# PROXIMATE AND ULTIMATE MECHANISMS ASSOCIATED WITH FEMALE SECONDARY COLORATION IN THE MEXICAN BOULDER SPINY LIZARD (*SCELOPORUS PYROCEPHALUS*)

by

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Presented to the Faculty of the Graduate School of

The University of Texas at Arlington

in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE IN BIOLOGY

THE UNIVERSITY OF TEXAS AT ARLINGTON

May 2006

ACKNOWLEDGEMENTS

I am indebted to my advisor, D. Formanowicz, and committee members, J. Campbell, D. Hews, and M. Roner, for their invaluable mentoring and unwavering support of both me and this project. I am very thankful to J. Boylan, who encouraged me to enroll in a graduate program to further pursue my passion for untangling the mechanisms behind biological phenomena. E. Smith was instrumental in aiding me in the commencement of this project, and I thank him for his many discussions throughout our two months of travel together in Mexico, and I also thank him as well as L. Canseco-Márquez, U. García-Vázquez, J. Malcom, J. Malone, P. Poncé, and many others for their help in the field.

This work would not have been possible without the omnipresent help and guidance of my committee member, D. Hews. D. Hews, along with her lab (M. Chakraborty, M. Thacker, and Z. Kabange), assisted me in the column chromatography, radioimmunoassay, and analysis of all hormone data. This work would also not have been possible without the help of parasitologists M. A. Arízmendi-Espinósa, E. Martínez-Salazar, and R. Mata-López from the Universidad National Autónoma de México, who collected and identified all parasites used in analyses.

I thank D. Brooks, R. Knapp, E. Martins, J. Mateo, J. Malone, K. McGraw, and M. Moore for discussions, D. Hawkins, J. Malone, J. Meik, S. Passy, and W. Schargel

for statistical input, N. Hubbard, M. Nelson, and K. McGraw for their advice on color analyses, and M.A. Michaós-Arías for permitting me use of his property to conduct a large part of this study. I am grateful to B. Fontenot, J. Malcom, J. Malone, K. McGraw, J. Meik, and my Master's advisor and committee for their valuable critiques of the chapters of this manuscript.

I thank J. Campbell, O. Flores-Villela, and V. León-Règagnon for funding support of this project (NSF DEB# 06423610), which allowed me and many other fortunate students to explore the incredible herpetofauna of México. This project was also funded by the East Texas Herpetological Society, the Southwestern Association of Naturalists, the Texas Academy of Science, and the University of Texas at Arlington Phi Sigma Society.

Finally, I thank my loving parents, D. and A. Calisi, sister, S. Calisi, and family members N., J., K., and M. Rodriguez, as well as urban-family members, J. Gillette, M. McGee, L. Pence, N. Thompson, and M. Watson, for their undying support of any and all my aspirations. I would like to especially thank my best friend and confidant, J. Malone, for not only guiding me, but for challenging me throughout all aspects of this project. He is the hardest working person I know, and his love and dedication to his research, as well as his amazing ability and willingness to both learn and teach, have been and will continue to be inspirational.

May 5, 2006

ABSTRACT

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Publication No. _____

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The University of Texas at Arlington, 2006

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Secondary sexual coloration is thought to be the result of runaway selection for signals that honestly communicate relative fitness. Much theoretical work suggests sexual selection has driven conspicuous secondary traits in males while natural selection has driven inconspicuous traits in females; however, in some species, bright female secondary coloration does exist. I used the Mexican boulder spiny lizard (*Sceloporus pyrocephalus*) as a model to examine secondary coloration in females. Adult females of *S. pyrocephalus* have conspicuously colored gular regions, ranging

from red, orange, to yellow, while adult males weakly express color in the gular region. Both sexes have dark stripes on the gular and venter areas and display these traits during same- and opposite-sex encounters. I examined sex steroid hormonal concentrations, reproductive state, and nematode abundance as predictors of secondary coloration, and how color can ultimately relate to resource holding potential.

My results suggest that color in females may be under hormonal control. Coloration in females changes with reproductive state, and hormonal changes associated with the female reproductive cycle may control this color change. Nematode loads are related to female color as well as circulating plasma testosterone concentrations; thus, color has the potential to serve as an honest signal of reproductive state and nematode load. Finally, while the ultimate implications of female coloration require additional study, larger areas covered by females with red gular regions and pale gular and venter stripes (color characteristics also indicative of high testosterone, late vitellogenic reproductive stages, and high nematode loads) could lead to the increased acquisition of resources, access to mates, and/or egg depositing sites, which in turn may result in greater fitness benefits. Conversely, these resource benefits may carry a cost of an increased predation risk, energy expenditure, and/or parasite exposure. Females of *S. pyrocephalus* may experience a tradeoff between sexual selection for secondary coloration and natural selection for survivorship. These results both illuminate and further necessitate new theoretical and empirical studies of female coloration to provide a more inclusive theory of sexual selection.

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## PLASMA HORMONE CONCENTRATIONS, REPRODUCTIVE STATE, AND MULTIPLE SEXUAL COLOR TRAITS IN MALES AND FEMALES OF *SCELOPORUS PYROCEPHALUS*


### 1.1 Abstract

Proximate mechanisms underlying color expression in males have been widely examined, but there has been less work on coloration in females. Adult female Mexican boulder spiny lizards (*Sceloporus pyrocephalus)* have conspicuously colored gular regions, ranging from red to yellow, while adult males only weakly express such color in their gular region. By contrast, both sexes have dark blue and black colored gular stripes and venter stripes. To characterize the proximate mechanisms influencing color in males and females of *S. pyrocephalus*, I examined three color variables (hue, saturation, and brightness) for the gular region, gular stripes, and venter stripes and their relationship with plasma steroid hormone concentrations of testosterone (T) and corticosterone (CORT) in males and T, CORT, and 17-β estradiol (E2) in females. I also examined the relationship between hormones and reproductive state (testes volume in males and vitellogenic follicles versus oviductal egg stages in females). High CORT may be related to dull gular stripes in males and dull venter stripes in females. Relationships may also exist between high T and E2 in females in association with red gular regions, pale grey gular stripes, and bright venter stripes. Male testes volume was

not related to color or hormone variables, but females with high E2 were carrying large vitellogenic follicles as opposed to oviductal eggs.

## 1.2 Introduction

Many species are sexually color dimorphic, in which males express bright secondary sexual coloration that can aid in their mate selection by females, which express dull coloration due to natural selection pressures. Variation in male secondary coloration can communicate, for example, receptivity periods (Cooper and Greenberg 1992), health (Zahavi 1975, Hamilton and Zuk 1982, Folstad and Karter 1992), and fighting abilities and resource acquisition (Sinervo et al. 2000, Calsbeek and Sinervo 2002, Zamudio and Sinervo 2003). The quality of these traits can honestly communicate the state of an organism, leading to variation in conspecific mate choice and thus fueling the evolution of the secondary characteristic (Fisher 1958, Kirkpatrick 1982, Andersson 1994).

Although much less common, secondary traits exist in females of some species. Female secondary sexual coloration has been documented in association with the reproductive cycle, including mammals (Dixson 1983, Setchell and Wickings 2004), fish (Rowland et al. 1991, Baird 1988), birds (Montegomerie and Thornhill1989, Roulin et al. 2001), and reptiles (Medica et al. 1973, Mitchell 1973, Ferguson 1976, Cooper et al. 1983, Cooper and Greenberg 1992, Watkins 1997, Cuadrado 2000, Hager 2001, Weiss 2002). The courtship stimulation hypothesis (Cooper and Greenberg 1992) suggests that secondary coloration in females may be advantageous to both sexes if

related to female fertilization receptivity. A female signaling unreceptivity to a male may reduce her costs commonly associated with courting and copulation, such as energy expenditure, predation risk, and alterations in thermoregulation and metabolism.

Several hormones, such as steroids testosterone (T), 17-β estradiol (E2), and corticosterone (CORT), can affect secondary color in reptiles (Cooper and Greenberg 1992, Hews and Moore 1995, Salvador et al. 1996, Hews and Quinn 2003). Reproductive state, related to steroid hormonal changes (Moore and Lindzey 1992, Whittier and Tokarz 1992, Crews 1979*a,b*), can also be associated with color change in some species.

Although numerous studies have examined hormone associations with one secondary color in one sex, few have examined hormone associations with multiple color traits in both sexes. The Mexican boulder spiny lizard, *Sceloporus pyrocephalus* (Cope 1864), is a oviparous species (Ramírez-Bautista and Olvera-Becerril 2004) that exhibits monochromatic masculinized coloration (Hews and Quinn 2003; Fig.1), in that both sexes exhibit blue/black gular stripes and venter stripes, but females express a conspicuous red gular region (varying from red to yellow; Fig. 2) while males only weakly express such color (pale yellow to white) in the gular region. Both males and females inflate their gular region and flatten their ventral area laterally when confronted with a conspecific (Calisi, personal observation), suggesting color of the gular region, gular stripes and venter stripes are visible to opposing company. Color perception in this species is unknown, but many reptiles have retinas that express cones involved in color perception (Fleishman et al. 1993, Yokoyama and Yokoyama 1996, Yokoyama

1997), and thus variation in male and female secondary coloration may be a trait on which selection has and/or is acting.

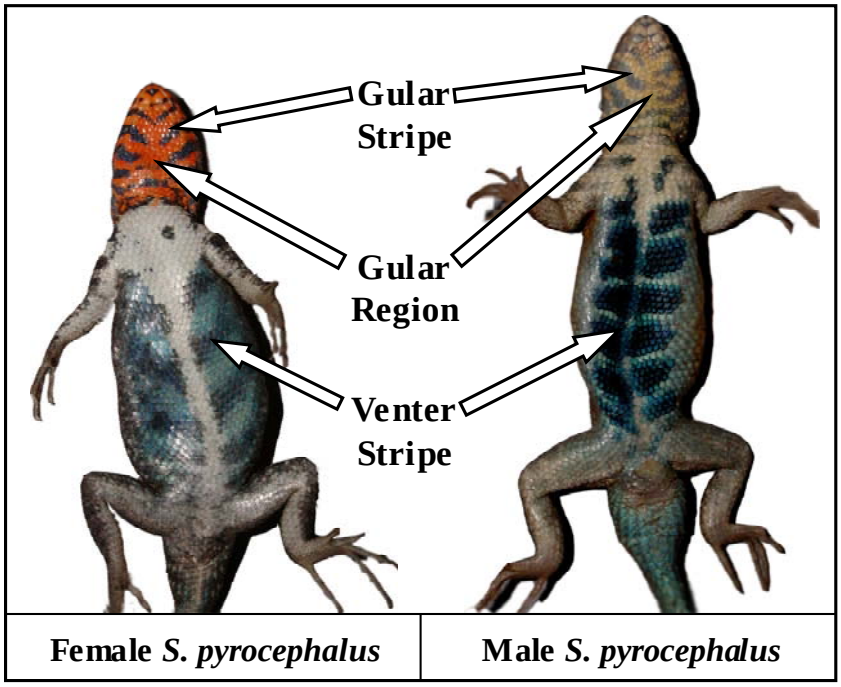


Figure 1.1 Male and female color variables.
Variables included the gular stripes, the gular region (which did not include the striped portion), and the venter stripe.

Figure 1.2. Color variation in female *S. pyrocephalus*.
Variation is evident in the gular region and gular stripe region (top row) and venter stripe region (bottom row).

I examined hormones T and CORT in males and T, CORT, and E2 in females of *S. pyrocephalus* and their relationship to color variation in the gular region, gular stripes, and venter stripes. I also examined the relationship between hormone concentrations and reproductive stages and discuss my results in the context of the courtship stimulation hypothesis (Cooper and Greenberg 1992).

## 1.3 Methods

Fieldwork was performed during the mid-breeding season of *S. pyrocephalus* (see Ramírez-Bautista and Olvera-Becerril, 2004) in tropical dry forest habitat during 17 June- 6 July, 2004. Twenty-eight mature female and 25 mature male *S. pyrocephalus* were sampled during the time of 1600-1800 hrs from eight different locations surrounding the small town of Lombardia, Michoacán, México (751m 19.17662N, 102.66351W).

The body size of each individual, as measured by snout to vent (SVL) length, was examined for any relationships with reproductive state, color, and/or hormonal variables. These variables were also examined for relationships to the time, day, and/or location of capture as well as the time it took to pursue and bleed each animal.

### 1.3.1 Reproductive stage

Lizards were necropsied to gain data on reproductive state. Female reproductive stage was categorized as to whether a female was carrying vitellogenic follicles versus oviductal eggs (follicles become eggs once they leave the ovary and enter the oviduct). All females collected were carrying follicles or eggs. Females in vitellogenic stages were all carrying relatively large follicles, and were thus determined

to be in late vitellogenic stages (vitellogenic follicles: 190.97mm$^2$ ± 43.23, $N$ = 9; oviductal eggs 251.26mm$^2$ ± 81.99, $N$ = 16). Late vitellogenic as well as oviductal stages are common for this time of year in this species (Ramírez-Bautista and Olvera-Becerril 2004).

