PRECOCIAL SEXUAL SELECTION
IN *CROTAPHYTUS COLLARIS*:
A FIELD STUDY

By

JUSTIN W. AGAN

Bachelor of Arts in Philosophy
Clayton State University
Morrow, Georgia
2013

Bachelor of Science in Biology
Clayton State University
Morrow, Georgia
2013

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Dissertation Approved:

Dr. Stanley F. Fox

Dissertation Advisor

Dr. Matthew B. Lovern

Dr. Jennifer L. Grindstaff

Dr. Timothy O’Connell

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Abstract: Color traits in animals can take on a variety of forms and serve many functions, including warnings to predators, advertisements to attract mates, and deterrents to rivals. Color is often sexually dimorphic and has variations in expression that can be used by adults to increase or decrease the fitness of the bearer because of sexual selection. Precocial sexual selection is proposed to occur when a sexually immature juvenile uses sexual dimorphism to increase its fitness as an adult. This study tests this idea by determining the development, costs, and benefits of the unique sexual dimorphism seen in juvenile collared lizards. Juvenile male collared lizards possess a sexual dimorphism in the form of dorsolateral orange bars that are lost upon reaching sexual maturity. To understand the development of the bars, we conducted feeding trials using carotenoids. We then conducted a four-year field study where we artificially modified the bars and aggression of male lizards to learn how the bars were used. Following this, we modeled the visual systems of predators and measured the survivorship of the lizards in the field study to show the costs of possessing the bars. Lastly, we calculated the fitness of lizards for the first year after reaching sexual maturity. We found that pteridines are likely the pigment used to express the orange bars and that higher egg incubation temperatures caused the color of the bars to be redder. We demonstrated that lizards use their bars and aggression as a linked signal and unmodified lizards retaliated against lizards that used an incongruent signal, meaning a lizard that had an increased amount of one signal without an increase in the other. We also found no difference in survival among our treatments despite the bars being conspicuous to the vision of predators. Lastly, we found that bars did not impact the fitness of the lizard, but instead found that more aggressive lizards were significantly more likely to produce offspring as yearlings. Overall, precocial sexual selection appears to favor more aggressive juveniles and the bars appear to operate as a socially maintained honest signal of the aggressiveness of the bearer.

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CHAPTER I

UNDERSTANDING THE ROLE OF CAROTENOIDS, PTERIDINES, AND INCUBATION TEMPERATURE IN THE EXPRESSION OF ORANGE BARS IN JUVENILE COLLARED LIZARDS

ABSTRACT

Color plays an important role in animal communication and animals can express colors in many ways. In reptiles, orange and red are common conspicuous signals. While most reptiles use pteridines, or metabolically derived pigments, to express color, some use carotenoids or use carotenoids in conjunction with pteridines. Only plants can produce carotenoids and so animals acquire them solely through their diet. Many speculate this process to play an important part in honest signaling, meaning that species that integrate carotenoids into a signal can produce a strong, bright signal only if they are superior foragers, i.e., an honest signal. We tested factors that may impact the expression of the orange bars seen in juvenile collared lizards (*Crotaphytus collaris*): geographic locality, egg incubation temperature, and diet (with and without supplemental carotenoids). Throughout the 12-week experimental period of dosing carotenoids, we measured the reflectance of the orange bars and calculated their brightness, saturation, and hue. We found that geographic locality had a significant impact on brightness and hue and that lizards incubated at higher temperatures produced redder bars than those incubated at lower temperatures. We also found that pteridines are likely the dominant pigment responsible for the orange color of the bars. Carotenoids might also play a small but additional role because lizards dosed with carotenoids produced the lowest peak wavelengths for hue. We do not know if the effect of the supplemental carotenoid is localized to the orange bars or alters coloration over the whole body. Overall, we found that dosing lizards with lutein does not impact orange bar expression, but locality of the mother and egg incubation temperature can impact how the bars are expressed.

INTRODUCTION

Males and females possessing different colors (sexual dichromatism) is an important part of sexual selection and often evolves through male competition or female choice (Andersson, 1994). In birds and mammals, these colors can be achieved by one sex depositing extra pigments, like carotenoids and melanins, into specific keratinized tissues, by reflectance of the nanostructures within the tissues, or a combination of the two (McGraw, 2004; Dufresne et al., 2009). In reptiles and other ectothermic vertebrates, the process is similar but instead of placing pigments into keratinized tissue, they deposit layers of chromatophores in the skin to absorb and reflect light to create dichromatism (Grether et al., 2004; Olsson et al., 2013). The dermal chromatophore unit contains xanthophores and erythrophores, iridophores, and melanophores and each layer plays its own role in absorbing and reflecting light to create a color. The xanthophores and erythrophores use carotenoid and pteridine pigments to create yellow to red coloration by filtering the light coming into the chromatophore unit. The iridophores reflect light back through the xanthophores and the color and intensity depends on the structure of the iridophores and the amount and types of pigments in the xanthophores. The melanophores contain melanin pigments that absorb light, causing the color to darken when more melanins are present (Grether et al., 2004; Olsson et al., 2013). Conspicuous coloration in reptiles often relies on carotenoid and/or pteridine pigments (Steffen & McGraw, 2007; McLean et al., 2017).

Carotenoids are a type of pigment that produces red, yellow, and orange colors seen in many animals. Plants produce carotenoids and animals cannot produce them *de novo*; they are ingested, absorbed, and sometimes modified through metabolic processes by animals (Hill, 2000; Olson and Owens, 2005; Peters, 2007). Animal characteristics with carotenoid pigments have received much attention because they serve as honest signals. As animals attain carotenoids only through ingestion, cheating is prevented, and the signal functions as an honest display of foraging success (Hill, 2000). Additionally, carotenoids may function as antioxidants, improve immune function, and can act as a signal for the health of the animal (Chew, Park, 2004; Peters, 2007; Svensson & Wong, 2011). Pérez-Rodríguez (2009) points out that these functions may not always be expressed together and there is much variation in the role of carotenoids within a taxon, and much more among taxa, so it is dangerous to extrapolate function across taxa.

Pteridines, unlike carotenoids, are synthesized in animal cells using guanosine triphosphate (Ziegler, 1965, Nichol et al., 1985), and development of pteridine-specific color traits are largely determined by genetics (Grether et al. 2005). Pteridines create similar colors as carotenoids without the cost of foraging associated with carotenoids. Some forms of pteridines also appear to affect immune function by being released when the immune system is activated (Huber et al., 1984) and can reduce oxidative stress by scavenging free-radicals (Oettl and Reibnegger, 2002). While the pteridine drosopterin is the most likely pteridine found in squamate color traits (Macedonia et al., 2000; Steffen and McGraw, 2009; Weiss et al., 2012; Haisten et al., 2015; McLean et al., 2017; McLean et al., 2019), the physiological impacts of ornamental pteridines have not yet been evaluated.

Carotenoids mediate production of orange color in many animals. Although the presence of carotenoid pigments is not well investigated in reptiles, carotenoids have been seen to play a role in the coloration of the orange dewlap of anole lizards (Steffen and McGraw 2007; Steffen and McGraw 2009), the orange colored frills of frillneck lizards (Hamilton et al. 2013; McLean et al., 2017), and the yellow-orange belly of the common lizard (Fitze et al., 2009). On the other hand, pteridines, not carotenoids, have been found to produce orange coloration in the throat patches of female *Sceloporus virgatus* (Weiss et al. 2012) and are used in conjunction with carotenoids to produce orange coloration in some anoles (Steffen and McGraw 2007; Steffen and McGraw 2009).

Collared lizards are sexually dimorphic lizards (males are larger than females) and dichromatic (males express blue/green and yellow/orange colorations) that use both differences to influence fitness (Husak et al., 2006a; Husak et al., 2006b; York, Baird, Haynie, 2014; Plasman et al., 2015). This species is an excellent model for study of carotenoids and pteridines because male juveniles develop conspicuous dorsolateral orange bars two to three weeks after hatching and lose them just prior to sexual maturity. Juveniles develop the bars in early fall after most adults have retreated for winter (Trauth, et al., 2004, Fox et al., 2020) and these juveniles appear to mimic the mating behavior of adult males by forming pair bonds with juvenile females while establishing and defending territories against other males, then use that pair recognition the next spring for mating (Fox et al., 2020). To understand the importance of the orange bars, we need to understand how and why the bars develop and what may influence their development. It is not yet known what drives the production and variation of the orange bars, but knowing this could help to identify a potential physiological cost that might help to explain the signal honesty.

Because lizards obtain carotenoids only from their diet, we can control the amount that is available to lizards in a laboratory setting. The role of this experiment was to determine if carotenoids in the diet of juvenile collared lizards impact production of the juvenile orange bars. Because collared lizards are thought to follow genotypic sex determination (GSD) that can be influenced by temperature (Santoyo-Brito et al., 2017; Wiggins et al., 2020), we incubated lizards at three different temperatures. We incubated lizards at different temperatures for three reasons. First, Santoyo-Brito et al. (2017) reported higher proportions of males produced when they incubated collared lizards at high and low temperatures. This would help us attain a higher sample size of males. Second, by utilizing different temperatures we could help space out the workload over a longer time as lizards that incubated at lower temperatures would take longer to hatch. Lastly, this would give us an opportunity to see if different incubation temperatures might alter carotenoid uptake and bar expression. We then conducted feeding trials on lizards beginning shortly after hatching to determine if juvenile males who received carotenoids produced different colored bars than those who received no carotenoids. We therefore proposed two hypotheses: 1) orange bar expression will increase in lizards dosed with carotenoids compared to controls, and 2) orange bars will be impacted by incubation temperature and show differences in expression related to temperature.

METHODS

Egg collection and incubation

During May and June 2018, we collected 15 gravid female lizards from the Glass Mountains (GM, Major Co.), Kaw Lake (KL, Kay Co.), and Sooner Lake (SL, Pawnee Co.) in northern Oklahoma. In a prior pilot study, we also collected 8 gravid females from GM and KL in May and June 2016. Their eggs were pooled with those of the main 2018 study to analyze for effects of parent locality and parent age on clutch size, and to see if incubation temperature affected the sex of the hatchlings. We did not include the offspring from the pilot study in the subsequent carotenoid dosing study or measurements of color. We housed all female lizards individually in large wooden cages (40 cm x 80 cm x 40 cm), each provided with sand substrate and bricks for basking/hiding. Every tank received illumination from a 100-watt light bulb for heat, a ZooMed ReptiSun 10.0 t8 light bulb for

UVB, and a nesting chamber that mimics nesting habitat in natural environments (Santoyo-Brito et al., 2012). As the females laid the eggs, we split each clutch of eggs evenly among three temperature groups: 27°C, 30°C, and 35°C. We placed eggs in covered individual jars on a moist, vermiculite substrate and monitored them until hatching. Upon hatching, we gave each lizard a unique toe clip and placed recently hatched lizards individually in 37.8-L terraria. Before starting the feeding trials, we waited until all lizards reached 4 weeks of age, after which we weighed each lizard and took bar reflectance measurements to record an initial color measurement. We continued weighing and measuring reflectance each week until the end of the 12-week dosing period. We fed lizards a mix of crickets and mealworms. We raised mealworms in-house and fed them oats, moistened flour, and calcium to prevent accidental carotenoid exposure to control lizards. We shipped in crickets from The Bug Company (Ham Lake, MN), which were set aside for a couple of days before being used as food to clear any carotenoids from their gut that may have been fed to the crickets by the breeder. We fed crickets potatoes and oats with care to prevent exposure to carotenoids.

Carotenoid dosing and color measurements

Half the lizards received a dose of carotenoids (lutein) and half received a water dose (control). We used lutein because preliminary work measuring the optical absorption spectra of the pigments in the bars presented a small peak that matched the wavelengths we should see for lutein (Agan unpublished data). While the peak was small overall, this informed us that carotenoids might influence bar expression. We gave carotenoid doses using Doctor’s Best Lutein with Lutemax, where each pill contained 20 mg of lutein, a type of carotenoid. We used a 10-100µl micropipette to orally dose the lizards three days a week. Each dose equated to 5 µg of lutein per gram of lizard. The dose was determined by reference to Weiss et al. (2012). Before dosing, we weighed each lizard at the end of each week, starting from the first week the initial color measurement was collected, and used that mass for the following week to administer the correct amount of lutein. Lizards receiving a water dose received their dose in the same way as the carotenoid-dosed lizards.

We measured reflectance of the lizards' orange bars using a UV-Vis spectrometer (USB 4000, Ocean Optics), deuterium-halogen light source (DH-2000-BAL, Ocean Optics), probe (QR400-7-SR-BX, Ocean Optics), and SpectraSuite software (Ocean Optics). We held the probe at a 90° angle from the skin and used an opaque hollow tube attached to the end of our probe to standardize our measurements to a 2-mm diameter collection field 7 mm from the skin. We took four reflectance color measurements of the orange bars on each lizard each week after the lizards reached four weeks of age. We took two measures of bar reflectance on the left and right side of the neck and two from the left and right dorsolateral bars about halfway down the body (Figure 1). We later used these measurements to calculate the color variables of brightness, saturation, and hue. We also took reflectance measurements of each subject prior to dosing to set a base reflectance without carotenoids. We started dosing after the first reflectance measurement and took the same measurements each week for 12 additional weeks, giving us 13 sets of measurements (one set one week before and 12 sets during dosing) in total for each lizard. At the end of the trial period, we used the CLR: Colour Analysis Program (v1.05; Montgomerie, 2008a) to create data files for use in the RCLR28.R program in R (Montgomerie, 2008b). Using RCLR28.R, we calculated B2 (mean brightness), S1R (saturation at high wavelengths), and H1 (hue at peak wavelength). We averaged B2, S1R, and H1 for all four measurements each week. B2 is the average of all percent reflectance values (R_i) from the minimum wavelength (λ_{min}) to the maximum wavelength (λ_{max}) and is useful as a metric of brightness as it can easily be compared to other individuals or species despite use of different wavelengths (Montgomerie, 2006). For saturation, we used S1R because it calculates chroma from 605 nm to λ_{max} (750 nm), which covers our orange-red spectrum of interest. We calculated S1R by dividing the total reflectance of the orange-red spectrum (605 – 750 nm) by the total reflectance for the entire reflectance window (400 – 750 nm). For hue, we chose H1 because we wanted to know if any of our explanatory variables would shift the spectral location, or the wavelength at the maximal percent reflectance ($\lambda_{R\max}$). One problem with H1 is that the variable is determined by a single location and is susceptible to potential random noise in the reflectance measurements during collection (Montgomerie, 2006). We tried to control for any random fluctuations by averaging together our four H1 measurements to calculate an average H1 for each lizard. We applied this same method to create average B2 and S1R variables so that each measurement was more of a measure of the orange bars across the body and not of a single location.

Initially, we used λ_{min} of 400 nm, as collared lizard orange bars do not show much ultraviolet reflectance (Agan et al., in review), and λ_{max} of 700 nm to be consistent with other studies that have looked at orange bar reflectance (e.g., Crosby, 2015; Wiggins, 2018; Agan, in review). Upon closer examination of the data, we found that lizards incubated at 35°C often had $\lambda_{R\max}$ in slight excess of 700 nm, therefore we raised our λ_{max} to 750 nm for all calculations. Using the higher λ_{max} captures the slightly higher wavelength peaks seen in some lizards, reduces artificial clustering that would make data analysis more difficult, and accounts for any potential reflected light between 700 nm and 750 nm.

Statistical analysis

For the analyses of clutch size and offspring sex ratios, we pooled the data from the main study and the pilot study. As the number of eggs produced was not normally distributed, even after applying transformations, we ran a non-parametric Kruskal-Wallis test to see if there was an effect of parent locality on clutch size and a Mann-Whitney U Test to see if age of the mother affected clutch size. Female age class was determined by the lizard's snout-vent length (SVL). The smallest adult females are approximately 84 mm SVL, and whereas some age-certain yearling female lizards can slightly exceed this size, the vast majority are smaller than 84 mm SVL (Agan and Fox unpublished data). We collected 8 gravid females in 2016 and 15 gravid females in 2018, and together they produced 117 eggs. There was a significant difference in clutch size depending on the age of the mother ($U = 127$, $p < 0.001$, $r = 0.822$), Adult mothers ($n = 13$) produced 6.23 eggs on average with a range of 5-7 and yearling mothers ($n = 10$) produced 3.6 eggs on average with a range of 3-5.

Because of this age effect, we included maternal age (and also locality) as independent variables in analyses of clutch size. We also included incubation temperature in analyses of sex ratios of hatchlings. We used Chi-squared tests to test for age differences between parent localities and to test for an effect of incubation temperature on the sex of the hatchlings. Although we included these data here, they are not our primary focus in the investigation, but were retained in case they were relevant to the principal results.

For the color analyses, we used data only from 2018 and ran linear mixed-effects models with offspring ID as a random effect, our color variables as the response variables, and dosing type (carotenoids or water), incubation temperature (27°C, 30°C, or 35°C), locality of parent, and week of measurement as the explanatory variables. We fit all models using a restricted maximum likelihood approach. We plotted a histogram of each model's residuals to check for normality and plotted the model's residuals versus the model's fitted values to check for variance in the data prior to running analyses. We used least square means for pairwise comparisons of values across different groups in our linear mixed-effects models. We ran all analyses in R version 4.1.0 (R Core Team, 2021) using the packages nlme (Pinheiro et al., 2021), car (Fox and Weisberg, 2019), and lsmeans (Length, 2016).

RESULTS

Egg collection and incubation

There was no significant difference in number of eggs laid by locality (H = 3.723, df = 2, p = 0.16); lizards from KL generally produced smaller clutches than those from GM and SL (mean 4.25 eggs/lizard at KL versus 5.33 at GM and 5.83 at SL). There was a slight, non-significant age difference in gravid females among localities, with KL having a yearling:adult ratio of 3:1, while GM was 1:2 and SL was 1:5 (x^2 = 5.367, df = 2, p = 0.068), so age differences of mothers among sites may explain part of the tendency of clutch size differences among localities. Females collected from

SL had the highest hatching success with 71.3% success, while the hatching success of GM females was 44.3% and KL females was 41.1%, and again these differences may partly be due to age differences of mothers among localities. Incubation temperature had a significant impact on the sex of the hatchling ($x^2 = 11.378$, df = 2, $p < 0.01$, Cramer's V = 0.373) with 78% being males at 27°C (20 of 26 lizards), 71.4% being female at 30°C (10 of 14 lizards), and a near even split between males and females at 35°C (17 of 31 lizards were male, or 55%).

