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 <sup>2</sup>Centre for Applied Conservation Research, University of British Columbia, 2424 Main Mall, Vancouver, British Columbia V6T 1Z4, Canada [r.germain@alumni.ubc.ca];
<sup>3</sup>Department of Biology, University of Ottawa, 30 Marie-Curie, Ottawa, Ontario K1N 6N5, Canada [gblouin@uottawa.ca];
<sup>4</sup>Present address: Department of Psychology, Neuroscience and Behaviour, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4L8, Canada [coconn@mcmaster.ca]

ABSTRACT. – Sexual dichromatism is common in many animal taxa, but little quantitative information on sexual dichromatism is available for turtles. We quantified sexual dichromatism in the postorbital spots of northern map turtles (*Graptemys geographica*) using reflectance spectrometry and examined the relationship between postorbital spot coloration and circulating testosterone among males. We found that the coloration of postorbital spots differs between the sexes, with adult males exhibiting brighter spots than adult females. However, adult males and juvenile females did not exibit significant differences in coloration, and testosterone levels did not explain the variation in postorbital spot coloration among males.

In a wide range of animal taxa, males are more conspicuously colored than females. Intersexual differences in coloration (sexual dichromatism) is typically interpreted as a consequence of sexual selection (Andersson 1994). Indeed, in many species females choose their mates based on some attributes of their coloration (e.g., McGraw et al. 2001; Stein and Uy 2006; Reudink et al. 2009). While many examples of spectacular sexual dicromatism exist, dichromatism that is subtle or even undetectable to the human eye can be functionally meaningful for communication and sexual selection (Mennill et al. 2003; Eaton 2005). Quantifying sexual dichromatism can thus provide insights into traits potentially involved in mate choice.

Sexual dichromatism is known in chelonians (reviewed by Moll et al. 1981), but there is little quantitative information available on dichromatism in this group. We quantified sexual dichromatism in the postorbital spots of the northern map turtle (*Graptemys geographica*). The function of these yellow spots is unknown, but they may be involved in intersexual communication and sexual selection because male northern map turtles bob their heads in front of females prior to copulation (Vogt 1980). We used reflectance spectrometry to quantify the coloration of postorbital spots and tested for the presence of sexual dichromatism in northern map turtles.

In addition to quantifying sexual dichromatism, we explored whether levels of circulating testoterone can explain part of the variation in postorbital spot coloration among male northern map turtles. Circulating testosterone may be an indicator of male quality and is positively correlated with elaborate color in males across a wide

Chelonian Conservation and Biology, 2013, 12(1): 187–192 © 2013 Chelonian Research Foundation

## Sexual Dichromatism in the Northern Map Turtle, *Graptemys geographica*

# GRÉGORY BULTÉ<sup>1</sup>, RYAN R. GERMAIN<sup>2</sup>, CONSTANCE M. O'CONNOR<sup>1,4</sup>, AND GABRIEL BLOUIN-DEMERS<sup>3</sup>

<sup>1</sup>Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada [gregory\_bulte@carleton.ca]; variety of taxa (Ligon et al. 1990; Rand 1990, 1992; see metanalysis by Roberts et al. 2004). Thus, we examined the relationship between male postorbital spot coloration and circulating testosterone to determine whether coloration in male northern map turtles is a testosteronedependent trait.

#### METHODS

During the mating season in 2009 (September), we captured 56 northern map turtles in Lake Opinicon (lat 44°34'N, long 76°19'W), Ontario, Canada. Turtles were captured by hand while snorkeling and brought back to the laboratory for standard morphological measurements, color measurement, and blood sampling. Turtles were separated into 3 classes: adult females (n = 9), juvenile females (n = 11), and adult males (n = 36). We did not include immature males. Sexual maturity in females was determined from plastron length; in our study population, the minimum size at maturity in females is 193 mm (Bulté and Blouin-Demers 2009).

