size, measured as snout-vent length (SVL), on subjective orange coloration categories assigned to individual male hatchling collared lizards, using a linear mixed-effects model to account for repeated measurements on individual lizards. Degrees of freedom and P-values were estimated using Satterthwaite's approximation.

Parameters	**Estimate**	**Std. Error**	**df**	**t value**	**P-value**
(Intercept)	-3.902	3.662	8.518	-1.066	0.3159
SVL*	21.838	12.058	9.044	1.811	0.1034
SVL^2	-18.569	9.577	8.808	-1.939	0.0852

* SVL was divided by 100, because at the original values the model would not converge due to the fact that the scales of SVL and SVL^2 were very different. This has no impact on parameter estimates, and dividing the estimates by 100 makes them applicable to the original SVL values.

Figure 1.1. Male hatchling *Crotaphytus collaris* showing orange bars.

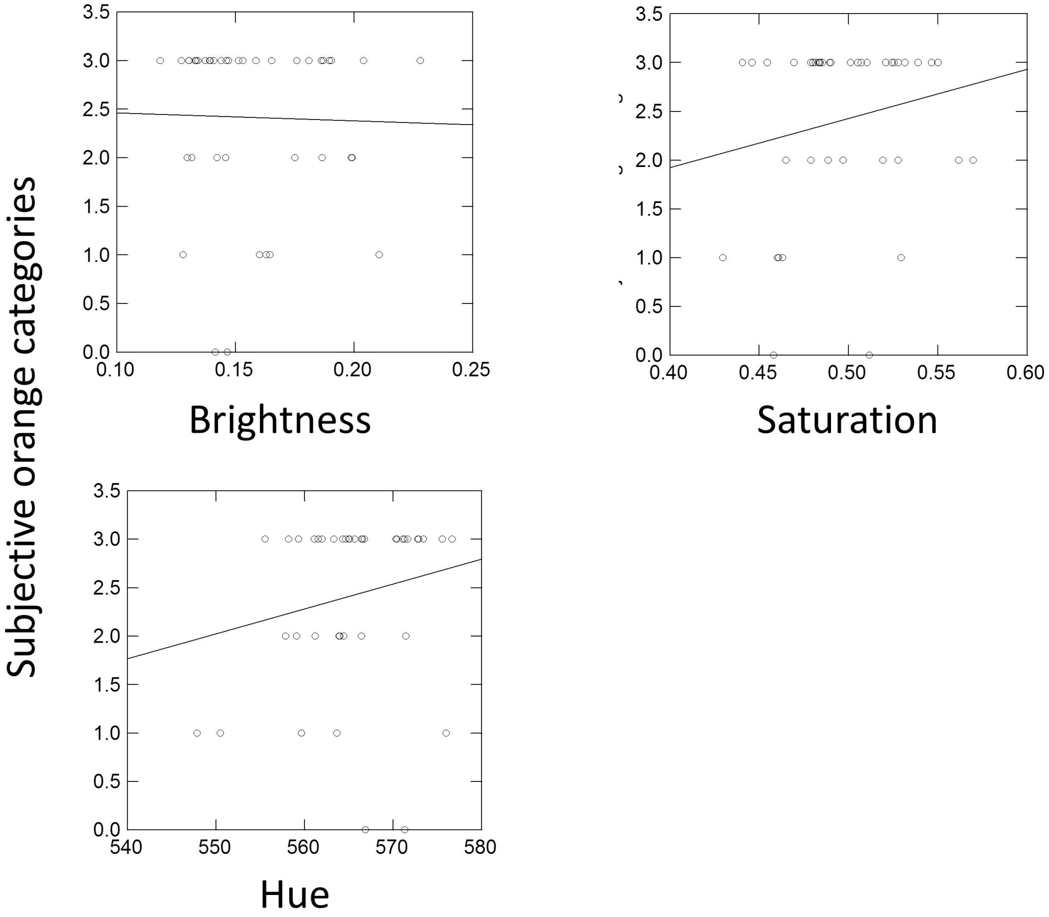

Figure 1.2. Correlation between subjective orange categories and spectral data for a sample of hatchling collared lizards re-measured multiple times over their life in 2013.

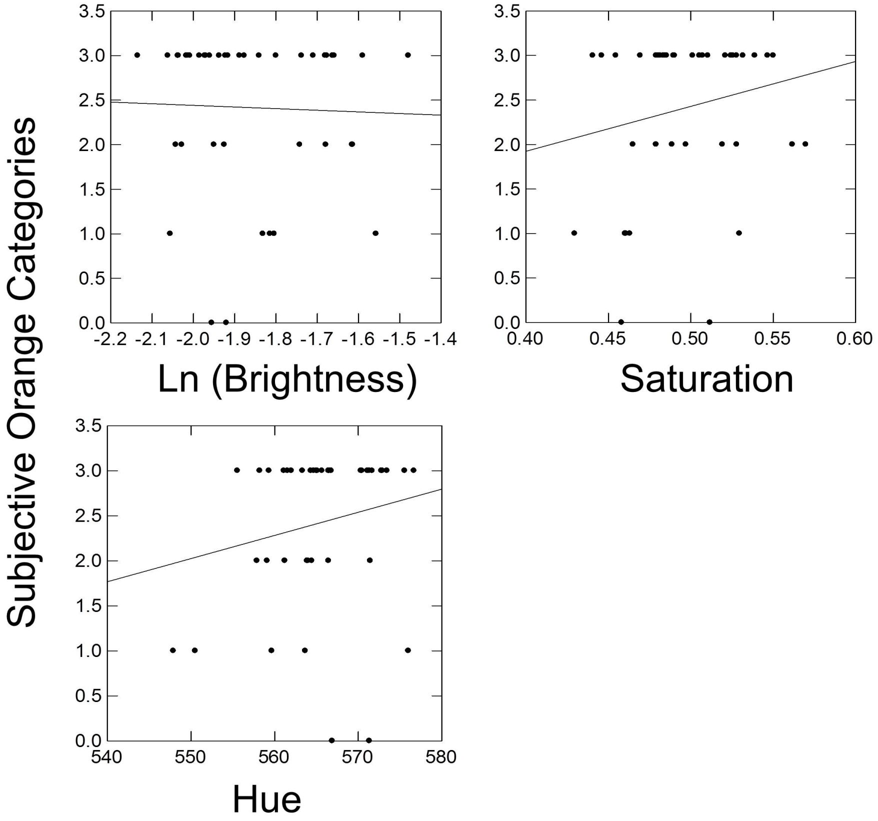

Figure 1.3. Regression of orange brightness, saturation and hue against body size (snout-vent length) for male hatchling collared lizards re-measured multiple times over their life in 2013.

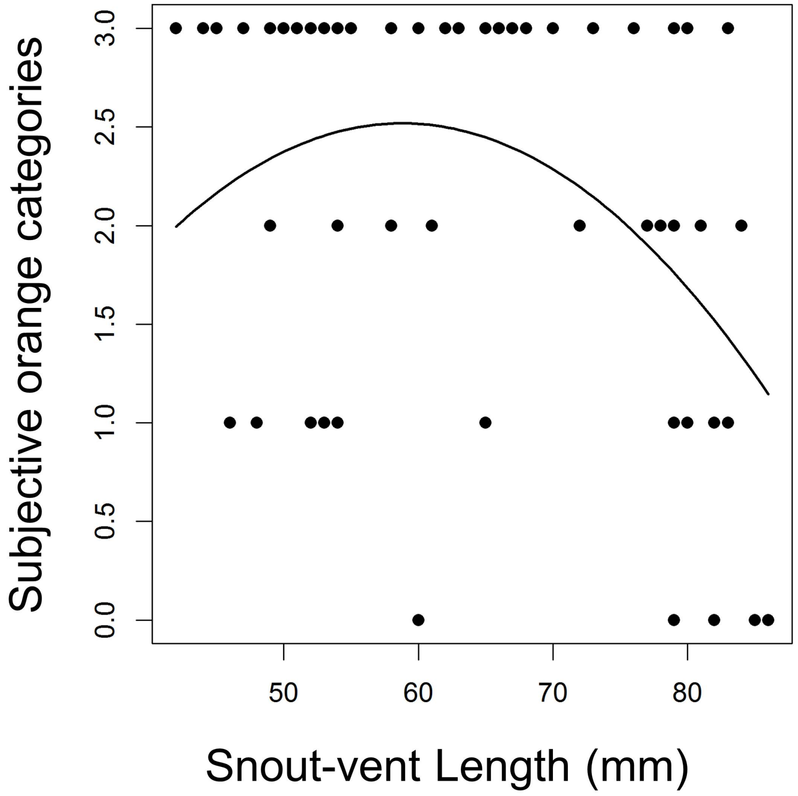

Figure 1.4. Change in subjective orange coloration over hatchling growth. The curve is a quadratic regression (see Table 1.2 for statistics).

CHAPTER II

EFFECTS OF EXOGENOUS ANDROGENS ON DIMORPHIC ORANGE COLORATION AND AGGRESSIVE BEHAVIOR IN HATCHLING COLLARED LIZARDS (*CROTAPHYTUS COLLARIS*)

Abstract

Collared lizards (*Crotaphytus collaris*) are unique in the sense that not only adults but also hatchlings present sexual dichromatism, despite hatchlings being sexually immature. Male hatchlings develop conspicuous dorsolateral orange bars that increase in intensity and later fade as adult coloration begins to develop. In many organisms, bright, sexually dichromatic coloration (usually present in adult males) has been positively related to androgen levels and aggressive behavior. It is unknown how androgen levels are related to the expression of the orange bars in male hatchling collared lizards. We hypothesized that male hatchlings with higher levels of androgens (i.e., testosterone and/or dihydrotestosterone) would show more orange coverage (measured as proportion of orange), higher orange saturation (color purity), and more aggressive behavior than male hatchlings with lower levels of these androgens. We collected 40 male hatchlings, size-matched them and arranged them in pairs. Each member of 9 pairs was implanted with DHT or control pellets, and each member of 11 pairs with T or control pellets. Thirty days after receiving the implants, for each lizard we measured snout-vent length, aggressive behavior, T and DHT levels, amount of orange, and the color variables brightness, saturation and hue. We found statistically significant relationships among androgens, orange coloration, and aggression. Collectively, our results suggest that orange coloration and aggression in hatchling males depends more on DHT than T. Orange saturation significantly increased with DHT levels (in lizards with DHT implants), proportion of orange significantly increased with DHT levels (in lizards with T implants), and aggression increased significantly with DHT (in lizards with DHT implants). Nearly 40% of the variation in aggression was explained by DHT levels, and less than 21% was explained by T. We propose that the orange coloration in male collared lizard hatchlings appears to be an activating effect since DHT, not T, had the stronger effect.

Introduction

In male vertebrates, high levels of circulating androgens can correlate with dominant, territorial behavior and enhances the expression of secondary sexual traits (Hau 2007). The effect of androgens such as testosterone on sexual behavior and coloration has been studied mainly in fish (Fernald 1976) and birds (Madsen et al. 2007). In lizards, hormones are related to physiological performance and immunity (Belliure et al. 2004; Gowan et al. 2010; Hau 2007; Huyghe et al. 2009), sexual dichromatism (Cooper Jr. and Greenberg 1992; Cox et al. 2005; Hews and Moore 1995), sexual dimorphism (Hau 2007; Husak et al. 2007), and aggression (Greenberg and Crews 1990; Sinervo et al. 2000; Woodley and Moore 1999). Male secondary sexual characteristics are often tightly associated with androgens such as testosterone (Hau 2007; Wingfield et al. 2001) and dihydrotestosterone (Hews and Moore 1995). In male *Sceloporus* lizards, seasonal color development is activated by testosterone (Cox et al. 2008; Rand 1992). Similarly, in *Uta stansburiana,* males with orange dewlaps are ultra- territorial and have a higher testosterone level than the other two color morphs, blue and yellow (Sinervo et al. 2000). The relation between androgens and aggression has been studied in adult collared lizards (Baird and Hews 2007) and it has been suggested that more than one androgen is involved in the sexual maturation of the species (Rand 1986). Dihydrotestosterone (DHT) functions in a capacity similar to testosterone (T). Both T and DHT bind to the same receptors but DHT has a potency five times that of testosterone, having stronger binding properties (Ahmad et al. 1973; Wright et al. 1996). DHT is synthesized from T in the prostate and adrenal glands by 5-alpha reductase (Nelson 2005). It has been shown in one case that T appears to be more important in organizing color differences but DHT appears to be more important in activating expression of underlying color differences (Hews and Moore 1995).

Despite the fact that ontogenetic changes have been commonly observed in reptiles, only a handful of studies have been done to address such occurrence in detail (Galan 2008; Germano and Williams 2007; Hawlena et al. 2006). In most crotaphytids, sexual dichromatism is present in adults, particularly those with polygynous mating systems. However, sexual dichromatism in sexually immature organisms is not expected. Male hatchling collared lizards develop a conspicuous orange coloration (orange dorsolateral bars) anywhere from 1 day to 3 weeks after hatching, while females maintain a dull brown coloration. The function of these hatchling orange bars is unknown, but it appears they relate to hatchling-hatchling communication and hatchlings use them to distinguish males from females (Fox et al., in prep.). From the results of experimental manipulation of the hatchling male bars in free-living hatchlings, it is known that the males treat other males more aggressively and repel them from their developing territories, yet allow the close presence of hatchling females. Fox et al. (in prep.) propose that the hatchling orange bars may be a sexually dimorphic trait advanced by precocial sexual selection, where the benefits are reaped once the lizards gain sexual maturity. They propose that males with more developed orange bars may have better success at repelling rival males (future competitors for mates) and better success at forming early pair bonds with females, which extend to the next spring when these yearlings can mate. It is unknown how androgen levels are related to the expression of the orange bars in male hatchling collared lizards. We hypothesized that young male hatchlings with higher levels of T or its metabolite, DHT, or both, would show more orange coverage, higher orange saturation, and more aggressive behavior than male hatchlings with lower levels of these androgens.

Methods

This project was conducted in the fall months of 2012 and 2013. In the first year, we collected 22 male hatchlings during August and September from Sooner Lake dam in north central Oklahoma (Pawnee Co.). Lizards were brought to the laboratory, measured and size matched within 1 mm SVL, and placed individually in glass terraria. A total of 11 pairs were set up. The next day, one member of each pair received a Testosterone (T) implant (treatment), and the other member an empty pellet (control). The minimum lizard size for implants was 39 mm SVL (or 2.2 g). Implants were inserted subcutaneously posterior to the left shoulder through a 5-mm incision. The incision was then sealed with

Vetbond^{TM} tissue adhesive. T pellets were made by mixing 676 mg of silicone sealant (Dow Corning; Midland, MI) with 155 mg of powdered T (Sigma-Aldrich). This mixture was expelled through a 1-cc syringe in a straight line onto wax paper, and cured overnight at room temperature. The next day, the total length of the mixture was measured (167 mm), producing a concentration of 0.928 mg T per mm. The cured extrusion was then cut into 3-mm pellets with a final concentration of 2.78 mg T per implant. Control implants consisted of only silicone also cut into 3-mm lengths. All implants were sterilized with 70% ethanol before being implanted.

After receiving the implant, each lizard was returned to its individual terrarium and left 3 days to recover. During this time, lizards had ad lib water and were fed 4-5 small crickets once a day. The third day after being implanted, lizards were transferred to outdoor pens (1.5 m wide x 3 m long x 1 m tall; walls were galvanized metal sheets. Substrate in each pen was half natural soil and grass, and half bare, coarse gravel. Pens were outfitted with water bowls, coverboards, and concrete bricks for lizards to bask on and to take refuge underneath. Lizards at the pens were fed 10-15 medium crickets 3 times a week. To avoid familiarity between members of each pair, each lizard in a pair was placed in a separate pen. While lizards were at the pens, one treatment and three control hatchlings died, and one control turned out to be a female. Thus, our sample size was reduced to 17 hatchlings, and from the initial 11 pairs only 6 remained for subsequent analyses.

