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Sexual Dimorphism and Seasonal Variation of Reproductive Hormones in the Pascagoula Map Turtle, *Graptemys gibbonsi*

Sean P. Graham¹, Chelsea K. Ward², Jennifer Shelby Walker³, Sean Sterrett⁴, and Mary T. Mendonça³

The Pascagoula Map Turtle (*Graptemys gibbonsi*) is a narrowly endemic species found only in the Pascagoula River drainage in Mississippi. It is among the most poorly known turtle species because of research taxonomic biases and this species' relatively recent recognition as a unique taxon. A recent petition requested protective status for *G. gibbonsi* under the U.S. Endangered Species Act. We describe population parameters, quantitatively assess sexual dimorphism of *G. gibbonsi*, and document hormone secretion patterns from the Chickasawhay and Leaf rivers in Mississippi. We demonstrate a significant male-skewed sex ratio and a female-biased size dimorphism in both carapace length and height. Males showed a bimodal peak of plasma testosterone in fall and spring, consistent with the pattern shown by many other southeastern turtles with late summer-fall spermatogenesis and mating during spring and fall. Females did not show seasonal variation in estradiol secretion, an unexpected result that was possibly due to our small sample size of females, none of which were gravid when captured. Although this observation may be due to our limited capacity to sample females, given the reproductive issues reported for *Graptemys flavimaculata* from the same drainage (e.g., reproductive hormone abnormalities, low nesting frequency and success), this finding warrants concern and necessitates additional research. Finally, in order to put our hormone data in context, we briefly review hormone and reproductive patterns in southeastern U.S. turtles. Our review includes the timing of follicular enlargement, ovulation and nesting, clutch frequency, and estradiol cycles. The review for male turtles includes details on the spermatogenic cycle, spermiation, and the timing and frequency of testosterone peaks.

TURTLES are among the most imperiled vertebrates resulting in part from many species having intrinsic life-history characteristics such as slow growth rates, delayed sexual maturity, long generation times, and low hatchling survivorship (Congdon et al., 1994), in combination with unsustainable human exploitation (Cheung and Dudgeon, 2006). Management decisions and conservation protocols developed for these threatened vertebrates rely heavily on information about their basic natural history and reproductive biology; however, these data are not available for many species. For example, a recent analysis determined that certain turtles have received a majority of scientific attention, while others are much less well known and only a few studies describe their basic biology (Lovich and Ennen, 2013). An additional problem is that taxonomic uncertainty makes some of the basic information available of limited value; studies on certain species may be an amalgamation of information attributable to multiple cryptic species.

The Pascagoula Map Turtle, *Graptemys gibbonsi*, is an example of a turtle species about which we know very little as a result of a lack of published studies in combination with past taxonomic uncertainty. Despite their attractiveness, high species richness, and fascinating morphological variation, turtles in the genus *Graptemys* are among the least studied turtles in North America (Lovich and Ennen, 2013). In addition, *G. gibbonsi* was formerly considered to belong to a more widespread Gulf Coast species (*G. pulchra*, *sensu lato*) thought to be distributed west to east from the Pearl River to the Escambia River drainage in Alabama and Florida (Cagle, 1952; Lovich, 1985). This complex was then recognized to

be made up of four species with distinctive genetic and morphological features, which range from west to east as: *G. pearlensis* (Pearl River drainage; Lovich and McCoy, 1992; Ennen et al., 2010), *G. gibbonsi* (Pascagoula drainage), *G. pulchra* (*sensu stricto*; Alabama River drainage), and *G. ernsti* (Escambia River drainage). Most of this taxonomic resolution has come fairly recently, and our knowledge about these turtles has not kept pace. Information about *G. gibbonsi* determined before this taxonomic reassignment has equivocal value, and little information is attributable specifically to this turtle (see Lovich and Ennen, 2013).