Color Measurement and Analysis. - We measured reflectance spectra for postorbital spots of all captured turtles using a PX-2 pulsed xenon light source attached to an Ocean Optics USB2000+ spectrometer (Dunedin, FL). We took 10 readings throughout the yellow region of each postorbital spot (left and right) for a total of 20 readings from each individual. We then grouped raw reflectance data into 10-nm bins from 380 to 700 nm using CLR version 1.0.3 (Montgomerie 2008) and averaged across the 20 measurements for each individual. For our analysis, we quantified the coloration of postorbital spots using the standard coloration variables of hue, saturation, and brightness. In general terms, hue (also known as "spectral location") is measured as the wavelength along the visible spectrum which contributes most to what we perceive as the "color" of an object and is a correlate of the shape of a reflectance spectrum (Endler 1990; Montgomerie 2006). Hue is calculated as the following:

Hue = arctan(
$$[\{\mathbf{R}_{v} - \mathbf{R}_{b}\}/\mathbf{R}_{t}]/[\{\mathbf{R}_{r} - \mathbf{R}_{e}\}/\mathbf{R}_{t}]$$
),

where R is the reflectance of each segment of the spectrum: yellow (y) = 550-625 nm, blue (b) = 400-475 nm, red (r) = 625-700 nm, green (g) = 475-550 nm, and total (t) = 380-700 nm (Saks et al. 2003). Chroma (the "purity" or "saturation" of a color) is the degree to which a color is composed of a single wavelength and is a function of how rapidly reflectance intensity changes with wavelength (Endler 1990; Montgomerie 2006). Chroma is most often used to describe the relative saturation of color within a specific region of interest in the visible spectrum, typically that with the steepest

reflectance slope (Endler 1990; Montgomerie 2006). Here, we calculated chroma in the yellow region of the visual spectrum by the following general equation:

Yellow chroma (saturation) = 
$$R_v/R_t$$

where y and t equal reflectance in the yellow region of the spectrum and total reflectance, as above (Montgomerie 2006). Finally, brightness (or "spectral intensity") is a measure of the total amount of light being reflected from a unit area, measured as the area under the spectral reflectance curve (Montgomerie 2006). Brightness is calculated in the following way:

Brightness = 
$$\sum_{\lambda_{max}}^{\lambda_{min}} = \mathbf{R}_i / n$$
,

where *i* is the reflectance (R) of each wavelength ( $\lambda$ ) divided by the total number (*n*) of wavelengths (Montgomerie 2006). We measured reflectance in the ultraviolet (UV) range (300–379 nm), but preliminary examination of the data indicated that both sexes exhibit minimal reflectance in this range (Fig. 1). We thus excluded UV reflectance from our calculations, limiting our measures of hue, saturation, and brightness to the 380–700-nm range of the light spectrum (i.e., the typical vertebrate trichromatic visual system).

Testosterone Analysis. - We collected 0.3-0.5 ml of blood from the cervical sinus of 32 males using heparinized 1-ml syringes mounted with 25-gauge needles to determine the concentration of circulating testosterone in the blood. Turtles were sampled within 1 hr of capture. Blood samples were held in ice-water slurries for no more than 1 hr and then centrifuged at  $10,000 \times g$  for 5 min (Compact II Centrifuge, Clay Adams, NJ). Plasma samples were flash-frozen in liquid nitrogen and stored at  $-80^{\circ}$ C until analysis. For measurements of circulating testosterone, plasma samples were extracted 3 times using 5 ml ethyl acetate and the dried extract was resuspended in phosphate-buffered saline (pH 7.6) containing 0.3% gelatin and measured in duplicate by <sup>3</sup>H-radioimmunoassay following the methods outlined in McMaster et al. (1992).