Carotenoid dosing and color measurements

We excluded all KL juveniles from the color analyses as we had only two successfully hatch in 2018 and only one that survived the full study period. All our models explained significantly more variation than the null hypothesis: B2 – Cox and Snell $R^2 = 0.376$, $p < 0.001$; S1R – Cox and Snell $R^2 = 0.638$, $p < 0.001$; H1 – Cox and Snell $R^2 = 0.603$, $p < 0.001$. For B2 and H1, we saw significant differences in the response variable based on locality of the lizard's parent ($p < 0.01$ and $p < 0.05$, respectively; Figure 2). The week of measurement was significant in all models and showed that as lizards aged, values for the color variables all increased (B2 – $p < 0.001$; S1R – $p < 0.001$; H1 – $p < 0.001$; Figure 3). Lizards incubated at 35°C had significantly higher peak wavelengths (i.e., bars were redder) than lizards incubated at lower temperatures ($p < 0.001$; Figure 4). The dosing treatment (carotenoid vs. water) did not have a significant impact in any of our models, although for offspring with parents from either GM or SL, carotenoid-dosed offspring exhibited slightly lower hues by the end of the dosing period (mean H1 of last four weeks, Figure 5).

DISCUSSION

Our results show that lutein did not play a significant role in the expression of juvenile orange bars in collared lizards and instead suggest that pteridines are likely more at play (or possibly some carotenoid other than lutein). That said, it did appear that lizards dosed with carotenoids expressed peak hues at a slightly lower wavelength than lizards dosed with water. We think that while pteridines (likely drosopterin) are the dominant pigments used to produce the juvenile orange bars, carotenoids may be allocated in small amounts to the bars and may alter the color in a minor way. In *Sceloporus virgatus,* the orange patches on the throats of females are shown to be made up predominately of drosopterin with small amounts of carotenoids, but levels of carotenoids in the orange patches did not differ from the levels found in the surrounding skin (Weiss et al., 2012). It may also be the case for our juvenile orange bars that the carotenoids we think are present in the bars are found at the same concentrations in the adjacent skin. We also found that lizards incubated at a higher temperature produced bars that were redder, and that parent locality impacted how the bars expressed, thus demonstrating how both environmental (temperature-caused shift in bar color) and genetic/environmental (expressed color based on population) factors can shape the juvenile phenotype. Lastly, as expected, we saw that ontogeny had a big impact on B1, S1R, and H1; as juveniles aged, the bars increased in all color variables. This reinforces the field observation that lizards increase their orange bar signals as they age (Crosby, 2015; JA, NB, SFF, personal observation).

Juvenile collared lizards, at some level, hormonally regulate the orange bars. Lizards that receive exogenous dihydrotestosterone (a testosterone derivative) produce proportionally larger orange bars that have a higher orange saturation (Crosby, 2015). Hormones play an important role in regulating individual variation, and androgens, in general, mediate color signals in multiple reptile species (e.g., Cooper and Ferguson, 1972a,b; Medica et al., 1973; Sinervo et al., 2000; Cox et al., 2005). Interestingly, adult female collared lizards produce similar orange bars and appear to use the bars in a similar fashion as males to attract the opposite sex (Baird, 2004; York and Baird, 2019). The female bars develop over a short period during the breeding season and reach maximal brightness around the same time the lizards start ovulation (Ferguson, 1976, but see Baird, 2004). Female bars appear to be induced by progesterone (Cooper and Ferguson, 1972a,b). Progesterone induction of orange bars also appears to be the case for female long-nosed leopard lizards (*Gambelia wislizenii*), a species within the Crotaphytidae family (Medica et al., 1973). Because of the rapid onset of orange in the female orange bars, it seems unlikely that the females are storing carotenoids for subsequent expression of the bars, and instead are likely producing the bars as needed from metabolically derived pteridines. Our juvenile males are likely operating in a similar way, where increases in specific hormonal cues activate the mechanisms for the allocation of pigments (mostly pteridines) to the skin. While this hypothesis explains the mechanism of expression of the orange bars seen in males and females, it does not explain the variation of orange seen in newly hatched lizards. Upon hatching, male and female lizards can have bars across much of their body that is entirely orange, entirely yellow, or a mix of the two with the orange being more intense nearer the head (JA, personal observation). The variation in color upon hatching could be a result of the maternal environment as females can deposit carotenoids into egg yolks during vitellogenesis and the amount deposited is variable based on the female’s diet (McGraw et al., 2005). This process has also been observed in some oviparous reptiles (Dierenfeld et al., 2002; Weiss et al., 2011). As juvenile females age, the orange and yellow quickly fade to the dull brown seen in juvenile females, but males retain the orange/yellow bars on the neck and body and gradually enhance the orange bars (Crosby, 2015; our results).

We did not think that locality would have had as strong an impact as it did. The SL site is 140 km east of the GM site with collared lizard populations presumably connecting the two. Despite this, we still saw that GM lizards produced brighter and less red bars. As adults, GM males have a more bluish body color, while adult males from SL have a more greenish body color, which is consistent with previous studies that show significant variation in sexual dichromatism among collared lizard populations (Baird et al., 1997; McCoy et al., 1997). Conversely, juveniles and adult females from GM and SL appear visually indistinguishable from each other (JA, personal observation). That said, we still saw a significant difference in juvenile orange bar expression between both populations. The effect of locality on color could be the result of two different processes. First, as mentioned earlier, the maternal environment could also have an impact on offspring development post-hatching. Females from both sites are exposed to different environments: GM is a natural environment, while SL is human-made and is maintained to prevent vegetation from growing along the embankment. Second, genetics could be playing an important role bar expression. We raised both populations of lizards under identical conditions after we brought females back to the laboratory, meaning the difference seen in locality could be from varying environmental selection pressures within each population. Regardless, if maternal effects or genetics are driving bar expression based on locality, the consistency of both populations in their expression helps us to better understand how an environmental variable (incubation temperature) modified the signal. While we do not know exactly what incubation temperatures are under natural conditions nor how incubation under a constant laboratory temperature (vs. variable temperatures in the field) affects development, we revealed that the juvenile orange bars are susceptible to environmental pressures.

The changes we saw in color of the orange bars could amplify other effects brought on by incubation of eggs at different temperatures, for example growth and foraging behavior (Siviter et al., 2019; Miller et al., 2020). The changes in color could have even broader implications in social structure and behavior. Collared lizards already take part in a complex juvenile social environment (Agan et al., in prep) and younger, but sexually mature, collared lizards take on submissive “sneaker” male roles when older, larger males are present on territories (York et al., 2014). Although we did not see variation in color saturation in our lizards, Crosby (2015) reported that juvenile collared lizards with higher saturation tended to be more aggressive, while lizards with proportionally more orange were significantly more aggressive. As adults, collared lizards show individual recognition (Husak and Fox, 2003). Individual variation in juvenile orange bar expression may play an important role in identifying specific males as well as assessing their social status.

While we did not seek to test the effects of incubation temperature on sex ratios, our results add support to those of Santoyo-Brito et al. (2017). They found that collared lizards, a species traditionally thought to have genotypic sex determination (GSD; Wiggins et al., 2020), seemed to follow an inversion of the temperature sex determination (TSD) II pattern. This means that lizards incubated at higher and lower temperatures produced mostly males, while middle incubation temperatures produced mostly females. They concluded that the species either follows a type of TSD or is GSD with temperature extremes causing sex switching. Our results do not exactly follow the pattern seen by Santoyo-Brito et al. (2017), but we did see a strong majority of males produced at the low temperature and a strong majority of females produced at the intermediate temperature. The part that does not fit into the inverted TSD II pattern in our study is that the lizards incubated at high temperatures, instead of having more males, produced an almost even number of males and females. This finding lends support to the idea that this species follows GSD but can be overridden by environmental pressures (Shine et al., 2002; Holleley et al., 2015; Santoyo-Brito et al., 2017; Dissanayake et al., 2021; Whiteley et al., 2021).

We showed that lutein did not play an important role in orange bar expression and while we do not know the exact pigment being used, we think pteridine pigments are likely being used to produce the signal. As we did not measure the carotenoids available in crickets used as food, which we ordered from a supplier, there is a small chance we may have been accidentally dosing our water group with higher doses of carotenoids than minimal amounts attained through crickets and mealworms raised on a carotenoid free diet. Even so, it is unlikely that this had a major impact on our results as any concentration available from the crickets likely would not have been enough to make a significant difference. We also demonstrated how the environment may impact orange bar expression by way of higher incubation temperatures shifting the hue to a redder color. There is much we still do not know about color expression, the physiological importance of pteridine pigments, and the importance of carotenoids in reptiles, but we now know that orange bars in collared lizards are a malleable, complex signal that can be shaped by an individual's genetics, hormones, and environment to create a unique ornament that plays an important role in both natural selection and precocial sexual selection (Fox et al., 2020).

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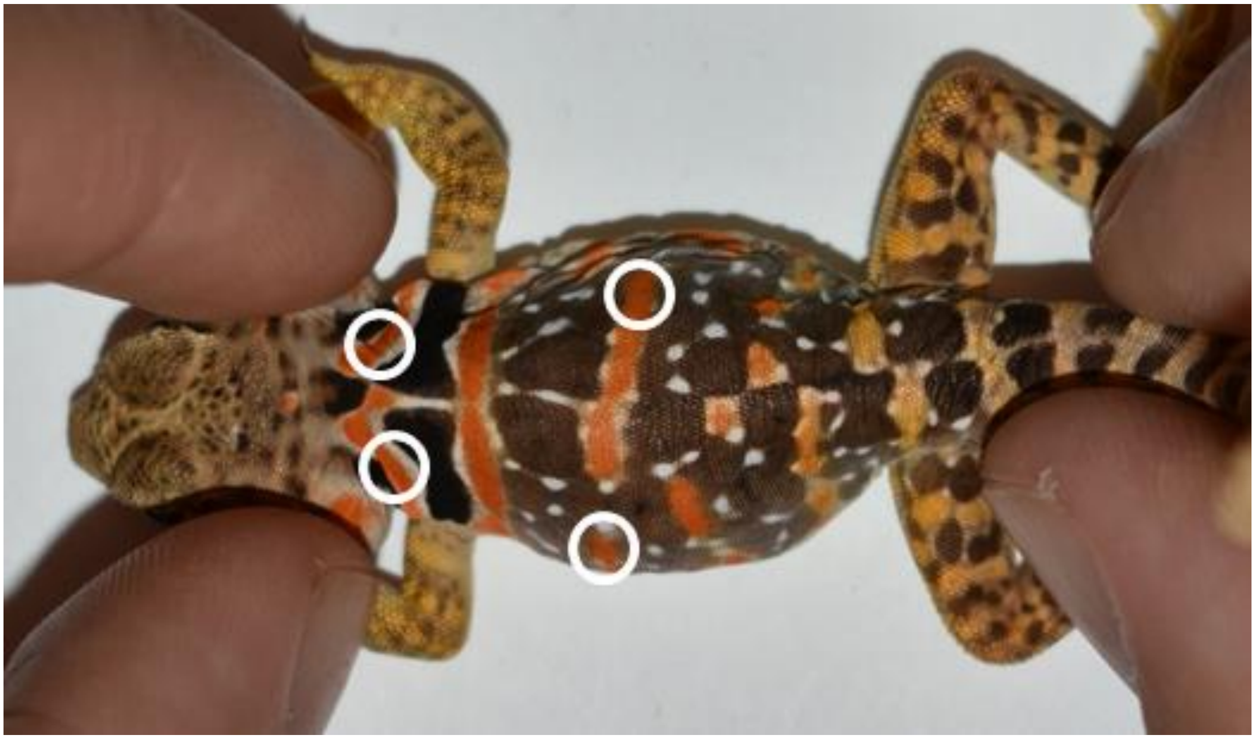

Figure 1. Photograph of juvenile male collared lizard with orange bars. Circles indicate locations where reflectance measurements were taken (not depicted to scale of probe).

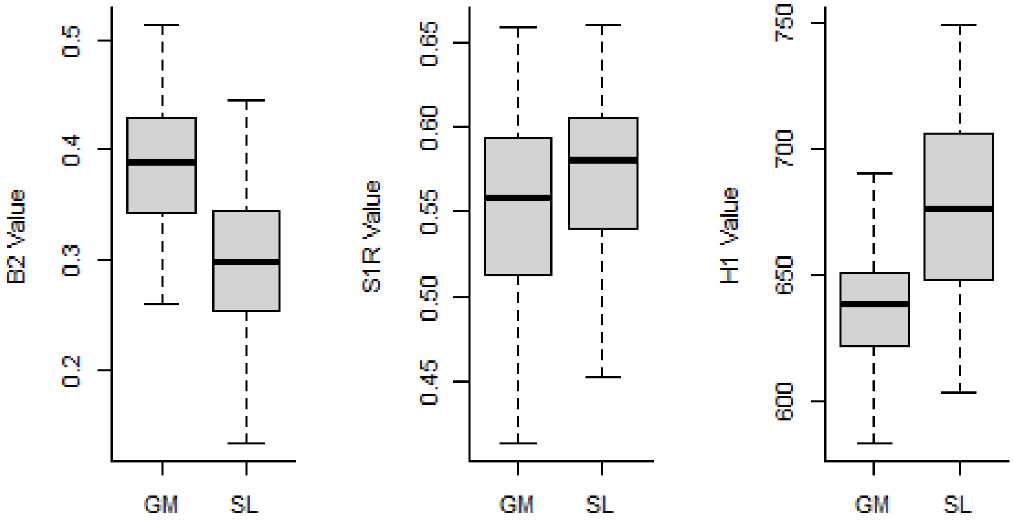

Figure 2. Box plots of color variables between juveniles collected at GM (Glass Mountains, Major Co.) and SL (Sooner Lake, Pawnee Co.) in northern Oklahoma. B2 is brightness, S1R is color saturation, and H1 is hue. Each box represents the central 50% of the data (i.e., the interquartile range; IQR) with a horizontal line representing the median. End caps of the error bars are placed at the minimum value within the range of the 1st quartile minus 1.5 IQR and the maximum value within the range of the 3rd quartile plus 1.5 IQR.

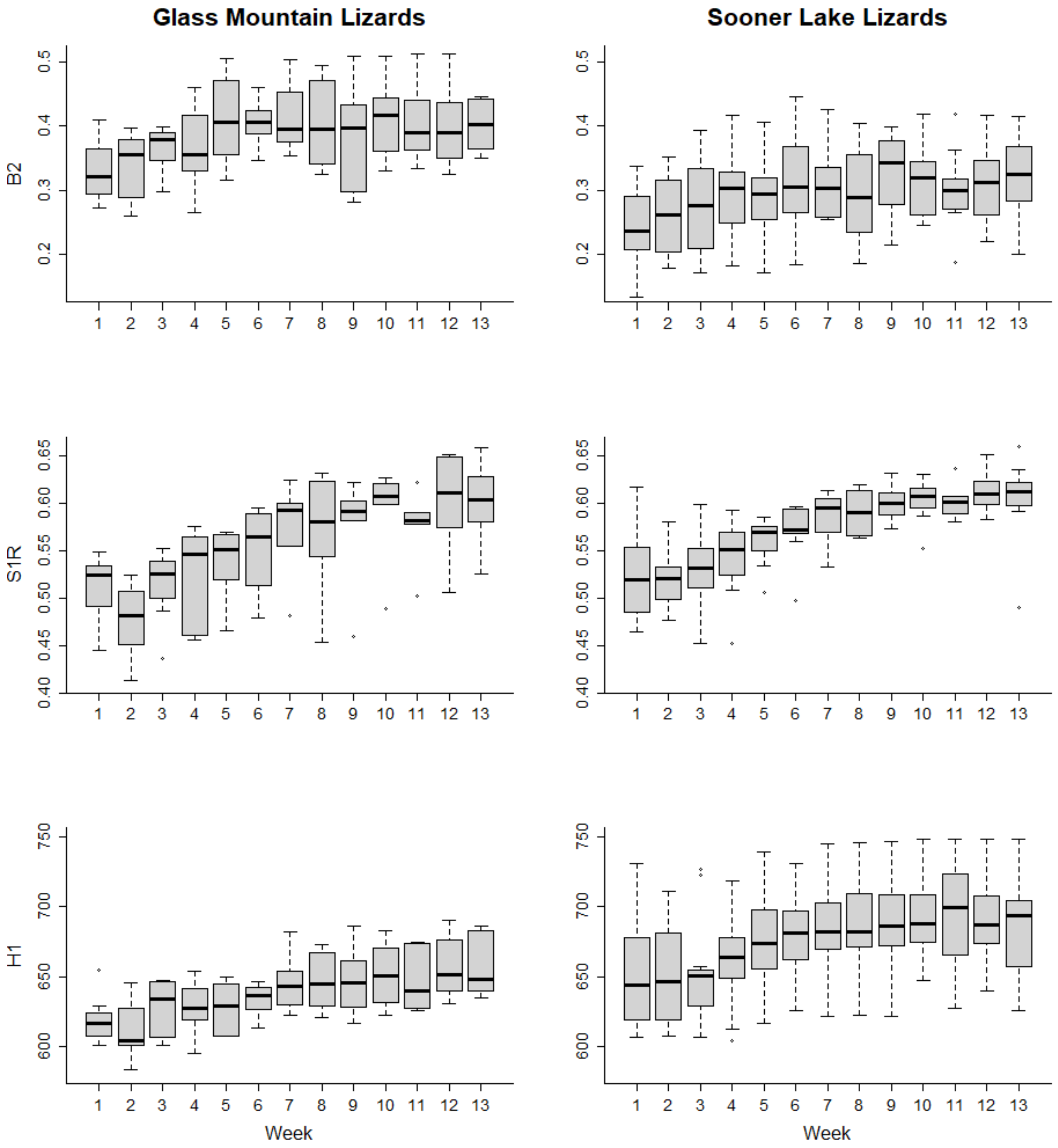

Figure 3. Box plots of color variables by the week of measurement. B2 is brightness, S1R is color saturation, and H1 is hue. Legend as in Figure 2. Outliers are depicted as open circles above or below the end caps.

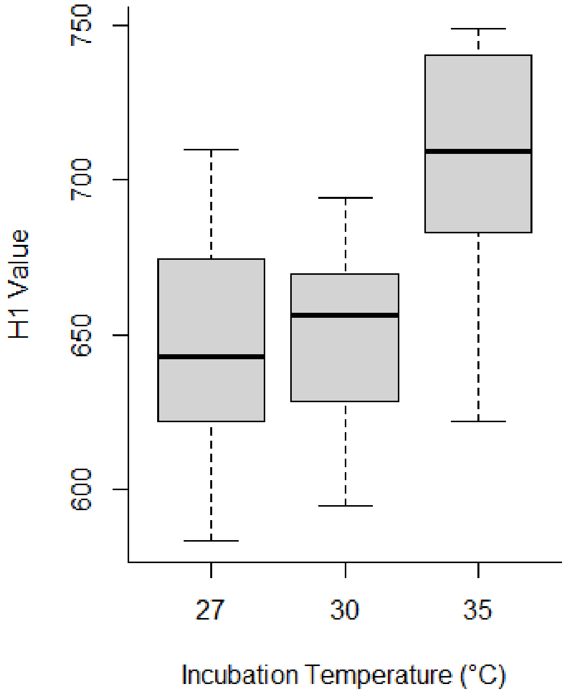

Figure 4. Box plots of H1 variable (hue) by the incubation temperature of the lizards. Legend as in Figure 2.