Thirty days after receiving the implants, lizards were brought back to the laboratory to quantify growth, aggression, spectral variables of orange coloration, and orange coverage. First, we recorded body measurements (SVL and weight). Then, hatchlings were left basking in their individual tanks for an hour to allow them to reach their preferred body temperature. We then quantified aggressive behavior by way of dyadic encounters in a neutral arena. The arena was a rectangular wooden box (60 cm long x 39 cm wide x 45 cm high) divided into two sections by a removable opaque partition and a pane of glass at the front to observe the encounter. The box was uniformly illuminated and heated to optimal temperatures of 28–30 ºC. We introduced male hatchlings into the neutral arena with the division in place, treatment on one side and control on the other side (randomly assigned) and left them undisturbed for five minutes to habituate. To distinguish the members of each pair during the encounter, we painted a blue dot of acrylic paint on the dorsum of treatment lizards and a white dot on control lizards. After this time period, we removed the partition and immediately recorded behavior during a 10-min focal observation. Following Husak and Fox (2003), we recorded agonistic behaviors of both hatchlings, distinguishing the following aggressive behavior (scaled responses): 3 = fight, 3 = attack, 3 = bite, 2 = superimposition, 2 = circle, 2 = lateral throat display, 2 = throat display, 2 = pushup, 2 = head bob and 1 = approach; and the submissive behavior -1= retreat. Following each behavioral trial, spectrometer readings were taken to quantify orange coloration. Spectral reflectance and color variables were processed as described in Chapter 1.

Since not only spectral qualities but also the total amount of orange bars displayed on the lizard's body could be playing a communicative social role in hatchlings, we quantified the areal coverage of orange bars using ArcGIS version 10.0 (ESRI, 2010). A digital color photograph was taken of the right side of each lizard as the sides appear to be the most prominent body region displayed to conspecifics during social interactions.

Each picture was taken in the same windowless room with the same camera and light conditions. Below each lizard, a ruler was placed for scale purposes. After loading the pictures into ArcGIS the first step was to generate a “Box Diagram” for reference in centimeters, which was done by creating a shape file from which coordinates could be derived in order to rectify the pictures. We used Albers Equal Area Conic USGS for the coordinate system for its properties that precisely maintain area. We then created a “link” file for the image and entered the X and Y coordinate values of identifiable points on the image, and the image was then rectified. Using the new rectified image, we delineated the outline of the body and each orange spot or bar on the body. The body area of interest was defined from the last stripe of the black collar to the point of insertion of the hind legs, and from the top of the back to the lowest point on the side without including the venter. We then summed all of the orange areas and computed the total area. Total area of orange spots and bars was divided by the body area to obtain the proportion of orange, and this was the variable used to analyze amount of orange.

Hatchlings were returned to the pens that same day after behavioral trials, spectrometer readings, and photographs were done. Three days later, we returned to the pens and collected blood samples for androgen analyses. To obtain baseline hormone levels, we punctured the post-orbital sinus of subjects with a hematocrit microcapillary tube that was placed between the posterior portion of the eye and the bony orbit. Gentle pressure on the capillary tube ruptured the membrane and allowed for blood collection. Approximately 25–50 µl of whole blood were collected per lizard bled. Samples were transferred from the microcapillary tube to 1.5-mL microcentrifuge tubes and kept on ice until return to the laboratory (within 1 h), where they were centrifuged for 5 min at 6000 rpm to separate the plasma fraction. Subsequently, plasma samples were stored at −20 °C until assays were conducted. Concentrations of T and DHT were measured by standard radioimmunoassay (RIA) techniques following extraction and chromatographic separation (Husak et al. 2007; Wingfield and Farner 1975). From each blood sample we measured T and DHT concentrations. After bleeding the lizards, the treatment subjects were euthanized and the control subjects were released back to the same spot where they were captured in nature.

In 2013 we captured 18 male hatchlings from the same location at the Sooner Lake dam and repeated the same methodology from the previous year. This time, instead of T, we used implants of DHT. To make the pellets containing DHT, we weighed 683 mg of silicone, which was mixed with 153 mg of powdered DHT. A total extruded line of 186 mm was cut into 3-mm long implants, having a concentration of 2.47 mg DHT per implant. All the hatchlings survived for the duration of the experiment, maintaining 9 pairs throughout the experiment.

Statistical analysis: We compared values of SVL, aggression, color variables (brightness, B; saturation, S; and hue, H), amount of orange, T, and DHT between treatment and control groups using independent two-sample t-tests for each year (2012 and 2013). To examine if androgens were dependent on SVL, we performed simple linear regressions of T and DHT levels as a function of SVL for both the T and DHT experiments. Since we learned from the results in Chapter 1 that orange saturation increases significantly with a lizard's growth, we removed the effect of size by regressing each of the color variables against SVL. The residuals of each regression were used as the new variables for brightness (B_2-res), saturation (S_1R-res) and hue (H_3-res). Since proportion of orange might also be correlated with the size of the lizard, we performed linear regression. Finding a positive relationship, we removed the effect of size by analyzing the residuals in subsequent analyses. Because color variables can be highly correlated to one another, we performed a Principal Components Analysis (PCA) for each year in order to identify which variables had the most explanatory power. To examine the effects of T and DHT on aggression, orange coloration, and proportion of orange, we pooled together treatment and control groups for each year and performed simple linear regressions.

Results

For the T experiment, neither T ($r^2 = 0.009$, $p = 0.710$, $n = 17$) nor DHT levels ($r^2 = 0.017$, $p = 0.615$, $n = 17$) were dependent on SVL. For the DHT experiment, likewise neither T ($r^2 = 0.106$, $p = 0.188$, $n = 18$) nor DHT levels ($r^2 = 0.095$, $p = 0.214$, $n = 18$) were dependent on SVL. As expected, both T and DHT implants increased the amount of circulating androgens. For the T implants experiment, T levels were significantly higher ($t = -7.408$, $df = 10.4$, $p < 0.001$) in the treatment group ($n = 11$, mean = 73.86, 1 SD = 30.69 ng/ml) than the control group ($n = 6$, mean = 4.66, 1 SD = 3.14 ng/ml). For this same experiment, DHT levels were elevated, but not significantly so ($t = -0.496$, $df = 15$, $p = 0.627$), in the treatment group ($n = 11$, mean = 8.67, 1 SD = 2.80 ng/ml) compared to the control group ($n = 6$, mean = 8.00, 1 SD = 2.46 ng/ml) (Figure 2.1). For the DHT implants experiment, T levels were significantly higher ($t = -3.494$, $df = 16$, $p = 0.003$) in the treatment group ($n = 9$, mean = 1.97, 1 SD = 0.83 ng/ml) compared to the control group ($n = 9$, mean = 0.74, 1 SD = 0.64 ng/ml). In addition, DHT levels were significantly higher ($t = -9.305$, $df = 8.4$, $p = 0.001$) in the treatment group ($n = 9$, mean =

36.89, 1 SD = 10.55 ng/ml) compared to the control group (*n* = 9, mean = 3.79, 1 SD = 1.60 ng/ml) (Figure 2.1). When comparing T and DHT values obtained from the implants to the baseline levels found on the 55 wild hatchlings (see Chapter 3), exogenously elevated levels of both androgens were outside the normal physiological range. In the experiment with T implants, mean T levels recovered from treatment lizards were 304% higher than the highest level measured in wild hatchlings. Conversely, when DHT was implanted, mean DHT concentrations found in treatment lizards were 1376% higher than the highest level measured in wild hatchlings.

Aggression was significantly higher in the treatment group than the control group for both T (paired *t* = 3.607, *df* = 5, *p* = 0.015) and DHT (paired *t* = 4.637, *df* = 8, *p* = 0.002) implants. Amount of orange did not show significant differences between treatment and control groups when T was implanted (paired *t* = 0.903, *df* = 5, *p* = 0.408); however, it was marginally higher in the treatment group (paired *t* = 2.038, *df* = 8, *p* = 0.076) when DHT was implanted. For the experiment with T implants, brightness was not significantly different (*t* = 0.779, *df* = 15, *p* = 0.448) between treatment (mean = 0.172, 1 SD = 0.020) and control (mean = 0.179, 1 SD = 0.014). Neither was saturation (*t* = -1.835, *df* = 15, *p* = 0.086) between treatment (mean = 0.536, 1 SD = 0.044) and control (mean = 0.499, 1 SD = 0.030); or hue (*t* = -0.727, *df* = 15, *p* = 0.479) between treatment (mean = 567.75, 1 SD = 7.731) and control (mean = 565.01, 1 SD = 6.90). For the experiment using DHT implants, brightness was not significantly different (*t* = 0.016, *df* = 16, *p* = 0.988) between treatment (mean = 0.176, 1 SD = 0.049) and control (mean = 0.177, 1 SD = 0.029). Neither was saturation (*t* = -1.572, *df* = 16, *p* = 0.139) between treatment (mean = 0.490, 1 SD = 0.046) and control (mean = 0.461, 1 SD = 0.029); or hue ($t = -1.055$, $df = 16$, $p = 0.307$) between treatment (mean = 560.642, 1 SD = 9.843) and control groups (mean = 554.877, 1 SD = 13.113). A PCA for brightness (B2-res), saturation (S1-res) and hue (H-res) showed, for both T and DHT implants, a positive correlation between brightness and saturation (Figure 2.2).

Androgen levels and orange intensity

In the experiment where T implants were tested ($n = 17$), saturation residuals increased non-significantly with T levels ($r^2 = 0.024$, $p = 0.550$, $n = 17$) and decreased non-significantly with DHT levels ($r^2 = 0.131$, $p = 0.154$, $n = 17$). Brightness ($r^2 = 0.001$, $p = 0.966$, $n = 17$) and hue residuals ($r^2 = 0.001$, $p = 0.987$, $n = 17$) showed no relation with T levels. With DHT levels, no significant relationships were found with brightness ($r^2 = 0.176$, $p = 0.094$, $n = 17$) or hue residuals ($r^2 = 0.097$, $p = 0.223$, $n = 17$) (Figure 2.3). In the experiment where DHT was implanted, saturation residuals increased non-significantly with T levels ($r^2 = 0.040$, $p = 0.428$, $n = 18$) and had a statistically significant regression with DHT levels ($r^2 = 0.248$, $p = 0.035$, $n = 18$). Brightness residuals showed no significant relationships with either T ($r^2 = 0.023$, $p = 0.544$, $n = 18$) or DHT levels ($r^2 = 0.131$, $p = 0.140$, $n = 18$). Hue residuals showed no relation with T levels ($r^2 = 0.005$, $p = 0.777$, $n = 18$) or DHT levels ($r^2 = 0.011$, $p = 0.680$, $n = 18$) (Figure 2.4).

Androgen levels and proportion of orange

For the T implants experiment ($n = 17$), proportion of orange increased non-significantly with T ($r^2 = 0.062$, $p = 0.335$) and significantly with DHT ($r^2 = 0.491$, $p = 0.002$; Figure 2.5). With the DHT implants experiment ($n = 18$), proportion of orange increased non-significantly with T ($r^2 = 0.002$, $p = 0.865$) and DHT levels ($r^2 = 0.081$, $p = 0.252$).

Androgen levels and aggression

In the experiment where T implants were used ($n = 17$), aggression increased non-significantly with T ($r^2 = 0.167$, $p = 0.103$) and DHT ($r^2 = 0.040$, $p = 0.443$). With the DHT implants experiment ($n = 18$), aggression increased significantly with DHT ($r^2 = 0.396$, $p = 0.005$) and marginally with T ($r^2 = 0.208$, $p = 0.057$; Figure 2.6)

Orange intensity and aggression

For the T implants experiment ($n = 17$), we found no correlation between aggression and residuals of brightness ($p= 0.755$), saturation ($p = 0.740$), or hue ($p = 0.905$). With the DHT implants experiment ($n = 18$), we found a positive correlation between saturation residuals and aggression ($r^2 = 0.163$, $p = 0.097$; Figure 2.7), but it was not a statistically significant one. No relationship was found between residuals of brightness ($p = 0.585$) and hue ($p = 0.153$) with aggression.

Proportion of orange and aggression

In the experiment where T implants were tested ($n = 17$), we found a positive non-significant correlation between aggression and residuals of proportion of orange. With the DHT implants experiment ($n = 18$), we found a significant positive correlation ($r^2 = 0.302$, $p = 0.018$; Figure 2.8).

Discussion

When assessing the development of the orange bars in chapter 1, we observed various natural degrees of orange intensity. Therefore, we conducted this experiment to exogenously increase the amount of circulating androgens to verify if orange intensity, amount of orange, and aggression were indeed triggered by T, DHT, or both.

A few studies have shown that some aspects of sexual dimorphism depend more on DHT than on T (Hews and Moore 1995; Rand 1986), while others have found that T is the main steroid hormone associated with social signals in lizards (Cox et al. 2008; McGlothlin et al. 2007; Sinervo et al. 2000). T and DHT are both androgens that bind to the same receptors. Circulating T is further converted into DHT, which is the more potent steroid and always present in lower quantities. From our study, we can conclude that both orange saturation and proportion of orange increase the most with higher levels of DHT compared to T. Also, DHT alone explained 40% of the variation in aggression, while T alone explained only 21%. This result supports that DHT is more potent, having a greater effect than T at increasing aggression and increasing the orange color signal of hatchling males.

In lizards, sexual dichromatism has been linked to behavioral tactics in several species, with certain colors associated with more aggressive male behavior (Olsson et al. 2007). Therefore, we hypothesized that hatchling males with more intense orange bars or more lateral coverage with orange bars would be more aggressive compared to hatchling males with less conspicuous/less lateral coverage of orange. Proportion of orange confirmed our hypothesis. Hatchlings implanted with DHT that had more orange for their size were significantly more aggressive. Our results also showed that with DHT implants, as individuals obtained more saturated orange coloration, their aggressive behavior tended to increase; however, a bigger sample size might be necessary to yield a statistically significant relationship.

In one case during our assessment of the development of the orange bars in the first chapter, we were able to collect two blood samples from a hatchling still showing the orange bars. This lizard was bled twice 14 days apart, at 65 and 77 mm SVL. We correlated the orange coloration to androgen levels and found that saturation increased from 0.51 to 0.57, DHT increased from 1.11 to 8.70 ng/ml while T decreased from 50.77 to 37.38 ng/ml. Despite being a single case, the positive association between orange saturation and DHT in an unmanipulated wild hatchling is consistent with our findings using exogenous DHT. Collectively, our results suggest that orange coloration (both orange saturation and proportion of orange) and aggression in male hatchlings of collared lizards depend more on DHT than on T.

Rand (1986) described the cellular events that occur within the testes of collared lizards during sexual maturation and correlated those events to circulating androgen levels. According to his results, *C. collaris* maintains a relatively high circulating androgen level during sexual maturation and there is a high probability that an androgen other than testosterone is circulating before this, during the early stages of development. The presence of DHT during amphibian (Callard et al. 1978; Pierantoni et al. 1986) and bird testicular steroidogenesis (Ottinger and Mahlke 1984), and the presence of high levels of androgens other than T, e.g., DHT, in prepubescent mammals (Gupta et al.

1975; Rand 1986) support the hypothesis that DHT is present in male collared lizard hatchlings during early stages of development (Rand 1986).

As indicated from our results with exogenous androgens, DHT is the more important androgen responsible for the expression of the orange bars, a sexual dichromatic signal in male hatchling collared lizards. Sex steroid hormones contribute to the development of sex differences, either through action early in life (organization), following sexual maturation (activation), or both. Organization usually refers to creating the differences that will later be activated (Nelson 2005), and can occur either during embryological development or very early post birth (Hews and Moore 1995). The time at which DHT is being expressed in male collared lizard hatchlings, however, does not match DHT time of action in other vertebrates. In humans for example, DHT is responsible for the formation of male primary sex characteristics while in the womb, and for the development of most male secondary sex characteristics during puberty (Nelson 2005). In contrast, in hatchling male collared lizards, DHT is active after hatching but long before the onset of sexual maturation.

Hews and Moore (1995) manipulated hormones via implants in tree lizards (*Urosaurus ornatus*) and found that T was important early in development for setting up morph differences between orange versus orange-blue males. DHT was important later on in producing (activating) those color differences, so that when DHT was present the color was expressed. Without the presence of T early in post-hatching development, there was no subsequent development of color differences (Hews and Moore 1995). However, if DHT was not present later in development, color differences that had been set up before were not expressed. In our study, we saw a temporary color that appeared to be an activation event, but occurred during a time period normally associated with organization (Hews and Moore 1995). Thus, we could propose that the orange coloration in male collared lizard hatchlings appears to be an activating effect since DHT, not T, has the stronger effect.

