

## MATURATIONAL CHANGES IN MALE SLIDER TURTLES (*TRACHEMYS SCRIPTA*) FROM ILLINOIS

ANNE M. READEL<sup>1,2,4</sup>, JONATHAN K. WARNER<sup>1</sup>, REBECCA L. HOLBERTON<sup>3</sup>,  
AND CHRISTOPHER A. PHILLIPS<sup>1,2</sup>

<sup>1</sup>Illinois Natural History Survey, Division of Biodiversity and Ecological Entomology, 1816 S. Oak St.,  
Champaign, Illinois 61820, USA

<sup>2</sup>University of Illinois, Ecology and Evolutionary Biology, 286 Morrill Hall, 505 S. Goodwin Ave., Urbana, Illinois 61801,  
USA

<sup>3</sup>Laboratory of Avian Biology, 221 Murray Hall, Department of Biological Sciences, University of Maine,  
Orono, Maine 04469, USA

<sup>4</sup>Corresponding Author, e-mail: [readel@uiuc.edu](mailto:readel@uiuc.edu)

**Abstract.**—Accurate identification of sexually mature individuals is essential for life-history studies. In emydid turtles, secondary sexual characters (SSCs) are most often used to identify male maturity while numerous other methods exist. In this study, we examined the association between body size, age, foreclaw length (FCL), preanal tail length (PTL), testis mass, and plasma androgen level in Slider Turtles (*Trachemys scripta*; n = 40) from multiple populations in Illinois. We determined the accuracy of these methods by dissecting male Slider Turtles and used the presence of sperm as a definitive test of sexual maturity. Male *T. scripta* matured at 98 mm plastron length, between 3-5 years old, and generally had FCLs > 7 mm, PTLs > 13 mm, and testis weights > 0.06 g. Androgen levels were significantly higher in mature males compared to immature males and immature females. Overlap in the individual raw values for androgens between categories limits their use for identifying maturity in this species. Overall, size and SSCs appear to be the least invasive and most accurate methods available to identify male maturity.

**Key Words.**—androgens; Emydidae; maturity; secondary sexual characters; Slider Turtle; testis mass; *Trachemys scripta*

### INTRODUCTION

Many male emydid turtles mature at younger ages and smaller sizes than females (Gibbons et al. 1981; Ernst et al. 1994) and different ecology, behavior, and life-history characteristics are often displayed between the sexes and life stages. Therefore, accurate identification of male maturity is essential for ecological and evolutionary studies. Secondary sexual characters (SSCs), such as elongated foreclaws and enlarged tails, develop rapidly during male maturation (Cagle 1948; Evans 1952; Gibbons and Greene 1990) and are often used by researchers to identify mature males (Cagle 1950; Ernst et al. 1994; Thomas 2002). Numerous other methods also exist to identify male maturity and include dissection and gonad histology (Van der Heiden et al. 1985; reviewed in Wibbels et al. 2000), laparoscopy (Rostal et al. 1994), radioimmunoassay of androgens (Diez and Dam 2003), and electroejaculation for sperm collection (Gist et al. 1990).

The Slider Turtle (*Trachemys scripta*) is widespread and well-studied (Ernst et al. 1994). Although many studies have identified the size and age of male maturity in *T. scripta* (Cagle 1948; Webb 1961; Gibbons et al. 1981; Gibbons and Greene 1990; Mitchell and Pague 1990), few have described the morphological and physiological changes associated with the onset of maturity (Cagle 1948, Gibbons and Greene 1990). Our