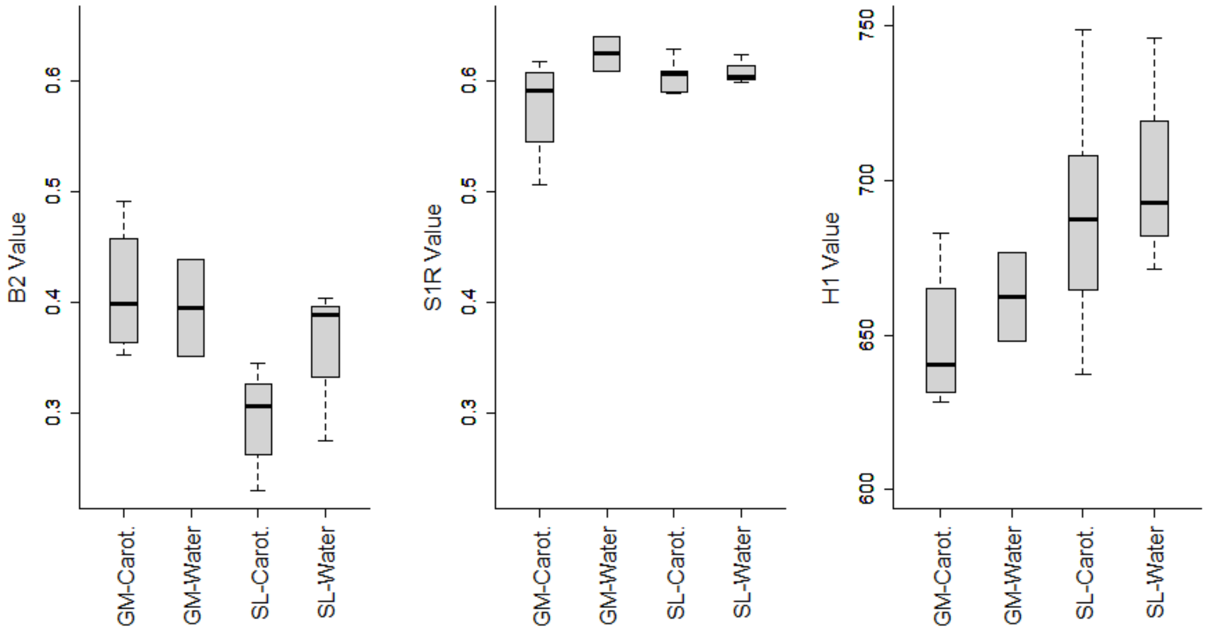

Figure 5. Box plots of color variables by dose treatment and parent locality using the average values for weeks 10-13. B2 is brightness, S1R is color saturation, and H1 is hue. Legend as in Figure 2.

CHAPTER II

JUVENILE MALE COLORATION, DOMINANCE, AND SOCIALLY MAINTAINED SIGNAL HONESTY IN THE LIZARD *CROTAPHYTUS COLLARIS*

The following chapter is formatted for *Behavioral Ecology*:

ABSTRACT

Sexual dimorphism and dichromatism in species are often evidence of positive selection (often sexual selection) and play important roles in signaling the bearer's quality, aggressiveness, or resource holding potential. These signals are considered honest by incorporating mechanisms to prevent individuals from deceiving or faking the signals, which by definition makes the signals costly to produce. In many systems, signal honesty is attained by costly physiological mechanisms, but not in all. In some cases, social policing is used to maintain honesty by evaluating multiple redundant signals, i.e., the signal incongruence hypothesis. This study tests the signal incongruence hypothesis to determine the role of male juvenile orange bars (JOB) in the lizard *Crotaphytus collaris*. In this species, juvenile males, but not females, exhibit JOB, which are lost upon reaching sexual maturation. To test the purpose of these JOB, we conducted a long-term field experiment in which we experimentally altered the expression of the JOB and also increased aggression (or not) via androgen implants in various treatment groups. We found that JOB and aggression are linked as signals of dominance to conspecific juvenile male receivers. When subjects expressed JOB discordant with their level of aggression, opponents responded with significantly increased aggression, i.e., lizards were socially policing each other based on the congruence of opponents’ aggression and visual signal. This system is an example of a juvenile-specific sexual dichromatism that relies on congruent, honest signaling in intrasexual agonistic interactions in the context of precocial sexual selection.

Key words: intrasexual interaction; sexual selection; color; androgen implants; incongruence hypothesis; collared lizard; dichromatism

INTRODUCTION

In most animals, males and females are nearly identical prior to sexual maturity (aside from primary sexual characteristics), but as they mature, they diverge in behavior and develop different secondary sex characteristics, including sexually dimorphic and dichromatic traits. Commonly, these sexually dimorphic traits (including behavior) are advanced and maintained by sexual selection (Andersson 1994; Stuart-Fox and Ord 2004). Many of these dimorphic traits and behaviors are caused by sex hormones that usually increase at sexual maturation (Cooke et al. 1998; Hau 2007). When a dimorphic/dichromatic trait is representative of the health and fitness of an individual, or its resource holding potential (RHP), the trait is called an honest signal and is linked to physiological costs for attaining the signal (Zahavi 1975; Zahavi 1977) or to social costs of agonism against cheaters (Számadó 2011). Honest signals have been documented in many animals; some examples include the color and level of toxicity of some frogs (Blount et al. 2009), the length of songs in male zebra finches (Spencer et al. 2003), and multiple color traits in adult male Dickerson’s collared lizards (Plasman et al. 2015).

One common use of these signals is territory defense/acquisition or dominance status to increase access to the limiting sex (Trivers 1972). These signals can take on various forms in order to achieve this goal. Signals are often used as weapons (Emlen 2008), where they can take form as exaggerated antlers, bills, teeth, etc. However, a signal does not necessarily have to be dangerous to be effective. Often, honest signals indicate to an opponent one's RHP without requiring a contest to decide the winner (Parker 1974). Such signals can save one or both individuals from sustaining injuries that might remove both of them from the contest (Tsubaki and Ono 1985).

While territoriality has been well studied, the relationship between ontogeny and territoriality is not as commonly examined. In birds, the natal territory has been studied to understand how it impacts juvenile development, e.g., a dominant sibling can have better staying potential on the parents' territory and be able to force weaker siblings to disperse first when they are not ready (Strickland 1991), or the dominant sibling will acquire more resources and be able to disperse sooner than the less dominant siblings (Ellsworth and Belthoff 1999). In amphibians without parental care, some juvenile terrestrial salamanders use intra- and interspecific aggression to competitively exclude others from a limited resource (Walls 1990; Nussbaum et al. 2016). For reptiles without parental care, Fox et al. (1981) suggested that in *Uta stansburiana*, more aggressive juveniles were able to acquire higher quality home ranges, which later converted to better territories. These lizards sometimes use the presence of an intact, non-autotomized tail as a status badge to signal dominance over lizards who have autotomized their tail (Fox and Rostker 1982). These studies demonstrate how an individual's ontogeny can have a lasting impact on its future, but do not, however, use early expressed male secondary sex characteristics as central to acquisition of territory or dominance.

In *Crotaphytus collaris*, the Eastern collared lizard, juvenile males emerge from eggs and develop juvenile orange bars (JOB) in early fall after most adults have retreated for winter (Trauth et al. 2004; Fox et al. 2020). These juveniles appear to use the same behavior as adult males to pair bond with juvenile females while establishing and defending territories against other juvenile males (Fox et al. 2020). The JOB are then lost upon reaching sexual maturation in the spring, or just prior to overwintering in late fall (Crosby, 2015). Husak & Fox (2003) showed that in *C. collaris*, adult lizards can individually recognize other lizards by showing decreased aggression toward resident neighbors compared to strangers and increased aggression when those neighbors were displaced to the other side of the resident's territory. Even outside the context of the territory, strangers received more aggression than familiar neighbors. We suggest that individual recognition used in pair bonding in juveniles in the fall carries over to the next spring when mating occurs upon emergence from overwintering, and perhaps also to recognition of rival males who were earlier driven away by the more dominant juveniles in the fall. We think the JOB play an important role in juvenile conspecific interactions that subsequently impact territory and mate acquisition the next spring. The JOB would operate in the same way and are susceptible to the same pressures as the sexually selected traits in adult collared lizards (Husak, Fox, et al. 2006; Husak, Macedonia, et al. 2006; York et al. 2014; Plasman et al. 2015).

This study follows the methods used by Rohwer and Rohwer (1978) by using paint and hormone implants to parse out the influence of the JOB on intra-specific social interactions prior to sexual maturation. Having modified the visual and aggressive signals in wild, free-ranging collared lizard juveniles, we subsequently engaged them in dyadic interactions in a neutral arena in the laboratory to determine how they behaved with their treatments and how unmodified lizards responded to the modified lizards. We hypothesize that lizards will need both enhanced bars and enhanced aggression to benefit from either enhanced signal during intra-specific interactions, just like in Rohwer and Rohwer (1978). From this, we predict that if lizards lack one or the other enhanced signal, they will not do as well during their social interactions as lizards with both signals.

METHODS

Study site and lizard capture

The study was carried out between early August and late October for four consecutive years starting in 2014 at Sooner Lake, Pawnee County, Oklahoma, and at Oklahoma State University. The study site was located on the property of the Oklahoma Gas and Electric Sooner Generating Station and consisted of a roughly one-mile stretch of stone-covered embankment between a gravel access road and the shoreline of Sooner Lake. During the months of August through October each year, we captured all lizards at the site using a noose tied to the tip of a 4-m fiberglass, telescopic pole. We assigned a unique toe-clip for permanent identification for every lizard caught on the site. When toe-clipping, we removed only the very distal phalanx from each digit being clipped and did not clip more than one toe tip per foot. Afterwards, we applied an antibiotic ointment to the digit to help stop any bleeding and to prevent infection. Every lizard also received a unique color code consisting of four small dorsal paint dots using non-toxic paint for visual identification until the next molt. We placed male hatchling lizards into one of three treatments and a control up to 2017, and in 2017 and 2018 we added a fourth treatment.

Treatment groups

Male lizards were placed into one of four treatments using implants with and without dihydrotestosterone (DHT) and painted with spectrally similar paint (Fox et al. 2020) to enhance or mask JOB, and a control group with a blank implant and painted with water—Blank+Bars: blank implant and enhanced JOB (n = 24); DHT+Mask: DHT implant and masked JOB (n = 20); DHT+Bars: DHT implant and enhanced JOB (n = 17); Blank+Mask: blank implant and masked JOB (n = 7); and Control: blank implant and painted with water to control for handling (n = 21).

DHT implants were made following the steps described in Lovern et al. (2004) to a concentration of 1 mg DHT/mm of implant with a total length of 2.5 mm per implant.

Due to the risk of implanting very small hatchlings, subjects were placed in treatment groups only after they reached at least 2.5 g or about 40 mm snout-vent length (SVL). When we captured lizards meeting this size requirement, we brought the lizards back to Oklahoma State University to be implanted. Prior to being implanted, lizards were anesthetized by inhalation of isoflurane at an estimated 5-7% airborne concentration (Carpenter 2005, Mader 2006). While under a fume hood, lizards inhaled the isoflurane vapor until they reached a state of unresponsiveness. Before we implanted the lizard, we cleaned the implant area with chlorhexidine and sterilized the PIT tagging needle used to insert the implant with a hot bead sterilizer (Fine Science Tools Keller Sterilizer 250). Implants were inserted subcutaneously into the left side of the lizards and anterior to the hind limb. After implanting, we used Vetbond Tissue Adhesive (3M; St. Paul, MN) to close the puncture site. After the lizards received the implant, we placed them in a clean 37.8-L aquarium with a paper towel substrate and held them for two days to ensure the implant stayed in place and the implant puncture did not reopen. Following a two-day holding time, we returned the lizards to their point of capture.

Behavioral trials and plasma collection

After males reached 60-mm SVL and had carried an implant for at least two weeks in the field, we recaptured and used them in dyadic interactions in a neutral arena in the laboratory. We placed a lizard from one of the four treatments or control into one side of a neutral arena (45.2 cm x 61.0 cm x 30.5 cm) divided by an opaque partition with a size-matched and novel stimulus male on the other side. Stimulus lizards were not modified and were caught from a nearby, off-site location with no prior interactions with the treatment lizards. From behind a blind, we acclimated the subject lizards for ten minutes to their side of the divided arena, remotely raised the dividing partition, and then gave lizards ten minutes to interact with each other after the first recorded behavior from the treatment lizard. We recorded dominant and submissive behaviors, and each behavior was given its own weight in the construction of a subsequent scaled score of aggression following Crosby (2015). Dominant behaviors (and respective weights) were bite (3), fight (3), attack (3), superimposition (2), push-up (2), circle (2), lateral throat display (2), throat display (2), head bob (2), approach (1), and tail switch (1). The one submissive behavior was retreat (-1). We computed an aggression score for each lizard of the dyad by summing the frequency of each behavior multiplied by its weight. At the conclusion of the trial, lizards were placed back into individual aquaria in the laboratory for a two-day recovery period. Each aquarium had a heating lamp, UVB bulb, water bowl (along with daily misting of the tank), and refuge to hide under. We secured the top of the tank with a wire mesh lid to allow the ultraviolet light to pass through and masked the sides of the tank to reduce stress on the lizards. After a recovery period of two days in the laboratory, we anesthetized lizards *via* inhaled isoflurane at an airborne concentration of 5-7% (Carpenter 2005, Mader 2006) to draw blood and measure each lizard's basal circulating hormone levels. We used a hematocrit microcapillary tube to puncture the post-orbital sinus to draw about 50 µL of whole blood (Moore 1986). We spun the whole blood in a centrifuge for 5 minutes at 6000 rpm to separate the blood cells from the plasma and stored the plasma in a -20°C freezer until analyzed in a radioimmunoassay (RIA) for plasma hormone concentrations.

Hormone analysis

We ran two separate analyses, one for the years 2014–2015 and another for 2016–2018, to determine our plasma concentrations for each of our hormones. Plasma concentrations of testosterone (T), DHT, estradiol (E2), and progesterone (P) were measured following extraction and chromatographic separation described by Wingfield and Farner (1975), Moore (1986), and Lovern, McNabb, and Jenssen (2000). We equilibrated all samples overnight at 5°C with 2000 dpm of each radioactive steroid hormone: ^{3}H-T:NET-370, ^{3}H-DHT:NET-453, ^{3}H-P:NET-381, and ^{3}H-E2:NET-317 (all from PerkinElmer Life Sciences, Inc.) for individual recovery determination. For each hormone, we prepared standards using hormones from Sigma (T:T1500; DHT:A8380; P:P0130; E2:E8875). We then extracted hormones from the samples twice using 2 ml of diethyl ether, then dried in a 37°C water bath and under N. Samples were reconstituted with 500 µl of 10% ethyl acetate:isooctane and let sit at 5°C overnight. We ran samples through columns for chromatographic separation to extract each hormone from every sample. Each of the columns consisted of an upper glycol phase (6:1.5:1.5 w:v:v), diatomaceous earth (Celpure P300:Sigma-Aldrich):propylene glycol:ethylene glycol, and a lower water phase (3:1 w:v), diatomaceous earth:distilled water. We eluted P with 1.5 ml of 2% ethyl acetate in isooctane, DHT with 2 ml of 10% ethyl acetate in isooctane, T with 2 ml of 20% ethyl acetate in isooctane, and E2 with 2.5 ml of 40% ethyl acetate in isooctane. Isolated hormone samples were dried under N in a 37°C water bath, resuspended in 300 µl of phosphate buffer solution, and placed in a 5°C refrigerator overnight. The mean recoveries for each assay were 1) T = 48%, DHT = 79%, E2 = 16%, P = 53% and 2) T = 52%, DHT = 50%, E2 = 52%, P = 48%. We ran the competitive binding RIA using ^{3}H radiolabeled hormones and their corresponding antisera (T:T-3003 from Research Diagnostics, Inc.; DHT:T-3003 from Research Diagnostics, Inc.; P:P5289 from Sigma; E2:7010-2650 from Biogenesis). The standard curve was made in duplicate from 1.95 to 500 pg. We then averaged both standard curves for each hormone and used them to calculate the individual recovery. Average coefficients of variation (CV) for each hormone were T = 32%, DHT = 21%, E2 = 37%, P = 16% for the first assay and T = 8%, DHT = 7%, E2 = 8%, P = 9% for the second assay.

Statistics

The data were analyzed in four steps. First, we analyzed the DHT levels of all lizards to ensure the implants increased DHT levels. Next, aggressive interaction data were analyzed from the perspective of the treatment lizards, then separately from the perspective of the stimulus lizards, and lastly, for both lizards considered together. Because we were not initially collecting behavior of the stimulus lizards, we had a reduced sample size for most of our treatment groups: Blank+Bars (n = 12); Blank+Mask (n = 7); DHT+Bars (n = 8); DHT+Mask (n = 12); Control (n = 12). As the hormone levels were not normally distributed (Shapiro-Wilk test, $p < 0.001$) even after transforming the data, we used a Mann-Whitney U test (2-tailed) to look for significant differences in hormone levels between DHT and blank implants. Generalized linear models (GLM) were constructed to predict how aggression was affected by treatment group, JOB color, four hormone levels (T, DHT, E2 and P), implant type, time to first aggression, and size difference (treatment lizard SVL minus stimulus lizard SVL). GLM were first constructed to explain aggression for treatment lizards, then for stimulus lizards. We then used Akaike's Information Criterion (AIC) with a sample size correction (AICc) to select the best-fitting model and any model with a ΔAIC_C of less than 2 considered as a viable model (Burnham and Anderson, 2002; Burnham, et al., 2011).

We combined the aggression scores of treatment and stimulus lizards to form a social polarity score (treatment aggression score minus stimulus aggression score). Because the data were not normally distributed (Shapiro-Wilk test, $p < 0.006$) even after a log transformation, we used a Kruskal-Wallis test to evaluate differences in the social polarity scores among the treatment groups. We followed this with post-hoc Mann-Whitney U tests (2-tailed) for between-group differences.

RESULTS

Hormone levels

Lizards that were given DHT implants had significantly higher DHT levels than lizards that received blank implants (Mann-Whitney U test; U = 183, $p < 0.001$, r = 0.674; Figure 1). Lizards with a DHT implant had a median plasma DHT level of 36.99 ng/ml (n = 35, IQR = 17.18 – 75.15), while blank implanted lizards had a median plasma DHT level of 1.29 ng/ml (n = 52, IQR = 0.32 – 2.78). There were no significant differences in T, E2, or P between those receiving a DHT implant and those receiving a blank.