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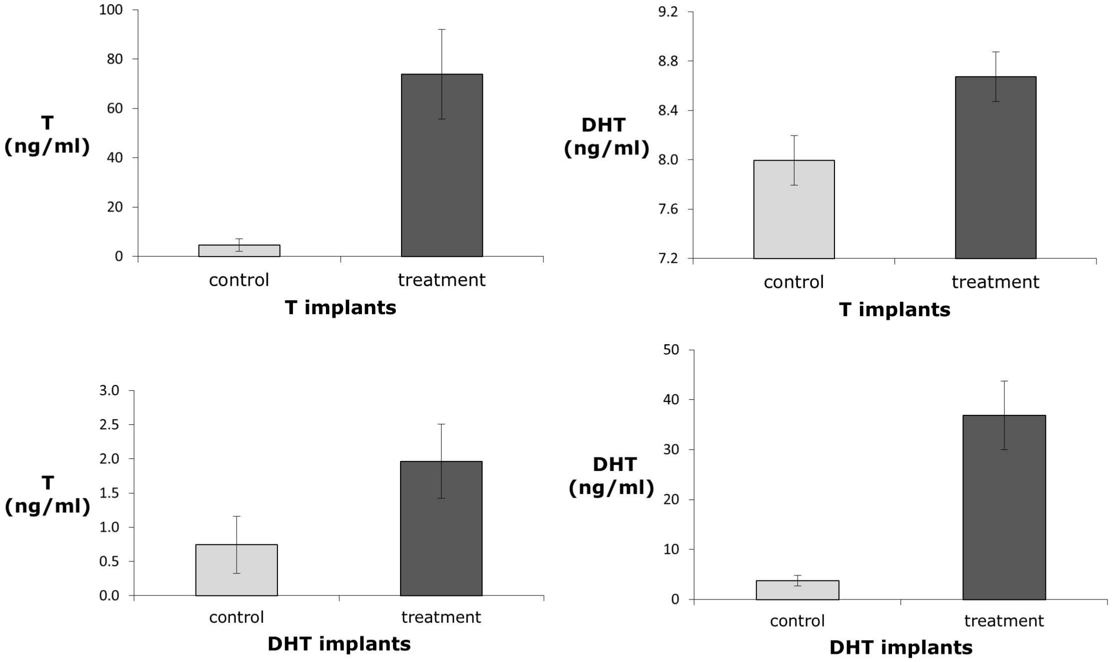

Figure 2.1. Top two panels: circulating androgen levels measured 30 days after T implantation in 2012. Bottom two panels: circulating androgen levels measured 30 days after DHT implantation in 2013.

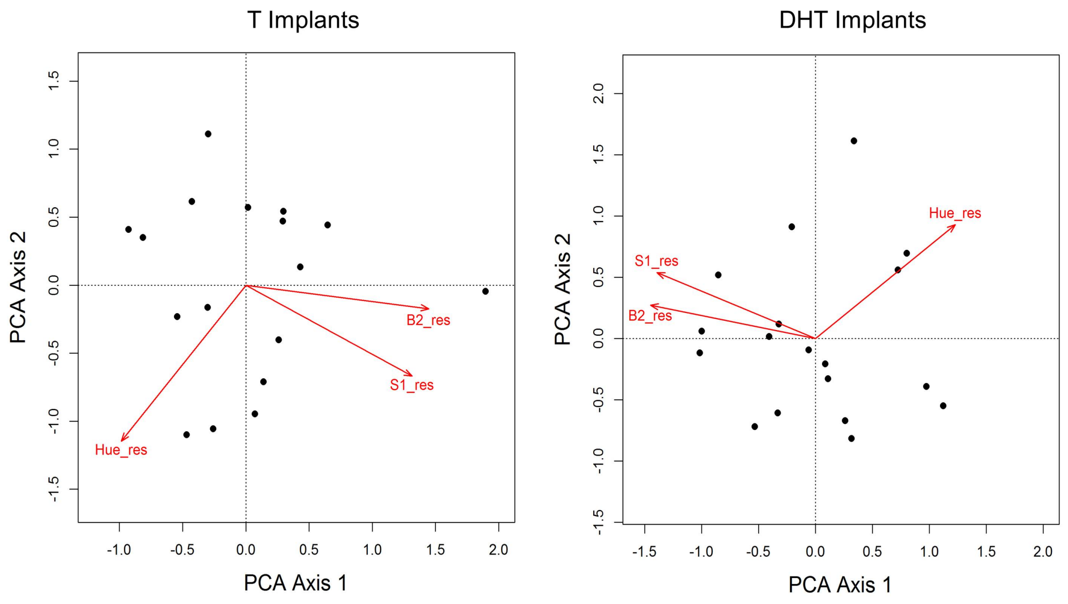

Figure 2.2. Principal Components Analysis (PCA) of residuals of brightness, saturation and hue for T (left panel, *n* = 17) and DHT (right panel, *n* = 18) implants. Treatment and control groups are pooled together. Each dot represents an individual subject lizard.

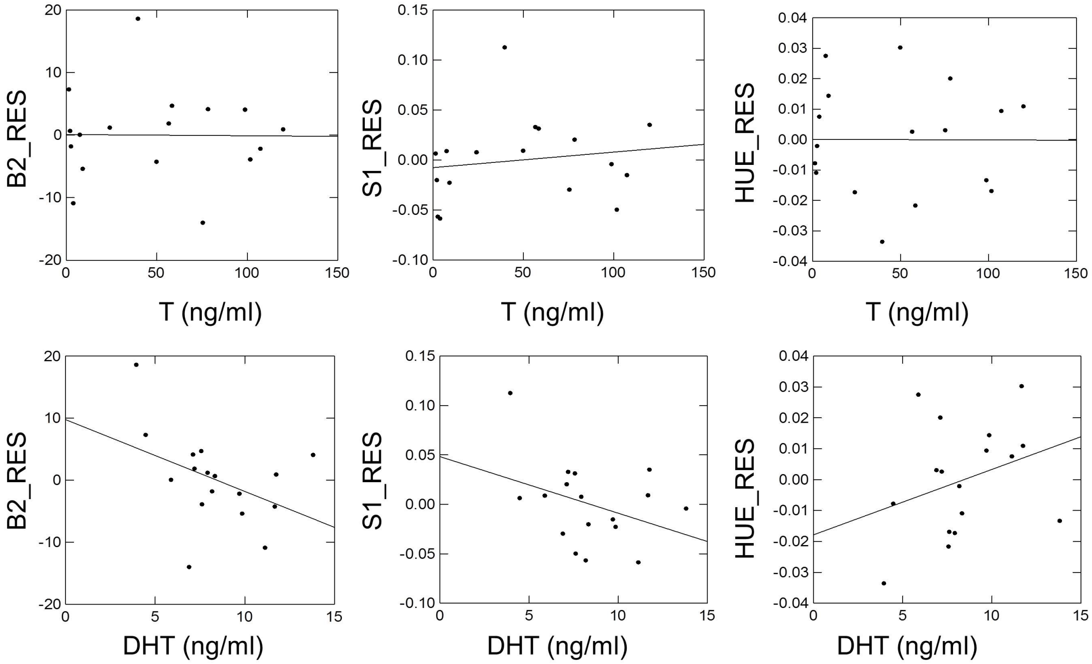

Figure 2.3. Simple linear regressions for the T implants experiment (*n* = 17) of residuals of brightness, saturation and hue against T levels on the top row and DHT levels on the bottom row.

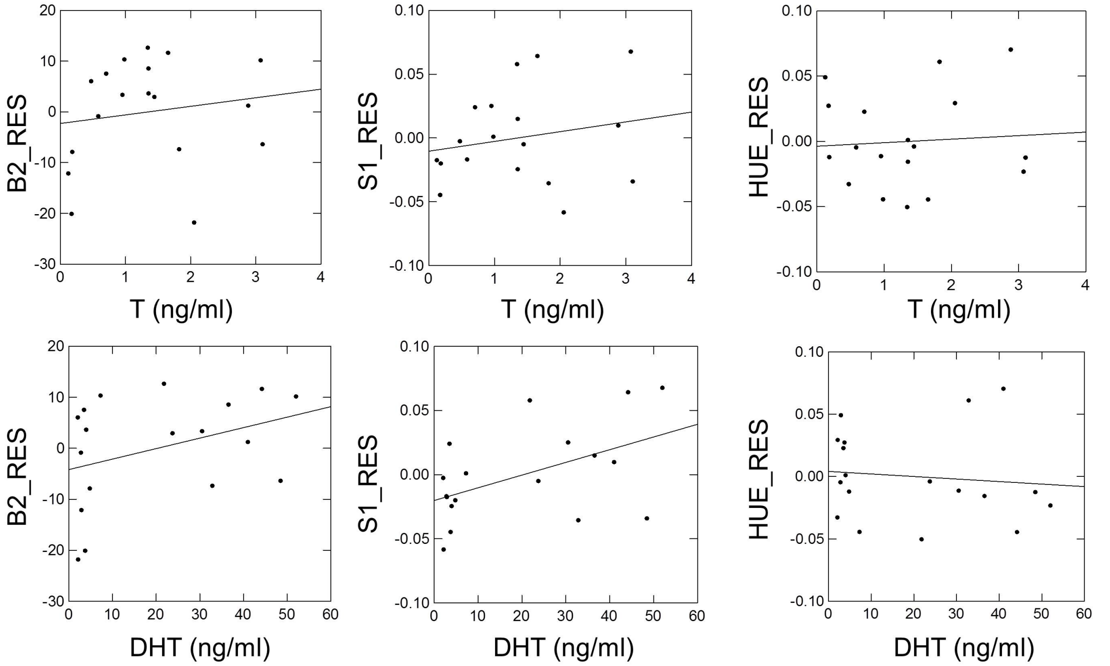

Figure 2.4 Simple linear regressions for the DHT implants experiment (*n* = 18) between residuals of brightness, saturation and hue against T levels on the top row and DHT levels on the bottom row.

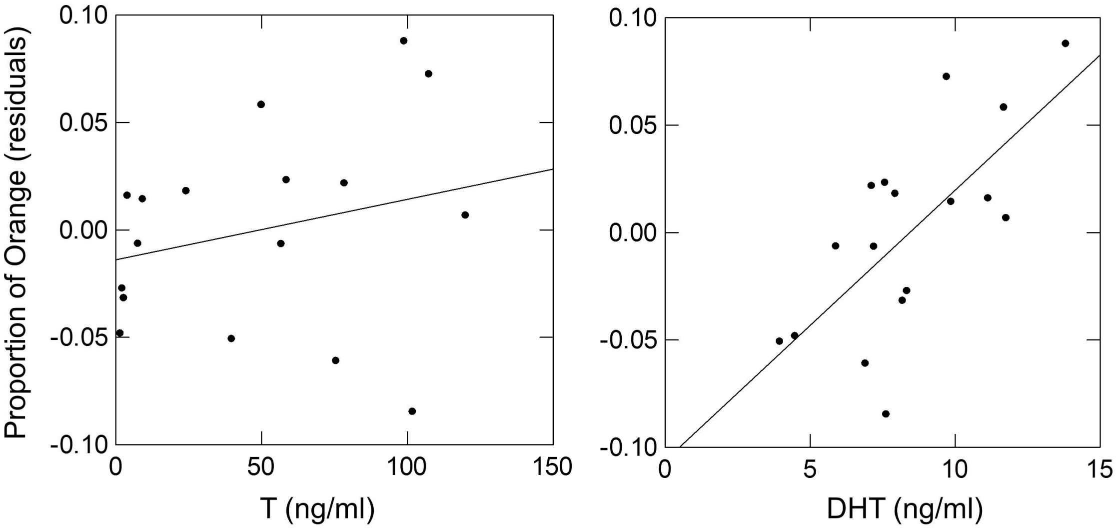

Figure 2.5. Simple linear regressions for T implants of proportion of orange against circulating T and DHT androgen levels. Proportion of orange was first regressed against SVL to correct for size; the residuals are then used in this and subsequent analyses.

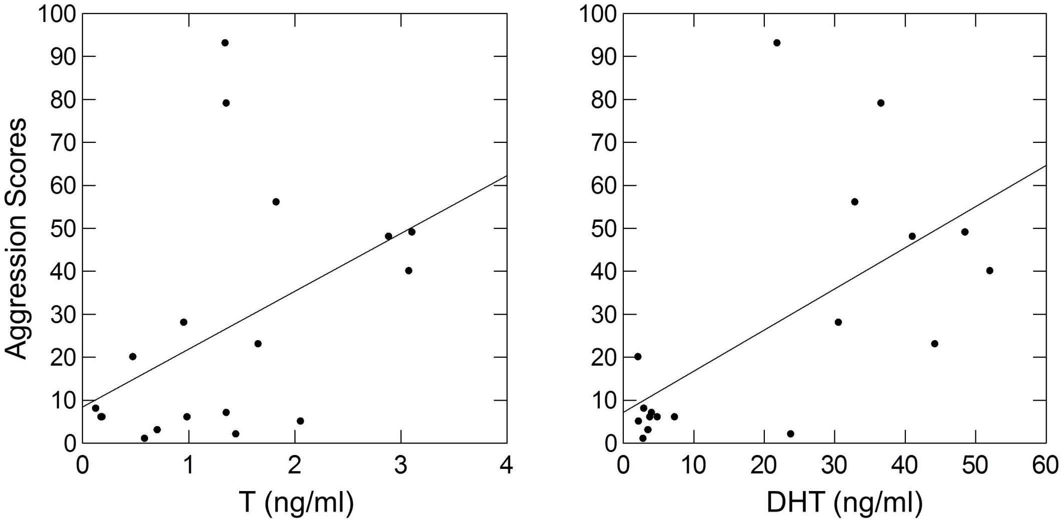

Figure 2.6. Simple linear regressions for DHT implants of aggression against circulating T and DHT androgen levels.

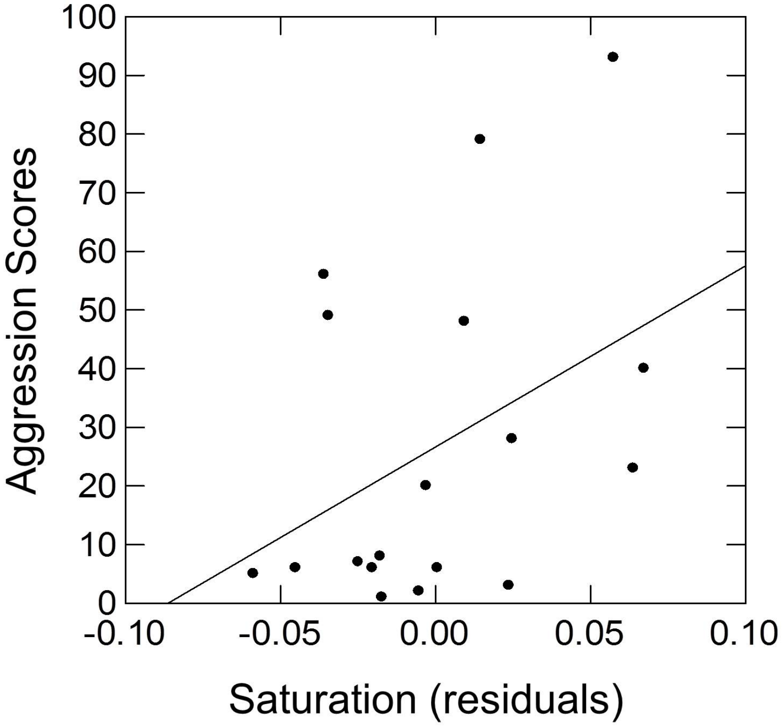

Figure 2.7. Simple linear regression for DHT implants of aggression against saturation residuals.

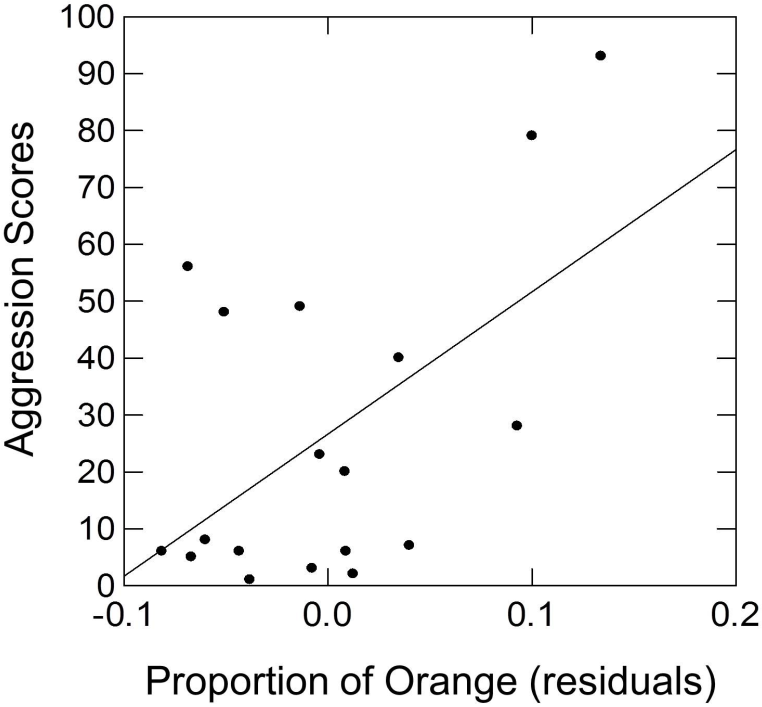

Figure 2.8. Simple linear regression for DHT implants of aggression against proportion of orange.