All males collected were determined sexually mature because they expressed waxy femoral pores and enlarged hemipenes. Male reproductive stage was determined by measuring testes size in males to the nearest 0.1mm; an average measurement was calculated from both testis measurements per male. The volume of each testis was estimated using the formula for a prolate spheroid:

$V=4/3\pi\ (length/2)*(width/2)^2$

Due to a simultaneous study involving the examination of the reproductive tract in these lizards, egg and testes size were not measured in one male and three females (randomly selected); thus, a total of 24 males and 25 females were used for analyses associated with reproductive state.

### 1.3.2 Blood sampling

I sampled blood from the retro-orbital sinus using heparinized microcapillary tubes. Blood samples were collected from 53 adults (25 males and 28 females) immediately following capture. The time that elapsed while catching the lizard and the time interval following the capture and during the collection of blood (capture and bleed time) were recorded and examined for correlations to hormone concentrations  Plasma

samples were kept in an ice cooler until hand centrifuged (within 12 hours) and then stored in liquid nitrogen until placed in a -20ºC freezer at the Universidad Autónoma de México (UNAM) until being transferred to Indiana State University (ISU) on dry ice for assaying.

### 1.3.3 Hormone analyses

I measured total plasma concentrations of testosterone (T), 17-B estradiol (E2), and corticosterone (CORT) in females and T and CORT in males by radioimmunoassay (RIA) after chromatographic separation on microcolumns of diatomaceous earth (following Wingfield and Farner 1975, as modified by Moore 1986 and Hews et al. 1994). Briefly, the Wingfield-Farner method involves first separating steroid hormones from each other and neutral interfering lipids using ether extraction and phase partition column chromatography. Then the purified steroid hormone fractions are dried, re-suspended in assay buffer, and assayed in triplicate using RIA. Individual samples are corrected for differences in plasma volumes and in individual recovery, which is assessed by adding a small amount of radiolabeled steroid hormone to each sample prior to the ether extraction.

Samples were analyzed in two assays. The first assay (assay 1) included blood plasma from females, and the second assay (assay 2) was performed on plasma from males. I first equilibrated plasma samples (usually 20 ul minimum 10 ul volume) overnight with 2400 cpm of each tritiated steroid for calculations of steroid recoveries from individual samples. I extracted the steroids from the plasma samples twice with diethyl ether (2 x 2ml), dried the ether phase in a 37 °C bath with nitrogen gas,

resuspended the steroids in 10% ethyl acetate pseudosaturated with ethylene glycol in isooctane, and refrigerated the samples overnight at 4 °C. For chromatographic separation of steroids I added the samples to celite microcolumns (celite: propanediol: ethylene glycol [4:1:1 w:v:v] in three aliquoits over celite:water [3:1 w:v] in one aliquoit). Neutral lipids, P (not assayed), T, E2, and CORT were eluted from the columns with increasing concentrations of ethyl acetate in isooctane to separate steroids according to polarity (neutral lipids, P, T, E2, and CORT in 3.0, 3.6, 4.5, 4.5, 4.0 and 4.0 ml of 0%, 1%, 10%, 20%, 40% and 52% of ethyl acetate in isooctane, respectively). Short columns were used for assay 2. Steroids were eluted from the columns with increasing concentrations of ethyl acetate in isooctane to separate steroids according to polarity: neutral lipids and DHT (not assayed), T, and CORT in 1.5, 2.2 and 2.8 ml of 10%, 20%, and 52% of ethyl acetate in isooctane, respectively.

After evaporating the organic solvents, I resuspended the steroids in phosphate buffered saline with gelatin and sodium azide and refrigerated the samples overnight at 4 °C before performing the radioimmunoassay. Duplicate aliquots of each sample were then incubated overnight at 4 °C with antibodies (testosterone, WLI-T3003, from RDI Division of Fitzgerald Industries Int., Concord MA; corticosterone, B3-163, from Esoterix Inc., Calabasas Hills, CA) and tritiated steroids (NEN Life Sciences, now PerkinElmer Life Sciences; testosterone NET 553, corticosterone NET 399). Unbound steroids were then removed by adding Dextran-coated charcoal and centrifuging after 10 minutes. I added a toluene-based scintillation fluid to the supernatant and counted radioactivity after a minimum 12-hr hold. Calculations of steroid concentrations were

corrected for individual sample recoveries and for plasma volumes. For assay 1, average individual recoveries were 85.0% for T, 84.3% for E2, and 53.1% for CORT. Within-assay coefficient of variation, based on six standard samples run with the plasma samples, was 2.0% for T, 6.3% for E2, and16.4% for CORT. For assay 2, average individual recoveries were of 84.8% for T, and 75.4% for CORT, and the intra-assay coefficient of variation of six standard columns was 2.0% for T and 9.4% for CORT.

### 1.3.4 Color measurements

Digital images were taken of the gular and ventral areas immediately on capture of each lizard using an Olympus C-700 digital camera in a shaded area. Lighting was standardized by using a camera flash, and each lizard was photographed on a neutral grey-colored cardstock board that served as a color standard. The distance between camera lens and lizard was standardized and ranged from 10-12 cm. Digital images were saved as TIFF files. I used this method to quantify color because it allowed for immediate documentation upon field capture (some lizards can change in color when being handled for long periods of time; Norris, 1967) and greater objectivity as compared to human-assigned values using color chips (Hamilton et al. 2005).

Color data were collected for several characteristics of each lizard. Two measurements were taken for the gular region; one of the larger reddish area in females or the yellow-white area in males (gular region), and the other for the dark, contrasting horizontal stripes on the throat (gular stripes). A separate color measurement was taken of the dark venter stripes ( Fig. 1). When needed, hue, saturation, and brightness were

adjusted using the background standard to maintain the same color standard values between digital images. This process was to ensure any difference in ambient lighting during photographing was greatly lessened or eliminated.

I measured color with Adobe Photoshop Elements, Version 3.0 software. Hue, saturation, and brightness values for the gular region, gular stripes, and venter stripes were documented by taking an average color sample from four arbitrarily chosen five by five pixel areas. For hue, measured in degrees, smaller values indicated more red (less orange-yellow) gular regions, darker gular stripes (more black/ blue), and darker venter patches (more black/dark blue). Saturation, given a percent value, measures the height and width, or sharpness, of the associated reflectance peak. In this species, it was observed that deep red colors had tall, narrow peaks, indicating high saturation. Oranges and yellows had shorter, wider peaks, and were therefore considered less saturated. The opposite was true for the dark blue/black coloration of the gular stripes and venter patches. There is no peak for a black (zero reflectance) spectral curve; therefore, dark black coloration is considered unsaturated, whereas lighter blue and grey shades express a higher degree of saturation. Brightness, given a percent value, captures the amount of white, or total reflectance, of a color, with higher values indicating brighter colors.

### 1.3.5 Statistical analyses

I checked for equality of variance and normality of $\log_{10}$ transformed data for all variables. A two-tailed student's t-test was used to compare T and CORT concentrations in males and females, and alpha was set at 0.05. I examined how T, E2,

and CORT were related in females and T and CORT relationships in males. I then assessed what hormones may be associated with reproductive state (vitellogenic versus oviductal stages) in females and testis size in males.

I initially used Principal Components Analysis (PCA) in SYSTAT 8.0 to extract the factor scores for the first principle component (Eigen value > 1) of hue, saturation, and brightness values for each body location of the gular region, gular stripe, and venter stripe areas. These first components were then used in multiple linear regression analyses (general linear model procedure: GLM) to model the relationship between the color trait value, plasma hormone concentrations, and testes volume or vitellogenic versus oviductal stages. I checked for confounding factors such as SVL, collection date, collection location, collection time (of day), and the time it took to capture and bleed a lizard (capture and bleed time).

I then took a univariate approach and analyzed hue, saturation, and brightness color variables for each location on the body (gular region, gular stripe, and venter stripe) independently with plasma hormone concentrations and testes volume or vitellogenic versus oviductal stages. Because this sample was taken from its natural setting, which may expose it to numerous confounding variables not examined in this study and thus make relationships more difficult to assess, and due to the general exploratory nature of these analyses, I was not conservative, and alpha was set at 0.05. Results from this study are intended to generate hypotheses for future testing in a controlled lab setting.

## 1.4 Results

Capture and bleed time ranged from two to nine minutes in males (mean ± SD = 4.57 ± 2.08) and females (4.75 ± 1.71). Plasma T concentrations in females (5.22 ± 16.78 ng/ml) were not significantly different from male concentrations (3.44 ± 8.56 ng/ml), but mean CORT concentrations in females (9.60 ± 0.33 ng/ml) were significantly higher ($t$ = -3.49, df = 54, $P < 0.001$) than concentrations in males (4.31 ± 4.75). Female E2 concentrations averaged 0.233 ± 0.327 ng/ml.

### 1.4.1 Males

The concentrations of T and CORT were not related ($F_{1,26} = 0.107$, $P = 0.747$). Concentrations of T and CORT were not related to testes volume ($F_{2,21} = 0.321$, $P = 0.729$). Principal components of coloration as well as gular region, gular stripe, and venter stripe hue, saturation, and brightness, were not related to testes volume.

#### 1.4.1.1 PCA and GLM

The first principle component of gular hue, saturation, and brightness (gular color) yielded an eigenvalue of 1.70 and explained 56.54% of the total variance. The first component for gular stripe hue, saturation, and brightness yielded an eigenvalue of 1.56 and explained 51.96% of the total variance. The first component for venter stripe hue, saturation, and brightness yielded and eigenvalue of 1.30 and explained 43.21% of the total variance. The second principle components yielded Eigen values less than one and were not considered in analyses.

The first principle component for gular coloration was related to collection date ($F_{5,12} = 7.302$, $P = 0.002$) and was thus controlled in analyses concerning gular color (Table 1.1).

Table 1.1 Male and female color associations with confounding variables. Male and female color variables and their relationships with possible confounding factors of snot-vent length (SVL), collection date, collection site, collection time of day, and capture and bleed time. Factor loadings from the first principle component in a multivariate PCA that include hue, saturation and brightness for each body location were assessed, as were simple models for individual color variables for each body location. The first principle components all had Eigen values greater than 1. Abbreviations: *, P < 0.05; $^{+}$, trend (P<0.10).

| **Dependent Variable: MALE** | **DF** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| Gular region first component | 5,12 | 0.163 | 0.741 | **0.002*** |
| Gular stripe first component | 5,12 | 0.540 | 2.817 | *0.066*$^{+}$ |
| Venter stripe first component | 5,12 | 0.096 | 0.256 | 0.929 |
| | | | | |
| Gular Region Hue | 5,12 | 0.840 | 12.566 | **<0.001*** |
| - collection date | 1 | | | **<0.001*** |
| - capture and bleed time | 1 | | | **0.025*** |
| Saturation | 5,12 | 0.548 | 2.909 | *0.060*$^{+}$ |
| Brightness | 5,12 | 0.392 | 1.550 | 0.247 |
| | | | | |
| Gular Stripe Hue | 5,12 | 0.342 | 2.909 | *0.060*$^{+}$ |
| Saturation | 5,12 | 0.578 | 3.292 | **0.042*** |
| - collection site | 1 | | | *0.070*$^{+}$ |
| Brightness | 5,12 | 0.151 | 0.425 | 0.822 |
| | | | | |
| Venter Stripe Hue | 5,12 | 0.211 | 0.641 | 0.673 |
| Saturation | 5,12 | 0.330 | 1.185 | 0.372 |
| Brightness | 5,12 | 0.262 | 0.854 | 0.538 |

| **Dependent Variable: FEMALE** | **DF** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| Gular region first component | 5,19 | 0.163 | 0.741 | 0.602 |
| Gular stripe first component | 5,19 | 0.214 | 1.032 | 0.427 |
| Venter stripe first component | 5,19 | 0.427 | 10.910 | **<0.001*** |
| | | | | |
| Gular Region Hue | 5,19 | 0.247 | 1.249 | 0.326 |
| Saturation | 5,19 | 0.237 | 1.178 | 0.356 |
| Brightness | 5,19 | 0.037 | 0.147 | 0.978 |
| | | | | |
| Gular Stripe Hue | 5,19 | 0.196 | 0.925 | 0.486 |
| Saturation | 5,19 | 0.226 | 1.110 | 0.388 |
| Brightness | 5,19 | 0.120 | 0.520 | 0.758 |
| | | | | |
| Venter Stripe Hue | 5,19 | 0.120 | 0.520 | 0.758 |
| Saturation | 5,19 | 0.449 | 3.099 | **0.033*** |
| - collection date | 1 | | | **0.001*** |
| Brightness | 5,19 | 0.726 | 10.088 | **<0.001*** |
| - collection site | 1 | | | **0.003*** |
| - collection location | 1 | | | **0.001*** |
| - collection time of day | 1 | | | **0.004*** |

Although the overall model examining the gular region's association with T and CORT, controlling for date collected, was significant ($F_{3,21} = 13.690$, $P < 0.001$), T ($P = 0.740$) and CORT ($P = 0.815$) were not related to the first principle component for gular color. Gular stripe ($F_{2,22} = 1.722$, $P = 0.202$) and venter stripe ($F_{2,22} = 0.297$, $P = 0.746$) areas also did not exhibit significant relationships (Table 1.2).