Treatment lizard aggression

Treatment lizard aggression was best predicted by whether the treatment lizard received a DHT or blank implant and its circulating DHT levels ($W_{AIC} = 1.0$; Table 1a). Lizards that received a DHT implant were more aggressive than lizards that received a blank implant (Figure 2). The effect of these two variables was strong enough to exclude all other variables.

Stimulus lizard aggression

Stimulus lizard aggression was best predicted by the model using color of the treatment lizard's bars, the size difference of the lizards, and the interaction of the two ($W_{AIC} = 0.871$; Table 1b). The model predicted increased aggression toward lizards whose bars were masked (Blank+Mask and DHT+Mask) and decreased aggression toward lizards with enhanced bars (Blank+Bars and DHT+Bars) and Control lizards (Figure 3). The model also predicted more aggression from stimulus lizards toward larger lizards and predicted an interaction between lizards with masked bars and size difference where stimulus lizards were more aggressive toward larger lizards with masked bars than toward larger control and larger enhanced-JOB lizards (Figure 4).

Social polarity

After subtracting the stimulus lizard's aggression from the treatment lizard's aggression to form our social polarity score, we found there was a significant overall difference among groups in social polarity ($\chi^2 = 31.903$, df = 4, $p < 0.001$, $\varepsilon^2 = 0.638$; Figure 5). When followed up with Mann-Whitney U tests, there was no significant difference between the DHT treatments of DHT+Bars and DHT+Mask, nor was there a difference between the non-DHT treatments of Blank+Bars and Blank+Mask. The DHT+Bars and DHT+Mask treatments had significantly higher social polarity than the control ($U = 4$, $p = 0.001$, $r = 0.76$; and $U = 19.5$, $p = 0.002$, $r = 0.621$, respectively), Blank+Bars ($U = 1$, $p < 0.001$, $r = 0.812$; and $U = 5$, $p < 0.001$, $r = 0.79$, respectively), and Blank+Mask ($U = 1$, $p = 0.002$, $r = 0.808$; and $U = 8$, $p = 0.004$, $r = 0.658$, respectively). The Blank+Bars and Blank+Mask treatments had significantly lower social polarity than the control ($U = 31$, $p = 0.018$, $r = 0.484$; and $U = 16$, $r = 0.505$, $p = 0.028$, respectively).

DISCUSSION

An individual's JOB and aggression together influenced the aggressive interactions between males. When one male encountered another male, it used the JOB as an indicator to how aggressive the opponent should be. When encountering a male with masked JOB, opponents responded significantly more aggressively. Even when masked lizards were very aggressive (made so with DHT implants), opponents retaliated against them because they did not display sufficiently intense JOB. When a male encountered another male with bright bars, he relied on that male's aggressiveness to determine how strongly to respond. Lizards who possessed enhanced bars, but did not act in accordance with their signal, received increased aggression from their opponents. In general, two situations brought on such retaliation in stimulus lizards: (1)

when lizards lacked the JOB but showed aggression, whether increased aggression due to the DHT implant or normal levels of aggression, and (2) when lizards had enhanced JOB and had normal aggression. In situation 1, stimulus lizards retaliated against treatments for having aggression greater than indicated by their signal, and in situation 2, stimulus lizards retaliated against lizards who were not as aggressive as their signal indicated. These results suggest that lizards can detect discordance between the signal and expressed aggression in opponents and react with more aggression when there is discordance, i.e., they are socially policing the JOB signals and using them to scale their agonistic response.

We did not anticipate that the hormone implants would increase the aggressiveness as strongly as they did. In hindsight, smaller doses of DHT or a slower mechanism of delivery may have moderated the aggressiveness of the implanted subject, but one benefit of the strong effect of the DHT implant was the surprisingly persistent aggressiveness of the stimulus lizards in response to the DHT+Mask treatment. Despite how aggressive the DHT+Mask lizard became, so much so that the treatment lizard score was often double or triple the scores of the stimulus opponent, stimulus lizards continued to retaliate against lizards for acting aggressive without the appropriate bars. Additionally, stimulus lizards were also more aggressive to lizards that were larger, especially larger opponents without JOB, meaning body size absent the signal was not enough to deter stimulus aggression. Turning to social polarity scores, we believe that because of the powerful effect of the DHT implant, we were not able to see a clear difference in the social polarity scores between the two DHT treatments, even though the social polarity was less polarized, but not significantly so, in the DHT+Mask treatment than the DHT+Bars treatment. This slightly lower social polarity score was due to elevated aggression by the stimulus lizard toward a very aggressive opponent who did not display bright JOB. Probably less potent implants would have better exposed the difference in response toward the lizards of the two DHT treatments. Regardless, the response of the stimulus lizards demonstrates how important it is for lizards bearing JOB of variable intensity to act in accordance with the intensity of that signal.

Webster et al. (2018) reviewed various biological systems for maintaining signal honesty and suggested that social mechanisms for honesty, or receiver-dependent ones, should be favored *per se* by selection, rather than because the traits might be physiologically costly to produce and maintain. Rohwer and Rohwer (1978) first demonstrated this with the signal incongruence hypothesis. They showed that in Harris' Sparrows (*Zonotrichia querula*), birds used the darkness of their feathers on their head and breast along with their behavior to establish their winter dominance hierarchy. Sparrows that had darker feathers attained a higher social status in winter (and thus fed better at feeders) than birds with paler heads and chests. The authors experimentally manipulated feather darkness and aggression and found that birds needed both increased aggression and the darker colors to gain and retain a higher social status; having just one of the two was not enough. Since then, other studies have shown how social systems can help to maintain signal honesty (e.g., Australasian Swamphen birds, Dey et al. 2014; *Zootoca vivipara* lizards, Martin et al. 2016; *Polistes dominulus* wasps, Tibbetts and Izzo 2010). Like other receiver-dependent signals, juvenile collared lizards use both aggression and the JOB to structure their social system and higher levels of one signal without concomitantly higher levels in the other is cause for retaliation from the receivers. Receivers retaliated against not just those with masked bars and normal/increased aggression, but also against those with enhanced JOB and normal aggression.

It has been demonstrated that color of the JOB is likely conspicuous to predators as well as conspecifics and that predation might select against orange bars (Agan et al., in review). Nevertheless, the benefit of well-developed JOB appears to be an advantage to the bearer of preference by juvenile females that carries over to preference toward those males even after the

JOB are lost upon reaching sexual maturity the next spring, and dominance in agonistic interactions with other male juveniles as territories are established (Wiggins 2018; Fox et al. 2020). On balance, males with well-developed JOB subsequently sire more offspring as yearlings the next spring compared to males with less-developed JOB (Wiggins 2018). We have shown that JOB are an important intraspecific, intrasexual signal, along with the corresponding level of aggression, and together function as a receiver-dependent signal to help juvenile male lizards determine how they should interact with each other. Peers socially regulate other lizards to prevent dishonest signals from occurring. This can potentially have lasting impacts on the lizards by potentially slowing growth through restricted access to better food patches. With substandard feeding, lizards may not grow enough to survive overwintering, a period with high mortality (Agan and Fox, unpublished data). Juveniles with brighter and more extensive JOB are more aggressive (Crosby 2015) and probably drive away juvenile rivals in the fall and form pair associations with more juvenile females, both of which can aid them as yearlings the next spring to secure additional matings. This is beneficial since most collared lizards rely on non-territorial alternative mating strategies in their first year because most males do not defend exclusive territories until their second year (Baird et al. 1996). More recently, York and Baird (2019) showed that females seek multiple mates and the more mates females have, the more offspring they produce. This would imply that if males stay nearer to females in the fall and pair-bond with them, this may increase male fitness. We think the JOB lead to enhanced fitness of the bearers because of precocial sexual selection, or sexual selection occurring prior to reproductive maturity (Fox et al. 2020).

Our work demonstrates that juvenile collared lizard social interactions are complex and likely have lasting impacts on the lizards as yearlings and adults, as winning and losing interactions as juveniles can impact their future territory, mates, foraging prospects, and survival to sexual maturity. Webster et al. (2018) stated that socially maintained signal honesty has received little attention over the last few decades, unlike the studies looking at physiologically costly traits, and more work should be done to explore the role of socially enforced honesty. We agree and add that the study of precocial signaling is even more in its infancy and can obviously have a lasting impact on an individual’s later fitness.

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Table 1. Model selection results for treatment and stimulus lizards. Table (a) shows the top two of 17 models for treatment lizard aggression. Table (b) shows the top three of nine models for stimulus lizard aggression.

(a) Model	***K***	**ΔAIC_C**	**W_{AIC}**
DHT Level + Implant Type + DHT Level*Implant Type	4	0.0	1
DHT Level + Implant Type	3	24.8	<0.001

(b) Model	***K***	**ΔAIC_C**	**W_{AIC}**
Color + Size + Color*Size	6	0.0	0.871
Color + Size + Time to First Aggression	5	3.9	0.126
Color + Size	4	11.3	0.003

Models are ranked by ΔAIC_C. *K* = the total number of parameters in each model. W_{AIC} = the Akaike weight or relative likelihood of the model. DHT = hormone level of respective hormone in treatment lizards, Implant Type = DHT or blank implant, Color = masking or enhancing juvenile orange bars (JOB) of treatment lizards, Size = size difference between treatment and stimulus lizards, Time to First Aggression = elapsed time to first aggression by treatment lizard after divider was lifted.

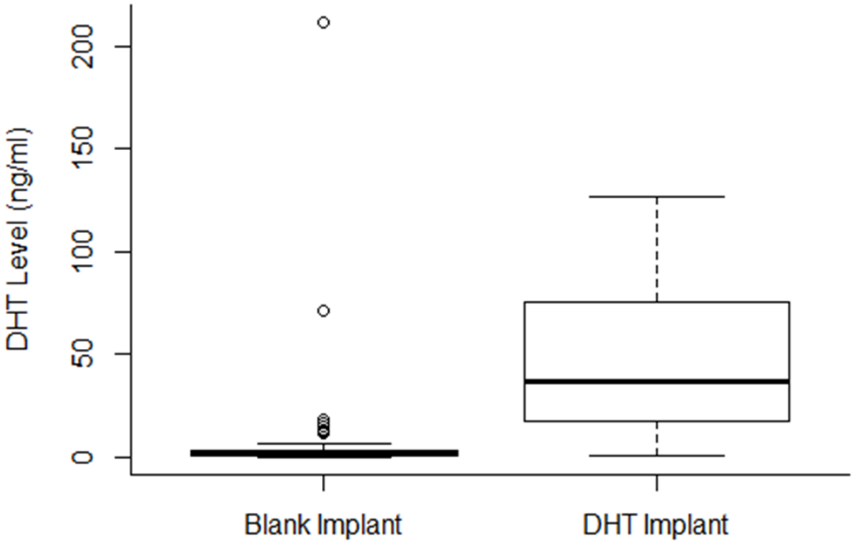

Figure 1. Box plots showing contrast between circulating DHT concentration of lizards with and without DHT implants. The central box accounts for the range of the central 50% of the data (i.e., interquartile range; IQR) with the horizontal line depicting the median. The end caps are shown at the minimum value within the range of the 1st quartile minus 1.5 IQR and the maximum value within the range of the 3rd quartile plus 1.5 IQR. Outliers are shown as open circles.

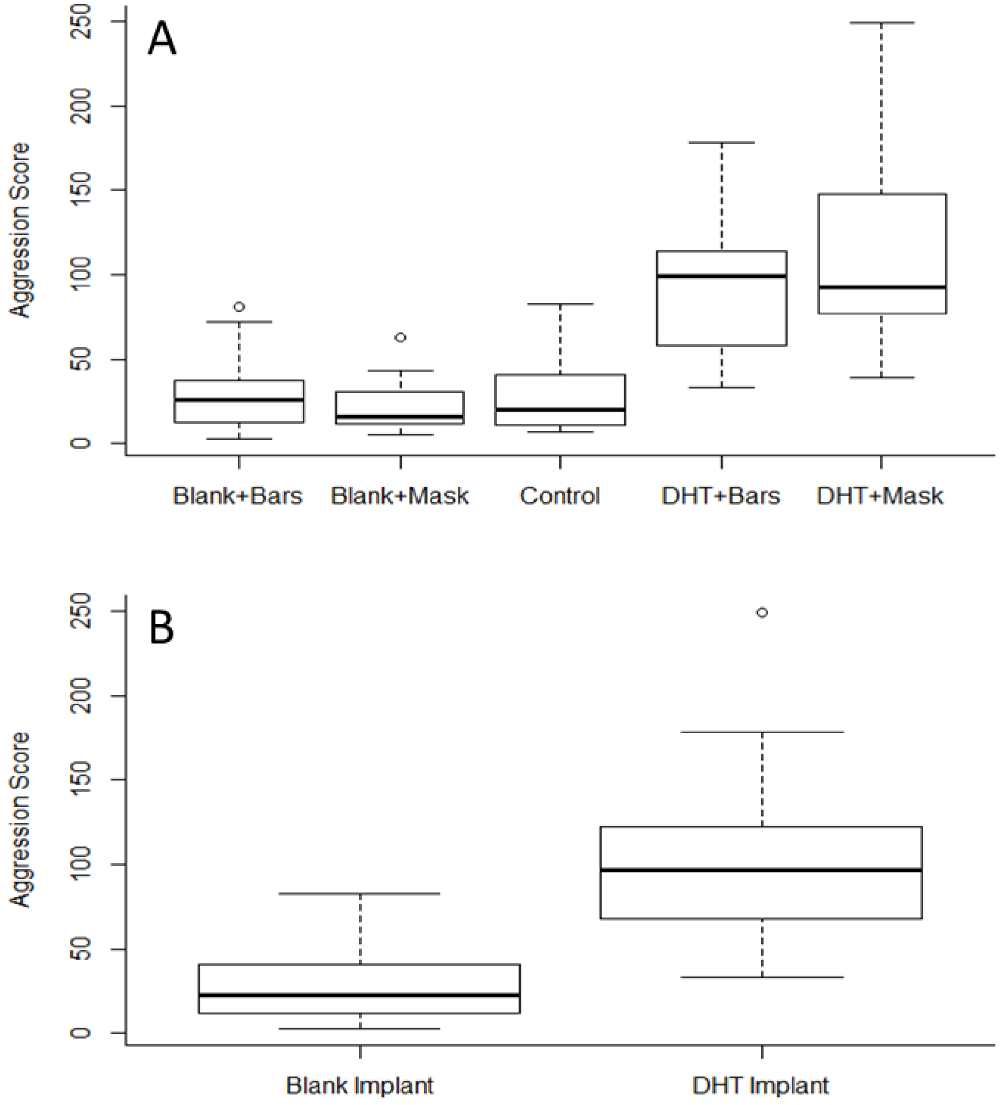

Figure 2. Box plots comparing treatment lizard aggression for (A) treatment and (B) implant type. The central box accounts for the range of the central 50% of the data (i.e., interquartile range; IQR) with the horizontal line depicting the median. The end caps are shown at the minimum value within the range of the 1st quartile minus 1.5 IQR and the maximum value within the range of the 3rd quartile plus 1.5 IQR. Outliers are shown as open circles.

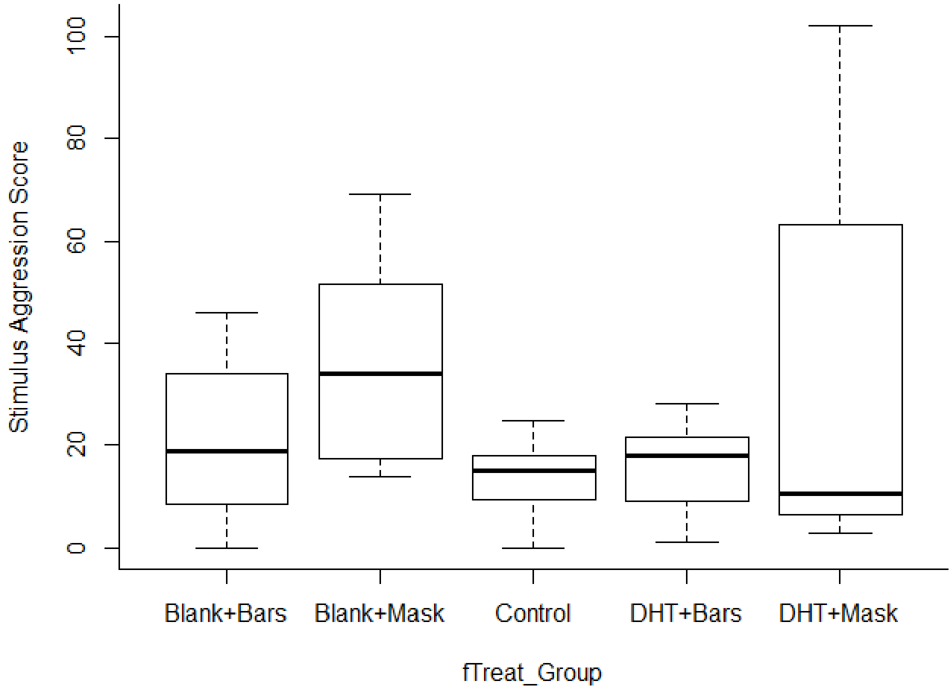

Figure 3. Box plots comparing aggression score of stimulus lizards in relation to JOB color and DHT implant status of treatment lizards. The central box accounts for the range of the central 50% of the data (i.e., interquartile range; IQR) with the horizontal line depicting the median. The end caps are shown at the minimum value within the range of the 1st quartile minus 1.5 IQR and the maximum value within the range of the 3rd quartile plus 1.5 IQR.

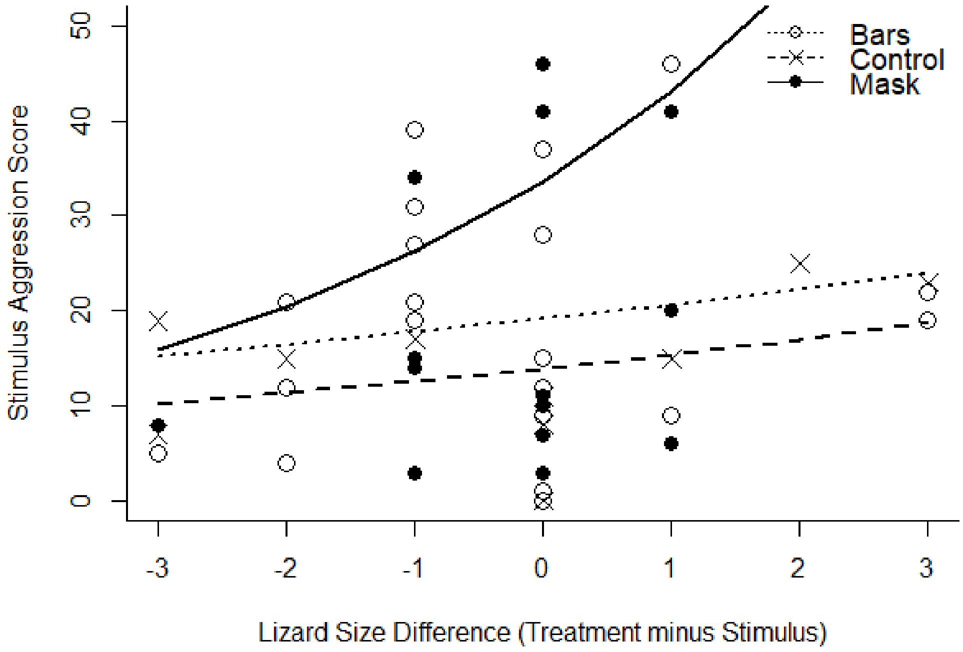

Figure 4. Stimulus aggression score plotted against the size difference of stimulus and treatment lizards (treatment SVL minus stimulus SVL). The datapoints and model predictions were plotted based on the treatment lizard’s JOB coloration. “Bars” includes Blank+Bars and DHT+Bars; “Mask” includes Blank+Mask and DHT+Mask.