Statistical Analyses. — For our analysis of coloration, we divided turtles into 3 groups: adult males, adult females, and juvenile females. We used a multivariate analysis of variance (MANOVA) to test for differences in coloration (hue, saturation, and brightness) among the groups. Because the group effect was significant in the MANOVA, we then performed univariate analyses to compare each of the color measures across the 3 age/sex groups. Finally, we determined if levels of circulating testosterone in males were related to coloration using correlation analysis. We performed all statistical analyses with JMP version 5.0.1a (SAS Institute 2002).



**Figure 1.** Percentage of reflectance of the postorbital spot as a function of wavelength for adult male (solid black line), adult female (solid grey line), and juvenile female (dashed grey line) northern map turtles from Lake Opinicon. Values are based on mean reflectance curves for all individuals of each sex. Error bars indicate 95% confidence intervals.

#### RESULTS

The complete MANOVA model comparing hue, yellow chroma, and brightness among the 3 groups (adult males, adult females, and juvenile females) indicated a statistically significant difference in coloration among groups (Wilk's  $\lambda_{6,102} = 0.36$ , p < 0.0001). Pairwise comparisons between groups indicated no significant difference between adult males and juvenile females  $(F_{3,51} = 2.07, p = 0.11)$ , but significant differences between adult males and adult females ( $F_{3,51} = 28.75$ , p < 0.001) and between adult and juvenile females  $(F_{3.51} = 12.74, p < 0.001)$ . Univariate analysis of variance followed by HSD-Tukey pairwise comparisons indicated that there were significant differences for all 3 color variables between adult males and adult females, no differences between adult males and juvenile females, and a difference in hue but not in yellow chroma or brightness between juvenile and adult females (Table 1; Fig. 2). The correlations between the level of circulating testoterone and the 3 color variables in male northern map turtles were nonsignificant (all p > 0.52).

#### DISCUSSION

The goal of this study was to identify sexual dichromatism in the postorbital spots of northern map turtles using spectral reflectance and to investigate the relationship between male postorbital spot coloration and levels of circulating testosterone. We found that postorbital spots were clearly dichromatic between adult males and adult females. The postorbital spots of adult males were significantly brighter and more saturated in yellow pigment than those of adult females and had significantly lower scores of hue. These results indicate that adult males express a brighter, richer, and more yellow-shifted coloration of their postorbital head spots

**Table 1.** Results of univariate analysis of variance testing the effect of groups (adult males, adult females, juvenile females) on color variables in northern map turtles.

Source	df	F	р	$r^2$
Brightness	2,53	9.00	$< {0.0004 \atop 0.0001 \atop 0.0170}$	0.25
Yellow chroma	2,53	40.61		0.60
Hue	2,53	4.37		0.14

than adult females. Sexually dimorphic traits are commonly involved in sexual selection (e.g., Salvador et al. 1997; McGraw et al. 2001; Stein and Uy 2005; Reudink et al. 2009). Male northern map turtles bob their heads in the vertical plane (Vogt 1980) in front of females during their courtship. Because they are dichromatic and located in an area that would be conspicuous during courtship, postorbital spots are potentially involved in intersexual selection.

We did not find evidence that the coloration of postorbital spots in males was associated with the levels of circulating plasma testosterone. Testosterone plays a key role in mating and sexual behavior, as well as territorial and aggressive behavior in both male and female animals (reviewed by Norris and Jones 1987). In many species, higher testosterone levels are associated with higher male quality (e.g., males that are more attractive, or better able to defend a resource) or with more elaborate coloration (see metanalysis by Roberts et al. 2004). In reptiles, elevated circulating testosterone is typically associated with more elaborate sexual ornamentation (e.g., Cooper et al. 1987; Moore et al. 1998). However, we did not find the predicted relationship between male coloration and testosterone levels in northern map turtles.