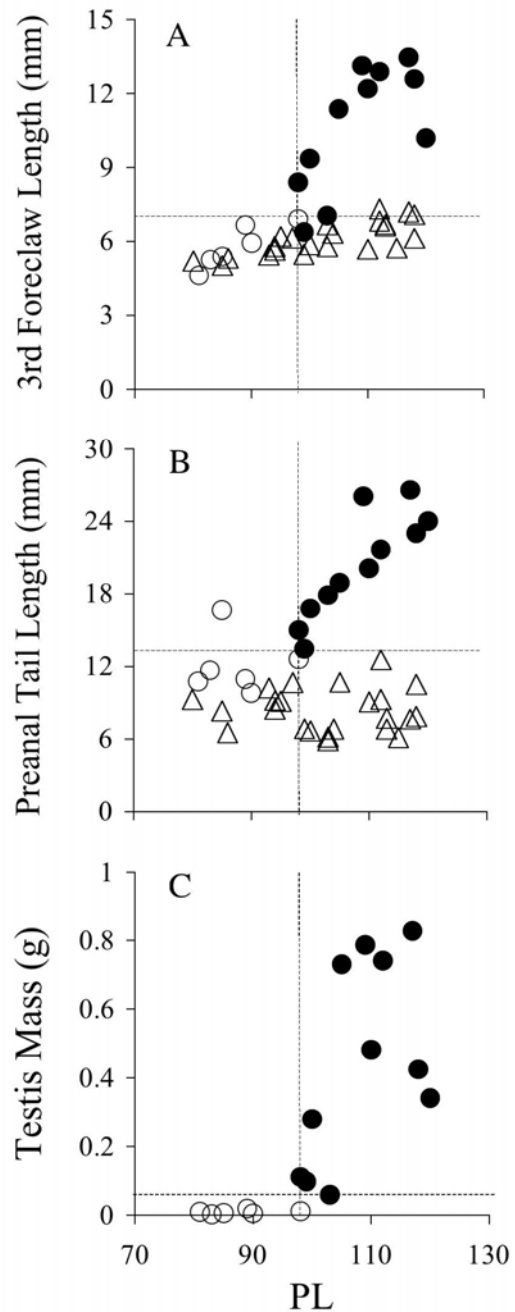
objectives were to describe the differences in body size, age, SSC development, testis mass, and androgen levels between mature (producing sperm) and immature (not producing sperm) males, and examine the utility of these variables in identifying mature males.

### MATERIALS AND METHODS

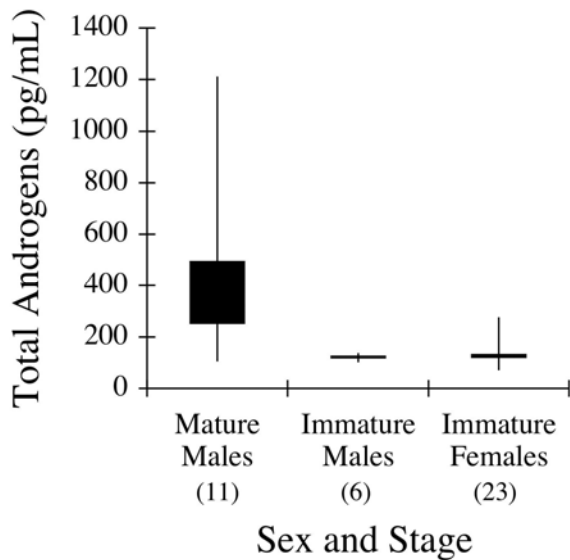
We captured turtles from multiple populations in Vermilion, Champaign, White, Gallatin, and Saline counties in Illinois using baited hoop traps from May to September 2005. Each turtle was marked with a unique notch pattern on the marginal scutes (Cagle 1939). We measured straight-line plastron length (PL) to the nearest mm using tree calipers. Only turtles that were 80-120 mm PL were brought back to the laboratory where they were housed in mixed sex groups and fed commercial turtle-food pellets every 2-3 days. We maintained all turtles at ~22°C with ~8 hours of artificial light for at least 24 days to minimize androgen (Licht et al. 1985; Cash 2000) and testicular mass variation (Gibbons 1968; Ernst 1971; Christiansen and Moll 1973) before sacrificing them between 12 September and 9 October 2005. Housing turtles in groups may result in higher androgen levels than housing turtles singly; however, the overall pattern of androgen secretion between housing strategies is similar throughout the year (Cash 2000).