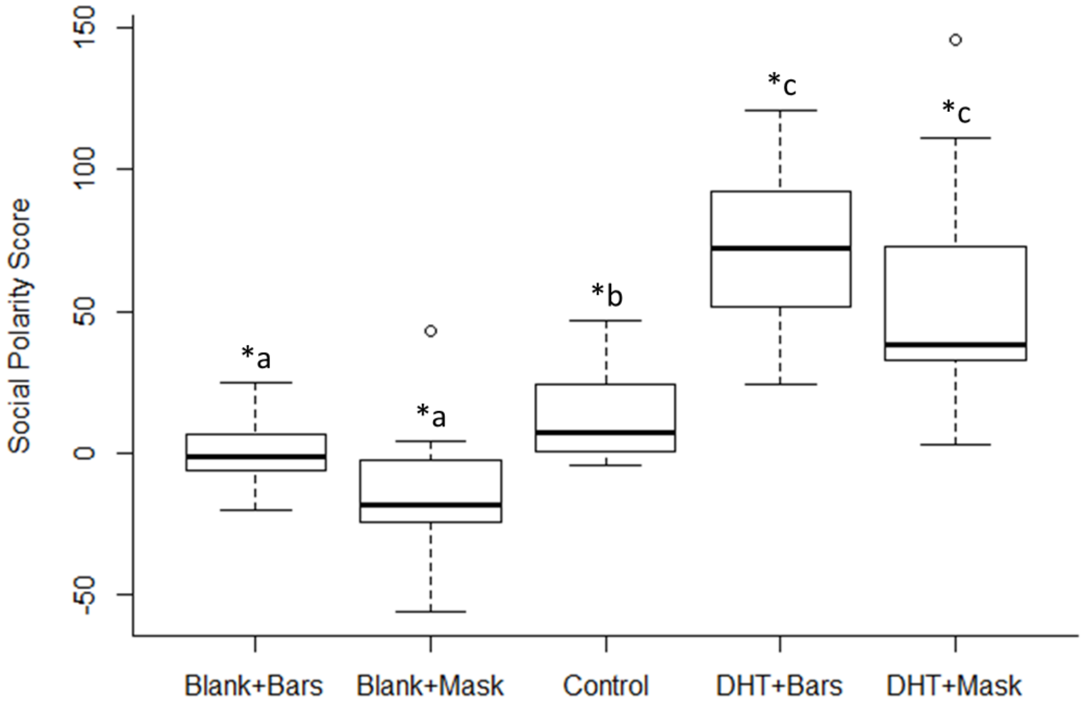

Figure 5. Box plots showing the social polarity scores by treatment. Each box has a letter over it where different letters denote significant between-group differences. The central box accounts for the range of the central 50% of the data (i.e., interquartile range; IQR) with the horizontal line depicting the median. The end caps are shown at the minimum value within the range of the 1st quartile minus 1.5 IQR and the maximum value within the range of the 3rd quartile plus 1.5 IQR. Outliers are shown as open circles.

CHAPTER III

VISUALLY MODELING THE CONSPICUOUSNESS OF A SEXUALLY DIMORPHIC JUVENILE TRAIT IN EASTERN COLLARED LIZARDS, *CROTAPHYTUS COLLARIS* (IGUANIA: CROTAPHYTIDAE)

The following chapter is formatted for the *Biological Journal of the Linnean Society*:

ABSTRACT

Juvenile collared lizards (*Crotaphytus collaris*) are sexually dichromatic. Dorsolateral bars are bright orange and showy in males but light tan and inconspicuous in females. These dorsolateral bars disappear as normal adult sexual dichromatism develops. As adult male collared lizards are known to suffer increased predation as a result of bright coloration, we hypothesized that juvenile males might be more detectable by predators due to the conspicuousness of their dorsolateral bars. To test this hypothesis, we measured the reflectance of the dorsolateral bars, non-bar background body regions, and the rocky habitat where the lizards live. We modeled the vision of collared lizards and their dominant predators to quantify and statistically compare the contrasts of male and female dorsolateral bars against the natural rock background. We found that male orange bars exhibited significantly higher chromatic contrast, but significantly lower achromatic contrast, than female bars when perceived through the visual models of predators and conspecifics. The dorsolateral background color of juvenile males and females did not differ significantly in chromatic or achromatic contrast with rocks in any visual model. Our findings indicate that juvenile male bars are conspicuous both to conspecifics and predators, and that the bars probably increase predation risk on juvenile males.

Keywords: achromatic contrast – chromatic contrast – color – prey detection – reptile – vision

INTRODUCTION

Conspicuous coloration in animals can be used to attract mates, secure resources, and deter rivals (Andersson, 1994). In many cases, males have evolved colorful traits that attract females and that are selected through female mate choice, i.e., intersexual selection (Darwin, 1871; Andersson, 1994). Male-male competition also can be a driving force in the evolution of conspicuous coloration, which males can use to repel reproductive rivals or to secure better territories or resources that grant them access to females, i.e., intrasexual selection (Andersson, 1994; Charles & Ord, 2012). In any case, the benefits of conspicuous coloration must outweigh the costs over the lifetime of the individual. For carotenoid-dependent conspicuous traits, the largest cost can be locating and securing prey with abundant carotenoids, as animals cannot synthesize carotenoids (Endler, 1980; McGraw & Ardia, 2003; Svensson & Wong, 2011). For other conspicuous traits, sometimes the cost is finding a sufficient amount of food to supply the energy needed to produce the traits (Hill, 2000; Scheuber et al., 2003; Barnett & Briskie, 2007). Often, however, the leading cost will be increased predation risk from exhibiting the conspicuous trait (Magnhagen, 1991; Zuk & Kolluru, 1998; Montgomerie et al., 2001; Macedonia et al., 2002; Godin & McDonough, 2003; Stuart-Fox et al. 2003; Husak et al., 2006; Ruiz-Rodríguez et al., 2013).

Despite the fact that natural selection typically favors cryptic coloration in response to predation pressure (Endler, 1978; 1980; 1991; Slagsvold et al., 1995; Husak et al., 2006), drivers other than sexual selection can underlie the appearance and maintenance of non-cryptic coloration (e.g., latitude – Karubian, 2013; habitat – Medina et al., 2017). For example, Cain et al. (2019) used 3D printed models of Fairy Wrens to demonstrate that dull-colored models were attacked at a rate similar to conspicuously colored models in a closed habitat, whereas in an open habitat models of females—both dull and conspicuous—had a higher rate of predation than models of conspicuous males. The authors suggest that this lack of predator attack bias toward conspicuous plumage could result from a complex interaction between conspicuousness against particular backgrounds, sex-specific vigilance, and predator behavior. This idea has some commonalities with the unprofitable prey hypothesis for conspicuous bird plumage, which predicts that predators will avoid conspicuous prey because conspicuousness advertises prey unprofitability and should shift predation pressure to more cryptic birds (Baker & Parker, 1979). Like the unprofitable prey hypothesis for birds, some lizards (and other taxa) demonstrate their unprofitability by presenting pursuit deterrent signals that alert the predator of the animal's readiness and competency to escape (Hasson et al., 1989; Cresswell, 1994; Clark, 2005; Font et al., 2012; York & Baird, 2016). Alternatively, other lizard species use conspicuous traits and colorful expendable tails to direct predator attacks away from vital parts of the body, thus allowing for escape (Castilla et al., 1999; Bateman & Fleming, 2009; Murali & Kodandaramaiah, 2016). Lastly, aposematic species, found in a variety of taxa, use conspicuous coloration to advertise their toxicity or harmfulness to deter predation (Blount et al., 2009; Summer et al., 2015). Regardless of the particular functions that conspicuous coloration may serve in interactions with predators, predation frequently is an important selective force in the development of color signals in prey.

In the genus *Crotaphytus*, various species possess both sexually dimorphic and dichromatic traits, with adult males being larger and possessing more colorful body patterns (McGuire, 1996). In *C. collaris* (Say, 1822), adult males use their exaggerated traits in displays to defend territories from other males and to secure mating opportunities (Baird et al., 1997; Husak, 2004; Baird, 2013). Nevertheless, male coloration in some *Crotaphytus* species has been shown to increase their conspicuousness to predators (Husak et al., 2006; Macedonia et al., 2009). Interestingly, juvenile male collared lizards also possess a sex-specific trait in the form of dorsolateral orange bars (Figure 1A), which juvenile females lack (McGuire, 1996). In *C. collaris,* the male orange bars are lost upon reaching sexual maturity, when males begin to develop their adult blue-green coloration (Fox et al., 2020). Juvenile females have similar bar patterns, but the bars are a light tan, inconspicuous color (female tan bars; Figure 1B). Fox et al. (2020) hypothesized that the male orange bars are favored via a form of precocial sexual selection. In this context, dorsolateral orange bars offer an early advantage to sexually immature males by helping them 1) secure juvenile territories (which later expand into breeding territories) and 2) attain pair-bonds with nearby juvenile females (which are retained into adulthood). Wiggins (2018) showed that juvenile males with more developed dorsolateral orange bars left significantly more offspring as adults than juvenile males with less developed orange bars. In the present work, we present findings that further our understanding of how predation pressure relates to the presence of male orange bars and female tan bars.

Our goal was to test the conspicuousness of juvenile collared lizard male orange bars against a natural background. Given that juvenile males present different sexually-selected conspicuous traits than do adults, and at a different time of the year, we wanted to learn if the same potential predation pressures exist for juveniles that exist for adults. We addressed this question by modeling reflectance spectra of the male orange bars and female tan bars using the visual systems of Eastern collared lizards (*C. collaris*) and their two dominant predators in Oklahoma, the Eastern coachwhip (*Masticophis flagellum* Shaw, 1802) and the greater roadrunner (*Geococcyx californianus* Lesson, 1829). We predicted that male orange bars would be significantly more conspicuous against a natural background than female tan bars when viewed through the eyes of conspecifics as well as predators.

METHODS

Measuring Lizard and Visual Background Reflectance

The study took place between August and October of 2019. We captured juvenile male and female lizards from the Oklahoma Gas and Electric Sooner Generating Station's dam along Sooner Lake in Pawnee County, Oklahoma. We used a telescoping fiberglass pole and noose to capture lizards that were found along the dam's concrete and stone embankment. Because the lizards had previously been used in a separate study, we used the lizard's toe clips to identify individuals and prevent using the same lizard twice in our study. As lizards achieve maximal dorsolateral bar development at a Snout-Vent Length (SVL) of 58 – 81 mm (Crosby, 2015), we collected lizards only within that range. We brought the captured lizards back to Oklahoma State University to measure spectral reflectance of the dorsolateral bars and body background coloration. After acclimating a lizard in a 37.9-L tank under a heat lamp for at least 1 hour to raise body temperature to a typical range found in the field (35 to 40 °C), we used a UV-VIS spectrometer (USB 4000, Ocean Optics), a deuterium-halogen light source (DH-2000-BAL, Ocean Optics), a reflectance probe (QR400-7-SR-BX, Ocean Optics), and SpectraSuite software (Ocean Optics) to collect the spectral reflectance data. We equipped the probe with an opaque hollow tube to standardize measurements from a 2-mm diameter target at a 90° angle and 7 mm from the surface. We took reflectance measurements of both the dorsolateral bars and the body background coloration on the lizard's right side. Beginning with the dorsolateral bar nearest the collar, we measured reflectance at the bar's widest point and continued caudally until we had measured three consecutive bars. We then took readings of body background reflectance, starting caudally to the first bar and repeating this measurement for the second and third bar while remaining centrally located between the two nearest bars. Several lizards (3 males and 10 females) did not have their body background reflectance recorded because we added this measurement after the study had begun. Reflectance spectra also were gathered from a sample of 20 rocks collected from our study area. The study area harbors little vegetation, and the lizards spend most of their time on top of the rocks in direct sunlight. Rocks at the dam therefore constitute the relevant visual background for the collared lizards and their potential ophidian and avian predators.

Visual Modeling

Birds and non-avian reptiles exhibit four classes of single cones. In lizards, these photoreceptors include ultraviolet-wavelength-sensitive (UVS), short-wavelength-sensitive (SWS), middle-wavelength-sensitive (MWS), and long-wavelength-sensitive (LWS) cones (Loew et al., 2002; Macedonia et al., 2009). Many avian taxa exhibit violet-sensitive (VS) cones rather than UVS cones. Methylation-specific PCR and opsin sequencing data (Ödeen & Håstad, 2003) indicate that VS cones are ancestral for Aves (Hart & Hunt, 2007) and that all raptors (Accipitridae & Falconidae) exhibit VS cones. UVS and VS cones are homologues that contain the same SWS1 opsin (Ödeen & Håstad, 2003; Lind et al., 2013; Toomey & Corbo, 2017), and we refer to the two types collectively as SWS1 cones.

As a rule, birds and non-avian reptiles also possess double cones that contain the LWS pigment in both members of the cone pair. In birds and diurnal lizards, the principal member of a double cone pair typically possesses an oil droplet with a shorter wavelength cutoff than the single LWS cones, the effect of which broadens the absorption range of the double cones (Bowmaker et al., 1997; Osorio & Vorobyev, 2005; Toomey & Corbo, 2017). In contrast to other birds, most raptor species studied to date (including the species used for our visual model) lack double cones (Mitkus et al., 2017; Perez-Martinez et al., 2020).

We generated Gaussian visual pigment absorptance curves using the peak absorptance (λ_{max}) value for each class of single cones and the photoreceptor template equations of Govardovskii et al. (2000). We then used the oil droplet template of Hart and Vorobyev (2005) to calculate cone oil droplet transmittance in our lizard and raptor visual models (snake photoreceptors do not possess oil droplets: Sillman et al., 1997; Simões et al., 2015). The reflectance spectra obtained from our lizard subjects, as well as the rocks that constitute their visual background, exhibit very low UV reflectance (≈ 5-8% from 330-700 nm, Figure 2). We therefore followed Fleishman et al. (2016a) in omitting UVS cones from our lizard (and snake) visual models and restricted our analyses of chromatic and achromatic contrast to 400–700 nm. Similarly, we omitted the homologous VS cone from calculations in our raptor visual model, as approximately 40% of this cone's absorptance from 330–700 nm lies in the UV (Lind et al., 2013).

Lizard Visual Model

Our lizard visual model, the Eastern collared lizard (*Crotaphytus collaris*), possesses a pure cone retina with four classes of single cones and a double cone (E. R. Loew, pers. comm.). Spectral sensitivity of individual photoreceptors and oil droplets in *C. collaris* was determined with microspectrophotometry (MSP) and is virtually identical to that of *C. dickersonae* (Schmidt, 1922; see Macedonia et al., 2009), with mean λ_{max} values for each of the four cone classes differing only 0–2 nm between the two species (*C. collaris*: UVS = 357, SWS = 459, MWS = 480, and LWS = 556; data courtesy of E. R. Loew, Cornell University).

Snake Visual Model

Most diurnal snakes exhibit two classes of single cones and one class of rods, where cones usually are UVS and LWS (Sillman et al., 1999; Simões et al., 2016) but in some cases are

UVS and MWS (Sillman et al., 1997). In contrast, our visual model, the coachwhip (*Masticophis flagellum*), possesses a pure cone retina with three cone classes: UVS, SWS, and LWS (Macedonia et al. 2009: their Figure 4B). The highly active coachwhip hunts both moving and sedentary prey and is the most dangerous ophidian predator of collared lizards where the distributions of the two reptiles overlap (Macedonia et al., 2002, 2009), including our study site (S. Fox & J. Agan, pers. obs.). Secor & Nagy (1994) determined in a metabolic study that about half of the coachwhip diet consisted of lizards. Spectral sensitivity in *M. flagellum* was determined with MSP from a specimen collected at Sooner Lake dam. This individual was one of two subjects whose visual pigment λ_{max} values were averaged in Macedonia et al. (2009: their Table 2). For the Sooner Lake specimen, the λ_{max} values were UVS = 359, SWS = 458, and LWS = 561 (data courtesy of E. R. Loew, Cornell University).

Avian Visual Model

The terrestrial greater roadrunner (*Geococcyx californianus*) may represent the most dangerous avian predator of collared lizards at our study site (J. Husak and S. Fox, pers. obs.), but spectral sensitivity has not been determined for this species. Given the distribution of VS and UVS cones in birds (Ödeen & Håstad, 2003), however, it is highly likely that *G. californianus* possesses a VS cone type. In the absence of *G. californianus* spectral sensitivity data, we deferred to using a raptor visual model. While raptors may not be as important as the greater roadrunner as predators at our study site, raptors are common in areas with collared lizards (including our study site) and can be found preying on lizards (Trauth et al., 2004; Husak et al., 2006; Ivanyi, 2009). For our raptor visual model we used cone pigment (λ_{max}) and oil droplet absorption data for the common buzzard (*Buteo buteo* Linnaeus, 1758) from Lind et al. (2013). The common buzzard has been used similarly in visually modeling avian predator threat for the Australian frilled dragon (*Chlamydosaurus kingii* Gray, 1825) (Perez-Martinez et al., 2020).

Cone Quantum Catch

To determine the photoreceptor output in response to a target spectrum, we calculated quantum catch for each cone class, Q_i, as:

$$Q_i = \int_{400}^{700} R(\lambda)I(\lambda)S(\lambda)d\lambda \qquad \text{(eqn 1)}$$

where $R(\lambda)$ is the reflectance spectrum of the target, $I(\lambda)$ is the irradiance spectrum illuminating the target, and $S(\lambda)$ is the spectral sensitivity of a given photoreceptor class, integrated over the wavelength range of interest $d\lambda$. To convert lizard and rock reflectance to radiance (as would be perceived by viewers in the field), each reflectance spectrum was multiplied by a mean (N = 3) downwelling irradiance spectrum measured previously with an irradiance probe under clear skies in an open location (near Punta Chueca, Sonora, Mexico: for equipment, procedural details, and conversion of raw spectra to units appropriate for vision, see methods and Figure 3A in Macedonia et al., 2009). We chose to calculate radiance from reflectance and irradiance rather than measuring it directly in the field to tightly control the lighting conditions under which we gathered spectral data for this study. We then simplified our lizard and rock radiance spectra for contrast calculations by extracting intensity values at 10 nm intervals from 400–700 nm (total of 30 values).