Interestingly, most of the work examining the relationship between coloration and testosterone levels in reptiles has been conducted in territorial species (e.g., Cooper et al. 1987; Moore et al. 1998; Salvador and Veiga 2000). To our knowledge, northern map turtles are not territorial and do not engage in male–male combat to secure mating opportunities. Since testosterone is important for both sexual and territorial behavior, it is possible that the relationship between elaborate traits and testosterone is stronger in territorial species. Further research exploring the relationship between elaborate coloration and testosterone in a variety of nonterritorial species is necessary to explore this possibility.

Although testorone did not explain the interindividual variation in coloration in males, it may be involved in the development of sexual dichromatism and in the difference in coloration between adult and juvenile females. Testosterone has been demonstrated experimentally to influence the development of male coloration and sexual dichromatism in lizards (Cox et al. 2005, 2008). In turtles, young females have also been shown to



Figure 2. Box plots of a) brightness, b) yellow chroma, and c) hue across 3 groups of age and sex (juvenile females: n = 11, adult females: n = 9, and adult males: n = 36) in northern map turtles. Age/sex groups with the same letters on the *x*-axis did not differ in multiple pairwise comparisons (Tukey's HSD test). Horizontal box lines represent the median, 25th, and 75th percentiles; horizontal 'whisker' lines represent the 10th and 90th percentiles. Open circles beyond horizontal lines represent outliers.

produce eggs with higher levels of yolk testosterone than old females, suggesting that young females have higher circulating levels of testosterone during vitellogenesis than old females (Bowden et al. 2004). Experimental manipulation of hormone levels is now indicated in northern map turtles to determine the influence of hormones such as testosterone on the development of sexual dichromatism and the apparent ontogenetic shift in coloration in female map turtles noted in the current study. Finally, it is possible that other, unmeasured hormones are also related to dichromatism in northern map turtles. For example, progesterone has been linked to female coloration in some species of lizards (e.g., Cooper and Clarke 1982). Other proximate factors may also influence the development of sexual dichromatism in northern map turtles. Incubation temperature is known to influence coloration in *Graptemys* (Vogt 1993). Because males and females are produced at different incubation temperatures, dichromatism may be partly a consequence of incubation temperature. However, we found dichromatism to be more pronounced between adult males and adult females than between adult males and juvenile females, suggesting that factors others than incubation must be influencing the extent of dichromatism. Diet could also influence the coloration of the postorbital spots in northern map turtles. The expression of carotenoid-based coloration is known to be diet-dependent in some species and may signal a

male's overall condition and ability to forage (Hill and Montgomerie 1994; Keyser and Hill 1999). Interestingly, adult males and juvenile females do not differ in diet in our study population, whereas adult males and adult females do, with the males eating relatively more insects and the females relatively more molluscs (Bulté et al. 2008). Although carotenoids are commonly involved in yellow coloration, other pigments can also produce the same coloration (McGraw et al. 2006). We did not determine which pigments are involved in the yellow coloration of the postorbital spots of northern map turtles. Testing for the presence of carotenoids would provide more insights into the factors affecting the conditiondependent expression of the yellow coloration in northern map turtles.

#### ACKNOWLEDGMENTS

We are grateful to the staff of the Queen's University Biological Station for their logistical support. Thanks to P.R. Martin for the use of his spectral equipment and to J.A. Mennigan and V.L. Trudeau for their assistance and support with testosterone assays. Thanks to P.R. Martin and S.J. Cooke for providing comments on earlier versions of the manuscript. Financial support for G.B. was provided by the Ministry of Research and Innovation and the Fond Québecois de Recherche sur la Nature et les Technologies. Financial support for C.M.O. was provided by the Ministry of Research and Innovation and by Natural Sciences and Engineering Research Council Discovery Grants to S.J. Cooke and K.M. Gilmour. Our procedures were approved by the Animal Care Committee at Carleton University (protocol B09-27) and permits were provided by the Ontario Ministry of Natural Resources (permit 1045605).

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Received: 31 January 2012

- Revised and Accepted: 13 September 2012
- Handling Editor: Peter V. Lindeman