Table 1.2 Male and female color and hormones.
After extracting the first principle components for the variables measuring hue, saturation, and brightness of each body location (gular region, gular stripe, and venter stripe), relationship of the factor loading scores with T (testosterone) and CORT (corticosterone) for males, and T, CORT, and E2 (17-beta estradiol) for females were assessed with GLM's, in which confounding variables were controlled for if they significantly explained variation. Abbreviations: *, $P < 0.05$; $^{+}$, trend ($P<0.10$).

| **MALES** | | | | |
|---|---|---|---|---|
| **Gular Region First Component** | **DF** | **$R^2$** | **F** | **Sig.** |
| MODEL: | 3,21 | 0.662 | 13.690 | **<0.001*** |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| T | -0.056 | -0.045 | -0.337 | 0.740 |
| CORT | -0.065 | -0.030 | -0.237 | 0.815 |
| collection date | 0.005 | 0.823 | 6.109 | **<0.001*** |
| **Gular Stripe First Component** | **DF** | **$R^2$** | **F** | **Sig.** |
| MODEL: | 2,22 | 0.135 | 1.722 | 0.202 |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| T | 0.204 | 0.166 | 0.832 | 0.414 |
| CORT | -0.679 | -0.315 | -1.585 | 0.127 |
| **Venter Stripe First Component** | **DF** | **$R^2$** | **F** | **Sig.** |
| MODEL: | 2,22 | 0.026 | 0.297 | 0.746 |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| T | -0.041 | -0.033 | -0.157 | 0.876 |
| CORT | -0.348 | -0.161 | -0.765 | 0.453 |
| **FEMALES** | | | | |
| **Gular Region First Component** | **DF** | **$R^2$** | **F** | **Sig.** |
| MODEL: | 3,24 | 0.127 | 1.164 | 0.344 |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| T | -0.451 | -0.299 | -1.307 | 0.204 |
| CORT | -0.808 | -0.198 | -1.025 | 0.316 |
| E2 | 0.137 | 0.234 | 1.030 | 0.313 |

Table 1.2 - continued

| **Gular Stripe First Component** | **DF** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 3,24 | 0.172 | 1.665 | 0.201 |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| T | -0.640 | -0.424 | -1.904 | *0.069*$^{+}$ |
| CORT | -0.150 | -0.037 | -0.195 | 0.847 |
| E2 | 0.246 | 0.419 | 1.894 | *0.070*$^{+}$ |

| **Venter Stripe First Component** | **DF** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 6,19 | 0.728 | 8.474 | **<0.001*** |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| T | -0.006 | -0.004 | -0.025 | 0.981 |
| CORT | 0.696 | 0.177 | 1.326 | 0.201 |
| E2 | -0.205 | -0.354 | -1.915 | *0.071*$^{+}$ |
| collection date | 224.800 | 0.455 | 2.855 | **0.010*** |
| collection location | -0.183 | -0.362 | -2.126 | **0.047*** |
| collection time of day | 0.004 | 0.437 | 2.991 | **0.008*** |

### 1.4.1.2 Exploratory GLM analyses

Gular hue was related to date of collection and bleed time ($F_{5,12} = 12.566$, $P < 0.001$); thus, these variables were controlled in analyses concerning gular hue (Table 1.3).

The model examining the relationship between gular hue and T and CORT, controlling for date of collection and bleed time, was significant ($F_{4,20} = 12.484$, $P < 0.001$), but T ($P = 0.622$) and CORT ($P = 0.982$) were not significant within the model.

Gular stripe brightness exhibited a trend with CORT (model: $F_{2,22} = 2.906$, $P = 0.076$; within model results for CORT: $P = 0.038$), with high CORT associated with dull gular stripes.

Concentrations of T or CORT were not related to venter stripe hue ($F_{2,22} = 0.109$, $P = 0.897$), venter stripe saturation, ($F_{2,22} = 0.455$, $P = 0.640$), or venter stripe brightness ($F_{2,22} = 0.651$, $P = 0.531$; Table 1.3).

Table 1.3 Analysis of color and hormones in males.

Results from exploratory GLM analyses individually examining the hue, saturation, and brightness of the gular region, gular stripe, and venter stripe in relation to plasma levels of T (testosterone) and CORT (corticosterone). Confounding variables were controlled for when they contributed significantly to explaining variation. Abbreviations: *, P <0.05; $^{+}$, trend (P <0.10).

| **Gular Region Hue** | **DF** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 4,20 | 0.714 | 12.484 | **<0.001*** |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| T | -0.011 | -0.065 | -0.501 | 0.622 |
| CORT | 0.001 | 0.003 | 0.023 | 0.982 |
| collection date | 0.001 | 0.871 | 6.710 | **<0.001*** |
| capture and bleed time | 0.014 | 0.221 | 1.785 | *0.090*$^{+}$ |

| **Gular Region Saturation** | **DF** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 2,22 | 0.035 | 0.401 | 0.675 |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| T | -0.041 | -0.143 | -0.681 | 0.503 |
| CORT | 0.055 | 0.110 | 0.524 | 0.606 |

| **Gular Region Brightness** | **DF** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 2,22 | 0.111 | 1.369 | 0.275 |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| T | 0.005 | 0.143 | 0.709 | 0.486 |
| CORT | -0.017 | -0.289 | -1.432 | 0.166 |

| **Gular Stripe Hue** | **DF** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 2,22 | 0.040 | 0.464 | 0.635 |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| T | 0.022 | 0.133 | 0.634 | 0.533 |
| CORT | 0.048 | 0.162 | 0.774 | 0.447 |

| **Gular Stripe Saturation** | **DF** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 2,22 | 0.079 | 0.950 | 0.402 |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| T | -0.019 | -0.097 | -0.472 | 0.641 |
| CORT | 0.088 | 0.257 | 1.252 | 0.224 |

| **Gular Stripe Brightness** | **DF** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 2,22 | 0.209 | 2.906 | *0.076*$^{+}$ |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| T | 0.008 | 0.152 | 0.798 | 0.433 |
| CORT | -0.037 | -0.419 | -2.202 | **0.038*** |

| **Venter Stripe Hue** | **DF** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 2,22 | 0.010 | 0.109 | 0.897 |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| T | -0.005 | -0.057 | -0.270 | 0.790 |
| CORT | 0.012 | 0.076 | 0.359 | 0.723 |

Table 1.3 - continued

| Venter Stripe Saturation | | DF | $R^2$ | F | Sig. |
|---|---|---|---|---|---|
| MODEL: | | 2,22 | 0.040 | 0.455 | 0.640 |
| | | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| | T | -0.008 | -0.052 | -0.248 | 0.806 |
| | CORT | 0.050 | 0.188 | 0.898 | 0.379 |
| **Venter Stripe Brightness** | | **DF** | **$R^2$** | **F** | **Sig.** |
| MODEL: | | 2,22 | 0.056 | 0.651 | 0.531 |
| | | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| | T | -0.032 | -0.106 | -0.511 | 0.615 |
| | CORT | -0.116 | -0.220 | -1.059 | 0.301 |

## 1.4.2 Females

The concentrations of T, E2, and CORT were related ($F_{2,25} = 5.474$, $P = 0.011$), with T and E2 exhibiting a positive relationship to each other, and CORT exhibiting a negative relationship with T and E2. Concentrations of E2 are high in females with vitellogenic follicles and low in females with oviductal eggs ($F_{1,23} = 10.772$, $P = 0.003$; Fig. 1.3). Principal components of coloration as well as gular region, gular stripe, and venter stripe hue, saturation, and brightness, were not related to reproductive stage.

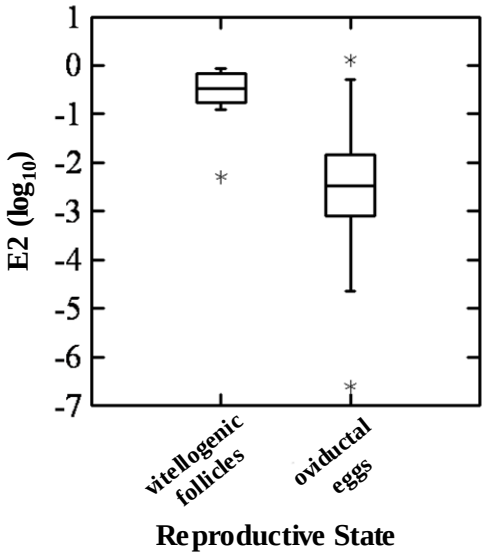


Figure 1.3 Box plot of E2 differences between vitellogenic and oviductal females.

### 1.4.2.1 PCA and GLM

The first principle component for gular hue, saturation, and brightness yielded an Eigen value of 1.86 and explained 61.82% of the total variance. The first component for gular stripe hue, saturation, and brightness yielded an Eigen value of 1.53 and explained 51.08% of the total variance. The first component for venter stripe hue, saturation, and brightness yielded and Eigen value of 1.71 and explained 57.02% of the total variance. The second principle components yielded Eigen values less than one and were not considered in analyses.

The first principle component for venter stripe coloration was related to collection date, collection location, and time of collection ($F_{5,19} = 10.910$, $P < 0.001$) and these were thus controlled in analyses concerning the venter stripe. Gular region ($F_{5,19} = 0.741$, $P = 0.602$) and gular stripes ($F_{5,19} = 1.032$, $P = 0.427$) were not associated with collection date, collection location, time of collection, SVL, or time to capture and bleed the lizard (Table 1.1).

The first venter stripe component exhibited a trend in its relationship to E2 (model: $F_{6,19} = 8.474$, $P < 0.001$; within model results for E2: $P = 0.071$). The first gular region component ($F_{3,24} = 1.164$, $P = 0.344$) and gular stripe component ($F_{3,24} = 1.665$, $P = 0.201$) were not associated with T, E2, or CORT (Table 1.2).

### 1.4.2.2 Exploratory GLM Analyses

Venter stripe saturation was related to the date and location of collection, and time of day of collection ($F_{3,24} = 10.088$, $P < 0.001$); thus, these variables were controlled in analyses concerning the venter stripe (Table 1.4).

Table 1.4 Analysis of color and hormones in females.
Results from exploratory GLM analyses examining the hue, saturation, and brightness of the gular region, gular stripe, and venter stripe in relation to plasma levels of T (testosterone), CORT (corticosterone), and E2 (17-beta estradiol). Confounding variables were controlled for when they contributed significantly to explaining variation. Abbreviations: *, P <0.05; $^{+}$, trend (P<0.10).

| **Gular Region Hue** | | **df** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|---|
| MODEL: | | 3,24 | 0.050 | 0.418 | 0.742 |
| | | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| | T | 0.069 | 0.151 | 0.632 | 0.533 |
| | CORT | -0.200 | -0.161 | -0.799 | 0.432 |
| | E2 | 0.017 | 0.094 | 0.398 | 0.694 |
| **Gular Region Saturation** | | **df** | **$R^2$** | **F** | **Sig.** |
| MODEL: | | 3,24 | 0.259 | 2.792 | *0.062*$^{+}$ |
| | | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| | T | 0.038 | 0.561 | 2.662 | **0.014*** |
| | CORT | -0.001 | -0.004 | -0.024 | 0.981 |
| | E2 | 0.013 | 0.484 | 2.312 | **0.030*** |
| **Gular Region Brightness** | | **df** | **$R^2$** | **F** | **Sig.** |
| MODEL: | | 3,24 | 0.093 | 0.816 | 0.497 |
| | | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| | T | -0.006 | -0.117 | -0.503 | 0.619 |
| | CORT | -0.039 | -0.269 | -1.364 | 0.185 |
| | E2 | 0.002 | 0.088 | 0.380 | 0.707 |
| **Gular Stripe Hue** | | **df** | **$R^2$** | **F** | **Sig.** |
| MODEL: | | 3,24 | 0.237 | 2.478 | *0.086*$^{+}$ |
| | | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| | T | 0.314 | 0.505 | 2.363 | **0.027*** |
| | CORT | -0.183 | -0.109 | -0.604 | 0.552 |
| | E2 | 0.103 | 0.426 | 2.008 | *0.056*$^{+}$ |
| **Gular Stripe Saturation** | | **df** | **$R^2$** | **F** | **Sig.** |
| MODEL: | | 3,24 | 0.263 | 2.848 | *0.059*$^{+}$ |
| | | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| | T | 0.044 | 0.542 | 2.576 | **0.017*** |
| | CORT | -0.034 | -0.156 | -0.879 | 0.388 |
| | E2 | 0.016 | 0.524 | 2.508 | **0.019*** |
| **Gular Stripe Brightness** | | **df** | **$R^2$** | **F** | **Sig.** |
| MODEL: | | 3,24 | 0.093 | 0.818 | 0.497 |
| | | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| | T | -0.013 | -0.180 | -0.772 | 0.447 |
| | CORT | -0.044 | -0.218 | -1.104 | 0.281 |
| | E2 | 0.006 | 0.192 | 0.830 | 0.415 |

Table 1.4 - continued

| **Venter Stripe Hue** | **df** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 3,24 | 0.149 | 1.398 | 0.268 |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| T | -0.027 | -0.107 | -0.474 | 0.640 |
| CORT | -0.192 | -0.286 | -1.497 | 0.147 |
| E2 | -0.017 | -0.178 | -0.792 | 0.436 |

| **Venter Stripe Saturation** | **df** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 6,19 | 0.685 | 6.901 | **0.001*** |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| T | 0.010 | 0.020 | 0.110 | 0.914 |
| CORT | 0.192 | 0.134 | 0.937 | 0.361 |
| E2 | -0.059 | -0.281 | -1.416 | 0.173 |
| collection date | 87.018 | 0.485 | 2.831 | **0.011*** |
| collection location | -0.090 | -0.490 | -2.677 | **0.015*** |
| collection time of day | 0.001 | 0.340 | 2.161 | **0.044*** |

| **Venter Stripe Brightness** | **df** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 3,24 | 0.358 | 4.456 | **0.013*** |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| T | 0.023 | 0.114 | 0.581 | 0.567 |
| CORT | -0.208 | -0.390 | -2.351 | **0.027*** |
| E2 | 0.029 | 0.381 | 1.956 | *0.062*$^{+}$ |

Gular saturation exhibited a trend in its relationship with T and E2 (model: $F_{3,24} = 2.792$, $P = 0.062$; within model results for T: $P = 0.014$; E2: $P = 0.030$), with high T and E2 associated with red gular regions.