Prior to calculation of a visual model's response to a calculated radiance input (dorsolateral bars, body background coloration, or rocks), we accounted for chromatic adaptation of the eye to the visual background as:

$$k_i = 1/\int_{400}^{700} A(\lambda)S_i(\lambda)d\lambda \qquad \text{(eqn 2)}$$

where $A(\lambda)$ is the adapting spectrum (rock radiance) and $S(\lambda)$ is the spectral sensitivity of a given photoreceptor class. The response of each cone in a visual model to a radiance input then can be summarized as q_i, where $q_i = k_iQ_i$. Our mathematical operations were performed in the following sequence. First, the spectral sensitivity of each cone was multiplied by the adapting spectrum and the outputs of each cone were summed. Second, we calculated the von Kries correction (Fleishman et al. 2016a,b) for the adapting spectrum as 1 divided by the sum of each cone output (i.e., equation 2). When multiplied by this quotient, the summed output of each cone equals 1, thereby satisfying the assumption that the neural stimulation of each cone class is equal in response to a 'white' stimulus (Fleishman & Persons, 2001; Fleishman et al., 2016a,b). Third, as chromatic contrast is computed using cone output proportions, the relative output of each cone class was calculated by dividing the von Kries-corrected sum of each cone class output by the combined sum of the cone class outputs. For example, in a visual model with four outputs (each summing to 1 when von Kries corrected), each proportional output is 0.25. Last, note that calculations of proportional cone outputs for the target spectra (dorsolateral bars and body background coloration) differ from those calculated for the adapting spectrum (rock radiance) in only one way: the von Kries correction factors generated in response to the adapting spectrum are the same values used to calculate the proportional outputs of the target spectra, as opposed to generating von Kries corrections for the target spectra de novo.

Two different modeling approaches frequently are used to estimate color perception in animals: the receptor noise model (Vorobyev & Osorio, 1998) and the Euclidean distance model (Endler & Mielke, 2005). In our study we used the simpler Euclidean distance method. Fleishman et al. (2016a) have shown that the two approaches produce equivalent results, and the Euclidean distance approach is preferable when the ratios of different types of cones are uncertain, as is the case for all of our visual models.

Chromatic Contrast Calculations

For each subject we calculated chromatic contrast as the Euclidean distance between the targets (e.g., dorsolateral bar or body background radiance proportional cone output) and the visual background (rock radiance mean proportional output). For example, in a tetrachromatic visual model Euclidean distance is calculated as:

$$CC = \sqrt{(W_a - W_b)^2 + (X_a - X_b)^2 + (Y_a - Y_b)^2 + (Z_a - Z_b)^2} \qquad \text{(eqn. 3)}$$

where "W" is the UVS-VS (SWS1) cone, "X" is the SWS cone, "Y" is the MWS cone, and "Z" is the LWS cone. Our omission of the SWS1 cone (see above) simplifies the tetrachromatic model to three terms for our tetrachromats (*C. collaris* and *B. buteo*) and two terms for our trichromat (*M. flagellum*). The subscript "a" represents the proportional cone output of the target spectrum, and subscript "b" represents the proportional cone output of the visual background spectrum.

Achromatic Contrast Calculations

As we lacked sufficient data for *M. flagellum* to use double cones in calculating achromatic contrast for the snake visual model, and because our raptor visual model (*B. buteo*) lacks double cones in its foveae, we followed Perez-Martinez et al. (2020) in summing only outputs of single cones for all of our visual models to calculate achromatic contrast. This quantity was calculated for each subject in each visual model as the mathematical difference between the perceived luminance of dorsolateral bar or body background radiance and the perceived luminance of rock radiance, divided by the sum of these two quantities:

$$AC_{DB} = (DB - R) / (DB + R) \text{ or } AC_{Back} = (Back - R) / (Back + R) \qquad \text{(eqn. 4)}$$

where "DB" is dorsolateral bar calculated radiance, "Back" is dorsolateral background calculated radiance, and "R" is rock calculated radiance. Positive values result in a visual model when the target is perceived as being brighter than the rock background, and negative values result when the target is perceived as being darker than the rock background.

Statistical Comparison of Contrast Between Lizard Body Coloration and Rocks

Preliminary data exploration (SPSS v. 21) revealed that our data distributions (grouped by sex and visual model) for chromatic contrast exhibited significant unequal variance in Levene's tests. Log_{10} transformation of the data alleviated the problem of unequal variance, but Shapiro-Wilk tests showed that several distributions were non-normal. We therefore used Mann-Whitney U tests for between-sex, within-visual-model comparisons of contrasts between dorsolateral bars (and body background coloration) against the visual background of rocks.

RESULTS

Contrast of Collared Lizard Dorsolateral Bars and Body Background Color Against Rocks

Juvenile male dorsolateral bars are perceived as having significantly greater chromatic contrast with rocks than are juvenile female dorsolateral bars in our lizard, snake, and raptor visual models (Mann-Whitney U range = 3 – 14, $P < 0.00001$ in all cases; Figure 3A). Conversely, juvenile male dorsolateral bars are perceived as having significantly less achromatic contrast with rocks than are juvenile female dorsolateral bars in all three visual models *(*Mann-Whitney U range = 102 – 164, $0.00004 < P < 0.009$; Figure 3B).

By comparison, juvenile male and female dorsolateral body backgrounds are not perceived as having significantly different chromatic contrast with rocks in our lizard, snake, and raptor visual models (Mann-Whitney U range = 135 – 145, $0.27 < P < 0.42$ in all cases; Figure 4A). Likewise, juvenile male and female dorsolateral body backgrounds are not perceived as having significantly different achromatic contrast with rocks in our three visual models (Mann-Whitney U range = 142 – 147, 0.37 < P < 0.45 in all cases; Figure 4B).

DISCUSSION

Our results show that the highly chromatic orange dorsolateral bars of male juvenile collared lizards are more conspicuous against a visual background of rocks than are the less chromatic tan dorsolateral bars of juvenile females. This interpretation follows from the fact that, for most visual systems, colors possessing strong chroma should be more detectable in natural environments than weakly chromatic colors (Endler, 1990). As anticipated, we found no differences in contrasts between male and female background body coloration as viewed against the rocky substrate of our study species' habitat. Somewhat surprisingly, female dorsolateral bars exhibited greater achromatic contrast than did male bars in all visual models. This result may stem from the larger difference between reflectance of the female bars and the rocks, compared to that of males, in the short and middle wavelengths (e.g., 425–550 nm: Figure 2).

Color vision plays important roles in ecological contexts such as foraging, intraspecific fighting, and mate choice, but highly chromatic visual signals favored by sexual selection in attracting mates or repelling conspecific rivals also can attract predators (Endler, 1978; Baker & Parker, 1979; Husak et al., 2006; Karubian, 2013; Cain et al., 2019). In juvenile collared lizards, territory defense and female pair bonding necessitate that males exhibit conspicuous behavior and approach the conspecific closely while displaying their striking dorsolateral bars (Fox et al., 2020). Unfortunately, frequent patrolling, intraspecific agonistic contests, and male courtship all increase exposure to potential predators (Andersson, 1994).

Juvenile females do not advertise or defend territories, but instead reside on the developing territories of juvenile males. The less frequent and less vigorous movements by females reduce the risk of being seen by a predator. By comparison, juvenile males need to find and pair bond with juvenile females (Fox et al., 2020). Females may pay a price, nevertheless, by interacting with or simply being in the vicinity of an active, conspicuous male (Dawkins & Guilford, 1991; Edomwande & Barbosa, 2020). For example, in guppies (*Poecilia reticulata,* Peters, 1859), Pocklinton and Dill (1995) showed that when a cichlid predator was presented with mixed-sex guppy pairs, they significantly targeted females first. The authors concluded that females were more profitable, as they were roughly three times the size of males and there was no difference in capture rate between the sexes. Pocklington and Dill (1995) speculated that in nature predators might shift their prey target to the female after initially detecting the conspicuous male color and behavior. In another study with sand lizards (*Lacerta agilis,* Linnaeus, 1758), predators tended to target cryptic female models over conspicuously colored male models, which suggests that increased visual conspicuousness is not necessarily an indicator of predation risk (Olsson, 1993).

In our study, we suspect that the comparatively stronger achromatic contrast of juvenile collared lizard female dorsolateral bars does not translate into female-biased predation risk for two reasons. First, as juveniles of both sexes are approximately the same size, it is unlikely that females are more profitable prey than are males. Juvenile females also survive better than males to the yearling stage (J. Agan & S. Fox, unpubl. data). Second, achromatic vision is important for mediating spatial resolution and motion detection (Gegenfurtner & Hawken, 1996; Fleishman & Persons, 2001; Jones & Osorio, 2004; Hill & McGraw, 2006; Olsson et al., 2018; Kaestner et al., 2019), and the rocks at our study site appear as a matrix of brownish-tan patches that vary in luminance. From the perspective of viewers, the array of large rocks within the field of view forms a patchwork scene of light and shadows. By comparison, our spectrophotometric measurements of rock samples were taken from a very small area of rock surface and all samples were illuminated equally. Dorsolateral bars that are brighter than a rock on which a collared lizard is perched therefore might be relatively inconsequential for detection by predators or conspecifics. It seems possible that the dorsolateral bars also could be functioning as disruptive coloration that break up the lizard’s outline against the rocks (e.g., Cuthill et al., 2005; Stevens & Merilaita, 2009).

As adults, females develop orange dorsolateral bars during the reproductive season that appear quite similar to those of juvenile males, and we speculate that they serve the same function of attracting the opposite sex. It was originally thought that this visual signal was used to deter male aggression and courtship after females became gravid (Carpenter, 1967; Cooper & Ferguson, 1972), and that the orange bars of juvenile males served to mimic adult female coloration. The proposed function of this mimicry was to deter aggression from adult males, which due to their larger size possess a much stronger bite force (Lappin & Husak, 2005) that could harm or even kill juveniles (Fox, 2009; Ivanyi, 2009; Sievert & Sievert, 2011). The adult female mimicry hypothesis was tested and rejected by Husak et al. (2004) when they showed that adult male aggression was not reduced by the presence of orange dorsolateral bars in juvenile males. It also has been shown that adult female orange bars stimulate, rather than deter, approach and courtship by adult males (Baird, 2004). York and Baird (2019) further showed that females benefit from multiple sires by increasing hatching success and number of surviving offspring, which might be limited if the orange bars inhibited male courtship.

To conclude, Fox et al. (2020) suggested that precocial sexual selection may have driven the development of dorsolateral orange bars in juvenile male collared lizards and continues to maintain them. Our visual modeling results have shown that these orange bars are likely to be conspicuous, not only to the target audiences of juvenile females and of juvenile males, but also to an undesired audience of predators. The benefit of this signal's attraction to prospective mates and repulsion of prospective rivals nevertheless suggests that it has outweighed the potential cost from predation over evolutionary time.

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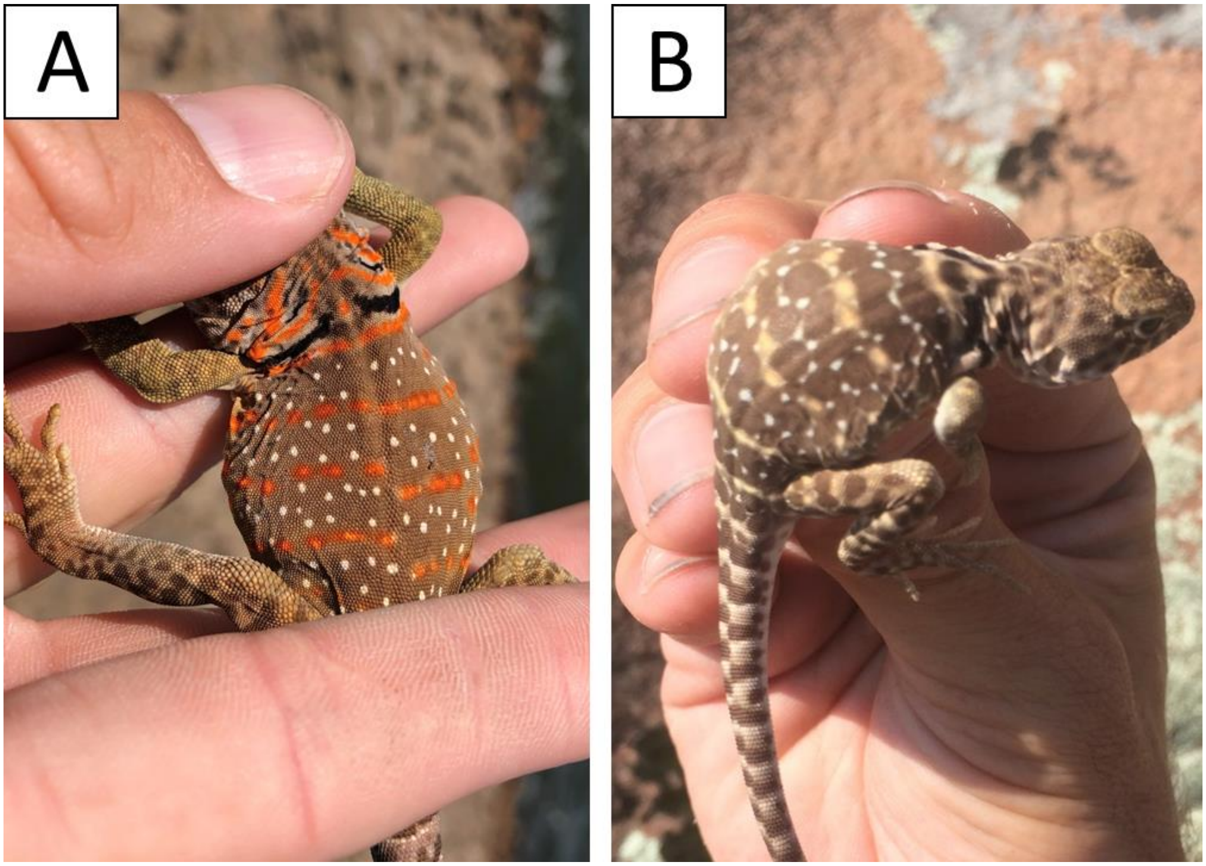

Figure 1. Photographs of juvenile male and female collared lizards showing differences in dorsolateral bars. The male (A) has conspicuous orange dorsolateral bars whereas the female (B) has inconspicuous tan dorsolateral bars.

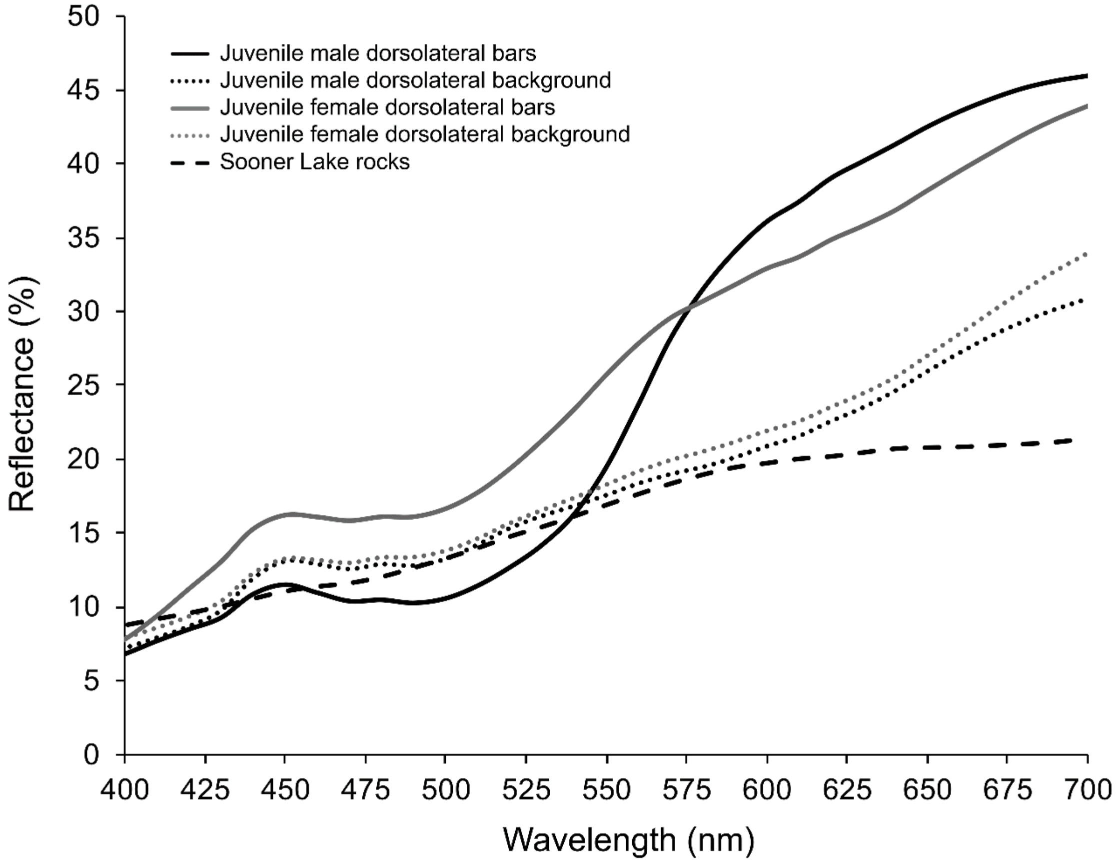

Figure 2. Mean reflectance spectra of juvenile *Crotaphytus collaris* and the rocks against which they are visually juxtaposed at the Sooner Lake study area. Sample sizes: male dorsolateral bars (N = 18: solid black line), male dorsolateral background coloration (N = 15: dotted black line), female dorsolateral bars (N = 33: solid gray line), female dorsolateral background coloration (N = 23: dotted gray line), Sooner Lake rocks (N = 20: dashed black line).