CHAPTER III

RELATIONSHIPS AMONG ANDROGENS, COLORATION, AND AGGRESSION IN WILD-RANGING HATCHLING COLLARED LIZARDS (*CROTOPHYTUS COLLARIS*)

Abstract

In lizards, the role of androgens on sexual dichromatism and aggression is well established. These relationships have been studied in adults, but mostly have not been examined in sexually immature individuals. Male collared lizard hatchlings develop conspicuous dorsolateral orange bars within the first 2 weeks of hatchling, thus making them an excellent model system to study the expression of secondary sexual characteristics in sexually immature individuals. We studied how androgen levels are related to the initial expression of orange bars in these male collared lizard hatchlings at a natural population in north-central Oklahoma. We hypothesized that testosterone (T) and dihydrotestosterone (DHT) levels would positively influence aggression, proportion of orange coloration on the body, and orange color saturation of the hatchling orange bars. Additionally, we hypothesized that male hatchlings with high levels of T and DHT, as well as intense and extensive orange coloration, would show high levels of androgens and intense breeding coloration later as yearlings and adults. Despite not being statistically

significant, our results showed a positive relationship between orange saturation and DHT. Male hatchlings with higher saturated orange bars tended to behave more aggressively than male hatchlings with less saturated orange coloration. Overall, aggression seems to be predicted by coloration more than by androgens. Our results also suggested that hatchlings with higher saturation of the orange bars had somewhat more saturated yellow throats as yearlings and especially as adults.

Introduction

During the development of an organism and then its life across seasons, levels of circulating hormones can show dramatic cycles of change (Hill 2010). Hormonal changes regulate changes in behavior, physiology and coloration, and sometimes behavior, physiology and coloration in turn affect hormone levels (Hirschenhauser et al. 2013; Nelson 2005). In birds, the effects of hormones on coloration have been studied mostly with respect to pigment-based coloration (Hill 2010). In lizards, the role of androgens on sexual dichromatism (Olsson et al. 2007) and aggression (Woodley and Moore 1999) has been well documented. However, these relationships have been overlooked in sexually immature individuals. Male collared lizard hatchlings develop conspicuous orange bars on their backs and sides just within a week or two after hatching and use this sexual signal to distinguish males from females and treat them differently (Fox et al., in prep.). Hatchling males are more aggressive to hatchlings with orange bars than without them, driving rival males away from their developing territories, while they accept the close presence of hatchling females without orange bars, perhaps to begin to form pair bonds with them, a possible expression of precocial sexual selection. Males lose these orange bars as they grow bigger, usually before their second summer (yearling stage). It has been shown that during sexual maturation in collared lizards relatively high levels of androgens (testosterone and dihydrotestosterone) are found circulating in the blood (Rand 1986). However, it is unknown if these androgens have an impact, and if so to what extent, in the expression of the orange bars and aggression of hatchlings. We wanted to determine if the relationship between androgens and coloration and aggression holds in male hatchlings as with sexually mature individuals. We hypothesized that elevated testosterone (T) and dihydrotestosterone (DHT) positively influence aggression and the intensity and areal coverage of the hatchling orange bars.

Additionally, we hypothesized that young male hatchlings with high levels of T and DHT, as well as intense and extensive orange coloration, will show high levels of androgens and intense adult coloration a year and two years later, when they are yearlings and adults, respectively. On the other hand, hatchlings with comparatively low androgen levels and dull, minimal orange bars will grow into yearlings and adults with low androgen levels and less intense adult coloration.

Methods

A total of 55 male hatchlings were captured at the Sooner Lake dam in north-central Oklahoma (Pawnee County) in late July to early October from 2011 to 2013 (14 in 2011, 17 in 2012 and 24 in 2013). Each time a lizard was captured for the first time, it was marked with a unique combination of toe clips and colored paint dots on the dorsum as explained in Chapter 1 to allow for individual-specific data collection. We collected and analyzed spectrometer readings as explained in Chapter 1 and digital color photographs and blood samples as described in Chapter 2. Ideally, to avoid confounding effects of daily fluctuations in plasma steroid concentrations, all samples should be collected at the same time of day. Due to weather and logistical constraints, however, samples were collected between 9:00 and 16:00 h. Every effort was made to take blood samples randomly during this time range. To minimize the effect of a rise in steroid levels due to handling stress, the procedure was timed from when we began attempting to capture the lizard to the moment we were finished collecting the blood sample. We made sure this process was completed in ≤ 10 minutes. Ideally, to relate androgen levels to the intensity and coverage of the orange bars as a hatchling grows, a blood sample would have been collected every time a lizard was captured and measured. However, blood samples are safe to obtain only when a hatchling weighs at least 9 g and subjects should not be bled more than once every 20 days to allow proper recovery. If we assume that 5-8% of a lizard's mass is blood volume, then a 9-g lizard minimally has 450 microliters of blood. In a hatchling, up to 10% of the volume can be safely removed, or up to 45 microliters. Due to the body size restriction and constraints on re-bleeding, we obtained repeated blood samples for only one hatchling.

For the purpose of following up changes in androgen levels and the transition of the orange bars of the hatchlings into the adult coloration of yearlings and adults, we recaptured as many survivors as we could find the following year. Hatchlings would grow into yearlings (one year old) and yearlings into adults (two years old). Of the 31 hatchlings collected in 2011 and 2012, 13 of them were found as yearlings the following year (6 in 2012 and 7 in 2013). From those 13 yearlings, 7 were recaptured the next year as adults (2 in 2013 and 5 in 2014). These yearlings and adults were bled for androgen analyses, and spectrometer readings were taken for color measurements. At the time of this writing, androgen levels only for adults recaptured in 2013 are available.

To analyze the coloration of yearlings and adults, three replicate spectral reflectance measurements were taken from the gular (GU), dorsolateral (DL), and ventrolateral (VL) regions of each lizard (McCoy et al. 1997). These regions were chosen because they are prominently displayed during the social interactions of collared lizards (Fitch 1956). To select the most appropriate index to quantify saturation, we looked at the reflectance curve and determined the appropriate range for each body region. We used S1R (λ 605-λ 700) for the throat and dorsolateral regions and S1Y (λ 550-λ 625) for the ventrolateral region. Brightness and hue were quantified using the same indices as used for hatchlings.

To quantify aggressive behavior in wild male hatchlings, we conducted staged intrusions in the field (Sooner Lake dam). We tethered a size-matched male intruder around the waist to one end of a 15-m pole. The intruder was placed ca. 1 m from the hatchling resident and immediately the observer backed away 15 m and started the trial. During 10 minutes we tallied counts of the following agonistic behavior patterns for the resident (weighted from 1-3 based on degree of escalation following Husak & Fox, 2003): 3 = fight, 3 = attack, 3 = bite, 2 = superimposition, 2 = circle, 2 = lateral throat display, 2 = throat display, 2 = pushup, 2 = head bob and 1 = approach; and submissive behavior patterns (weighted as -1): -1 = retreat, -1 = flee. Additionally, we recorded elapsed time to first aggression by the resident.

Statistical analysis: We performed simple linear regressions to analyze the relationships between androgen levels and orange coloration. To remove the effect of body size on color variables, we first regressed each dependent variable (brightness, saturation, and hue) against the independent variable SVL. The residuals were then used as the new variables for coloration. Body size also had a significant effect on the proportion of orange; therefore, proportion of orange was first regressed against SVL and the residuals were then used as the new variable.

When pooling together residuals for coloration measurements and proportion of orange for data from 2011, 2012 and 2013, we used standardized residuals (the residual divided by its standard deviation) to adjust for the fact that residuals might have different variances in different years.

We did not use residuals to analyze yearling and adult coloration since neither brightness, saturation nor hue varied significantly with SVL. The relationship between aggression and coloration was assessed using a multiple linear regression. We performed paired t-tests and used simple linear regressions to examine if androgen levels of hatchlings were an indicator of androgen levels when they became yearlings (one year later). To analyze the change in coloration (saturation) on the same lizards from hatchling to yearling and to adult ages, we used simple linear regressions.

Results

We obtained androgen baseline levels for the 55 male hatchlings captured during the hatchling seasons of 2011 through 2013. Natural values of T ranged from 0.13 to

55.68 ng/ml (18.261 ± 2.05; mean ± 1 SE) whereas DHT values varied from 0.22 to 18.76 (2.50 ± 0.44; mean ± 1 SE).

Androgen levels and orange coloration

When examining the levels of brightness, saturation, and hue in relation to androgen levels in wild hatchlings captured from 2011 through 2013 pooled, neither T nor DHT explained a significant amount of variation in coloration ($n = 55$); however, we observed the strongest relationship (and positive) between saturation and DHT levels ($r^2 = 0.029$, $p = 0.215$, $n = 55$) (Figure 3.1).

The relationship between androgens and coloration was also assessed one year later when 13 of these wild hatchlings were recaptured as yearlings. Since yearlings have already lost the orange bars and gained their adult coloration, we measured brightness, saturation and hue in the gular , dorsolateral, and ventrolateral body regions. We did not find any significant correlation between brightness, saturation or hue with T (Figure 3.2) or DHT (Figure 3.3). Despite not finding a significant relationship between androgens and coloration in yearlings, we observed a pattern of saturation in the ventrolateral body region increasing with T and DHT (Figures 3.2 and 3.3).

Androgen levels and proportion of orange

Androgen levels measured during three consecutive years (2011-2013 pooled) did not significantly explain the variation in proportion of orange in wild hatchlings (Figure 3.4). Proportion of orange showed a weak negative correlation with T ($r^2 = 0.033$, $p = 0.186$, $n = 55$) and DHT ($r^2 = 0.014$, $p = 0.391$, $n = 55$).

Androgen levels and aggression

Aggressive behavior measured in the field through staged intrusions in wild hatchlings (n = 11) did not show a significant relationship with T ($r^2 = 0.034$, $p = 0.589$, $n = 11$) or DHT ($r^2 = 0.001$, $p = 0.946$, $n = 11$) (Figure 3.5).

Orange coloration and aggression

In an analysis of multiple regression with the three color variables of brightness, saturation, and hue as predictors of aggression, we found no statistically significant relationship ($r^2 = 0.278$, $p = 0.489$, $n = 11$). Out of the three color variables, saturation contributed the most ($\beta = 297.25$, $p = 0.382$), followed by brightness ($\beta = 229.40$, $p = 0.566$) and hue ($\beta = -0.67$, $p = 0.602$).

Proportion of orange and aggression

The relationship between aggression and proportion of orange in wild hatchlings was not significant ($r^2 = 0.020$, $p = 0.678$, $n = 11$).

Progression of androgens and coloration through ontogeny

We compared androgen levels measured in males captured as hatchlings (T, 16.805 ± 4.325 ng/ml, $n = 13$; DHT, 1.607 ± 0.435 ng/ml, $n = 13$; $\bar{x} \pm 1$ SE) with the androgens levels measured in the same lizards recaptured as yearlings a year later (T, 32.358 ± 4.560 ng/ml, $n = 13$; DHT, 6.321 ± 1.616 ng/ml, $n = 13$; $\bar{x} \pm 1$ SE). One-tailed paired t-tests showed statistically significant differences in T levels ($t = 2.918$, $p = 0.006$, $df = 12$) and DHT levels ($t = 3.154$, $p = 0.004$, $df = 12$) between hatchlings and yearlings. Using linear regressions, we then compared how T and DHT levels had changed a year after in the same lizard (Figure 3.6). We found a positive but non-significant correlation in T ($r^2 = 0.079$, $p = 0.352$, $n = 13$) and DHT ($r^2 = 0.164$, $p = 0.171$, $n = 13$) levels in male lizards growing from hatchlings to yearlings.

We compared brightness, saturation and hue of the orange bars in hatchlings with brightness, saturation and hue of the yellowish coloration of the gular region when these same hatchlings grew into yearlings and adults. We found no significant correlation between brightness ($r^2 = 0.051$, $p = 0.457$, $n = 13$), saturation ($r^2 = 0.028$, $p = 0.588$, $n = 13$) or hue ($r^2 = 0.026$, $p = 0.599$, $n = 13$) from hatchlings to yearlings, nor in brightness ($r^2 = 0.292$, $p = 0.210$, $n = 7$), saturation ($r^2 = 0.142$, $p = 0.405$, $n = 7$) or hue ($r^2 = 0.117$, $p = 0.453$, $n = 7$) from hatchlings measured again as adults (Fig. 3.7). However, we did observe that hatchlings with more saturated orange bars had slightly more saturated yellow throats when yearlings. This tendency was stronger two years later when hatchlings were now adults (Figure 3.7).

Discussion

Despite not having found a statistically significant relationship between androgens and the strength of the orange coloration in hatchlings, our results showed a moderate positive trend between saturation and DHT during the 3 years combined. Such pattern could be an indicator of a subtle role that DHT has in regulating the expression of the orange coloration. There are a few arguments that could explain why in wild ranging hatchlings, elevated T and/or DHT did not have a bigger effect on amount of orange bars and on the strength of the orange coloration, particularly saturation (spectral purity), as we had hypothesized and as we found in Chapter II when we elevated androgens with exogenous implants. First, hatchlings could have had high androgen levels in the tissues and not circulating in the blood, which is from where we collected the blood samples to quantify T and DHT through a radioimmunoassay assay (RIA).

We should also consider diet. In the laboratory (Chapter II) we gave lizards a fixed diet (crickets). Conversely, hatchlings in the wild had access to a wider variety of invertebrates including spiders, beetles, butterflies, grasshoppers and other insects (Ivanyi 2009). The orange coloration of the bars suggests that this signal could be reliant on carotenoids. If the presence and development of the orange bars is to some degree determined by carotenoids acquired in the hatchlings' diet, the difference in diet between wild and captive lizards could explain differences in intensity and amount of the orange bars in the two groups.

The energetic trade-off that hatchlings face in their natural habitat could also explain the low contribution of androgens in increasing orange coloration. Traits regulated by carotenoids can be costly (Olson and Owens 1998). Thus, if the orange bars are indeed regulated to some extent by carotenoids, the additional costs of living in the natural environment might have diminished the relationship between DHT and orange coloration as seen in captive hatchlings (Chapter II). Furthermore, the captive hatchlings had artificially elevated DHT via exogenous implants.

We found a positive non-significant relationship between aggression and two measures of coloration (brightness and saturation), and a negative correlation with hue. Hatchlings with higher saturated orange bars tended to behave more aggressively than male hatchlings with less saturated orange coloration. We suggest that a larger sample size could yield statistically significant results. We did not find a significant relationship between aggression and androgens (and the relationship for both androgens and aggressive score was slightly negative, not positive), and DHT explained a very small percentage of the variation in saturation of the orange bars. Thus, aggression seems to be predicted by coloration more than by androgens.

Recapture of former hatchlings as yearlings allowed us to follow changes in androgen levels and body coloration. We expected that both androgen levels and color development would be correlated in hatchlings growing into yearlings, and possibly into adults. Our results show a pattern supporting this hypothesis for the development of coloration, as hatchlings with more saturated orange bars had somewhat more saturated yellow throats as adults. Our findings also showed a supporting pattern for our hypothesis regarding androgen changes from hatchlings to yearlings. However, the pattern of change in specific androgens from hatchlings to adults remains to be understood. In a previous work, Rand (1986) found a significant correlation between total androgens and stage of maturation. Male collared lizards in stage 2 of sexual maturation (yearlings collected in mid-April to mid-June), exhibited total androgen levels significantly lower than total androgen levels of lizards in stage 4 (adults collected from late May to mid-June). However, androgen levels of hatchlings were not significantly different from yearlings and adults. Total androgens for hatchlings collected in late August to early September were slightly higher than in yearlings collected in May and similar to adults collected in May.

In summary, most of our hypotheses regarding the influence of androgens T and DHT on hatchling aggression and on the intensity and areal coverage of the hatchling orange bars were supported, but only weakly. None were statistically significant. Only the relationships of proportion of orange and T and DHT, and the relationships of aggression and T and DHT were negative (but not significantly so), and seemed to contradict our hypotheses. Consequently, we predict that larger sample sizes might show clearer relationships that reach statistical significance. Our results are suggestive, but not definitive.