Gular stripe hue exhibited a trend with T and E2 (model: $F_{3,24} = 2.478$, $P = 0.086$; within model results for T: $P = 0.027$; E2: $P = 0.056$), with high T and E2 associated with pale grey gular stripes. Gular stripe saturation also exhibited a trend with T as well as E2 (model: $F_{3,24} = 2.848$, $P = 0.059$; within model results for T: $P = 0.017$; E2: $P = 0.019$), with high T and E2 also associated with pale grey stripes.

Venter stripe brightness was related to CORT and exhibited a relationship trend with E2 (model: $F_{3,24} = 4.456$, $P = 0.013$), with high E2 and low CORT associated with bright venter stripes (Table 1.3).

## 1.5 Discussion: males

The first principle components extracted from hue, saturation, and brightness variables from the gular region, gular stripe, and venter stripe areas did not show a relationship to steroid concentrations. However, exploratory analyses indicated that high male CORT could be related to dull gular stripes. Chronically high CORT may have negative effects on immune function (Dhabhar and McEwen 1997, French et al. 2006). Should male venter stripe brightness be an honest indication of health, females may differentially choose to mate with healthy over unhealthy males, thus evolutionarily propagating this trait.

Male testis volume was not related to concentrations of T or CORT. Although testis size is known to increase in this species during its breeding season (Ramírez-Bautista and Olvera-Becerril 2004), testis volume may have been maximized in this population during the time of collection, therefore limiting volume variation and thus its association with steroid concentration output.

A relationship was not evident between color and reproductive stage in males. Color may experience hormonal organization during critical periods of development, rather than be temporarily activated after sexual maturation (Arnold and Breedlove 1985, Hews and Quinn, 2003). Therefore, reproductive activity associated color may be nonexistent or vary minimally within a sexually mature individual.

## 1.6 Discussion: females

The first principle component for venter stripe color may be related to concentrations of E2. Results from exploratory analyses show mostly trends suggesting

high E2 and low CORT are associated with bright venter stripes. High T and E2 also may be related to red (as opposed to yellow) gular regions and pale grey (as opposed to black) gular stripes.

Concentrations of E2 were high in females carrying vitellogenic follicles, while E2 was low in females carrying oviductal eggs. Although T and CORT were not significantly related to reproductive stage, they, and E2, were related to each other. Concentrations of T and E2 were positively related to each other and negatively related to CORT. One explanation for these associations could be steroid involvement in follicle maturation accompanied by an interplay between the hypothalamic-pituitary-adrenal (HPA) axis, responsible for CORT release, and the hypothalamic-pituitary-gonadal (HPG) axis, responsible for T and E2 release. Concentrations of E2 typically show a positive correlation to ovarian follicle size (Whittier and Tokarz 1992, Crews 1980). An increase in T could facilitate an increase and/or maintain high concentrations of E2 if converted via aromatase enzymes (Whittier and Tokarz 1992, Baum 2002). Due to possible negative interactions between the HPA and the HPG axes, an increase in CORT may inhibit production of sex steroid hormones like T and E2 (Greenberg and Wingfield 1987, Tokarz 1987, Rivier and Rivest 1991, DeNardo and Licht 1993). However, contrary to my results, some studies show a positive interaction between the HPA and HPG axis during extended times of reproductive activity (Wilson and Wingfield 1994, Knapp and Moore 1997, Moore et al. 2000), suggesting testosterone may involve certain energetic costs that require CORT mobilization of energy stores (Wingfield et al. 1995).

Color was not directly related to reproductive stage but may exhibit an indirect relationship. Concentrations of E2 are high in vitellogenic females, E2 and T are positively related, and E2 and T may be high in females expressing a red gular region, pale grey gular stripes, and bright venter stripes. This indirect relationship may be the result of a time lag involved with color change. For example, a female may experience an increase in T and E2 to aid in follicle maturation; thus, her gular region is affected and changes from yellow to red. When T and E2 are highest, the gular region is most red. Once the follicles enter the oviduct and perhaps become fertilized, T and E2 drop, no longer needed to mature the follicle, and albumin hardens the eggs for deposition. While T and E2 may drop relatively quickly, color change/reduced pigment deposition occurs relatively slowly, and a female with fertilized, oviductal eggs may still express a red gular region due to this time lag.

Little is known about the mating system of *S. pyrocephalus*. Fertilization most likely takes place as the vitellogenic follicle leaves the ovary and enters the oviduct. A female *S. pyrocephalus* may then have the ability to signal fertilization receptivity, as she is most red in her gular region, pale in her gular stripe area, and bright in her venter stripe area, during these late vitellogenic stages. A yellow gular region, dark gular stripes, and dull venter stripes may thus signal unrecptivity. In accordance with the courtship stimulation hypothesis (Cooper and Greenberg 1992), signaling unrecptivity to a male may reduce the many costs associated with courting and copulation to both the male and female, thus evolutionarily propagating the trait.

# CHAPTER 2

## FEMALE SECONDARY COLORATION IN *SCELOPORUS PYROCEPHALUS* IS ASSOCIATED WITH NEMATODE LOAD AND REPRODUCTIVE STATE

### 2.1 Abstract

Male secondary sexual traits have been shown to facilitate female choice of a potential mate's quality, such as the ability to resist detrimental parasitic infection, thus selecting for parasitic resistance in her future offspring. Some studies have revealed that males carrying relatively high parasite numbers, or loads, can constrain the exaggeration of their traits, but few studies have examined the effects of parasites on female traits. Female Mexican boulder spiny lizards, *Sceloporus pyrocephalus*, express a red throat, or gular, region and black gular stripes in association with the reproductive cycle, as well as blue ventral stripes. Females of *S. pyrocephalus* visually display these color traits during same- and opposite-sex aggressive encounters by inflating their gular region and flattening their ventral area laterally. If parasites are adversely affecting their female host, energy allocated into producing traits may be redistributed to combat antigen invaders. Therefore, an individual fostering a relatively high parasite load, may not be able to allocate energy to fully express secondary traits, and these traits thus become honest signals of an organism's condition. Parasitic infection could be an indirect effect of high concentrations of the gonadal sex steroid testosterone, because

testosterone, while related to the development of secondary characteristics such as color, may also indirectly reduce immunocompetence. High testosterone is associated with late vitellogenic follicles as well as red gular regions and pale grey gular stripes in females of *S. pyrocephalus*. I examined 1) if female reproductive cycle color change varies in hue, saturation, and brightness in association with high nematode loads, and 2) if high testosterone is associated with high nematode loads. My results indicate that 1) high nematode loads of particular areas of the body are related to dull red gular regions and dull pale grey gular stripes, and 2) high plasma testosterone concentrations may be related to high nematode loads. Females could be most vulnerable to nematodes during late reproductive stages because of energy allocated to corresponding high concentrations of circulating testosterone and associated secondary color change. This suggests that females with secondary sexual coloration have the ability to provide an honest signal of nematode loads which may lead to sexual selection for coloration in females. I discuss the relationships between nematode loads, secondary sexual color, and reproductive stage and testosterone in the context of behavior during the reproductive period in *S. pyrocephalus*.

## 2.2 Introduction

Theoretical and empirical studies suggest that sexual selection has driven conspicuous traits in males while natural selection has driven inconspicuous traits in females (Darwin 1871, Fisher 1954, Lande 1980), but some species (which do not exhibit sex-role reversal) do exhibit bright female coloration. Female coloration could

be the result of a genetically correlated response to selection on male coloration (Lande 1980); however, coloration in females is broadly indicative of the reproductive cycle, as seen in fish (Rowland et al. 1991, Baird 1988), reptiles (Medica et al. 1973, Mitchell 1973, Ferguson 1976, Cooper et al. 1983, Cooper and Greenberg 1992, Watkins 1997, Cuadrado 2000, Hager 2001, Weiss 2002, Calisi 2006), birds (Montgomerie and Thornhill 1989, Roulin et al. 2001), and mammals (Dixson 1983, Setchell and Wickings 2004). Reproductive cycle associated coloration may be due to advantages experienced by both sexes: a female signaling nonreceptivity to a male may reduce courtship and copulation associated costs such as energy expenditure, predation risk, and altered thermoregulation and metabolism (Cooper and Greenberg 1992), thus selecting for the trait. However, while female secondary coloration may serve as an indication of female reproductive receptivity, could secondary coloration simultaneously indicate female health, or "quality"?

Male secondary sexual traits have been shown to communicate resistance for parasitic infections, and females that choose mates based on this signal may be able to confer genetic resistance to offspring (Hamilton and Zuk 1982). Some studies which have investigated parasitic effects on male traits revealed that relatively high parasite loads can act as a constraint on character exaggeration (Zuk et al. 1990). For example, ciliate parasitization in male three-spined stickleback fish was related to a significant decrease in male red color intensity as well as in physical condition, and females prefer more intensely red colored males (Milinski and Bakker 1990). Assuming many parasites are adversely affecting their host, energy generally allocated into producing

secondary sexual traits may be redistributed to combat antigen invaders. Therefore, an organism fostering a relatively high abundance of parasites (high parasite load), may not be able to allocate energy to fully express secondary traits, and these traits thus become honest signals of an organism's condition (Johnstone 1995).

Relatively few studies have examined parasitic effects on female quality. One study of barn owls suggests males may choose female mates based on their spottiness, and more spotted females are usually less parasitized (Roulin et al. 2001). Another study has shown that female bar-tailed godwit breeding plumage signals cestode infestation (Piersma et al. 2001), but separate examinations of variations in color associated with reproductive state were not conducted.

Female Mexican boulder spiny lizards, *Sceloporus pyrocephalus*, express different color patterns that covary with the reproductive cycle. *Sceloporus pyrocephalus* is an oviparous species (Ramírez-Bautista and Olvera-Becerril 2004) with monochromatic masculinized coloration (Hews and Quinn 2003), in that both sexes exhibit a blue/black throat, or gular, stripes and venter stripes, but females express a conspicuous red gular region (varying from red to yellow) while males only weakly express such color in the gular region. Red coloration of the female gular region and pale grey gular stripes are generally indicative of late vitellogenic follicles, while yellow gular coloration and black gular stripes are related to early vitellogenic stages. Both males and females exhibit same- and opposite-sex aggression by inflating their gular region and flattening their ventral area laterally when confronted with a conspecific (Calisi, personal observation), suggesting color of the gular region, gular

stripes, and venter stripes are visible during encounters with opposing individuals. Color perception in this species is unknown, but many reptiles have retinas that express cones involved in color perception (Fleishman et al. 1993, Yokoyama and Yokoyama 1996, Yokoyama 1997), and thus variation in male and female secondary coloration may be a trait on which selection has and/or is acting.

Parasitic infection could be an indirect effect of high concentrations of the gonadal sex steroid testosterone, because testosterone, while related to the development of secondary characteristics such as color, may also reduce immunocompetence (Folstad and Karter 1992). High testosterone is associated with mature follicles as well as red gular regions and pale gular stripes in females of *S. pyrocephalus*. I investigated female secondary sexual color variation in association with both the reproductive cycle and nematode load in *Sceloporus pyrocephalus*. Secondly, I examined a subset of females for the relationship between plasma testosterone concentration data and nematode load. I predicted that 1) female reproductive cycle color change would vary in hue, saturation, or brightness in association with high nematode loads, and 2) high testosterone would be associated with high nematode loads.

## 2.3 Methods

Fieldwork was performed in tropical dry forest in Michoacán, México, during the breeding season months of June-July (Ramirez-Bautísta and Olvera-Becerril, 2004) in 2004 and 2005. Fifty-two mature females (13 in 2004, 39 in 2005) of *S. pyrocephalus* were captured by noosing or hand between 1400 and 1800 hrs from eight different locations within a 40km radius of the town of Lombardia, Michoacán, México

(elevation 751m; 19.17662º N, 102.66351º W). The snout to vent length (SVL) was measured with a ruler to the nearest 1.0 mm. I sampled blood from the retro-orbital sinus using heparinized microcapillary tubes. Blood samples were collected from a subset of 11 females in 2004 immediately following capture. All females were taken from the same location on the same day during the time between 1600-1730 hrs. The time that elapsed while catching the lizard and the time interval following the capture and during the collection of blood were recorded and examined for possibly confounding correlations to testosterone concentrations. Blood samples were kept in an ice cooler until hand centrifuged (within 12 hours), and then plasma was stored in liquid nitrogen until placed in a -20ºC freezer at the Universidad Autónoma de México (UNAM). Samples were transferred to Indiana State University (ISU) on dry ice for assaying.