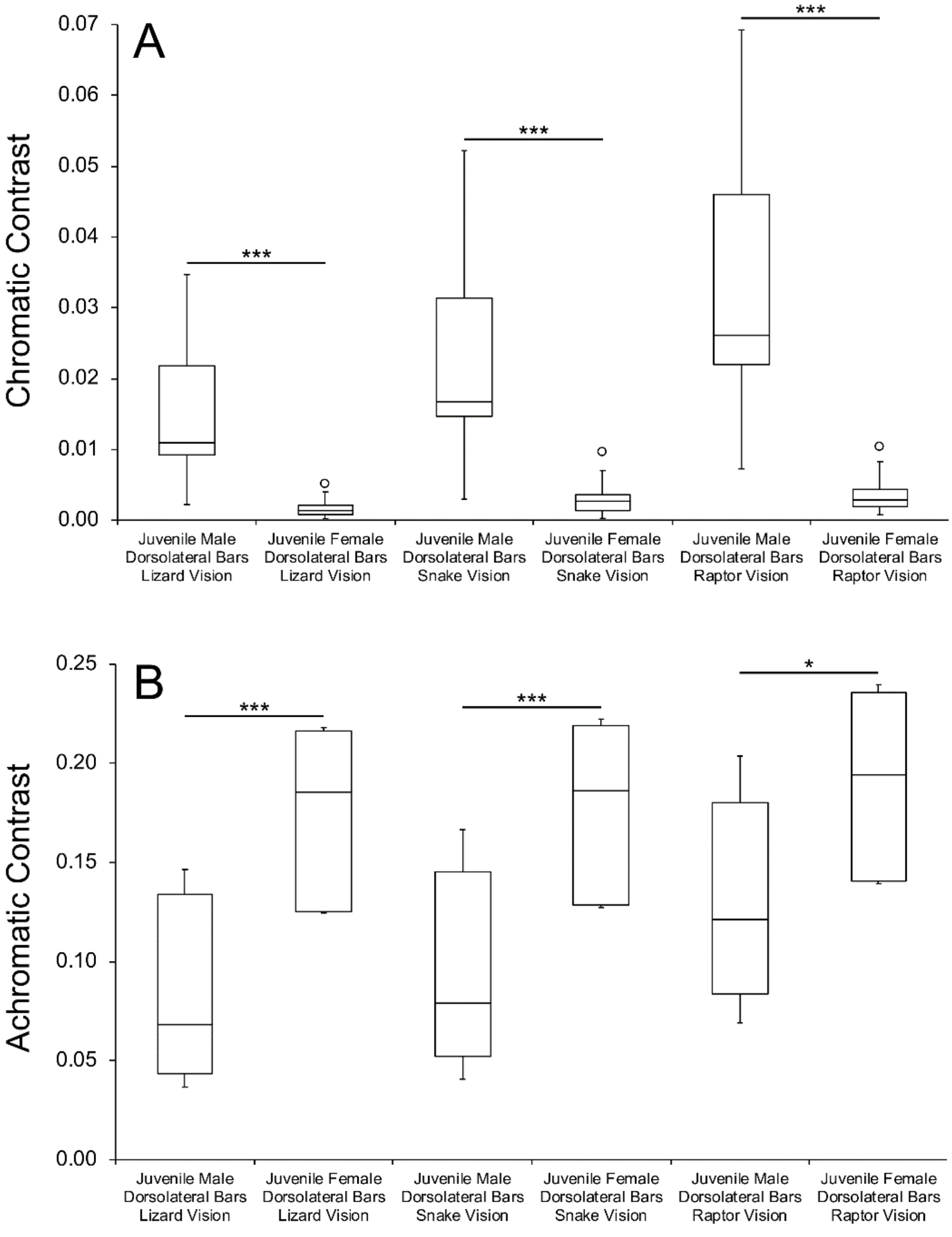

Figure 3. Box plots of (A) chromatic and (B) achromatic contrasts between juvenile dorsolateral bars and rocks. In each box, the central 50% of the data (i.e., the interquartile range; IQR) are

contained inside the box and the horizontal line depicts the median. The end caps of the error bars are placed at the minimum value within the range of the 1st quartile minus 1.5 IQR and the maximum value within the range of the 3rd quartile plus 1.5 IQR. Outliers depicted as open circles. One outlier not shown for achromatic contrast: juvenile male dorsolateral bars, lizard vision = –0.096. *$P < 0.05$; ***$P < 0.001$.

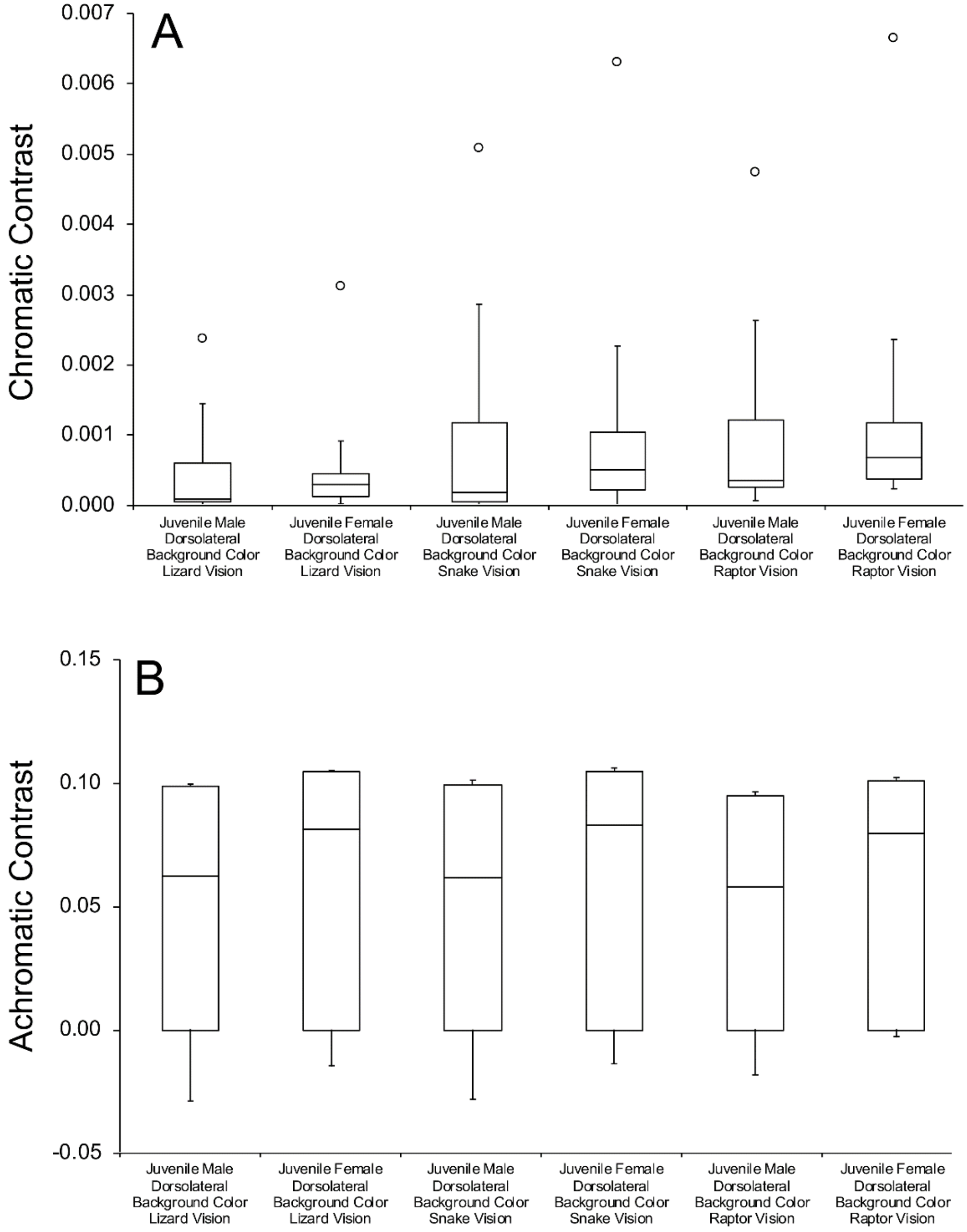

Figure 4. Box plots of (A) chromatic and (B) achromatic contrasts between juvenile dorsolateral background coloration and rocks. One outlier not shown for achromatic contrast: juvenile female

dorsolateral background color, raptor vision = –0.16. Legend as in Figure 3. No comparisons statistically significant.

CHAPTER IV

INFLUENCE OF JUVENILE ORANGE BARS AND AGGRESSION ON MALE SURVIVORSHIP AND FITNESS IN THE COLLARED LIZARD (*CROTAPHYTUS COLLARIS*)

ABSTRACT

The juvenile stage of an animal's life is important as it allows the individual to not only adapt to its environment to meet current challenges but also to prepare it for future challenges. Juvenile responses can impact an animal in many ways, including influencing social status, survival, and fitness. We modified a prominent juvenile dichromatic signal and aggressiveness in a field experiment and subsequently measured survivorship to the yearling stage and genetic fitness as yearlings began reproduction. Juvenile male collared lizards, *Crotaphytus collaris*, received a combination of dihydrotestosterone (DHT) or blank implants and enhanced or masked dorsolateral orange bars and maintained those treatments throughout the juvenile stage in a multi-year experiment. We recorded survivorship each year and conducted parentage analyses for all offspring produced. We found no survival differences among our treatments but confirmed that females survived better to sexual maturity than males. The inferior male survivorship is likely caused by a combination of display of conspicuous male behaviors and dimorphic coloration that

increases predation on male juveniles. Nevertheless, juvenile males who received DHT implants (and subsequently displayed more conspicuous behaviors) produced more offspring as yearlings than those who received blank implants. We believe that more aggressive juvenile males are favored by precocial sexual selection and the juvenile orange bars serve as a visual badge of the juvenile male's aggressiveness.

INTRODUCTION

Early-life interactions are important for juvenile animals as they can shape how an individual relates to its environment, including conspecifics, and can have life-long effects on fitness. While social and environmental stressors can hinder food intake and result in reduced growth and immune function (Bernier and Peter, 2001; Gapp et al., 2014), social interactions themselves can play an important positive role during development by altering the way individuals respond to their environment. The absence of social interactions during development can alter behavior, social status, and even influence gene expression (Taborsky, 2016; Nyman et al., 2017; Solomon-Lane and Hofmann, 2019). In songbirds, social interactions are important to juvenile birds for song development as they listen to and repeat the songs of those around them (Brainard & Doupe, 2002). While reptiles, traditionally but incorrectly regarded as largely asocial (Doody et al., 2013; 2021), can engage in social behavior via parental care (Somma, 2003; Munch et al., 2018; Santoyo-Brito et al., 2021), individual recognition (Cooper, 1996; Husak & Fox, 2003; Martin et al., 2020), and territory defense (Fox et al. 2003; Baird, 2018), they can also be impacted by social interactions during development (Fox et al. 1981; Fox and Rostker 1982, Ballen et al., 2014). Fox et al. (2020) proposed that sexual selection can act on juveniles prior to reaching sexual maturity. Selective pressures (precocial sexual selection: Fox et al., 2020; environmental selection: Johnson and Johnson, 2014) or even exclusion from social environments early in life can have lasting impacts by influencing development and later fitness (Heidbreder et al., 2000; Lukkes et al., 2009; Ballen et al., 2014) and means that regardless of the taxon, studying the early life environment can be important to understanding future fitness.

Another important factor in juvenile development and behavior is their hormones. Sex steroids specifically help regulate social behavior (Goodson, 2005; Newman, 1999) and are often responsible for mediating the long-term effects of early-life experiences as juveniles (Adkins-Regan, 2009; Brown and Spencer, 2013). In males, testosterone (T) is produced in the testes and can be correlated to the level of sexual and aggressive behavior (Tokarz, 1986; Ketterson & Nolan, 1992; Hau, 2007), and these traits are often higher in males with strongly dichromatic traits (Roberts et al., 2003; Husak et al., 2007). Circulating T levels increase during breeding seasons in some animals (Jenson et al., 2001; Wingfield et al., 2001), a time when sexually selected traits would be most developed and breeding behaviors are prevalent. Studies have also linked circulating T levels to the development of certain traits in animals that act as honest signals (Hews et al.,1994; Salvador et al., 1997; Cox & John-Alder, 2008), meaning T can help regulate the expression of many honest signals as well as their behaviors. That said, even though T is important in developing male-typical colors and behaviors, dihydrotestosterone (DHT) and estradiol can also play a large role (Arnold & Breedlove, 1985). This is especially true for DHT in collared lizards as it is tightly associated with development of secondary sex characteristics (Hews and Moore, 1995; Crosby, 2015). Increasing the available DHT has also been demonstrated to increase territorial behaviors in lizards (Huang & Crews, 2012; Crosby, 2015; Agan et al., in prep).

Collared lizards, *Crotaphytus collaris*, are sexually dimorphic and dichromatic lizards that use both differences to influence fitness (Husak et al., 2006a; Husak et al., 2006b; York et al., 2014; Plasman et al., 2015). Prior studies have shown that *C. collaris* has individual recognition by showing reduced aggression to resident neighbors and increased aggression when those neighbors were displaced (Husak & Fox, 2003). Even without the spatial context of a territory, residents treated strangers more aggressively than neighbors (Husak & Fox, 2003). Individual recognition allows males to use a combination of size and aggression to drive off intruding males while maintaining a more amicable relationship with neighbors. Maintaining territories allows adult males to develop pair associations with females to enhance their future reproductive success. In this species, copulation occurs early in the breeding season and then pair bonding later, apparently for pairs to bond socially over the active season to promote early copulation the next spring. This means that adults reinforcing pair associations throughout the summer likely remember and recognize their pair mates for early copulation the next spring.

As juveniles, males, but not females, develop a conspicuous color pattern that is different from the sexually dichromatic traits seen in adults. Juvenile males develop a set of dorsolateral orange bars along the body within a couple of weeks of hatching and maintain the signal until reaching sexual maturity at the end of the fall season before starting brumation or in early spring after brumation (Trauth et al., 2004; Fox et al., 2020). Agan et al. (in prep.) found that the juvenile orange bars, in accordance with aggression, act as a receiver dependent signal and have a significant impact on intraspecific interactions. In their study, lizards enhanced with only one signal, aggression or enhanced orange bars, triggered retaliation from their male peers. Juvenile males lose their orange bars in late fall to early spring upon reaching sexual maturity, a time when most lizards are not large enough to compete with resident adult males and instead take on furtive, submissive roles and make use of alternative mating strategies (York et al., 2014). Females also benefit from the different mating strategies as females that mate with multiple individuals produce more offspring (York & Baird, 2019). By associating with juvenile females prior to maturity, juvenile males putatively use their bars and aggression to increase their potential of reproducing immediately upon reaching sexual maturity (Fox et al. 2020).

This study seeks to build on the growing body of literature looking at the effects of early-life environment by parsing out the role of juvenile orange bars and aggression on survivorship and subsequent genetic fitness in male *C. collaris* lizards. In a wild population, we experimentally altered orange bar expression by masking or enhancing the bars for the duration of the juvenile stage and implanted each male with blank or long-lasting, slow-release DHT implants. We predict lizards with masked bars will survive to the yearling stage better than lizards with enhanced bars as they will be less conspicuous to predators and enjoy reduced predation risk. We also predict that even though the bars disappear by the next year, sexually mature yearling males will retain access to the females they paired with as juveniles and the territories they established during that time. Because of the retained access, we predict that those that had more developed orange bars and higher aggression as juveniles will end up with higher fitness, represented by the number of offspring they sire.

METHODS

Field work and Study Site

We conducted the study between May 2014 and July 2018 on the property of the Oklahoma Gas and Electric generating station at Sooner Lake, Pawnee County, Oklahoma. Every spring, we surveyed the site to capture and mark all lizards and tally which lizards survived overwintering. To record lizard locations, we set numbered flags 11.45 meters apart at the site, which was a linear strip of rocky habitat from a gravel road above to the shoreline of the lake below. The width of the strip was between 5.7-12.9 m. We used a four-meter telescopic, fiberglass pole with a string or monofilament noose tied to one end to capture lizards. During the fall, we captured neonate hatchlings and assigned unique toe-clips and color codes in the form of non-toxic latex paint dots to their dorsal surface. We placed juvenile males into one of four treatments or a control group. Treatments enhanced the bars by painting on full bars that spectrally matched the natural bars at their maximal brightness or masked the bars by matching the paint to the non-bar background (Fox et al., 2020), and treatments received either a blank or hormone implant. We made the DHT implants following the procedures used by Lovern et al. (2004) to make 1.0 x 2.5-mm implants at a concentration of 1 mg DHT/mm and gave a DHT implant or blank implant to enhance levels of aggression or to keep aggression unchanged. Blank+Bars received a blank implant and enhanced bars (n = 42), DHT+Mask received a DHT implant and masked bars (n = 42), DHT+Bars received a DHT implant and enhanced bars (n = 42). The control group (Control) received a blank implant and had their bars painted with distilled water (n = 37). Throughout the season, we caught the lizards as needed to reapply paint and to ensure the implant was still present.

Survivorship

Because we captured every lizard in the fall as a juvenile and again in the next and subsequent spring/summer seasons, we were able to measure the survivorship of the lizards in each treatment. We measured survivorship by assigning an age category in relation to when the lizard hatched. We classified as juveniles those immature subjects caught between August and November. Yearling lizards were those that emerged from brumation in early spring following the juvenile stage until they started brumation again in late summer to early fall. Adults were those that emerged after brumation as yearlings, with no difference given to first- or second-year adults (or older). We calculated survivorship in R (R core team, 2019) for each treatment as the percent of individuals who survived to reproductive age (yearling stage) and used a Pearson’s chi-square test to determine if treatments had a significant impact on survivorship. We also compared male versus female survivorship using the same methods.

Parentage Analysis

While clipping the toes during the assignment of the unique toe-clip, we collected and stored a couple of drops of blood on Whatman FTA Classic Cards for subsequent parentage analysis using eight previously identified microsatellite markers of *C. collaris* (Hutchison et al., 2004; Husak et al., 2006a; Sites, 2013; York et al., 2014). Following the GE Healthcare protocol for DNA isolation using Chelex 100 resin, we took a 3-mm diameter punch from the dried blood on the cards and used a 20-gauge needle to macerate the punch in a DNase-free 1.5-ml microcentrifuge tube. We washed the disc by adding 1 ml of sterile water and incubating at room temperature for 10 minutes while occasionally vortexing. We then moved the water to a fresh tube, repeated the wash of the punch an additional time, added the second wash to the first, then discarded our tube with the macerated punch. We centrifuged the water 3 minutes at 20,000 x g and discarded the supernatant. We added 200 µl of Chelex 100:sterile water (5% w/v) and incubated the sample at 56°C for 20 minutes. After incubating, we vortexed the tube for 15 seconds and incubated the sample again at 100°C for 8 minutes. We then vortexed the sample, repeated the centrifuging step to separate the Chelex 100 resin, and transferred the extracted DNA supernatant into a fresh tube.

We used an Eppendorf thermocycler to run our polymerase chain reaction (PCR) to amplify all our loci with primers identified by Hutchinson et al. (2004). For all loci but Org25, we used 0.5 µl of 1 µM forward and reverse primers, 4 µl of nuclease-free water, 9 µl TrueAllele, and 1 ul extracted DNA. For Org25, we reduced the water to 3.25 µl and included 0.75 µl of 0.25M $MgCl_2$. Using the PCR product, we multiplexed E48 + O6 and E21 + O21, with each analysis reaction containing 9 µl formamide (Hi-Di), 0.5 µl GeneScan 400HD Rox size standard, and 1 µl PCR product for each locus. We visualized the lengths using an Applied Biosystems 3130 or 3037 genetic analyzer through capillary electrophoresis and used GeneMapper 4.0 to determine the allelic size.