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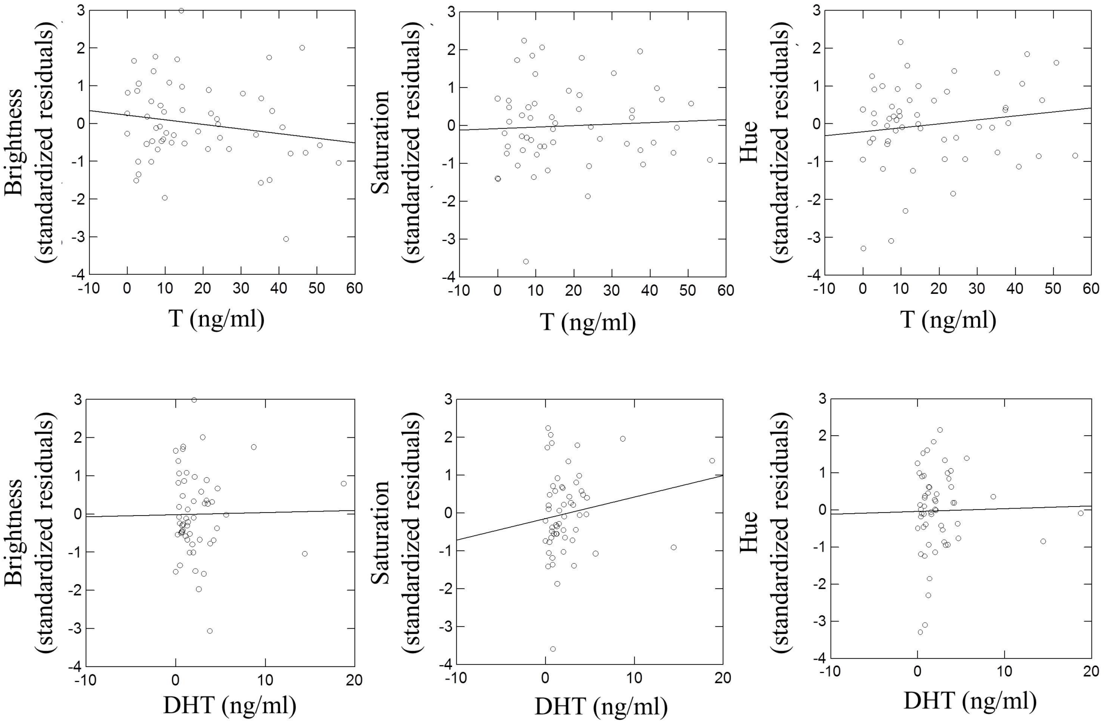

Figure 3.1. Simple linear regressions between coloration and androgen (T and DHT) levels from wild hatchlings captured during 2011, 2012 and 2013 pooled ($n = 55$).

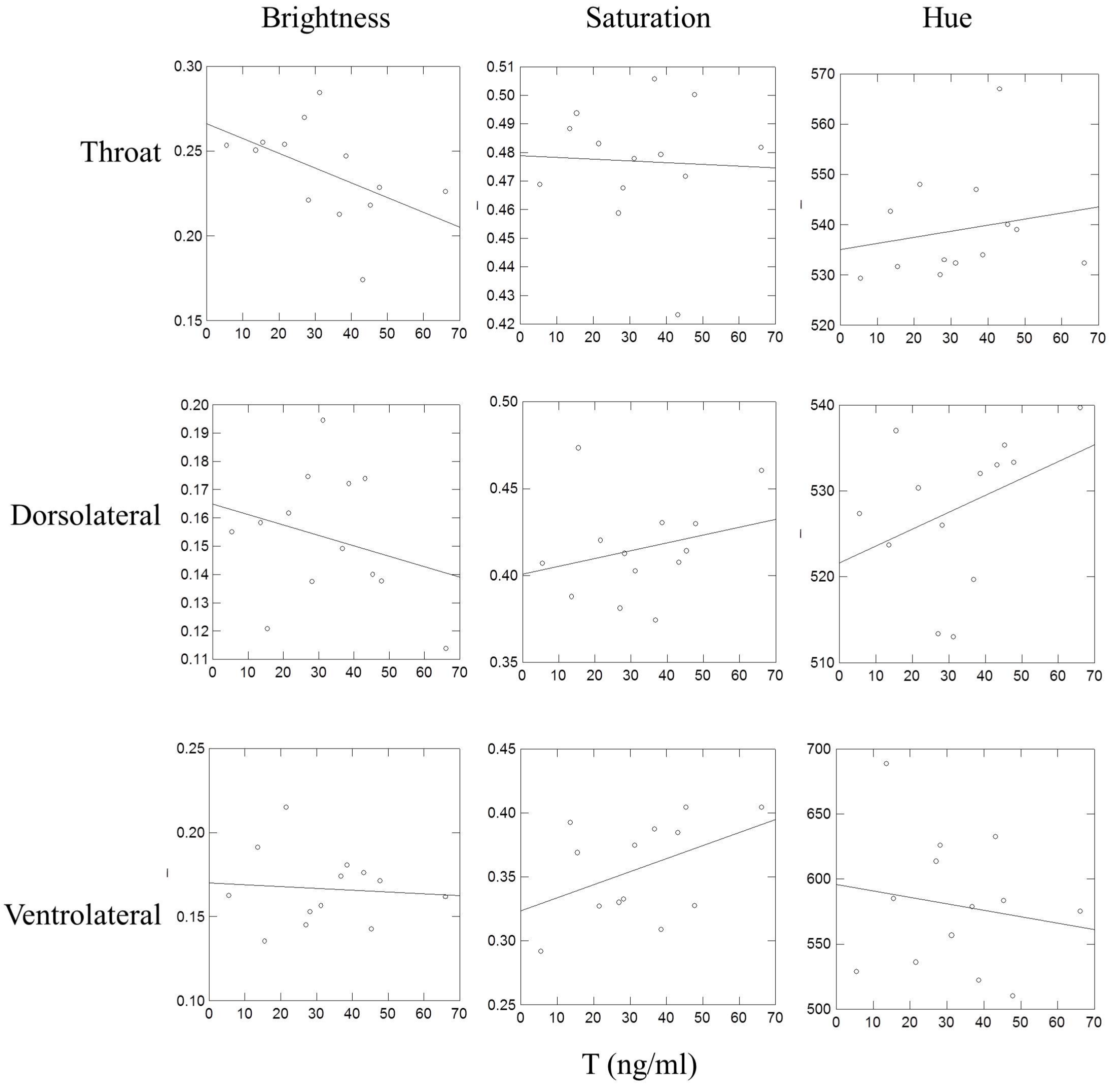

Figure 3.2. Simple linear regressions between coloration and T levels from wild yearlings captured during 2012 and 2013 (*n* = 13).

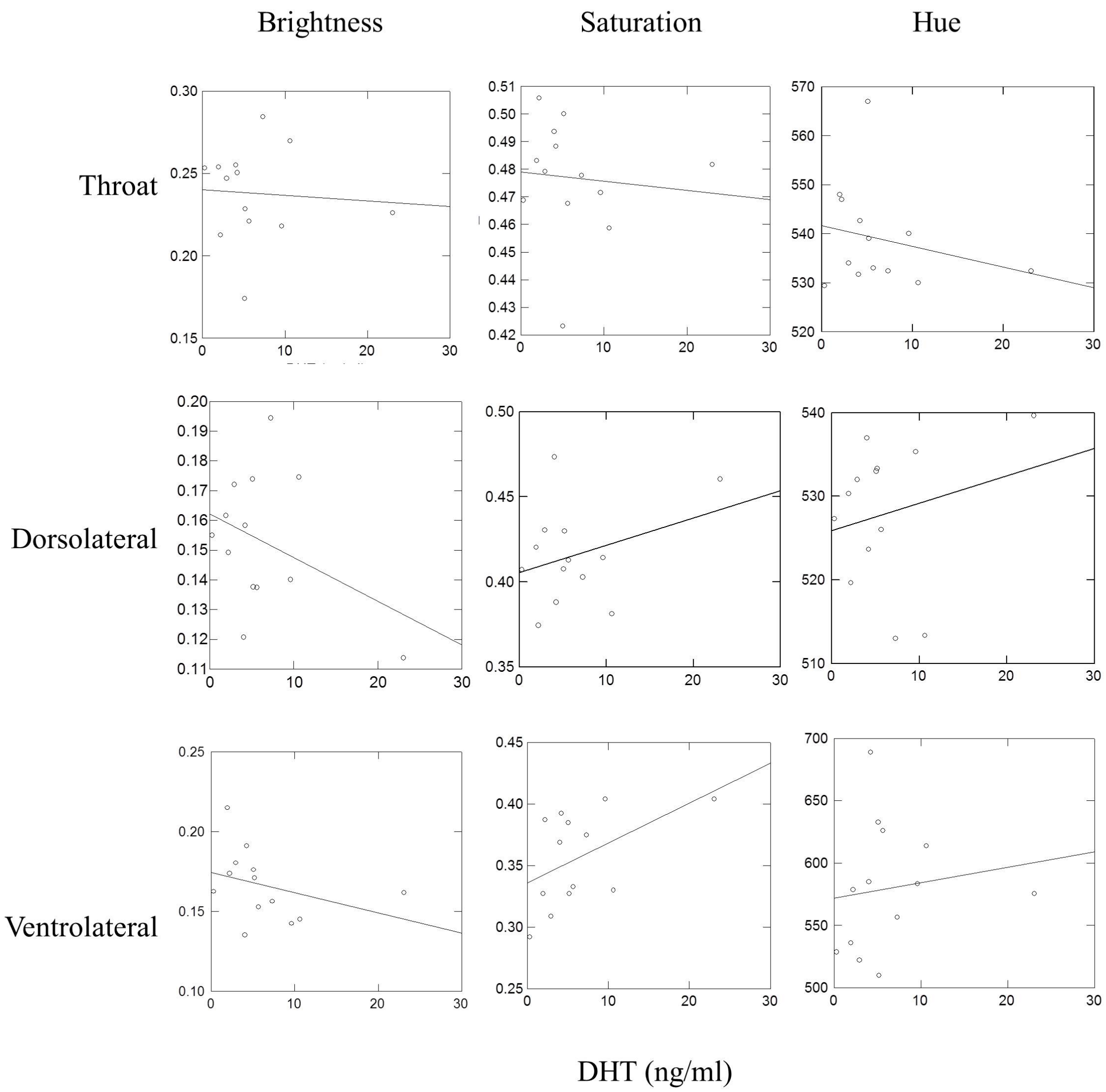

Figure 3.3. Simple linear regressions between coloration and DHT levels from wild yearlings captured during 2012 and 2013 (*n* = 13).

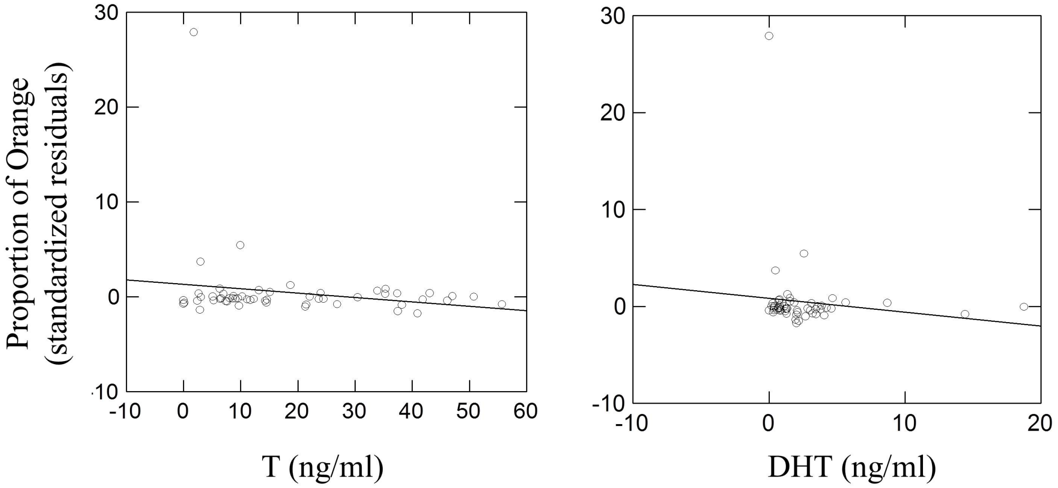

Figure 3.4. Simple linear regressions of proportion of orange against androgen levels from wild hatchlings for years 2011-1013 pooled (*n* = 55).

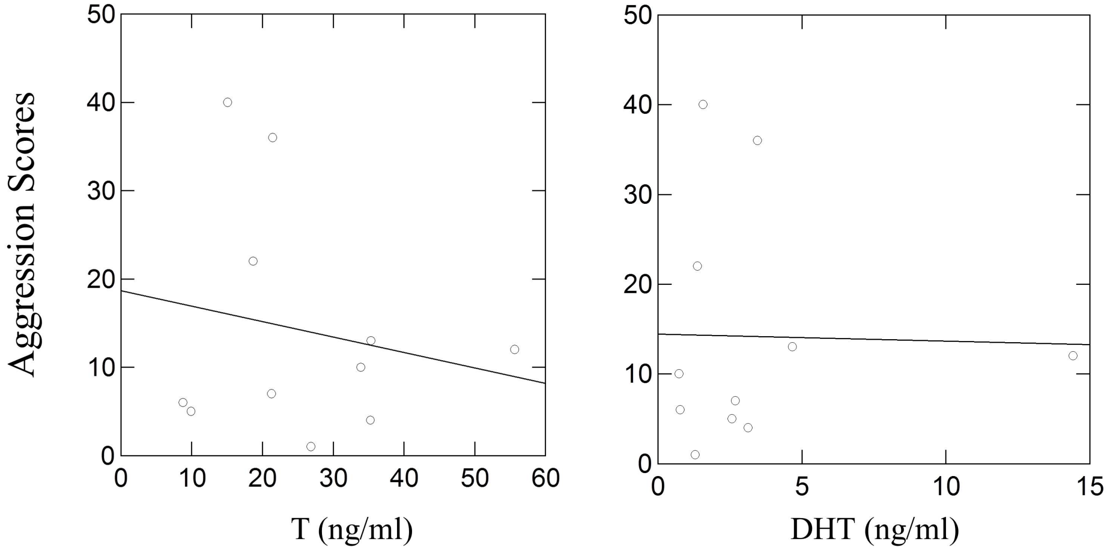

Figure 3.5. Simple linear regression of aggression against androgen levels in wild hatchlings (*n* = 11).

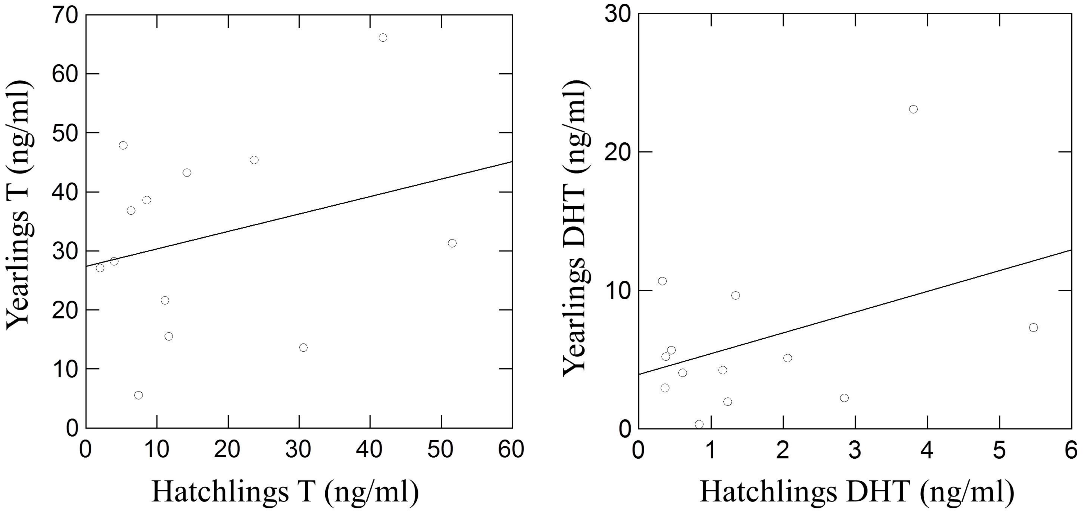

Figure 3.6. Simple linear regression of androgen levels on the same wild lizards when hatchlings and then when yearlings (*n* = 13).