### 2.3.1 Color measurements

Digital images were taken of the gular and ventral areas immediately following capture of each to measure color hue, saturation, and brightness of the gular region, gular stripes, and venter stripes (see Chapter 1 Methods, section 1.3.4 Color measurements).

### 2.3.2 Parasite load

Lizards were euthanized by an intraperitoneal injection of Nembutal® (sodium pentobarbital), and the mouth, peritoneal cavity, and all internal organs were examined for internal helminth parasites (*sensu* Mata-López et al. 2002) at the Universidad Nacional Autónoma de México, México City, by parasitologists E. Matínez-Salazar,

M.A. Arízmendi-Espinósa, and R. Mata-López in the lab of V. Léon-Règagnón. Roundworms (Phylum: Nematoda) were generally the only macroscopic parasite found, although one individual contained large amounts (100+) of cestode larvae and no nematodes; this individual, considered an outlier, was not used in analyses. The location of nematodes within the body, the number of individuals that had nematodes (nematode prevalence) and the number of nematodes per individual (nematode abundance) were documented.

Four common assumptions are generally made and should be kept in mind when interpreting the effects of parasite load (McLennan and Brooks 1991): 1) the frequency of parasite exposure was equal among lizards, 2) parasites could adversely affect the health of *S. pyrocephalus*, 3) secondary color traits could be reflections of health, and 4) variability observed in trait relationships with nematode parasites is a consequence of an evolutionary arms race between immune effects and subsequent trait alterations (see Getty 2002). My study was more exploratory in nature and these assumptions were therefore not strictly tested.

### 2.3.3 Reproductive state

Females were necropsied to determine their stage of reproduction. The volume of each egg/follicle was also estimated using the formula for a prolate spheroid:

$$V=4/3\pi\ (\text{length}/2)*(\text{width}/2)^2$$

Egg volume and position within the body cavity after fertilization is unknown in lizards of *S. pyrocephalus*; however the point of fertilization may occur when follicles enter the oviduct (now termed "eggs".) Females were categorized both visually and by using their average follicle/egg volume into having pre- ($<10mm^3$), mid- ($10\text{-}60mm^3$), late- ($>60mm^3$) vitellogenic follicles, or oviductal eggs.

### 2.3.4. Hormone analysis

I measured total plasma concentrations of testosterone by radioimmunoassay (RIA) after chromatographic separation on microcolumns of diatomaceous earth (following Wingfield and Farner 1975, as modified by Moore 1986 and Hews et al. 1994, see Chapter 1, section 1.3.3 Hormone analysis). Briefly, the Wingfield-Farner method involved first separating steroid hormones from each other and neutral interfering lipids using ether extraction and phase partition column chromatography. The purified steroid hormone fractions were dried, re-suspended in assay buffer, and assayed in triplicate using RIA. Individual samples were corrected for differences in plasma volumes and in individual recovery, which was assessed by adding a small amount of radiolabeled steroid hormone to each sample prior to the ether extraction. Average individual recoveries in my assay for testosterone were 84.8%, with a 2.0% intra-assay coefficient of variation.

### 2.3.5 Statistical analyses

SYSTAT 8.0 (SPSS Inc., 1998) software was used for all analyses. I checked for normality in the data, with $\log_{10}$ transformations of follicle and egg volume, color measurements, SVL, parasite load, and testosterone concentrations, successfully

fulfilling normality and equal variance assumptions. I used general linear regression analyses (GLM) to model relationships between color, reproductive state, testosterone, and parasite load. Color variables (hue, saturation, and brightness for the gular region, gular stripe, and venter stripe) and parasite loads (of the cloaca, intestine, stomach, and a total load of these three areas) were individually checked for possible confounding relationships by the independent variables of the year of collection, date of collection, location of collection, time of day of collection, and SVL. Each color variable was then examined in a model with independent variables including parasite loads of the cloaca, intestine, stomach, and/or the total loads. Many times, the overall model, but not individual independent variables, were significant. In this case, I reduced the model to locate the independent variable that was explained by the dependent color variable. Linear regression was also used to examine the relationship between testosterone concentrations and total parasite loads. Because of the exploratory nature of these analyses, alpha was set at 0.05, although most results involving multiple comparisons were significant at $P < 0.01$.

## 2.4 Results: reproductive state and color

Gular hue was related to reproductive stage in females ($F_{3,48} = 4.572$, $P = 0.007$). Early vitellogenic follicles were associated with orange-red gular hues, mid-vitellogenic follicles with orange hues, late-vitellogenic follicles with red hues, and oviductal eggs with a range from red-orange hues (Figure 2.1). Gular saturation and brightness, as well as hue, saturation, and brightness for the gular stripe and venter stripe regions were not related to reproductive state (DF = 3,48; gular saturation: $P =$

0.203, brightness: $P = 0.394$; gular stripe hue: $P = 0.260$, saturation: $P = 0.321$, brightness: $P = 0.368$; venter stripe hue: $P = 0.374$, saturation: $P = 0.157$, brightness: $P = 0.074$).

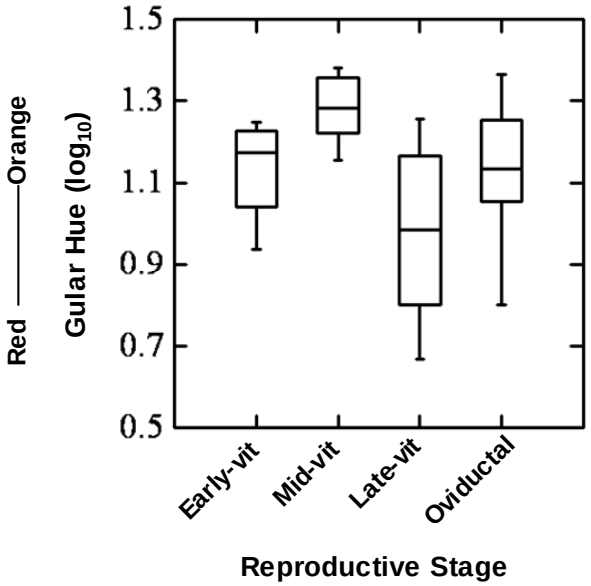


Figure 2.1 Box plot of female gular hue in relation to reproductive stage. Females are most red during late-vitellogenic stages, which could possibly signal fertilization receptivity to males.

### 2.4.1 Nematode load and color

Mean total nematode abundance was seven and mean totals per body area examined (cloacal, intestinal, stomach) were four, two, and four, respectively, for females (Table 2.1). Neither color variables nor parasite loads were related to the date of collection, location of collection, time of day of collection, or SVL (DF = 5,46; gular hue: $P = 0.192$, saturation: $P = 0.779$, brightness: $P = 0.639$; gular stripe hue: $P = 0.339$, saturation: $P = 0.683$, brightness $P = 0.746$; venter stripe hue: $P = 0.074$, saturation: $P = 0.274$, brightness: $P = 0.206$; nematodes in the cloaca: $P = 0.300$, intestine: $P = 0.474$,

stomach: $P = 0.870$, total: $P = 0.312$). Certain color variable and nematode load relationships were apparent from results of reduced models (for full and reduced model results, see Table 2.2).

Table 2.1 Female nematode loads.
Female nematode loads of the cloaca, intestine, stomach, and the total of these areas.

| | Nematode Loads | | | |
|---|---|---|---|---|
| | **Total** | **Cloacal** | **Intestinal** | **Stomach** |
| **Nematode Mean Abundance** | 7 | 4 | 2 | 4 |
| **Standard Deviation** | 18 | 15 | 4 | 15 |
| **Nematode Prevalence** | 81% | 52% | 31% | 21% |

Table 2.2: Female color and nematodes.
Results of full and reduced models depicting relationships between color variables and nematode loads. When full models were significant, the models were reduced to determine the specific body area(s) of nematode load related to the color variable in question. Abbreviations: *, P <0.05; $^{+}$, trend (P <0.10).

| **Gular Hue** | **DF** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 4,47 | 0.319 | 7.807 | **<0.001*** |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| Total | -0.621 | -1.307 | -1.990 | *0.052*$^{+}$ |
| Cloaca | -0.717 | -1.273 | -2.313 | **0.025*** |
| Intestine | 0.564 | 0.809 | 1.820 | 0.075 |
| Stomach | 0.262 | 0.485 | 0.937 | 0.353 |
| **Gular Hue: reduced model** | **DF** | **$R^2$** | **F** | **Sig.** |
| MODEL: | 2,49 | 0.277 | 9.400 | **<0.001*** |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| Total | -0.229 | -0.483 | -3.526 | **0.001*** |
| Cloaca | -0.298 | -0.530 | -3.867 | **<0.001*** |
| **Gular Saturation** | **DF** | **$R^2$** | **F** | **Sig.** |
| MODEL: | 4,47 | 0.260 | 4.139 | **0.006*** |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| Total | 0.147 | 0.412 | 0.566 | 0.574 |
| Cloaca | 0.081 | 0.191 | 0.312 | 0.756 |
| Intestine | 0.098 | 0.258 | 0.381 | 0.705 |
| Stomach | 0.129 | 0.232 | 0.557 | 0.580 |
| **Gular Saturation**: reduced model | **DF** | **$R^2$** | **F** | **Sig.** |
| Cloaca | 1,50 | 0.250 | 16.643 | **<0.001*** |

Table 2.2 - continued

| **Gular Brightness** | **DF** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 4,47 | 0.211 | 3.144 | **0.023*** |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| Total | -0.063 | -1.121 | -1.490 | 0.143 |
| Cloaca | 0.060 | 0.906 | 1.436 | 0.158 |
| Intestine | 0.063 | 0.759 | 1.490 | 0.143 |
| Stomach | -0.029 | -0.448 | -0.755 | 0.454 |
| **Gular Brightness**: reduced model | **DF** | **$R^2$** | **F** | **Sig.** |
| Stomach | 1,50 | 0.173 | 10.433 | **0.002*** |

| **Gular Stripe Hue** | **DF** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 4,47 | 0.060 | 0.752 | 0.562 |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| Total | -0.395 | -0.498 | -0.606 | 0.547 |
| Cloaca | 0.310 | 0.329 | 0.478 | 0.635 |
| Intestine | 0.328 | 0.281 | 0.505 | 0.616 |
| Stomach | 0.524 | 0.582 | 0.899 | 0.373 |

| **Gular Stripe Saturation** | **DF** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 4,47 | 0.191 | 2.783 | **0.037*** |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| Total | 0.038 | 0.139 | 0.183 | 0.856 |
| Cloaca | 0.100 | 0.311 | 0.488 | 0.628 |
| Intestine | 0.043 | 0.107 | 0.208 | 0.836 |
| Stomach | 0.042 | 0.136 | 0.227 | 0.821 |
| **Stripe Saturation**: reduced model | **DF** | **$R^2$** | **F** | **Sig.** |
| Cloaca | 1,50 | 0.190 | 11.729 | **0.001*** |

| **Gular Stripe Brightness** | **DF** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 4,47 | 0.166 | 2.336 | *0.069*$^{+}$ |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| Total | -0.053 | -0.633 | -0.818 | 0.417 |
| Cloaca | 0.043 | 0.432 | 0.666 | 0.509 |
| Intestine | 0.024 | 0.197 | 0.377 | 0.708 |
| Stomach | 0.010 | 0.104 | 0.171 | 0.865 |
| **Stripe Brightness**: reduced model | **DF** | **$R^2$** | **F** | **Sig.** |
| Cloaca | 1,50 | 0.115 | 6.519 | **0.014*** |

| **Venter Stripe Hue** | **DF** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 4,47 | 0.025 | 0.295 | 0.879 |
| | **Coef.** | **Std. Coef.** | **t** | **Sig.** |
| Total | -0.100 | -0.210 | -0.251 | 0.803 |
| Cloaca | 0.116 | 0.207 | 0.294 | 0.770 |
| Intestine | 0.132 | 0.189 | 0.334 | 0.740 |
| Stomach | 0.016 | 0.030 | 0.046 | 0.964 |

| **Venter Stripe Saturation** | **DF** | **$R^2$** | **F** | **Sig.** |
|---|---|---|---|---|
| MODEL: | 4,47 | 0.049 | 0.600 | 0.664 |

Table 2.2 - continued

| | Coef. | Std. Coef. | t | Sig. |
|---|---|---|---|---|
| Total | 0.070 | 0.106 | 0.128 | 0.899 |
| Cloaca | -0.229 | -0.292 | -0.421 | 0.676 |
| Intestine | -0.030 | -0.030 | -0.054 | 0.957 |
| Stomach | -0.079 | -0.105 | -0.161 | 0.873 |

| Venter Stripe Brightness | DF | $R^2$ | F | Sig. |
|---|---|---|---|---|
| MODEL: | 4,47 | 0.023 | 0.267 | 0.892 |

| | Coef. | Std. Coef. | t | Sig. |
|---|---|---|---|---|
| Total | 0.018 | 0.076 | 0.090 | 0.928 |
| Cloaca | 0.008 | 0.029 | 0.042 | 0.967 |
| Intestine | -0.050 | -0.140 | -0.248 | 0.805 |
| Stomach | -0.020 | -0.074 | -0.113 | 0.911 |

### 2.4.1.1 Gular region

The models consisting of gular hue ($F_{4,47} = 7.807$, $P < 0.001$), gular saturation ($F_{4,47} = 4.139$, $P = 0.006$), and gular brightness ($F_{4,47} = 3.144$, $P = 0.023$), and their relationship to cloacal, intestinal, stomach, and total nematode loads were significant overall. Reducing these models indicated a negative relationship between gular hue and both total and cloaca nematode load ($F_{2,49} = 9.400$, $P < 0.001$), a positive relationship between gular saturation and cloaca nematode load ($F_{1,50} = 16.643$, $P < 0.001$), and a negative relationship between gular brightness and stomach nematode load ($F_{1,50} = 10.443$, $P = 0.002$). In general, high cloaca nematode loads are related to highly saturated red, though less bright, gular regions.