We ran the parentage analysis for samples from 2015 and 2016 using data collected in 2014 – 2016. We did not run parentage for 2014 as that was the first year we put hatchling lizards into the treatment groups. We used Cervus 3.0 to assess allele frequencies in the current and previous years and to assign parentage to the offspring (Kalinowski et al., 2007; Marshall et al., 1998). We assigned parentage based on the log-likelihood statistic (LOD scores) and delta scores of father-mother-offspring trios and then father-offspring pairs if we did not identify a parent based on the trio LOD score. For each year, we selected the candidate parents as those adults or yearlings that had ever been sighted within 30 flags (343.5 m) of where the offspring was sighted at its earliest age. We ran simulations using the loci of both parents and offspring to assess the confidence levels of the delta values at 80% and 95% confidence.

We constructed generalized linear models (GLMs) in R (R core team, 2019) with our variables and used Akaike information criterion with a small sample size correction (AICc) to compare the models (Anderson, 2008). We included in the models the variables of bar treatment (masked, enhanced, or unchanged) and implant type (DHT or blank) to explain the number of offspring produced. Because we did not have enough years to adequately analyze fitness in the adult age category for treatment lizards, we compared fitness only among yearlings; thus, we excluded age as a variable.

RESULTS

Survivorship

Treatments had no impact on survival to the yearling stage ($x^2 = 0.974$, df = 3, $p > 0.05$; Figure 1). This was also true for survival to the adult stage ($x^2 = 3.19$, df = 3, $p > 0.05$). Juveniles had a 50:50 sex ratio, with 163 males and 167 females over the four-year period. Females survived to be yearlings significantly better than males (Pearson's chi-square test with Yate's continuity correction, $X^2 = 4.21$, df = 1, $p < 0.04$; Figure 2). This differential survival by sex did not carry on for yearlings surviving to be adults ($x^2 = 0.948$, df = 1, $p > 0.05$).

Parentage Analysis

The top model from the AICc analysis included implant type as the only explanatory variable predicting the number of offspring a yearling produced at the 80% confidence of parentage (Table 1). Those with DHT implants produced the most offspring (Figure 3). For the 95% confidence level, the best supported model also included only implant type (Table 1). Again, those with DHT implants produced the most offspring (Figure 3). However, the null model, or model created by randomly sampling from the distribution of the number of offspring, although less supported, had a delta AICc less than two, meaning it could not be discarded as a valid model (Table 1).

DISCUSSION

We predicted that lizards with masked bars would have a higher survivorship due to being less conspicuous without the orange bars. Instead, we saw that alterations made to the bars and/or aggression did not impact survival. Agan et al. (in revision) suggested that conspicuous *C. collaris* male behavior, such as territory defense and pair bonding, may contribute more to overall conspicuousness than just color. While we did not see increased survivorship of those with masked bars, some of those lizards with masked bars also carried a DHT implant. Perhaps this exogenous androgen increased aggressive behavior and patrolling, which put males at more risk of predation and therefore mitigated any gains that lizards with masked bars could have made by hiding the trait. In hindsight, it would have been better to also include a Blank+Mask treatment to see if covering the bars while maintaining normal aggression could have impacted survivorship. We must temper our conclusions on survivorship somewhat as during the second and third year of the study we saw an increase in the number of Greater Roadrunners (*Geococcyx californianus*) at the site. To prevent the reduction of our population size too much (roadrunners are very efficient predators on collared lizards), we relocated several roadrunners off site. We did this in conjunction with another study in which another lizard predator, coachwhip snakes (*Masticophis flagellum*), were captured and removed from an adjacent site; meaning that we suppressed the predation pressure to some extent. Despite potentially reducing the predation pressure, our survival was still low overall with only 28.2% of males and 39.5% of females surviving to be yearlings. Predator removal likely did not impact survival of lizards based on body size or overwintering site. Regardless of the cause, we still found field evidence of predation and in some cases observed actual attacks, but the pressure from predation was likely less than had we not removed some roadrunners and coachwhips. Nevertheless, females survived significantly better than males, likely because females are more cryptic and move around less than males.

We predicted that male lizards with enhanced orange bars and increased aggressiveness as juveniles would produce more offspring as yearlings than those with only enhanced bars or only elevated aggression. Instead of needing both signals to increase fitness, we found that lizards with increased aggression alone due to the DHT implants (DHT+Mask as well as DHT+Bars) produced more offspring as yearlings than lizards with blank implants. Agan et al. (in prep) showed that juvenile male lizards with DHT implants were more aggressive, but still suffered retaliation from rival males when they did not have the associated orange bars. The authors concluded that the orange bars and aggression together are a paired signal and lizards socially policed individuals and retaliated against those with one enhanced signal but not the other. Wiggins (2018) showed that lizards with naturally brighter orange bars were able to produce more offspring as yearlings than lizards with naturally duller orange bars. Our results showed that more aggressive lizards (those given DHT implants) produced more offspring, suggesting that aggression was the more important of the two signals for offspring production. This does not mean that the orange bars of males serve no purpose; we think in an unmanipulated population, the orange bars are likely used as an honest signal to advertise the bearer's aggressiveness to females and rivals (bars and aggression are linked) and therefore may be indirectly affecting the bearer's fitness as a result.

It may be that intersexual selection favors more aggressive juveniles, but intrasexual selection drives the production of the orange bars. Olsen et al. (2010) showed something similar with the evolution of plumage badges in tidal-marsh sparrows (*Melospiza georgiana nigrescens)* and inland-marsh sparrows (*M. g. georgiana*). They found that the plumage on the head was a result of both inter- and intrasexual selection. In both coastal and inland subspecies, the extent of black plumage on the forehead was a result of male competition and was correlated with aggression, while the rusty plumage on the cap was a product of female selection as the rusty cap was correlated with the amount of male parental care. The study demonstrated that the coastal birds had darker heads overall as there was likely stronger intrasexual selection occurring. That said, sexual dichromatism can be selected through means other than direct sexual selection (Andersson, 1994). Sexual color differences can arise from males utilizing different niches (Shine, 1989), from differing environmental pressures (Karubian, 2013; Medina et al., 2017; Price and Eaton, 2014), and from other selective pressures outside of sexual selection (Wallace, 1889; Hofmann et al., 2008). One could argue that the orange bars and aggression of juvenile collared lizards help secure limited resources to grow and survive better and thus lead to more offspring via natural selection instead of requiring the bars and aggression to be selected through sexual selection (e.g., Walls, 1990; Nussbaum et al., 2016). If this were true, however, we would have likely seen a difference in survival among the treatments as the groups that produced the most offspring would have also survived better, and we did not.

The exact way that the orange bars have come to appear in males and not females is not known. Instead of males evolving orange bars, the juvenile collared lizard dichromatism could have been gained through the loss of orange bars in females, perhaps due to an outside pressure like predation. Both males and females usually hatch with orange coloration, but soon after hatching males increase the expression of orange bars while females reduce it, almost always completely (Agan, personal observation). More research is needed to determine if males evolved orange bars or if the bars were lost in females, but juvenile orange bars are currently used as a signal of male aggression (Agan et al., in prep), used to attract females (Wiggins, 2018), likely costly to possess (Agan et al., in revision), and can be used as a predictor of future reproductive success (Wiggins, 2018).

This study adds to a growing body of evidence that shows the juvenile stage is an important time for not only growth and survival, but for social development. In collared lizards, this life history stage is important as the lizards engage in intraspecific aggression for territory formation and begin to associate with specific females with whom they later mate as yearlings. If lizards can secure better territories and form early pair-associations with females before reaching sexual maturity, they can be more prepared to mate should they survive to be yearlings. The yearling stage is a very important time for reproduction as only a minority of lizards survive to become first-year adults and then use their fully developed adult secondary sex characteristics and size to defend territories harboring resident females with whom they breed. York et al. (2014) showed that many male yearlings take on a “sneaker” role as adults are larger and are better able to exclude yearling males, but yearlings are quick to take over in the absence of adults. In combination with the fact that females benefit by copulating with multiple males (York and Baird, 2019), there is a complex social system that rewards males that survive to become yearling males, and first-year and first-year+ adults. While we showed that juvenile aggression is likely more predictive of future offspring production than the presence of orange bars, it is important to note that orange bars are naturally tied to male aggression and the two traits together impact future ability to reproduce.

Our results give us a snapshot of the importance of the juvenile orange bars and aggression for later fitness. We hypothesized that lizards with both enhanced bars and increased aggression would produce the most offspring, while lizards missing one of the signals would not produce as many offspring, if any. Instead, we found that aggressiveness alone appears to have the largest impact for future offspring production, while the bars themselves are likely signaling the aggressiveness of the bearer to its conspecifics. That said, because of low sample-size and power, we would need more data to conclude this definitively. We agree with Fox et al. (2020) that juvenile collared lizards are likely subject to precocial sexual selection favoring more aggressive juvenile males, and the linked orange bars have evolved to serve as an indicator of the lizard's aggressiveness. Our work helps to demonstrate the importance of considering early-life effects on an individual and we believe more studies should focus on early development and how juvenile traits can impact subsequent fitness.

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Table 1. AICc model selection results for yearling lizards assigned offspring at 80% confidence and 95% confidence.

	ΔAICc	***K***	***W_{AIC}***
Parents assigned at 95% confidence			
Implant Type	0.0	2	0.502
Null Model	1.4	1	0.251
Bar Type	2.6	3	0.137
Implant Type + Bar Type	4.4	4	0.055
Implant Type + Bar Type + Implant Type*Bar Type	4.4	4	0.055
Parents assigned at 80% confidence			
Implant Type	0.0	2	0.699
Null Model	3.6	1	0.115
Bar Type	4.7	3	0.068
Implant Type + Bar Type	5.0	4	0.059
Implant Type + Bar Type + Implant Type*Bar Type	5.0	4	0.059

Models are ranked by ΔAIC_C. *K* = the total number of parameters in each model. W_{AIC} = Akaike weight of the model. Implant Type = DHT or blank implant, Bar Type = color of treatment lizard's bars (Enhanced, Masked, or Unaltered).

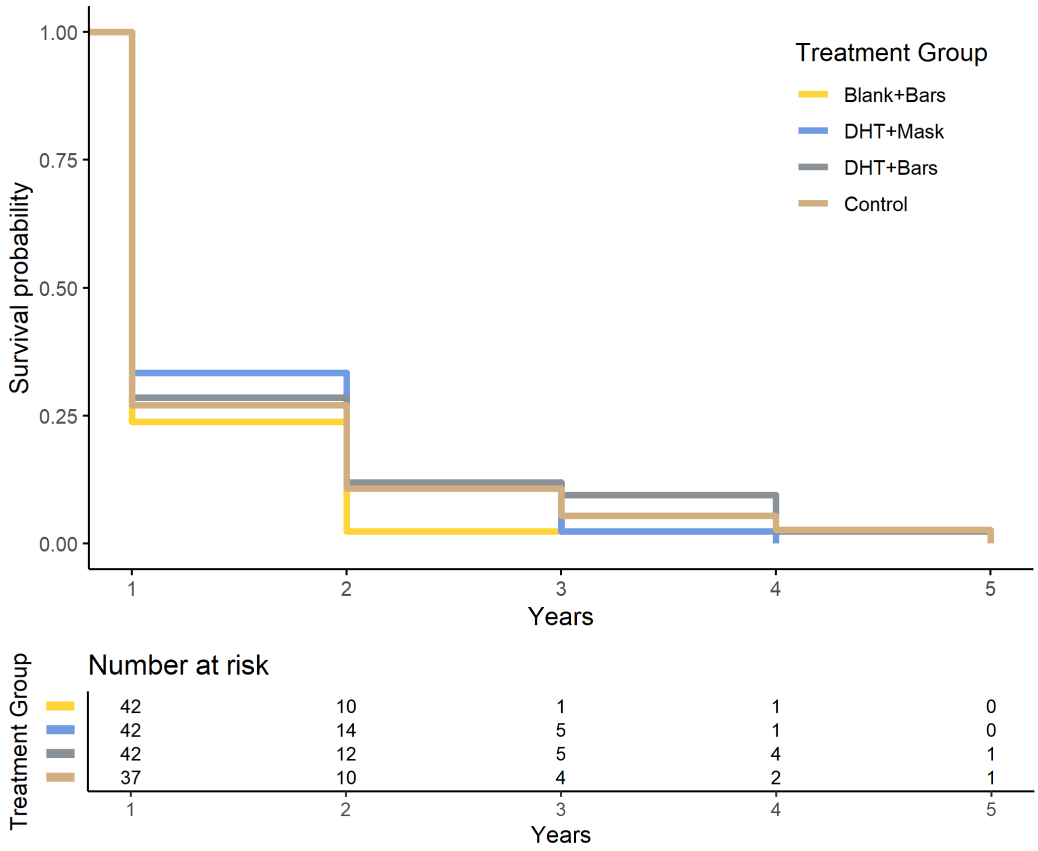

Figure 1. Each line demonstrates the proportion of a treatment group surviving to the next year. The juvenile stage occurs during the first year, the yearling stage occurs during the second year, the first-year adult stage occurs during the third year, the second-year adult stage occurs during the fourth year, and the third-year adult stage occurs during the fifth year. Risk table shows number of individuals in each treatment alive at the start of each year.

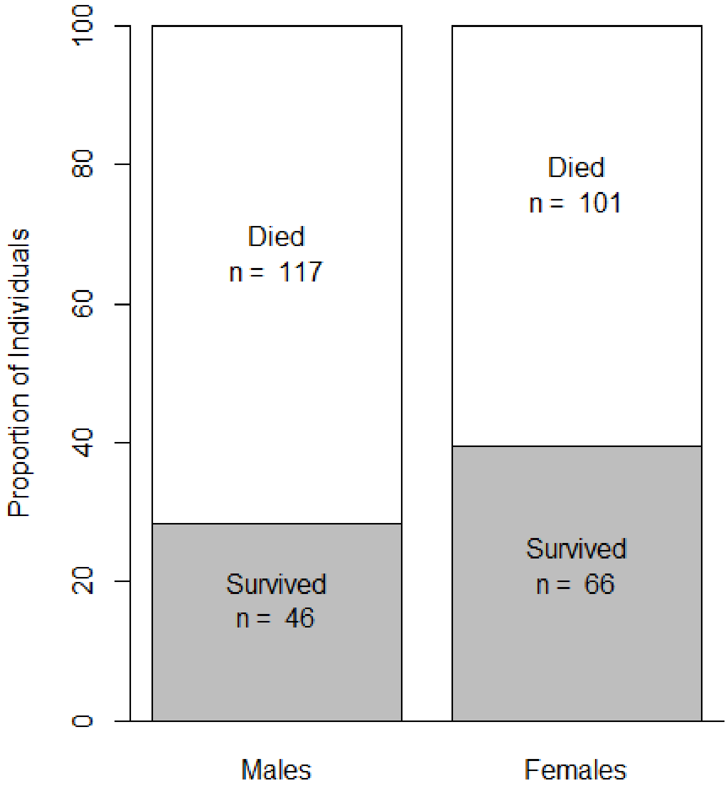

Figure 2. Number of males and females that survived to be yearlings and those who died.

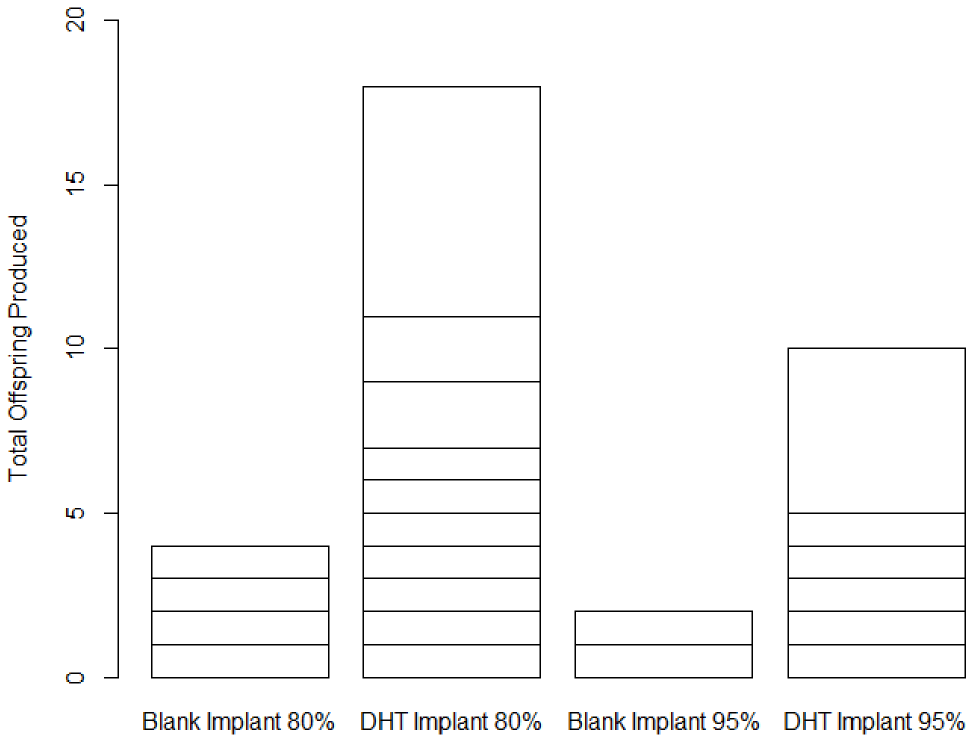

Figure 3. Number of offspring assigned to yearling parents at 80% confidence and 95% confidence. Each box within the bar accounts for the number of offspring produced by each yearling parent, minimum of 1.

VITA

Justin W. Agan

Candidate for the Degree of

Doctor of Philosophy

Dissertation: PRECOCIAL SEXUAL SELECTION IN *CROTAPHYTUS COLLARIS*: A FIELD STUDY

Major Field: Zoology

Biographical:

Education:

Completed the requirements for the Doctor of Philosophy in Zoology at Oklahoma State University, Stillwater, Oklahoma in July, 2021.

Completed the requirements for the Bachelor of Science in Biology at Clayton State University, Morrow, Georgia in May, 2013.

Completed the requirements for the Bachelor of Arts in Philosophy at Clayton State University, Morrow, Georgia in May, 2013.

Experience:

Graduate Research Assistant: 2014 – 2018
Graduate Teaching Assistant: 2015 – 2017
Venomous Snake and Fish Curator: 2016 – 2021
OSU Collection of Vertebrates, Collection Manager: 2018 – 2021

Professional Memberships:

American Society of Ichthyologists and Herpetologists
Herpetologists' League
Society for the Study of Amphibians and Reptiles
Southwestern Association of Naturalists