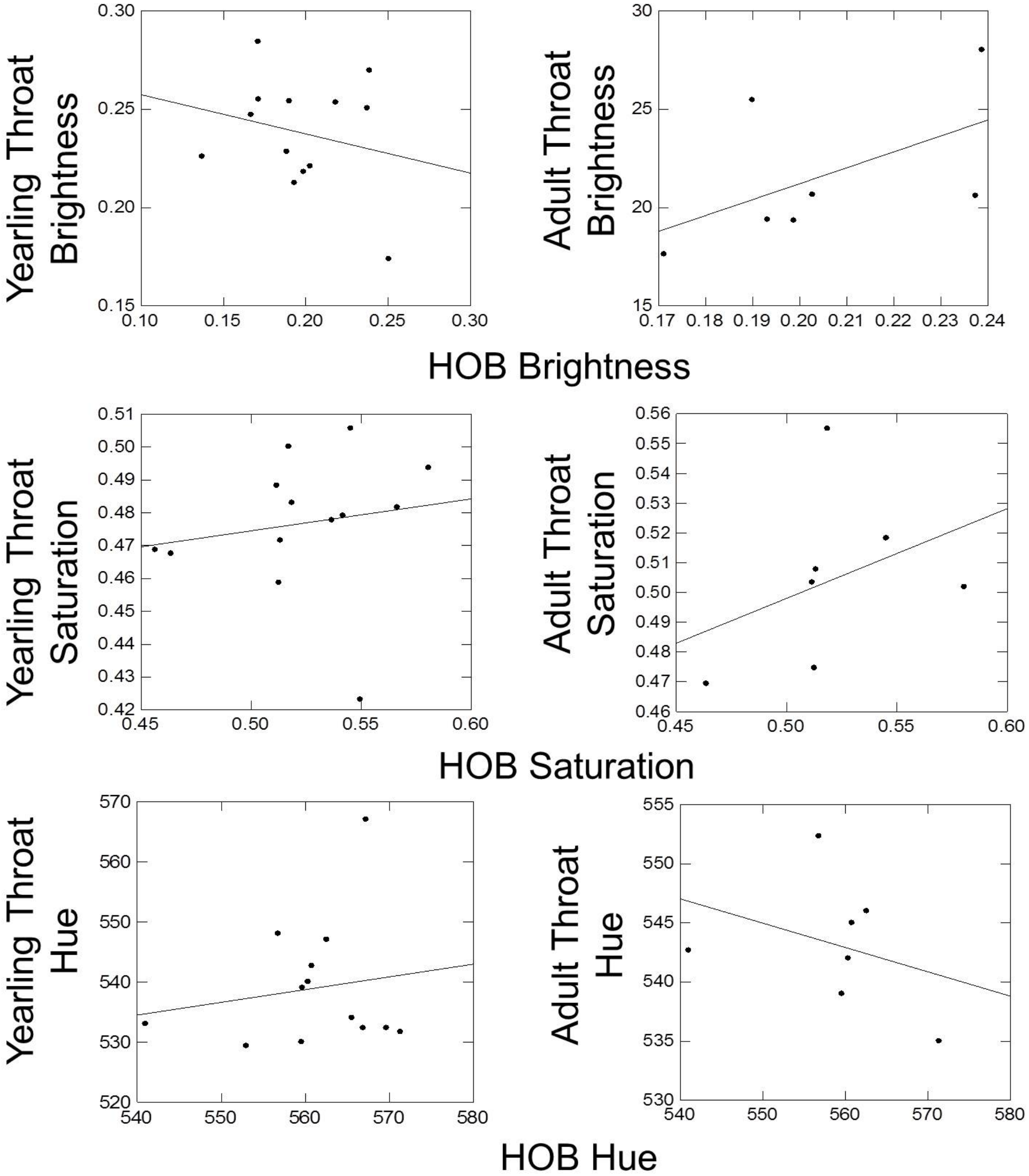

Figure 3.7. Simple linear regressions between brightness, saturation and hue of hatchling orange bars (HOB), and brightness, saturation and hue of the gular region on the same lizards one year later when yearlings (*n* = 13) and two years later when adults (*n* = 7).

CHAPTER IV

HATCHLING ORANGE COLORATION AND FITNESS IN THE COLLARED LIZARD (*CROTAPHYTUS COLLARIS*)

Abstract

The reproductive benefits that secondary sexual characteristics (e.g., conspicuous coloration) yields have been studied primarily in adult organisms because there is no apparent reason for the presence of these sexual traits in sexually immature individuals (i.e., juveniles). Adult collared lizards (*Crotaphytus collaris*) are polygynous, highly territorial, philopatric, and sexually dichromatic. Male collared lizard hatchlings also show sexual dichromatism in the form of bright dorsolateral orange bars that usually fade by the end of their first season. The adaptive significance of this precocial sexual trait is unclear, but preliminary field observations suggest that male hatchlings might be repelling future male rivals and establishing social bonds with hatchling females. Thus, male hatchling collared lizards may be securing (future) mating opportunities related to their coloration. We hypothesized that sexually mature collared lizards that had higher color saturation in orange bars and higher proportion of orange as hatchlings would be more likely to: a) maintain spatial associations with females established as hatchlings, 2) show less displacement from their average location when hatchlings, and 3) have a higher

inferred fitness. Our results were not statistically significant, but suggested that hatchlings with more orange coloration had a higher probability of pair-bonding, displaced less from their natal locations, had larger home range areas (as adults, but not as yearlings), and had higher inferred fitness (as adults, but not as yearlings). Thus, it is suggested but not confirmed that precocial sexual signaling in hatchlings is positively related to estimated fitness in adulthood as estimated from adult home range size and number of females overlapped spatially, but not so in yearlings when yearling males likely gain their fitness by sneaking female copulations and not by including and defending them in their territories like adults. It would be instructive to know if the true fitness of yearlings derived from sneaking copulations is related to their orange coloration as hatchlings.

Introduction

Secondary sexual characteristics are the traits that arise as a result of competition over mates (Anderson, 1994). These secondary sexual characteristics can take the form of songs in birds, antlers in ungulates, and horns in beetles to name a few. One very common form is conspicuous coloration in one sex, usually males, which is referred to as sexual dichromatism. The reproductive benefits that conspicuous coloration yields have been studied only in sexually mature individuals (adults), and because secondary sexual characteristics are thought to be expressed and influenced by sexual selection only when animals are able to reproduce, there is no apparent reason for the presence of these sexual traits in sexually immature individuals (juveniles). Nevertheless, sexual dichromatism in sexually immature individuals has been observed in a couple of cases. In juveniles of Bearded Tits the plumage remains the same between the sexes but males develop a bright orange beak about a week after fledging (Marin et al. 1994). In European Bee-eaters, juvenile plumage seems to be a significant indicator of sex (Kapun et al. 2011), males having a yellowish-brown back and females green back feathers. The adaptive significance of these sexually dimorphic traits is unclear. For sexual selection to favor the presence of a morphological trait such as a secondary sexual characteristic in sexually immature individuals, there must be a fitness advantage. In adults, the reproductive benefits of sexual dichromatism are often seen to be immediate. In juveniles, however, sexual dichromatism is not expected since no immediate reproductive benefits are obtained. For sexual selection to favor the presence of a secondary sexual characteristic in sexually immature individuals, the trait must promote future mating opportunities (i.e., if the trait increases mating success later in life when the individual gains sexual maturity).

Collared lizards (*Crotaphytus collaris*) are territorial reptiles that have a polygynous mating system in which adult males defend near-exclusive territories from other males (Lappin and Husak 2005). A single male territory usually entirely overlaps the home ranges of multiple females, allowing the male to restrict access of other males to those females (Fitch 1956; Lappin and Husak 2005). In Oklahoma, the breeding season for collared lizards begins in late-March to mid-April, when adults and yearlings come out of hibernation. Mating behavior reaches its peak in early summer, with most females laying one or two clutches of eggs every season (Trauth et al. 2004), although some females have been observed to lay up to three clutches in some years (Baird 2004; Baird and Timanus 1998; York et al. 2014). The hatchling season initiates in late July when the first hatchlings appear and continues through October (Trauth et al. 2004), after which hatchlings retreat underground for the winter. Hatchling *C. collaris* males display sexual dichromatism in the form of very conspicuous dorsolateral orange bars that usually fade by the end of their first season (late October), when hatchlings enter hibernation. Histological analyses show no evidence that hatchlings reach sexual maturity during their birth year (Rand 1986), nor do hatchlings have hemipenes capable of being everted (Crosby, Fox, and Baird, pers. observ.), which makes the occurrence of secondary sexual characteristics unusual.

In collared lizards, even though a hatchling is far removed temporally from sexual activity, it could benefit hatchling males to drive away other hatchling males (current resource competitors and future reproductive competitors) and accept the presence of nearby female hatchlings (future mates). Adult collared lizards appear to establish and strengthen social bonds with females and repel rival males in summer after spring breeding, probably for mating advantages early the next spring (Baird and Fox, pers. observ.). Hatchlings appear to follow the same pattern. Recent field observations, such as aggressive displays between 3-4-week-old males and courting attempts toward equally young females (when there are few adults and yearlings present with whom to compete), suggest that male hatchlings are driving away hatchling male neighbors and displaying to and possibly establishing social bonds with hatchling females. It is possible that this non-aggressive behavior toward hatchling females is to attain potential mating advantages with them as yearlings the next spring, and that the aggressive behavior toward hatchling males is to diminish sexual competition with them in the future. If male hatchlings are indeed securing their future mates as pre-reproductive juveniles (and repelling future mating rivals), this will be the first time this mating strategy is observed in vertebrates.

Besides physical size, coloration can be a strong determinant in male mating success and fitness of adults. Because coloration is often used as an honest signal in mate choice, males with large body areas of intense coloration are often the most successful in securing mating opportunities, for example in collared lizards (Baird et al. 1996). Male hatchling collared lizards may also secure (future) mating opportunities related to their coloration. The presence of conspicuous coloration in sexually immature males with the purpose of securing future mating opportunities can be called precocial sexual signaling, a recently observed phenomenon that might have been overlooked in the past in social species. Thus, the presence of the conspicuous coloration in male collared lizard hatchlings offers a unique opportunity to study precocial sexual signaling.

Since collared lizards are highly territorial and philopatric, it is possible to mark and recapture the juveniles and track them over time. Information on the locations and social interactions of identified individuals and patterns of space use is necessary in order to estimate mating relationships (Baird et al. 1996). By following the lizards' space use during each season and movements from one year to the next, it is possible to observe how their developing territories change over time, which hatchlings are maintaining (and expanding) their natal territory, and which ones are not.

Our overall research goal was to examine if hatchlings' greater coverage and intensity of orange bars, a secondary sexual trait, conferred male hatchlings greater fitness benefits as yearlings and adults, when they become sexually mature (Baird and Timanus 1998). Our first objective was to determine if early pair-bonding between male and female hatchlings occurs when they are still sexually immature. We hypothesized that those yearlings (and maybe adults) that had the most intense orange bars and areal coverage of orange bars as hatchlings would be more likely to maintain the associations with the females with whom they spatially overlapped as hatchlings. Our second objective was to establish if the orange coloration of male hatchlings is correlated with changes in their spatial location among hatchling, yearling, and adult stages. We hypothesized that hatchlings with more orange would tend to show less displacement from their average location when hatchlings while males with less orange would be forced to move around more and the next year would be found in a different place. Our third objective was to examine how coloration in male hatchlings impacts mating opportunities as yearlings and adults. We hypothesized that males with more orange as hatchlings would have more mating opportunities and higher potential reproductive output (PRO) compared to males with less orange coloration when hatchlings.

Methods

This study was conducted at Sooner Lake dam in Pawnee County, Oklahoma. Starting in 2011 from August to October, we captured the first cohort of hatchlings on which we would base this study. As described in the first chapter, for each lizard we recorded morphometric measurements, took spectrometer reading of orange bars, collected pictures to quantify area of the body covered by orange bars, and marked them permanently with a unique combination of toe clips and temporarily (until they next molted) with a unique combination of colored paint dots on their backs. Additionally, every time we observed a male and a female interacting either through a courtship display, close mutual presence (within a meter), or actual copulation, we identified each individual and recorded such interaction as consorting. To adjust for body size, the area of orange bars was quantified as the proportion of lateral body area between neck and hind limbs on one side of the body covered by orange (“proportion of orange;” chapter 2). The toe clips allowed us to identify each lizard the following year and after a molt in the same year, while the colored dots were used to identify lizards from a distance through binoculars during the same field season.

The location of every lizard was carefully recorded along the length of the dam using a numerical system of numbered flags. Flag numbering consisted of sequential numbers marked on the rail bordering the road, from one end of the dam to the other. The corresponding numbers on the rail were also painted on the rocks (in a straight line perpendicular to the road) where the lizards would be basking. The distance (in meters) between flags was known for the entire linear length of the dam. The width of the dam was measured at four different sections and averaged; dam width was very uniform. Thus, our study area comprised a total length of 1677.16 m and an average width of 12.94 m.

We examined the space use of individual lizards through daily sightings. During each sighting the location of the lizard was recorded relative to the two flags on either side (e.g., the location of a lizard halfway between flags 4 and 5 was recorded as 4.5). Using the distance from the beginning of the dam to the flag where the lizard was found, we calculated the minimum, maximum and average locations (in meters) for every individual. The length of the home range (x-length) was calculated by subtracting the minimum from the maximum location. The home range area (m^2) was then calculated by multiplying the length of the home range by the average width of the dam. From here forward, we refer to these areas of occupation as “home ranges” since we did not make the necessary observations needed to determine if the areas of occupation were defended (i.e., territories), despite that both adult males and females are known to defend territories (Baird et al. 1996; Baird and Sloan 2003; Husak and Fox 2003) and it is likely that hatchlings are setting up territories. If the x-length for a hatchling was greater than 100 m and the sequential locations indicated an abrupt change in space use, we assumed the lizard had switched its home range. Two home ranges were then assigned for that lizard and the home range where the orange was the strongest was selected for the analyses.

When assessing space use for yearlings and adults, we did not assume a home range shift for lizards with home ranges greater than 100 m in length because these larger home ranges might indicate lizards sneaking into another lizard's home range or simply a lizard with a very large home range. When estimating space use and fitness from overlaps, we did not use all the sightings for each adult and yearling for which we were making an estimate. Since the location of a yearling or adult in the fall does not relate to its potential mates, we used the sightings recorded only during the breeding season. We considered this season to be from the first spring sighting of the year until the end of June.

In subsequent years (2012 to 2014), as soon as the weather was warm enough for lizards to emerge from hibernation (~ late April; breeding season), we made every effort to recapture all previously marked hatchlings that survived to become yearlings as well as yearlings not captured as hatchlings the previous autumn. We then recorded all sightings of marked yearlings to determine home range size and location. We repeated this process through 2014, and thus a new cohort of hatchlings was added each year and tracked through subsequent years. In 2013 we were able to re-capture adults that had been hatchlings in 2011, yearlings that hatched the year before, and a new and final set of hatchlings. In 2014 we attempted to recapture all yearlings and adults that had survived from the previous season. To evaluate shifts in home range location, we calculated the absolute value of the difference between the average location of each individual between 1) when it was a hatchling and the following year when it was a yearling, and 2) when it was a hatchling and two years later when it was an adult, as a measure of displacement. Once home range area was calculated for each lizard, we measured overlap with other lizards' home ranges by constructing a matrix where the home range area of each lizard was compared against all other lizards. To examine pair-bonding, we recorded which female lizards had home ranges that overlapped with the same male during two or more years. We considered pair-bonding to have occurred if at least one female overlapped with the same male in both years.

Annual mating success and potential reproductive output (PRO) of males were the two estimates we used as an indirect measurement of fitness. Annual mating success was estimated by counting the number of females whose home ranges overlapped the home range of each male. Following Lappin and Husak (2005), the PRO of each yearling male was estimated by adding the PRO of each yearling and adult female whose home range overlapped with that male's home range in the spring breeding season. The PRO of each female was calculated using the regression formula for clutch size (CS) as a function of snout-vent length (SVL). For *Crotaphytus collaris*, $CS = 0.2105\ (SVL) - 11.45$ (Ballinger and Hipp 1985; Trauth 1978). The mean SVL of each female over its measurements in the breeding season was used for SVL. If a female's home range was overlapped by > 1 male, her PRO was divided equally among the number of males with whom she overlapped. Yearling females at Sooner Lake produce one clutch in the season while adult females can have two to three clutches in the same year (McCoy et al. 2003). To adjust for this variation, the PRO of adult females was doubled before being added to the PRO of each sexually mature male. To be included in the analyses, a male needed to have orange data recorded (spectrometer readings and photograph taken) as a hatchling, and have been recaptured the following year during the breeding season. As explained in Chapter 1, out of the three indices of coloration generated by the spectrophotometer readings (hue, saturation and brightness), we considered saturation to be the most biologically relevant for describing the development of the orange bars. Results from Chapter 2 confirmed that saturation was the color index most affected by androgen levels, and showed a statistically significant positive relationship between saturation and aggression. Therefore, we used saturation as the coloration variable that best showed the adaptive significance of the orange bars. Both estimates of orange coloration, proportion of orange and saturation, had a statistically significant relationship with SVL, so to remove the effect of size of the lizard, we regressed proportion of orange and saturation against SVL and used the residuals of each as the independent variables.