### 2.4.1.2 Gular stripe

The model consisting of gular stripe saturation and its relationship to cloacal, intestinal, stomach, and total nematode loads and gular stripe brightness was significant overall ($F_{4,47} = 2.783$, $P = 0.037$). The model consisting of gular stripe brightness showed a trend in its relationship to parasite loads ($F_{4,47} = 2.336$, $P = 0.069$). Reducing these models indicated a positive relationship between gular stripe saturation and cloaca

nematode load ($F_{1,50}$ = 11.729, $P$ = 0.001), and a negative relationship between gular stripe brightness and stomach nematode load ($F_{1,50}$ = 6.519, $P$ = 0.014). These relationships demonstrated associations between high cloaca nematode loads and pale grey gular stripes, and high stomach nematode loads and dull stripes. Gular stripe hue was not related to nematode loads ($F_{4,47}$ = 0.752, $P$ = 0.562).

#### 2.4.1.3 Venter stripe

The models consisting of venter stripe hue ($F_{4,47}$ = 0.295, $P$ = 0.025), venter stripe saturation ($F_{4,47}$ = 0.600, $P$ = 0.664), and venter stripe brightness ($F_{4,47}$ = 0.267, $P$ = 0.892), and their relationship to cloacal, intestinal, stomach, and total nematode loads were not significant.

### 2.4.2 Testosterone and nematode load

Total nematode load was positively related to plasma testosterone concentrations ($F_{4,47}$ = 6.333, $P$ = 0.033; Fig. 3) The time it took to capture and obtain a blood sample did not affect testosterone concentrations ($F_{1,9}$ = 0.004, $P$ = 0.950).

## 2.5 Discussion

My results indicate that some secondary color variables in females of *S. pyrocephalus* are correlated with parasite load as well as reproductive state. Females with bright red gular regions and dull, pale grey gular stripes had generally high nematode loads and were in late vitellogenic stages of reproduction. Late vitellogenic stages, a red gular region ,and pale grey gular stripes have been associated with high plasma testosterone concentrations in this species, and I show that high testosterone may be related to high nematode load.

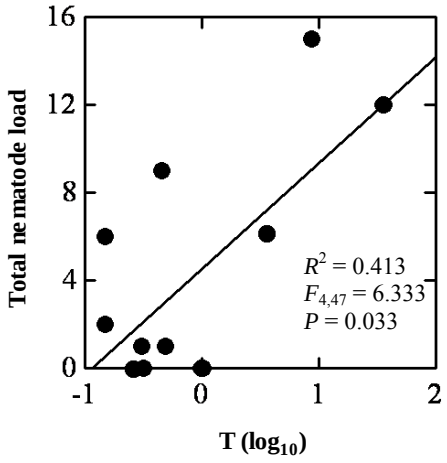


Figure 2.2 Female testosterone is related to total nematode load.

Females of *S. pyrocephalus* exhibit aggressive intra and inter-sexual behavioral interactions during the breeding season. Like males, females inflate their gular region and flatten their ventral area laterally when confronted with a same-sex conspecific. Also like males, females lunge and bite at the dorsal areas of opposing same-sex conspecifics, usually ending with one lizard leaving/being driven off the particular boulder of the occurrence, and they do this both in and out of the presence of a male. In terms of mating, males court females with push-ups and multiple series of head bobs, finally grasping the back of the female's neck, dragging her into a rock crevasse, and copulating; females are mostly, and sometimes successfully, resistant to male courting and copulation attempts, lunging and biting at the male. These behaviors may ensure that only a strong male (which could be indicative of good quality) can subdue a female and father her offspring, which in turn could provide strong, or good quality, sons ("the

sexy son hypothesis", Weatherhead and Robertson 1979). Inter- and intra-conspecific interactions appear to be energetically costly to both sexes, and thus selection may have acted to reduce these costs via honest signaling (Cooper and Greenberg 1992).

I demonstrate that female gular region coloration varies with reproductive stage, thus making available a mechanism for which males can discriminate between reproductive and non-reproductive females. Female gular regions appear most red during late-vitellogenic stages, when both sex steroids testosterone and estradiol are high. Although the point of fertilization is unknown in this species, it may be assumed that mature follicles about to leave/leaving the ovary may be receptive to fertilization. Females carrying oviductal eggs are less red (more orange) and have significantly lower testosterone and estradiol in comparison with females carrying late-vitellogenic follicles, as egg maturation may no longer require external hormonal aid from the mother and eggs are soon to be deposited.

Hormone physiology can ultimately yield fitness benefits by aiding in honest reproductive signaling, but costs may accompany these benefits. I show that testosterone, while related to female color and the reproductive cycle in *Sceloporus pyrocephalus*, also has the propensity to be positively related to nematode load. High concentrations of testosterone are associated with high estradiol concentrations and follicle maturation in lizards of *S. pyrocephalus*. Generally, reptile estradiol concentrations are positively correlated with ovarian follicle size (Crews 1980, Whittier and Tokarz 1992). An increase in testosterone could be explained because it is a precursor to estradiol and can convert estradiol via aromatase enzymes (Whittier and

Tokarz 1992, Baum 2002), thus maintaining high levels of estradiol necessary for follicle maturation. High concentrations of testosterone could benefit a female by increasing her reproductive efforts and altering her color, allowing honest fertilization receptivity signaling. Energy allocated to this signaling may also reduce immunocompetence and increase susceptibility to parasitic infection (Grossman 1985, Folstad and Karter 1992, Peters 2000, Roberts et al. 2004), as seen with malarial parasites in female birds (*Parus major;* Oppliger et al. 1996, Oppliger et al. 1997) and haematozoan parasites in female lizards (*Lacerta vivipara;* Sorci et al. 1996). Blood parasites of *L. vivipara* have also been linked to other physiological costs such as low levels of hemoglobin, decreased resting oxygen consumption, reduced running speed (Oppliger et al. 1996, Sorci et al. 1996), as well as reduced ability to regenerate the tail (Oppliger and Clobert 1997). However, as mentioned, costs can be accompanied by benefits to reproductive strategy; for example, male lizards of *Psammodromus algirus* given testosterone implants not only had higher tick numbers relative to controls, but they expressed larger breeding color patches (Salvador et al. 1996).

Increases in testosterone have sometimes been correlated with increased mobility in lizards (Sinervo et al. 2000, Olsson et al. 2000), and females *S. pyrocephalus* with dark red gular regions (high testosterone and large late-vitellogenic follicles) cover greater distances, possibly associated with holding larger territories, as opposed to females with yellow-orange gulars (low testosterone and small pre-vitellogenic follicles). This increased area coverage may allow females increased exposure to parasites such as nematodes, thus explaining high nematode loads in

association with high testosterone. Females may be covering greater distances at this point in their reproductive stage to increase the probability of encountering more resources, such as mates, food, and/or egg depositing sites. If female-female competition exists for these sites, and this appears to be the case in females of *S. pyrocephalus*, females may benefit by both signaling and perceiving a competitor's fighting ability to conserve energy expenditure and avoid unnecessary predator risk. A female *S. pyrocephalus* with a high nematode load will have a red, and yet dull, gular region as well as pale, dull gular stripes. I believe that red coloration may serve as a signal of female fertilization receptivity to males, while the brightness of the overall gular area (both the gular region and the gular stripes), may serve as indication of female quality to other females, as females engage in aggressive, same sex encounters during the reproductive period. Female color could serve as a mechanism to honestly determine an energetic output response in relation to the quality of the female competitor, and benefits gained from energy conservation and possible predator avoidance may have driven the evolution of this trait.

In conclusion, secondary coloration in females of *S. pyrocephalus* appears to be the result of complex interactions between physiological changes associated with follicle development and the influence of nematodes. Further manipulative studies are needed to better understand nematode influence, proximate mechanisms controlling female coloration, and their ultimate implications. Behavioral studies examining ultimate implications should assess the signaling capabilities of

female coloration to males, and the characteristics of a victorious female in a female-female interactions.

# CHAPTER 3

## BODY SIZE AND COLORATION CORRELATE WITH AREA USE IN FEMALES BUT NOT MALES OF *SCELOPORUS PYROCEPHALUS*


### Abstract 3.1

Few data link female secondary sexual traits to fitness surrogates such as resource acquisition. Mexican boulder spiny lizards, *Sceloporus pyrocephalus*, are sexually dimorphic for body size and sexually dichromatic in color but do not appear to exhibit sex-role reversal. I examined body size, and secondary gular and venter colors of both sexes to assess if these traits were predictors of area coverage. There was a positive correlation between body size and area coverage in females, but not males. Female gular and venter stripe coloration, but not male coloration, were also related to area coverage. Females with red gular regions and pale gular and venter stripes covered more area than females with orange gular regions and dark gular and venter stripes. Females that simultaneously had redder gular regions and pale gular stripes covered more area, suggesting evidence for a multicomponent signal in the female gular region. To my knowledge, this is the first report supporting a relationship between female secondary color and area coverage. I hypothesized that the larger areas occupied by females with secondary sexual coloration could lead to the increased acquisition of resources, which in turn may result in greater fitness

benefits. Conversely, these resource benefits may carry a cost of an increased risk of predation. Females of *S. pyrocephalus* may illustrate a tradeoff between sexual selection for secondary coloration and natural selection for survivorship.

## 3.2 Introduction

Variation in secondary sexual traits could ultimately lead to sexual selection (Darwin; 1871). Secondary sexual traits, such as color ornaments or large body size, may have evolved via processes like male contest competition and female choice (Darwin 1871, Andersson, 1994, Andersson and Iwasa, 1996). Theoretical work on sexual selection suggests that secondary color ornaments may provide clues to the variation in success of individuals during contests for resources (Andersson, 1982, 1994). Variation in these traits could therefore fuel differential and assortative mating.

Relationships of male territory size to male secondary characteristics have been studied mainly in birds ( Krebs 1977, Keyser and Hill 2000, Pryke et al., 2001); however, studies of male lizard coloration have linked territorial behavior with body size (Ruby 1984, Jenssen and Nunez 1998, Jenssen et al. 2005) and particular color morphs (Sinervo et al., 2000, Calsbeek and Sinervo 2002. Zamudio and Sinervo 2003). Although much understudied, female territorial behavior has been observed in many species such as fish (Beeching et al. 1998), birds (Gowaty and Wagner 1988), amphibians (Jaeger et al. 1982), and reptiles (Woodley and Moore 1999). Could female territorial behavior, as in males, be linked to secondary characteristics?

Lizards of the genus *Sceloporus* are generally sexually dimorphic (Fitch 1978, Ramírez-Bautista and Olvera-Becerril 2004) and have an array of color patterns that

vary among species and between the sexes (Wiens 1999, Hews and Quinn 2003). One species, the Mexican boulder spiny lizard (*Sceloporus pyrocephalus*), is a potentially useful model for testing hypotheses related to sexual selection, as females and males of this species have patterns of color that vary intra- and intersexually.

Lizards of *S. pyrocephalus* inhabit tropical dry forests in western México, and males and females are commonly observed perching, foraging, competing, displaying, and mating on all areas of small boulders. Sexually mature males are generally larger than sexually mature females, with males ranging from 50-75mm snout-vent length (SVL) and females ranging from 47-62mm (Ramírez-Bautista and Olvera-Becerril, 2004). Both sexes express bluish-black colored ventral stripes, and females express a conspicuous reddish gular region that varies with reproductive state while males do not have a conspicuous gular region. In other species of lizards, variation in color is often attributed to specific population color morphs (Moore et al. 1998; Sinervo et al. 2000), but my observations of lizards of *S. pyrocephalus* over several years suggest that color variation is continuous and not segregated into various color morph patterns.

Conspicuous coloration in females can be indicative of sex role reversal (Trivers 1972, Andersson 1994, Eens and Pinxten 2000). *Sceloporus pyrocephalus* do not exhibit role reversed characteristics because males court and compete over females, and it is unlikely that either sex provides parental care to offspring. This unusual coloration pattern might arise if different selection pressures are acting on each of the sexes, and empirical work on a parrot species *Eclectus roratus* has provided some support for this hypothesis (Heinsohn et al. 2005).