For hormone analysis, we collected blood from the subcarapacial sinus and held it in lithium heparinized Capject tubes (Termuno) on wet ice for up to two hours (Cash et al. 1997; Cash 2000). We then centrifuged samples at 6000 rpm for five minutes, and the plasma was stored in microcentrifuge tubes at  $-80^{\circ}\text{C}$ . We took samples within 10 min of the researcher entering the room where the turtles were housed. Samples taken within 10 minutes should represent the best estimate of a pre-disturbance baseline sample (Cash et al. 1997; Cash 2000). While “captive stress” can alter physiological profiles, wild *T. scripta* held in long-term captivity and under adequate conditions did not experience a chronic rise in corticosterone (stress hormone), and exhibited normal patterns of gonadal activity in response to photoperiod (Cash 2000). Plasma samples were analyzed by radioimmunoassay for total androgen (testosterone and dihydrotestosterone) concentration following the methods developed for corticosterone (described in Cash et al. 1997) and modified for total androgen. The androgen antibody (Endocrine Sciences/Esoterix T3-125) used in this assay has high specificity ( $> 95\%$ ) for testosterone but more than 44% cross reactivity with dihydrotestosterone. We therefore report total androgen concentration. The sensitivity based on the standard curve was 1.7 pg/mL.

We measured PL, third foreclaw length (FCL; Legler 1990) and preanal tail length (PTL; Cagle 1948) to the nearest 0.1 mm using digital calipers and then immediately dissected turtles to determine sex and maturity. We removed one testis from each male, blotted it to remove excess moisture, and then weighed it to the nearest 0.001 g (Denver Instrument Company, Model XE-100A, USA). To determine if turtles were mature (producing sperm), we examined testis smears microscopically. Any testes that appeared to be in the transition period to maturity (those that were not obviously producing sperm but appeared larger than other immature testes) were examined histologically. Maturity was confirmed for these individuals when histological slides contained differentiated spermatocytes and mature spermatids. Finally, we estimated age using scute ring counts only when age zero (areola) was present. Scute rings form during a cessation of growth as the epidermal lamina die back, forming a scar (Moll and Legler 1971). In temperate regions during the winter months deeper scars are formed (Moll and Legler 1971; Legler 1960). Although ageing with scute rings is contentious, critics do warrant its use when ageing young turtles (Germano and Bury 1998; Litzgus and Brooks 1998; Wilson et al. 2003). In Illinois, juvenile *T. scripta* added one scute ring annually (Cagle 1946) and rings of immature *P. concinna* showed strong corroboration with growth histories (Dreslik 1997); however, scute rings were only reliable up to age four in Oklahoma (Stone and Babb 2005). Age was only



**FIGURE 1.** The relationship between A) third foreclaw length; B) preanal tail length; and C) testis mass and plastron length (PL) of *Trachemys scripta*. Mature males are represented by filled circles, immature males by hollow circles, and immature females by hollow triangles. Dotted vertical lines demark the size that males started becoming sexually mature (PL = 98 mm), horizontal dotted lines demark the A) foreclaw length cutoff for mature males (7 mm); B) the preanal tail length cutoff for mature males (13 mm); and C) the testis mass cutoff for mature males (0.06 g). One mature male had a foreclaw length that was  $< 7$  mm, and one immature male had a preanal tail length  $> 13$  mm. Sex and maturity were validated by dissection.



**FIGURE 2.** Total androgen concentrations of *Trachemys scripta* by sex and reproductive stage. Mature males had significantly higher androgen concentrations than immature males and there was no significant difference between immature males and immature females. Box and whisker plots show the  $\pm 1$  SE of the mean (box) and range (vertical lines) of total androgens. Numbers below plots are number of individuals sampled.

estimated when clear ring formation was present and growth occurred beyond the last ring (as evidenced by lighter coloration in the plastron). In addition, no age estimates were made past seven years because growth slows and individual rings become more difficult to distinguish.

We graphed SSC lengths and testis mass against PL to determine the sizes of these characters at male maturity. Because we could not normalize the data, we used two-sided Wilcoxon Rank Sums tests to determine if androgen levels could be used to determine sex in immature individuals (immature males vs. immature females) and male maturity (mature males vs. immature males). To describe the association among PL, age, FCL, PTL, testis mass, and total androgen concentration, we used Spearman rank correlations because the joint distributions of all comparisons were not normal. We performed all statistical tests with SAS® software (SAS Institute, Cary, NC) and used Bonferroni corrections such that nominal alpha levels of  $\alpha \leq 0.025$  and  $\alpha \leq 0.003$  were significant ( $P \leq 0.05$ ) for Wilcoxon Rank Sums tests and Spearman rank correlations, respectively.