All variables followed a normal distribution except for PRO, which was log transformed to normalize it. We used Ln (PRO + 1) as the log transformation to account for any zeros in the dataset as recommended by Zar (1999). First, to determine the effect of the orange coloration on pair-bonding (when the same female overlaps with the same male during two or more in both years), we performed logistic regressions using proportion of orange and saturation separately as independent variables. To assess the relationship between orange coloration and consorting, we did logistic regressions between the dependent variable consorting (recorded as 1 when consorting was observed and 0 when it was not) and the independent variables proportion of orange and saturation. To examine correlations between proportion of orange and saturation in hatchlings and their potential fitness and space use, we did simple linear regressions between the dependent variables (home range area (m^2), the number of females whose home ranges overlapped a male's home range, PRO, and displacement), and our independent variables (residuals of proportion of orange and saturation). All statistical analyses were done using SYSTAT 10.1.

Results

Pair-bonding

Throughout the breeding seasons of the three consecutive years, we were able to recapture 50 male yearlings out of 143 male hatchlings marked the previous year (25 yearlings in 2014 out of 67 hatchlings marked in 2013, 13 yearlings in 2013 out of 33 hatchlings from 2012 and 12 yearlings in 2012 out of 43 hatchlings from 2011). From the 50 males recaptured as yearlings, 24 had proportion of orange and saturation measured when they were hatchlings; however, one of these yearlings was recaptured outside the breeding season and was not used in the analyses. Thus, of 23 yearlings used in the analysis, 15 were found to overlap with females in their home range in the following fashion: 10 maintained pair-bonding (i.e., overlapped with at least 1 of the same females when yearling or adult as when hatchling), and 5 did not maintain pair-bonds. The 8 remaining lizards in the analysis were not found to overlap with any females as yearlings. The logistic regression for pair-bonding in yearlings showed a positive, but not statistically significant, correlation with proportion of orange ($\chi^2 = 2.332$, $df = 1$, $p = 0.127$, $n = 23$; Fig. 4.1A). No correlation was found between pair-bonding and saturation

(χ^2 = 0.000, *df* = 1, *p* = 0.987, *n* = 23; Fig. 4.1B). Of the 23 yearlings, 7 were recaptured as adults. All these adults had females overlapping their home range but only one adult maintained pair-bonding, overlapping with the same female when hatchling and when adult.

We recorded consorting information for 14 males: 5 hatchlings, 3 yearlings and 6 adults. We were not able to document any males consorting with the same female in 2 or more years. Four of these yearlings and adults (3 adults and 1 yearling) were among the subset we had been following since they were hatchlings and for which we had orange data collected. We did not find a relationship between consorting and proportion of orange (χ^2 = 0.070, *df* = 1, *p* = 0.792, *n* = 23) or saturation (χ^2 = 0.204, *df* = 1, *p* = 0.651, *n* = 23).

Space Use

During the breeding seasons of 2012, 2013 and 2014 we marked a total of 76 yearling and 37 adult males. Of those yearlings, 48 had females overlapping their home range; 34 of the males had between 2 and 9 overlapped females, one male had 14 females in its home range, and the remaining 13 males had only one female overlapping their home range. Sightings for male yearlings during the breeding season ranged from 1 to 35 with an average of 5 sightings per lizard. Yearlings with only one sighting (*n* = 28) could not have their home range area calculated. The average home range area for all 48 male yearlings with more than one sighting was 691.5 m^2 (SE = 78.6); with a minimum and maximum of 15.5 and 2343.5 m^2, respectively. The number of yearling males overlapping a female's home range varied between 1 and 7.

Of the 37 adult males, 32 had females overlapping their home range (from 1 to 9 females). The five adults without female overlap only had one sighting recorded; therefore, home range area could not be calculated for these individuals. We recorded between 1 and 30 sightings with an average of 12 sightings per adult. The average home range area for all adults was 980.9 m^2 (SE = 87.6), with a minimum and maximum of 15.8 and 1885.6 m^2, respectively. The number of adult males overlapping a female's home range varied between 1 and 4.

For the yearling males analyzed in this study (i.e., with ≥2 sightings), home range area was not significantly correlated with proportion of orange ($r^2 = 0.011$, $p = 0.693$, $n = 16$; Fig. 4.2A) or saturation ($r^2 = 0.033$, $p = 0.498$, $n = 16$; Fig. 4.2B). Yearling males moved an average of 49.7 m (SE = 10.4) from their average location when hatchlings to the average location where they were found when yearlings. Displacement ranged from 1.3 to 118.1 m and was not significantly correlated with either proportion of orange ($r^2 = 0.017$, $p = 0.628$, $n = 16$; Fig. 4.2C) or orange saturation ($r^2 = 0.020$ $p = 0.598$, $n = 16$; Fig. 4.2D).

The home range area for the seven males whose orange coloration was quantified at hatchling age and who survived to adult age ranged from 650.7 to 1577.1 m^2 with a mean of 1141.7 m^2 (SE = 152.3). Home range area increased steeply but was only marginally significant with proportion of orange ($r^2 = 0.519$, $p = 0.068$, $n = 7$; Fig. 4.3A). The relationship between home range area and saturation was also positive but less pronounced ($r^2 = 0.008$, $p = 0.850$, $n = 7$; Fig. 4.3B). From their average location as hatchlings to their average location when adults, males displaced an average of 90.4 m (SE = 23.8) with a minimum and maximum of 8.0 and 190.8 m, respectively. Adults with a higher proportion of orange when hatchlings showed a lower (but not significant) displacement as adults ($r^2 = 0.198$ $p = 0.317$, $n = 7$; Fig. 4.3C). No relationship was found between displacement and saturation ($r^2 = 0.001$ $p = 0.969$, $n = 7$; Fig. 4.3D).

Inferred Fitness

The PRO for male yearlings ranged between 7.8 and 48.0 (mean = 18.2; 1 SE = 3.2; n = 15). For male yearlings, proportion of orange was not significantly correlated with the number of females whose home ranges were overlapped by that male ($r^2 = 0.025$, $p = 0.573$, $n = 15$; Fig. 4.4A), or PRO ($r^2 = 0.223$, $p = 0.075$, $n = 15$; Fig. 4.4C). Orange saturation followed the same tendency, showing no statistically significant correlations with number of females with overlapped home ranges ($r^2 = 0.002$, $p = 0.876$ $n = 15$; Fig. 4.4B), or PRO ($r^2 = 0.042$, $p = 0.461$, $n = 15$; Fig. 4.4D). All the regressions for yearlings were negative. The PRO for male adults varied from 4.1 to 29.8 (mean = 14.6, 1 SE = 3.6, n = 7). No significant correlation was found between proportion of orange and number of overlapped females ($r^2 = 0.001$, $p = 0.957$, $n = 7$; Fig. 4.5A). Individuals with greater proportion of orange coloration when they were hatchlings tended to have a higher PRO when adults ($r^2 = 0.158$, $p = 0.377$, $n = 7$; Fig. 4.5 C). Hatchlings that had more saturated orange bars, showed a positive but non-significant relationship with the number of females whose home ranges they overlapped ($r^2 = 0.091$, $p = 0.510$, $n = 7$; Fig. 4.5B) and their PRO when adults ($r^2 = 0.056$, $p = 0.608$, $n = 7$; Fig. 4.5D). All the regressions for adults were positive.

Discussion

In hatchling collared lizards, the presence of orange bars in males is used as a precocial sexual signal in inter- and intra-sexual social interactions (S. F. Fox, unpublished data). Therefore, the next question to explore, i.e., the present study, was to find if this phenomenon of precocial sexual signaling carries over as sexual selection after they are sexually mature, which could be described as selecting their mate ahead of time. Despite orange saturation and proportion of orange not being statistically significant between males overlapping with the same females and those that did not, the fact that 66% of the males maintained the spatial associations with females established when they were hatchlings allows us to suggest that early pair-bonding is likely to be occurring in these lizards. We found a positive association between proportion of orange in male hatchlings and the probability of maintaining spatial associations with females in future years. The ability to maintain a pair bond over multiple breeding seasons can translate into having more mating opportunities (i.e., receptive females are present in a male's home range at the beginning of the season rather than the male having to search for them). Female collared lizards can lay up to 3 clutches per season and the first clutch laid has a better chance of offspring survival (York et al. 2014), possibly due to hatchlings attaining larger sizes before going into hibernation. Thus, if early pair-bonds are maintained, it could be very beneficial for increasing lifetime fitness. A finding of a significant association between orange coloration and pair bonding could demonstrate a new mating strategy where male hatchlings are securing mates prior to reaching sexual maturity.

Our results linking orange coloration in hatchlings and subsequent space use as adults, despite not being statistically significant, did show support for our hypothesis, particularly with regard to proportion of orange. Our findings in chapter 2 indicated that hatchlings with higher proportion of orange are significantly more aggressive compared to hatchlings with lower proportion of orange. Also supporting our results, previous studies have shown that more colorful males possess higher levels of androgens (i.e., testosterone), which in turn increase their aggressiveness (Baird et al. 1996; Korzan et al. 2008; McGlothlin et al. 2008; Olsson et al. 2007). Therefore, coloration can also be a measurement of a male's capacity for defending a territory. This suggests that males that had more orange when hatchlings would tend to displace less and be more successful at defending bigger territories. This tendency should become more obvious in their second year when adult males are known to be highly territorial (York et al. 2014). On the other hand, adults with a lower proportion of orange when hatchlings displace longer distances and then maintain smaller home range areas. Additionally, given that collared lizards are philopatric, hatchling males with more orange coloration may have a higher probability of maintaining a pair bond through time due to lower displacement. This is consistent with our results for pair bonding where we observed a non-significant but positive relationship between pair bonding and proportion of orange (Fig. 4.1A).

Our results support the hypothesis that males with more orange as hatchlings would have more mating opportunities (i.e., more female overlaps) and higher PRO when adults, compared to hatchlings with lower amount of orange. We observed the same positive associations between orange saturation as hatchlings and number of female overlaps and PRO as adults. One possible explanation for these positive relationships with orange saturation is female preference for brighter adult male coloration, which has been shown in Oklahoma collared lizards (Baird et al. 1997). From our findings in chapter 3 we know that hatchlings with higher saturation of orange resulted in adults with brighter coloration, specifically, more saturated yellow coloration on their dewlap. A common social display of male collared lizards is the extension of the dewlap, which has been positively correlated with courtship frequency (Baird 2013). Additionally, not only the conspicuousness of the color signal seems to be important but also the proportion of the coloration. In male guppies, for example, Houde (1987) found that females preferred males with larger total area of orange. Hence, the significance of the positive association between hatchling orange bars and brighter adult coloration. Another aspect to consider in order to explain the relationship between orange in hatchlings and fitness as adults is the importance of a brighter coloration in adult males as an honest signal. It is possible that coloration of the orange bars in hatchlings and yellow dewlap in adults is carotenoid-dependent. This statement remains to be tested, but if that is the case; the development of the orange coloration may well signal condition of the hatchling and serve to enhance the effectiveness of precocial sexual selection (Hill 1990). In guppies, for example, the red coloration of the males depends on carotenoids and the brightness of the carotenoid color depends on diet (Kodric-Brown 1989). Therefore, bright carotenoid spots may be an indicator of foraging success and generally better physiological condition in the males (Endler 1980; Endler 1983).

Our results did not show statistically significant support for any of our hypotheses about the relationship between orange coloration in male collared lizard hatchlings and space use and potential fitness as yearlings or adults. Looking at each regression by itself, we cannot draw any conclusions because the patterns they shown could be due to random sampling variation rather than a real trend. However, if we consider simultaneously the relationships for yearlings between proportion of orange with displacement, home range size and female overlap, the three regressions together seem to tell an intriguing story: higher proportion of orange seems to correlate with lower displacement, smaller home range size; and, therefore, less female overlap (Fig. 4.2C, 4.2A and 4.4A). The third of these three regressions shows an apparent negative trend between proportion of orange and the number of females whose home ranges were overlapped by a male, interpreted as annual mating success (Lappin and Husak 2005). Number of females decreased (but not significantly) with proportion of orange. We had hypothesized that males with more orange as hatchlings would have higher fitness when sexually mature, and that this would be reflected in higher female overlap and could be interpreted as more mating opportunities. However, there may be a distinct difference in what the number of female home ranges overlapped means for yearlings versus adults. In male yearlings, a large number of females overlapping its home range does not necessarily mean increased mating opportunities. Male yearlings could have large displacement and show large home ranges if they are being socially excluded from adults' (and more aggressive yearlings') territories (Baird and Timanus 1998) and act in a subordinate way. Thus, their potential fitness would actually be lower, despite the fact that their home range might overlap with a greater number of females. Therefore, for yearlings, large home range area correlates positively with female overlap, but that does not necessarily indicate higher fitness. Because our estimate of male PRO is based primarily on number of females overlapped, it may actually be a misleading estimate of fitness in yearling males. This train of logic is supported by the positive regressions of adults for inferred fitness measures and orange coloration as hatchlings (Fig. 4.5) and the negative regressions for yearlings (Fig. 4.4). Even more telling are the findings of York et al. (2014) and York and Baird (2015), who showed that yearling males at Arcadia Lake in Oklahoma follow a much different mating strategy than adults. In this case, rather than defending territories, yearling males were adopting a submissive behavior and avoiding adult males defending their territories (Baird and Curtis 2010), and thus were able to sneak copulations (Husak et al. 2008) and perhaps inherit the adult male's territory when it was vacated (Smith and Arcese 1989; Woolfenden and Fitzpatrick 1978). These yearling males showed an unexpected high fitness, which could also be partially due to maintaining pair-bonds through low displacement. If this is the case, this could help explain how smaller home range (and thus lower female overlap) as a yearling might translate into higher overall fitness. On the other hand, this could simply be a locally-adapted behavior due to habitat features at Arcadia Lake (York et al. 2014), but probably not; both sites are human-made dams and spillways in central and north-central Oklahoma and are very similar. Thus, we suggest that the same measurement of fitness cannot be applied for yearlings and adults, and the findings of York et al. (2014) support this explanation. It would be instructive to know if the true fitness of yearlings derived from sneaking copulations is related to their orange coloration as hatchlings.

Taken together, with a very small sample size, this is the first time that a precocial sexual signal seems to be positively related to estimated fitness in adulthood. Given that the orange coloration may be an indicator of higher fitness, the importance of this precocial sexual signaling can be seen in maintaining early pair bonding. This precocial sexual signal can also be helping to avoid male-male contests and reducing the energy required to attract females and repel other males. This use of precocial sexual signaling to help secure mates prior to sexual maturity, and thus create a carryover effect that improves fitness later in life, is a newly observed strategy that offers new insights into potential mechanisms of sexual selection.

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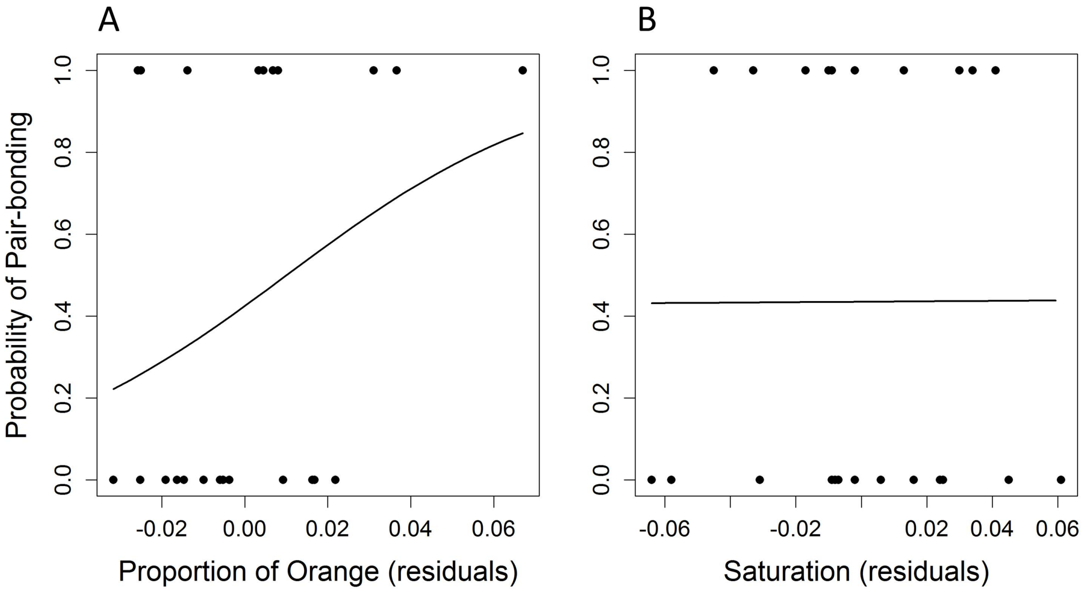

Figure 4.1. Logistic regressions for the probability of pair bonding related to (A) proportion of body area with orange coloration as hatchlings and (B) orange color saturation as hatchlings. Circles show the observed values and lines the estimated probability of pair-bonding (*n* = 23).