Female secondary coloration is correlated to reproductive state in various taxa, such as in mammals (Dixson 1983, Setchell and Wickings 2004), fish (Baird 1988, Rowland et al. 1991), birds (Montegomerie and Thornhill1989, Roulin et al. 2001), and reptiles (Medica et al. 1973, Mitchell 1973, Ferguson 1976, Cooper et al. 1983, Cooper and Greenberg 1992, Watkins, 1997, Cuadrado 2000, Hager 2001). Females of *S. pyrocephalus* carrying small follicles (in early stages of reproduction) express a yellow-orange hue while females carrying mature follicles express a red hue. If color is a reproductive indicator in females of this species, then one color/reproductive state may be correlated with a specific activity pattern. This pattern may be important to the mating strategy of the species.

Gular color and dark venter patches in other *Sceloporus* species can serve as signals of aggressive behavior or dominance status (Cooper and Burns 1987, Quinn and Hews 2000, Hews and Quinn 2003). Competitive behavior is common in lizards of *S. pyrocephalus*, as males attack and chase other males, and females attack and chase both females and males from rocks. Males copulate with females by biting the back of their necks and dragging them into rock crevasses. These behaviors could be related to home range/resource (including mate acquisition) and/or territorial defense. Therefore, the color variation in this species may be a good predictor of resource holding potential. I tested the hypothesis that variation in color of the conspicuous gular region, gular stripes, and venter stripes in male and female lizards of *S. pyrocephalus* is correlated with increased area coverage.

## 3.3 Methods

I studied *S. pyrocephalus* at a site located 2.0 km north of Lombardia, Michoacán, México (751 m; 19.17662 N, 102.66351 W) for an 11 day period during the mid-breeding season of July, 2004. Thirty-two lizards (14 male, 18 female) were captured and marked during the 11 day period. All individuals collected were sexually mature, which I confirmed from femoral pore secretions in both sexes as well as enlarged hemipenes in males; lizard body sizes also fell within the range variation for sexually mature lizards of *S. pyrocephalus* (Ramírez-Bautista and Olvera-Becerril 2004).

The study site was approximately three hectares and consisted of dry tropical forest with numerous boulder outcrops. I walked transects through the study area every one to two hours between 0730 and 2030 hrs to capture lizards, document areas occupied by lizards, and note the times lizards were observed. Lizards were captured by hand or noose, and each lizard was marked with non-toxic white paint on the lower back. I measured the snout to vent length (SVL) to the nearest 1.0 mm with a ruler, and the mass of each lizard was measured to the nearest 0.1 g using a Pesola scale.

Digital images were taken of the gular region, gular stripe, and venter stripe upon immediate capture of each lizard using an Olympus C-700 digital camera in a shaded area. Lighting was standardized by using a camera flash, and each lizard was photographed on a neutral grey-colored cardstock board that later served as a color standard. The distance between camera lens and lizard was standardized and ranged from 10-12 cm. Boulders on which lizards were sighted were marked numerically, and

a heuristic map of the boulder field was made. The length and width of each rock was measured to the nearest 0.1 m using a tape measure, and the distances between occupied rocks were measured to produce estimates of the area covered by each lizard. During subsequent transect walks, I documented re-sightings of lizards by noting the position of each re-sighted lizard. All lizards were photographed two or three times, and repeatability of measurements was confirmed to be reasonably consistent.

### 3.3.1 Area coverage

Relative sizes of boulders were made by calculating the ellipse area of each boulder according to the formula:

$$A=\pi(\text{width}/2)(\text{length}/2)$$

Area covered was calculated as the sum of the estimated area of each occupied boulder. Lizards of *S. pyrocephalus* rarely use the ground unless moving from boulder to boulder; therefore, methods such as minimum convex polygon are inappropriate, and occupied area is best estimated by the area of the boulder(s) on which the lizard resided.

My estimate of the area occupied by lizards can be strongly dependent on either the span of days between the first and last observation for a lizard or the number of total times a lizard was observed. I explored these relationships by regressing boulder area on the number of days between the first and last observation for a lizard and the number of times a lizard was observed. The boulder area for males ($R^2 = 0.45$, $F_{2,11}=4.61$, $P=0.03$) and females ($R^2 = 0.46$, $F_{2,11} = 4.77$, $P=0.03$) was positively correlated with both

confounding variables. Because of these relationships, I corrected the boulder area occupied by each lizard by including the number of days between the first and last observation and the total number of times lizards were observed in all subsequent analyses.

One female was a statistical outlier in multiple cases involving color relationships to area coverage. This may be explained by the female's abnormally large boulder area coverage, low sighting frequency, and/or its interaction with a male. The average lizard was observed four times over the 11 day study period. Statistical outlier, female seven, was captured and marked on day two but was only observed twice (the average female lizard captured on day two was observed seven times). Female seven also covered the greatest rock area ($11m^2$; average female rock area coverage was 4.9 $m^2$). The first time female seven was observed, she was being courted by a male; other females observed on days one and two were not seen being courted. Other than these differences, I have no reason to eliminate female seven from the data set; therefore, female seven was included in all remaining analyses unless otherwise indicated.

### 3.3.2 Color measurements

Digital images collected in the field were saved as TIFF files. Condensation and/or dirt on the camera lens caused three female digital images to be discolored; therefore, I chose not to include those individuals in color analyses. I report color data results on twenty-nine individuals (14 males and 15, as opposed to 18, females). Color data were collected on several characteristics of each lizard. The three secondary colors

analyzed consisted of the hue, saturation, and brightness of the gular region, gular stripe, and venter stripe (see Chapter 1, section 1.3.4 Color measurements).

A contrast value between gular region and gular stripe colors may help to elucidate if a multicomponent factor is related to habitat coverage. Color contrast between gular hue and gular stripe hue were examined by taking the difference of their values. This difference value, or throat contrast value, was then compared to area coverage.

### 3.3.3 Statistical analyses

A student's *t*-test was used to determine if SVL significantly differed between males and females, which would confirm dimorphism in body size at the study site. A one-way ANOVA was employed to examine differences between area coverage for males and females. I used general linear regression (GLM) to model the relationships between area coverage, SVL, and color variables using SYSTAT 8.0 to test the hypothesis that color variation was attributed to the area occupied by lizards. I used Shapiro-Wilk tests for normality in SAS, Version 8.0; data were considered normal with equal variances at $P>0.05$. Color values for gular saturation and gular stripe saturation in males, and gular hue, gular stripe hue, and venter stripe saturation in females required $\log_{10}$ transformations to achieve normality. A reciprocal transformation (-1/Y) for gular stripe saturation and a cube transformation for venter hue in females were used. Normality was confirmed after all transformations. Due to the exploratory, two-tailed nature of this study, significance was set at $P < 0.05$ in all analyses, and I report, unless otherwise indicated, mean ± standard variation for variables used in the analyses.

## 3.4 Results

Males were about 0.2 times larger than females (males = 66.4 ± 4.2 mm; females = 57.3 ± 2.0 mm; $P<0.001$). Neither male SVL ($F_{3,10}=1.114$; $P=0.389$; Fig 3.1a) nor female SVL ($F_{3,11}=3.368$; $P=0.059$; Table 3.1) were related to boulder area covered. This relationship in females changed when outlier number seven was removed from the data set ($F_{3,10}=8.763$; $P=0.004$; Fig.3.1b). Mean boulder area was 5.7 ± 2.75 $m^2$. There were sex differences in space use; males occupied larger boulder areas than females (6.8 $m^2$ versus 4.8$m^2$).

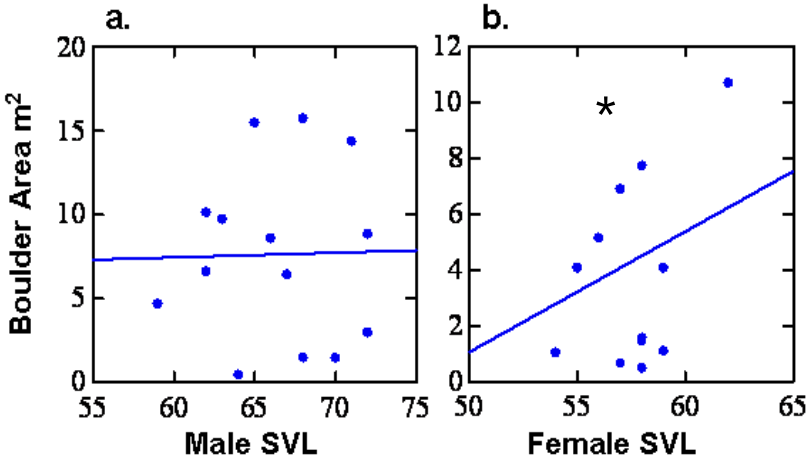


Figure 3.1. Boulder area coverage and body size relationship in males and females. Linear regression of the relationship between boulder area coverage and body size in (a) males and (b) females. Boulder area coverage was not related to body size in males but was related to female body size. An asterisk was used to indicate outlier number 7.

Table 3.1. Boulder area coverage relationship to body size and color variables in females.

Statistical hypothesis tests for the independent variables used in this study compared to the amount of boulder area occupied by females. Female lizard number seven was an outlier in multiple cases; therefore, when a trend was apparent, models were run with and without this lizard. Both results are indicated. Abbreviations: *, P <0.05; $^{+}$, trend (P<0.10).

## FEMALES

| Source | n | X ± SD | DF | $R^2$ | SS | MS | F | Sig. |
|---|---|---|---|---|---|---|---|---|
| SVL | 15 | 57.14 ± 1.99 | 3 | 0.47 | 97.72 | 32.57 | .368 | *0.059*$^{+}$ |
| -eliminating outlier | 14 | 57.15 ± 2.07 | 3 | 0.72 | 112.8 | 37.61 | 8.763 | **0.004*** |
| Total Gular Hue, Sat., & Bright. | 14 | 56.15 ± 7.37 | 5 | 0.72 | 137.9 | 27.58 | 4.208 | **0.036*** |
| -eliminating outlier | 13 | 18.00 ± 7.14 | 3 | 0.71 | 103.8 | 34.61 | 7.495 | **0.008*** |
| Gular Saturation | 14 | 84.45 ± 8.02 | 3 | 0.67 | 128.5 | 42.85 | 6.931 | **0.008*** |
| Gular Brightness | 14 | 66.54 ± 6.92 | 3 | 0.47 | 89.87 | 29.95 | 2.981 | *0.083*$^{+}$ |
| -eliminating outlier | 13 | 66.96 ± 7.01 | 3 | 0.71 | 103.8 | 34.62 | 7.506 | **0.008*** |
| Tot. Gular Stripe Hue, Sat., & Bright. | 14 | 65.91 ± 19.97 | 5 | 0.72 | 137.6 | 27.52 | 4.172 | **0.037*** |
| Gular Stripe Hue | 14 | 81.56 ± 43.12 | 3 | 0.58 | 111.4 | 37.16 | 4.711 | **0.027*** |
| Gular Stripe Saturation | 14 | 62.62 ± 11.04 | 3 | 0.62 | 118.6 | 39.56 | 5.519 | **0.017*** |
| Gular Stripe Brightness | 14 | 53.56 ± 5.75 | 3 | 0.49 | 93.9 | 31.31 | 3.247 | *0.069*$^{+}$ |
| -eliminating outlier | 13 | 53.37 ± 5.94 | 3 | 0.72 | 105.4 | 35.14 | 7.914 | **0.007*** |
| Tot. Venter Hue, Sat., & Bright. | 14 | 76.75 ± 18.74 | 5 | 0.69 | 132.0 | 26.40 | 3.618 | *0.052*$^{+}$ |
| -eliminating outlier | 13 | 77.07 ± 19.31 | 5 | 0.79 | 115.2 | 23.04 | 5.347 | **0.024*** |
| Venter Hue | 14 | 169.64 ± 28.32 | 3 | 0.46 | 88.72 | 29.57 | 2.909 | *0.080*$^{+}$ |
| -eliminating outlier | 13 | 169.86 ± 29.47 | 3 | 0.72 | 105.1 | 35.04 | 7.828 | **0.007*** |
| Venter Brightness | 14 | 35.66 ± 8.17 | 3 | 0.52 | 100.3 | 33.45 | 3.716 | *0.050*$^{+}$ |
| -eliminating outlier | 13 | 35.29 ± 8.38 | 3 | 0.75 | 109.7 | 36.57 | 9.227 | **0.004*** |
| Absolute Throat Hue Contrast | 14 | 65.77 ± 44.57 | 3 | 0.61 | 117.0 | 39.00 | 5.317 | **0.019*** |
| Stripe - Gular Area Hue Contrast | 14 | 64.11 ± 47.10 | 3 | 0.59 | 112.6 | 37.54 | 4.829 | **0.025*** |

### 3.4.1 Color and area coverage

Male hue, saturation, and brightness were not significantly correlated with area coverage in the gular ($F_{5,8}$=1.545; $P$=0.278), the gular stripe ($F_{5,8}$=1.068; $P$=0.444), or the venter stripe ($F_{5,8}$=0.966; $P$=0.491) regions.