### RESULTS

We dissected 40 individuals (11 mature males, six immature males, 23 immature females). Males  $> 98$  mm were sexually mature (Fig. 1). Of the sexually mature males, 82% ( $n = 9$ ) had FCLs  $> 7$  mm, all had PTLs  $> 13$

mm, and all had testis masses  $> 0.06$  g (Fig. 1). One immature male had a PTL  $> 13$  mm (Fig. 1), however. We were only able to confidently determine the age for 10 males and the estimated age of immature males ranged from 3-5 years ( $n = 4$ ), and 3-7 years for mature males ( $n = 6$ ). The mean estimated age of mature and immature males was 4.4 and 3.8 years, respectively. The total plasma androgen concentration range was 73.8-238.6 pg/mL for immature females, 104.7-133.6 pg/mL for immature males, and 107.8- 1209.1 pg/mL for mature males. There was no difference in the plasma androgen levels between immature males and immature females ( $Z = 0.135$ ,  $df = 1$ ,  $P = 0.893$ ; Fig. 2); however, mature males had significantly higher androgen levels than immature males ( $Z = 2.362$ ,  $df = 1$ ,  $P = 0.018$ ; Fig. 2). PL, FCL, PTL, testis mass, and androgen levels were all significantly correlated with each other but age did not correlate with any other variable (Table 1).

### DISCUSSION

All male *T. scripta* in this study reached maturity by 98 mm. This size at male maturity corresponds to those of other studies on Slider Turtles (Cagle 1948; Webb 1961; Gibbons et al. 1981; Gibbons and Green 1990; Mitchell and Pague 1990). Although our sample size was small, size appears to be an accurate measure of sexual maturity in our study.

Maturity relies on the interaction of environmental and genetic factors (Stearns and Koella 1986), however, that can result in variation in the size and age of maturity within and between populations (Webb 1961; Gibbons et al. 1981; Gibbons and Green 1990). In this study, the age at maturity varied considerably with one turtle producing sperm at age 3, while one remained immature at age 5.

Mature males in our study typically had FCL  $> 7$  mm (82% met this cutoff size) and PTL  $> 13$  mm, which were similar to findings in other studies of *T. scripta* (Cagle 1948; Gibbons and Greene 1990). Another study found that male *T. scripta* had FCLs more than double those of similar-sized females when examining turtles over 100 mm PL (Warner et al. 2006). The PTLs of mature males in our study appear similar to those in other studies of *T. scripta* in Illinois (Cagle 1948). The cutoff sizes for FCL and PTL that were found in this study should be used with caution, however, due to the small sample sizes and variation observed.

As far as we are aware, this is the first study that has described the differences in testicular weights between immature and mature male *T. scripta*. Studies on other turtle species have demonstrated that testicular size and weight correlates with PL (Gibbons 1968; Mitchell 1985) and can vary seasonally (Gibbons 1968; Ernst 1971; Christiansen and Moll 1973); thus, testicular sizes at maturity may vary between populations and seasons. Therefore, validating sperm production or performing a

**TABLE 1.** Spearman correlation coefficients among body size (plastron length; PL), age, foreclaw length (FCL), preanal tail length (PTL), testis mass, and androgen levels for male *Trachemys scripta*. Spearman correlation coefficients are displayed in the lower-left half of the table, and corresponding *p*-values in the upper-right half. Significant correlations (all  $P \leq 0.003$ ) and are bolded.

	PL	Age	FCL	PTL	Testis Mass	Androgens
PL	-----	0.068	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
Age	0.63	-----	0.068	0.241	0.285	0.084
FCL	0.89	0.63	-----	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>
PTL	0.9	0.43	0.9	-----	<b>&lt;0.001</b>	<b>0.003</b>
Testis Mass	0.85	0.4	0.96	0.88	-----	<b>&lt;0.001</b>
Androgens	0.8	0.61	0.82	0.68	0.83	-----

histological evaluation of testes remain better methods than using testicular weight to determine male maturity.