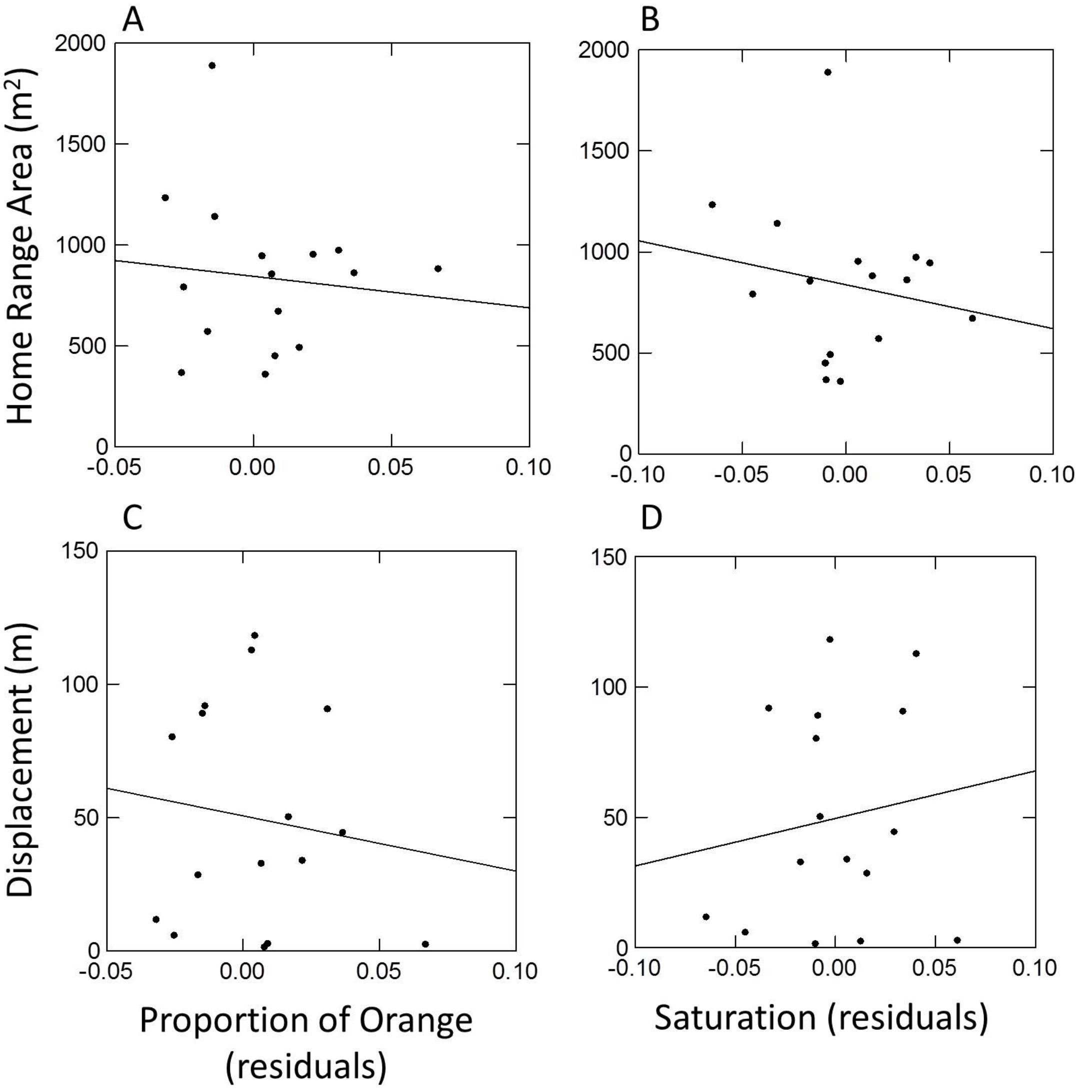

Figure 4.2. Relationships between proportion of orange (A, C) and saturation (B, D) as hatchlings with yearling home range area (m^2) and displacement (m) from their average location when hatchlings to their average location when yearlings (*n* = 16).

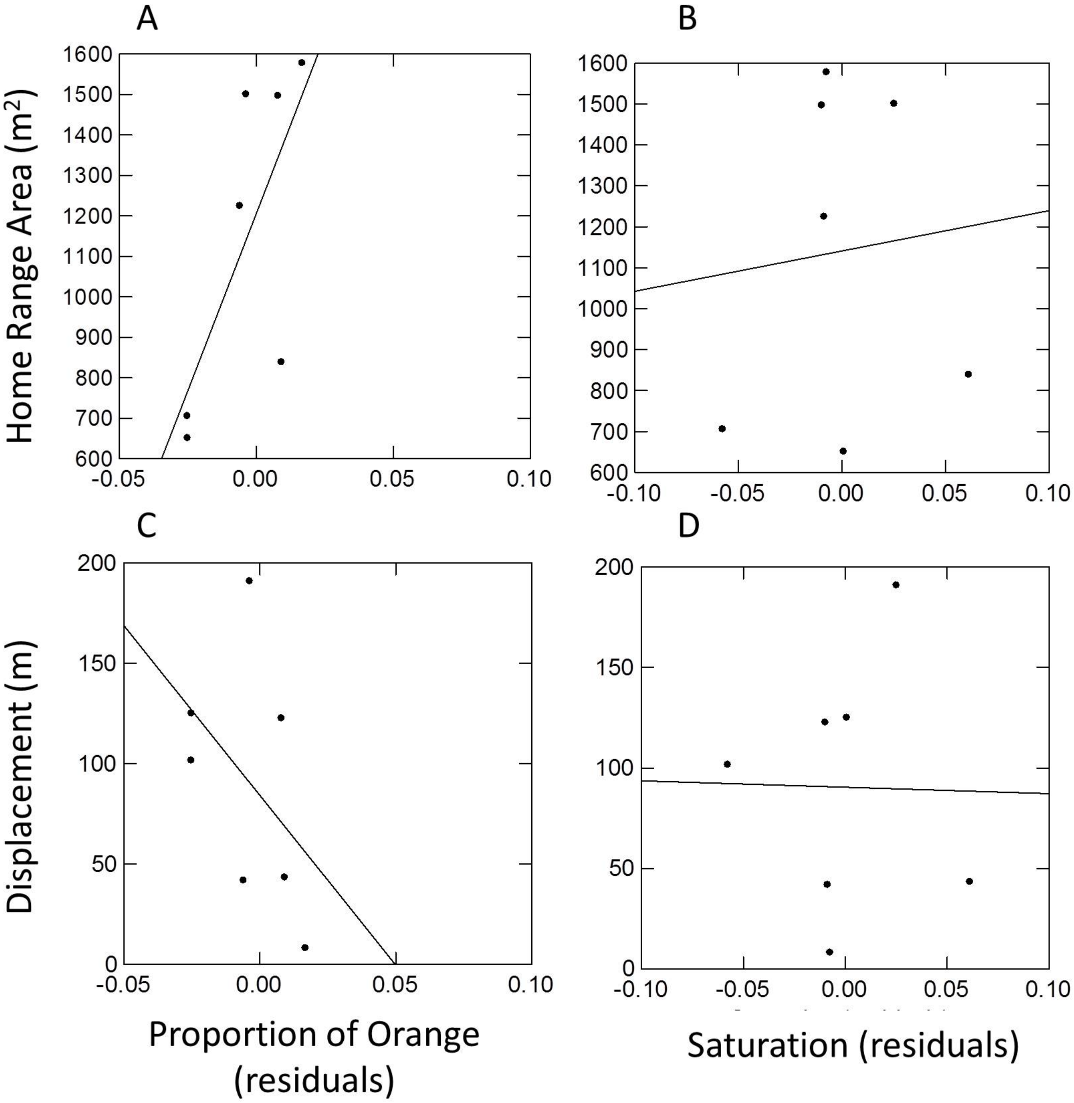

Figure 4.3. Relationships between proportion of orange (A, C) and saturation (B, D) as hatchlings with adult male home range area (m^2) and displacement (m) from their average location when hatchlings to their average location when adults (*n* = 7).

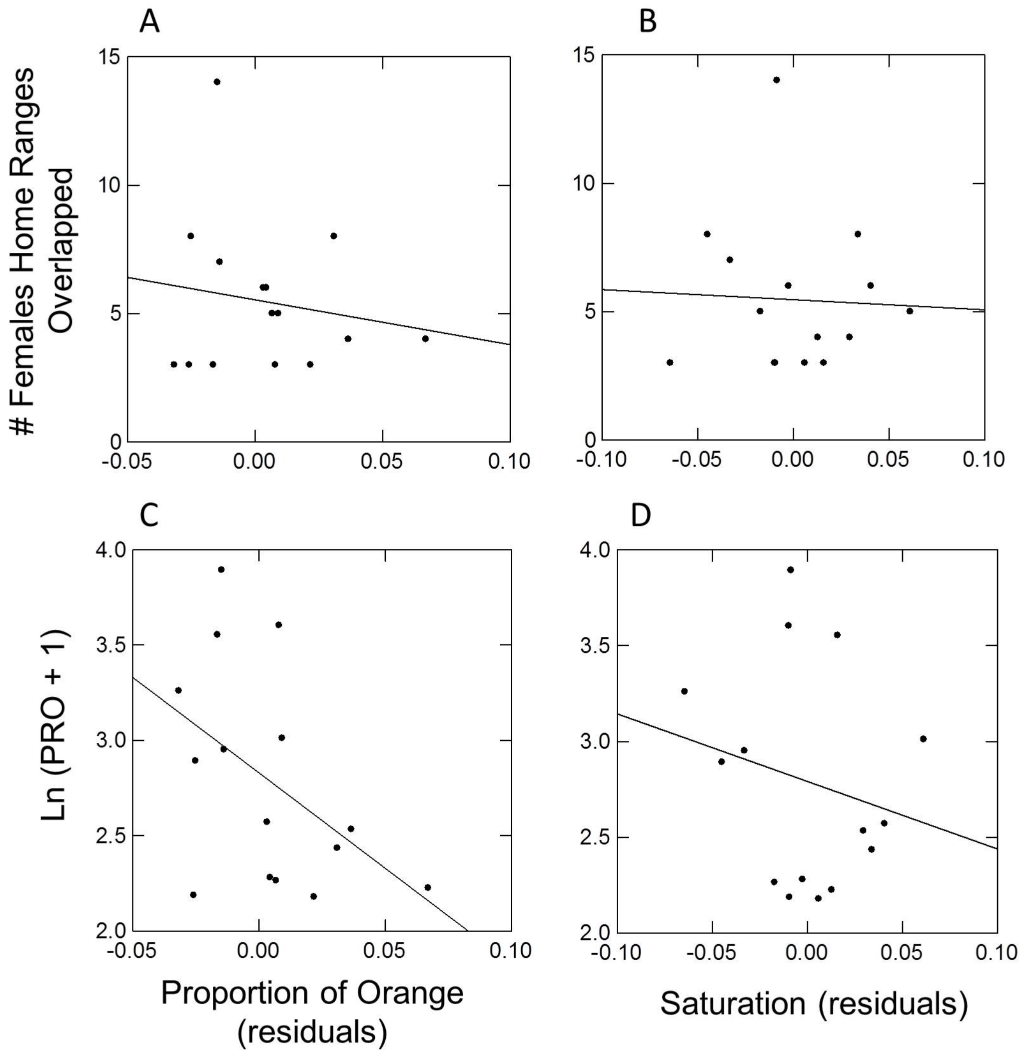

Figure 4.4. Relationships between proportion of orange and saturation as hatchlings with yearling estimated mating success (A, B), and potential reproductive output (PRO; C, D). Only males with two or more sightings (i.e., with female overlap) were included in these analyses (*n* = 15).

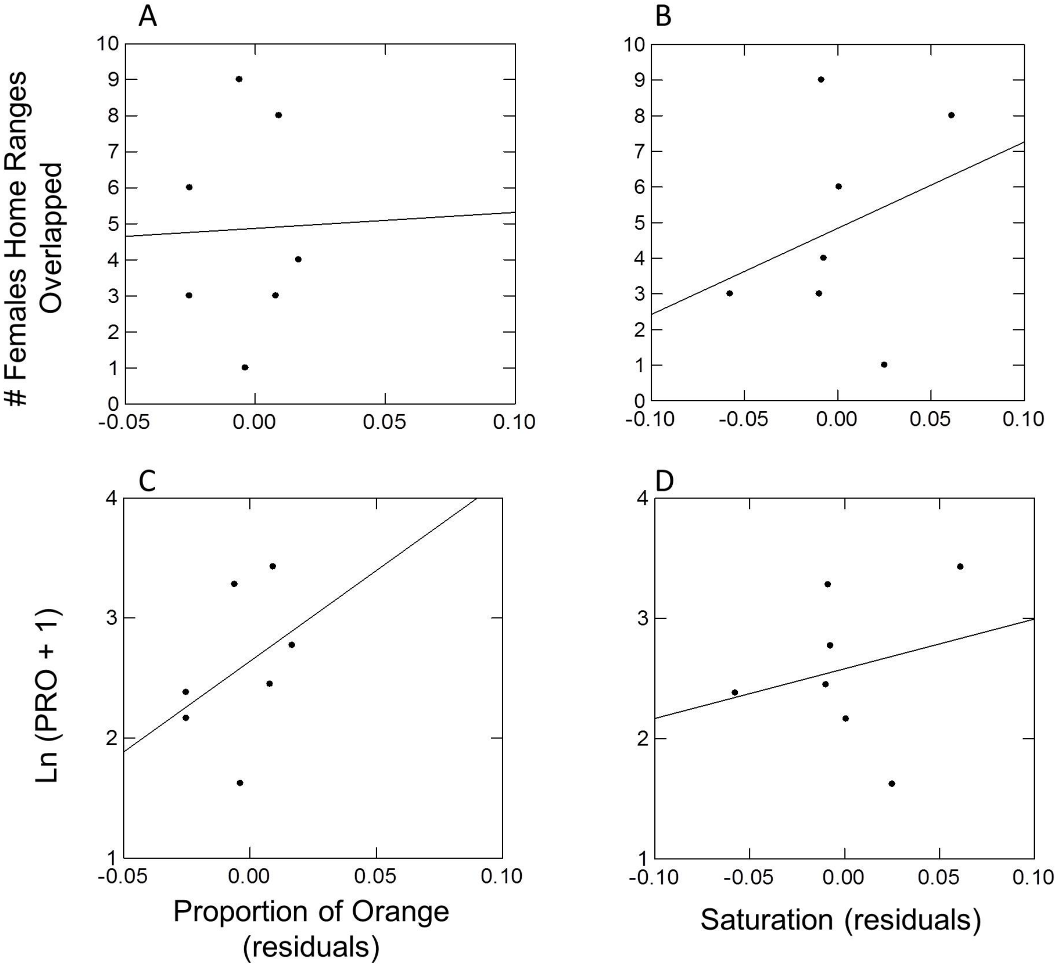

Figure 4.5. Relationships between proportion of orange and saturation as hatchlings with number of females overlapped (A, B), and potential reproductive output (PRO; C, D) when adults (*n* = 7).

VITA

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Doctor of Philosophy

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Biographical:

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- Completed the requirements for the Master of Science in Biology at University of Texas at Arlington, Arlington, Texas, in 2009.

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I served as collections manager while a doctoral graduate teaching assistant. During this time I catalogued and managed collections, administered specimen loans and information requests, conducted education and outreach activities, trained and managed undergraduate employees and volunteers.
As a part of my master's thesis, I worked on the phylogenetic relationships of the genus *Sceloporus* (Squamata: Phrynosomatidae), *formosus* group. Since previous molecular analyses of the group had been conducted using mainly mitochondrial markers, such as ND4, I incorporated nuclear markers, such as C-mos and ODC.
For my undergraduate thesis I conducted a pioneer study of the spatial distribution and habitat preference of an endemic species of coral (*Dendrogyra cylindrus*) for the Archipelago of San Andrés and Providencia, Colombian Caribbean.

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