Female gular hue, saturation, and brightness were correlated with area coverage ($F_{5,8}$=4.208; $P$=0.036). When the color variables were examined independently, low values of gular hue ($F_{3,9}$=7.495; $P$=0.008, without outlier number seven, Fig 3.2a), and gular saturation ($F_{3,10}$=6.931; $P$=0.008, Fig 3.2b), which indicated more red as opposed to orange-yellow gulars, were related to greater area coverage. Female gular brightness ($F_{3,9}$=7.506; $P$=0.008, without outlier number seven, Fig 3.2c) was also related to greater area coverage.

Gular stripe hue, saturation, and brightness were related to area coverage ($F_{5,8}$=4.172; $P$=0.037), with paler hue ($F_{3,10}$=4.711; $P$=0.027, Fig 3.2d), saturation ($F_{3,10}$=5.519; $P$=0.017, Fig 3.2e), and brightness ($F_{3,9}$=7.914; $P$=0.007, without outlier number seven, Fig 3.2f) being related to greater area covered; in this case. All relationships between venter stripe hue, saturation, and brightness were significant overall ($F_{5,9}$=5.347; $P$=0.024) and separately (hue: $F_{3,9}$=7.828; $P$=0.007, Fig 3.2g; saturation: $F_{3,9}$=7.465; $P$=0.008, Fig 3.2h; brightness: $F_{3,9}$=9.227; $P$=0.004, Fig 3.2i) when outlier female seven was removed.

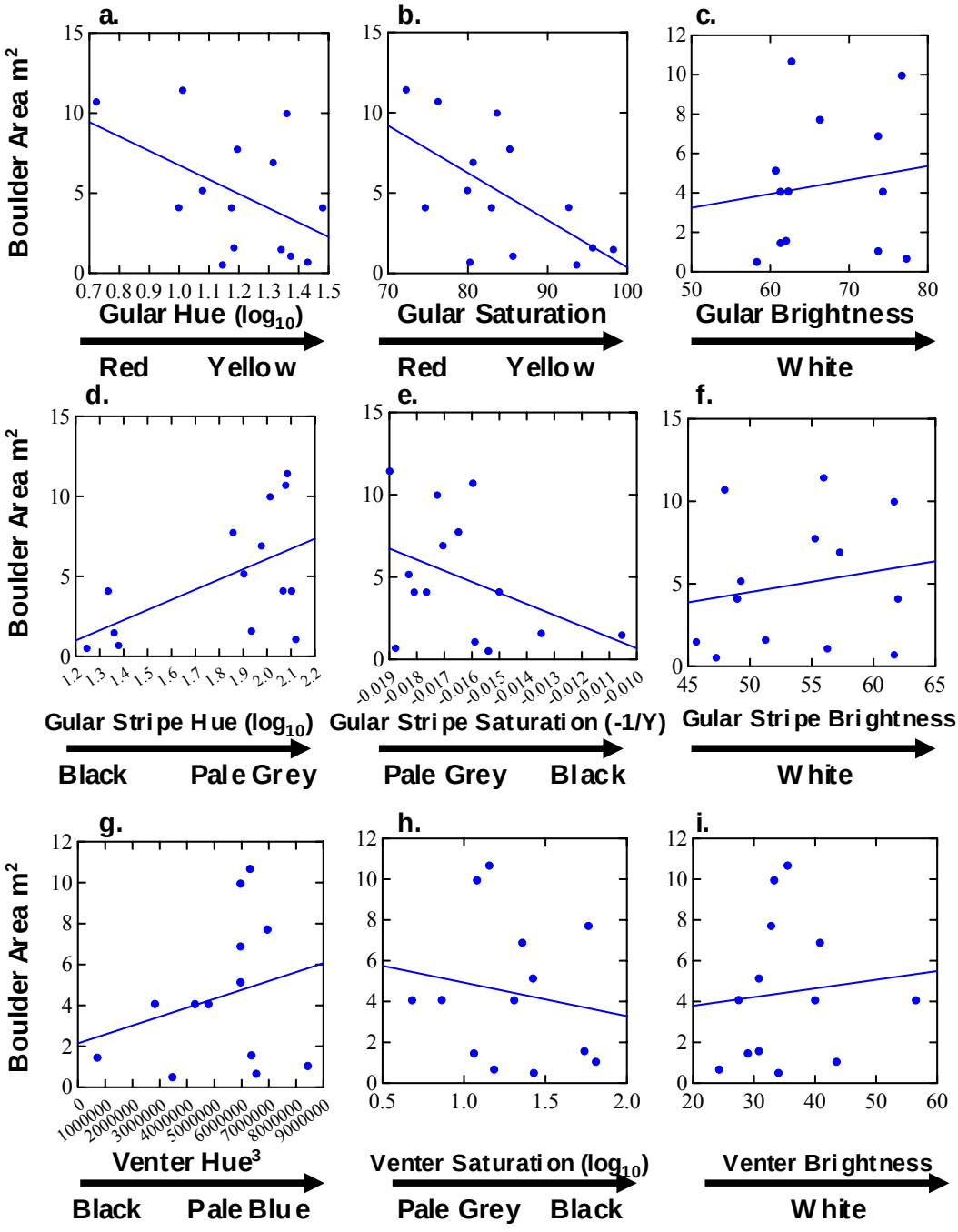


Figure 3.2. Female coloration in relation to boulder area coverage. Linear regression demonstrated that the following were related to large area coverage in females: red gular hue (a) and saturation (b), and bright gular regions (c), pale grey gular stripe hue (d) and saturation (e) and bright gular stripes (f), and pale venter stripe hue (g) and saturation (h) and bright venter stripes.

Male throat contrast (absolute value of gular hue subtracted from gular stripe hue) did not demonstrate a relationship with area coverage ($F_{3,10}$=1.095; $P$=0.396). Female throat contrast ($F_{3,10}$=4.829; $P$=0.025, Fig 3.3) was positively related to area coverage.

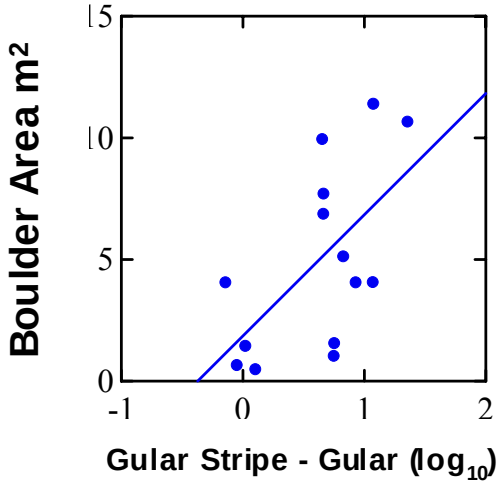


Figure 3.3.Female throat hue contrast in relation to boulder area coverage. Linear regression demonstrated that female throat hue contrast was positively related to boulder area coverage.

## 3.5 Discussion

Color characteristics in females of *Sceloporus pyrocephalus* are conspicuous and are atypical, not only in females of most species of *Sceloporus*, but for most species of vertebrates. Generally in *Sceloporus* and many other lizards, males express conspicuous and brilliant colors while females do not, although some females can express blue ventral coloration similar to males and/or orange color absent in males (Cooper 1984, Hews and Quinn 2003). When females express conspicuous coloration,

typically this is explained by sex-role reversal (Trivers 1972, Andersson 1994, Eens and Pinxten 2000); however, females of *S. pyrocephalus* do not show these characteristics. Males still court females via physical display, which includes a series of push-ups and head-bobs common of male courting in lizards, and there is no evidence of parental care by either sex. This unique system in which females express color, as well as males, but do not exhibit sex-role reversal, presents a novel case in which sexual selection theory, specifically pertaining to color, can be tested.

I found differences in the area occupied by each sex of *S. pyrocephalus* and correlated the size of this area to several characteristics of color in females but not in males. Sex differences were apparent, with males covering more boulder area than females. This relationship was maintained even when controlling for body size variation between the sexes, and therefore females generally occupied less area as compared to males. Larger area occupied by males may provide a mechanism for increasing encounters with multiple females, although data indicate similar opposite-sex overlap by both sexes.

Testosterone can have significant effects on male secondary sexual coloration (Salvador et al. 1996, Olsson et al. 2000), and increases in testosterone have been correlated with increased mobility (Olsson et al. 2000, Sinervo et al. 2000). However, no significant relationship between male color and area coverage in *S. pyrocephalus* was detected. Perhaps neither body size nor color related to area coverage in males because a relationship does not exist, or differences may be so subtle as to require larger sample sizes for further assessment.

While males generally occupied larger areas compared to females, there was no relationship between body size of males and boulder area coverage, but larger females generally occupied larger areas compared to smaller females. Aggression appears to be a very prominent characteristic in both sexes of this species (Calisi, unpublished data), and larger females may be able to out compete smaller ones. Better competitors can generally obtain increased resources (Andersson 1982, 1994), in this case, space. Larger male lizards can be superior competitors in intrasexual contests (Tokarz 1985, Olsson 1992, Jenssen et al. 2005), and results suggest that this mechanism could be operating in females of *S. pyrocephalus* in terms of the area occupied by females. Future work should focus on determining the outcome of female-female interactions in lizards of *S. pyrocephalus* as related to body size and to color characteristics as well as how area may relate to distribution of resources.

All hue, brightness, and saturation measurements were related to female area coverage (but some only revealed a significant relationship when I removed outlier female seven from the analysis). Gular hue was redder and the gular stripes and venter patches were paler in females that covered greater areas. Highly saturated female gular, gular stripe, and venter stripe regions correlated with greater area coverage. When saturation was compared with gular hue, in general, females with redder, more saturated gular regions occupied more area. This observation might be explained if an increase in pigment deposition, thought in this case to be pterin pigments (Macedonia et al. 2000, McGraw 2005), is associated with the yellow-orange to red color change, with increased pigment deposition resulting in greater purity, or saturation of the color.

However, a reciprocal relationship existed for gular stripe and venter stripe regions, suggesting that the reddish gular region is not controlled by the same pigmentary or structural mechanisms as the grey, blue, and black gular stripe and venter stripe regions.

Brighter females, both in gular, gular stripe, and venter stripe regions, covered more area. Brighter females of *S. pyrocephalus* are less parasitized by nematodes, and perhaps brightness may be indicative of health and subsequently a female's ability to compete for and/or cover greater area. Brightness of these secondary traits may therefore serve as an overall honest indication of area coverage and resource holding potential.

Females of *S. pyrocephalus* with red gular regions have large, mature follicles as compared to females with yellow-orange gular regions, which carry smaller, immature follicles. Reptilian estradiol concentrations are positively correlated with ovarian follicle size (Crews 1980, Whittier and Tokarz 1992), and increases in testosterone could facilitate increases and/or maintain high concentrations of estradiol if converted via aromatase enzymes (Whittier and Tokarz 1992, Baum 2002). Females of *S. pyrocephalus* exhibit high testosterone and estradiol in late stages of follicle maturation. If increased testosterone concentration is also correlated with increased mobility (Olsson et al. 2000, Sinervo et al. 2000), then females with red gular regions may be covering greater area to increase the probability of encountering a potential mate(s), searching for foraging sites, and/or searching for egg depositing sites.

The inverse color relationship of female gular hue and the gular stripe hue suggests that throat color contrast may be an important predictor of female area

coverage. The greater the throat contrast, the more area a female covered. Redder females with pale-grey stripes covered more area, while yellow-orange females with black stripes covered less area. The relationship between the contrast of the what may be the most conspicuous color trait in females of *S. pyrocephalus* suggests the interesting possibility of a multicomponent signal. This multicomponent signal, like other single component signals, may provide information regarding the reproductive/hormonal state and/or or immune function (Folstad and Karter 1992, Rowe 1999, Roberts 2004, McGraw 2005). Multiple signals could have a pleiotropic effect, with different traits amplifying the same communicative variable (Brooks and Couldridge 1999, Whiting et al. 2003), or may communicate multiple variables (Moller and Pomiankowski, 1993, Johnstone 1995, Whiting et al. 2003). Such an honest multicomponent signal may conserve the energy output of the signaler. Both mating and competing can be risky, with movements inviting predator attention, and a possible waste of energy in both males and females; thus, an honest color signal that can spare individuals these risks may be a sexually and/or naturally selected variable. Female color signal(s) may also communicate health status, as seen in males (Zahavi 1975, Hamilton and Zuk 1982, Folstad and Karter 1992), and could be valuable information during female-female competition assessment and/or male courting efforts. The possible resulting dynamic between signaler and receiver could therefore be adaptive.

Females of *S. pyrocephalus* offer an excellent system to address how sexual and natural selection may be operating on traits typically attributed to males. The consequences of greater area coverage in females remain unknown, but I hypothesize

that the amount of area covered may in part be related to reproductive state in females. Expression of color has also been correlated with female aggressive behaviors in lizards (Woodley and Moore 1999, Hews and Quinn 2003) and color(s) in females of *S. pyrocephalus* may serve as a predictor of female-female aggressive interaction outcomes similar to males of other lizard species (Cooper and Greenberg 1992, Whiting et al. 2003). Larger areas available to females could lead to the increased acquisition of resources, which in turn may result in greater fitness benefits. Conversely, these resource benefits may carry a cost of an increased risk of predation. Females of *S. pyrocephalus* may illustrate a tradeoff between sexual selection for secondary coloration and natural selection for survivorship.

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