The androgen levels we documented were slightly higher than those in hatchling *T. scripta* (Rhen et al. 1999), and lower than larger mature male *T. scripta* (Cash 2000). To our knowledge, this is the first study to document changing androgen levels with male maturity in this species. Androgen levels did not differ significantly between immature males and immature females, but mature males had androgen concentrations that averaged 250 pg/mL higher than those for immature males. Although researchers have used androgen levels to identify sex and maturity in turtles (Diez and Dam 2003; Geis et al. 2005), plasma androgen concentrations were unable to differentiate the sex of immature individuals, and were weak predictors of male maturity for *T. scripta* due to overlapping concentrations between mature and immature males. It is possible that threshold androgen levels could be used to distinguish one sex or maturity stage from the other, but they cannot confirm sex or maturity when levels are low. In this study, only mature males had androgen levels greater than 239 pg/mL so androgen levels above 239 pg/mL may signify a mature male; however, levels less than 239 pg/mL could be immature females, immature males, or mature males. We do not recommend that future studies use this threshold value, unless it is independently validated, due to the small sample size and dynamic nature of androgen levels. Other studies have reported that androgen levels can be questionable for sexing turtles due to their dynamic and highly variable nature (Schroeder and Owens 1994; Braun-McNeill et al. 2007). For instance, androgen levels can fluctuate throughout the day (Gancedo et al. 1997), vary seasonally with breeding activities (Rostal et al. 1998), and even differ within species at different research sites (Owens 1997).

Although dissection and gonad histology is accurate to identify male sexual maturity (Van der Heiden et al. 1985; Wibbels et al. 2000), it is not usually feasible for studies of threatened species or for long-term studies. Size appeared to be the most definitive and least invasive measure of sexual maturity. Measured SSC lengths may also prove useful, especially in populations that vary in the size of sexual maturity. At this time, androgen analyses do not appear as useful in identifying maturity in this species; however, their potential value as a non-

invasive measurement in use with threatened species or populations and species that do not display SSCs is high and should be investigated further in other emyids.

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**ANNE READEL** is a Ph.D student in Ecology, Evolution, and Conservation Biology at the University of Illinois in Urbana/Champaign where she also received her B.S. in Animal Sciences in 2003. Her dissertation research focuses on the impact of anthropogenic habitat degradation on the physiology, immunology, and infectious diseases of aquatic turtles in Illinois. She received the John Knauss Marine Policy Fellowship in 2006, and worked for a year in Washington, DC, at the National Oceanic and Atmospheric Administration (NOAA). Her interest in environmental policy has led her to apply to law school. Photographed by Tony L. Goldberg.



**JONATHAN WARNER** is a postgraduate student at the University of the Witwatersrand in South Africa. His research interests are in the distribution, ecology, and conservation of African herpetofauna. He is currently studying the ecology of the Gaboon Viper (*Bitis gabonica*) in southern Africa. He is also involved with establishing a biodiversity atlas in the Simangiliso Wetland Park, a UNESCO World Heritage Site. Jon received his B.S. in 2004 from the University of Illinois in Natural Resources and Environmental Sciences. Photographed by Xander Combrink.

**CHRISTOPHER A. PHILLIPS** (left) is an Associate Professional Scientist in the Division of Biodiversity and Ecological Entomology, Illinois Natural History Survey, Champaign. He received his Ph.D. from Washington University St. Louis in 1989 and his B.Sc. from Eastern Illinois University in 1983. His current research interests are in the fields of ecology and population genetics. Current questions focus on North American amphibians and reptiles. He is especially interested in population structure of wide ranging species and population viability. Chris is a current member of the Illinois Endangered Species Protection Board. Photographed by Joel Dexter.



**REBECCA L. HOLBERTON** (Not pictured) is an Associate Professor of Biology at the University of Maine in Orono. Her research integrates behavior, ecology and physiology by combining field and laboratory studies. Basic and applied research includes studies on how environmental conditions and habitat characteristics affect survivorship and reproduction of animals. Rebecca is an elective member and a Fellow of the American Ornithologists' Union and has served on the councils for various scholarly ornithological societies. She is currently a Bullard Fellow at the Harvard Forest. Rebecca is an Associate Editor for many journals, including *The Auk*, *Journal of Ornithology*, and *Physiological and Biochemical Zoology*. Her lab is expanding its large database of hormone and metabolite information on North American Birds.