Graptemys gibbonsi (*sensu stricto*) is a riverine species endemic to the Pascagoula River drainage of Mississippi. It belongs to the megacephalic group of turtles, species that exhibit extreme female-biased sexual dimorphism in carapace length and head size (Lindeman and Sharkey, 2001). Females have grossly enlarged heads and carapace lengths are distinctly more than twice the carapace length of males (Gibbons and Lovich, 1990; Lindeman, 2008; Ernst and Lovich, 2009), and most of the species in this group show narrow and drainage-specific endemism (Lamb et al., 1994; Lindeman, 2013). Due to this species' limited distribution (Lamb et al., 1994; Ernst and Lovich, 2009) and various potential threats ranging from commercial harvest, recreational shooting, and over-collection for the pet trade, a recent petition requested listing of *G. gibbonsi* under the Endangered Species Act (ESA; Center for Biological Conservation, 2010). This petition is supported by studies demonstrating that this currently unprotected turtle is less common than the federally threatened species *Graptemys*

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Table 1. Reproductive cycles of male non-marine, southeastern U.S. turtles. Aestival spermatogenesis = post nuptial spermatogenesis; occurs during the summer preceding mating. Year-round spermiation indicates spermatozoa present in epididymes/vas deferens year round, bimodal spermiation indicates spermatozoa present in epididymes/vas deferens immediately after peak spermatogenesis and maintained until the following spring. Unimodal peaks of testosterone coincide with peak spermatogenesis; bimodal peaks occurs in late summer (coincident with spermatogenesis and mating) and spring (coincident with mating). *Indicates spermatogenic cycle inferred from testis size variation.

| Species | Spermatogenic cycle | Spermiation | Testosterone cycle | Mating season (Ernst and Lovich, 2009) | According to |
|------------------------------------|---------------------|-------------|----------------------|--|---|
| <i>Chelydra serpentina</i> | aestival | year round | unimodal late summer | | White and Murphy, 1973; Mahmoud and Licht, 1997 |
| <i>Chrysemys picta</i> | aestival | bimodal | bimodal | | Callard et al., 1976 |
| <i>Chrysemys picta dorsalis</i> | aestival | bimodal | | | Moll, 1973 |
| <i>Deirochelys reticularia</i> | aestival* | | | | Gibbons, 1969; Ewert et al., 2006 |
| <i>Graptemys ernsti</i> | aestival* | year round | | late summer/fall | Shealy, 1976 |
| <i>Graptemys flavimaculata</i> | | | bimodal | | Shelby et al., 2000; Shelby and Mendonça, 2001 |
| <i>Graptemys geographica</i> | | | | bimodal | |
| <i>Graptemys gibbonsi</i> | | | bimodal | | this study |
| <i>Graptemys nigrinoda</i> | aestival | bimodal | | | Lahanas, 1982 |
| <i>Graptemys oculifera</i> | aestival* | | | spring | Kofron, 1991 |
| <i>Graptemys ouachitensis</i> | aestival | | | bimodal | Vogt, 1980 |
| <i>Graptemys pseudogeographica</i> | aestival | | | bimodal | Torres-Orozco et al., 2002 |
| <i>Malaclemys terrapin</i> | | | | spring | |
| <i>Pseudemys nelsoni</i> | aestival* | | | Oct-Mar; possibly year round | Jackson, 2006 |
| <i>Terrapene carolina</i> | aestival | year round | bimodal | bimodal | Altland, 1951; Currylow et al., 2013 |
| <i>Trachemys scripta</i> | aestival | year round | equivocal | bimodal | Lovich et al., 1990; Garstka et al., 1991 |
| <i>Kinosternon baurii</i> | aestival* | | | bimodal | |
| <i>Kinosternon subrubrum</i> | aestival | bimodal | | spring | Mahmoud and Klicka, 1972 |
| <i>Sternotherus carinatus</i> | aestival | bimodal | | spring | Mahmoud and Klicka, 1972 |
| <i>Sternotherus minor</i> | aestival | year round | | bimodal | Etchberger and Stovall, 1990 |
| <i>Sternotherus odoratus</i> | aestival | bimodal | bimodal | bimodal | McPherson et al., 1982 |
| <i>Gopherus polyphemus</i> | aestival | | unimodal late summer | bimodal | Ott et al., 2000 |
| <i>Apalone ferox</i> | aestival | bimodal | | | Meylan et al., 2002 |
| <i>Apalone mutica</i> | aestival | bimodal | | bimodal | Plummer, 1977 |
| <i>Apalone spinifera</i> | aestival | bimodal | | spring | Robinson and Murphy, 1978 |

flavimaculata found in the same drainage (Lindeman, 1999; Selman and Qualls, 2009). *Graptemys gibbonsi* and other turtles in the Pascagoula River drainage are also threatened by industrial pollution, which is thought to contribute to reproductive abnormalities in *G. flavimaculata* (Shelby and Mendonça, 2001; Shelby-Walker et al., 2009). This makes comparative reproductive information about *G. gibbonsi* from the same drainage especially valuable.

It has been over 30 years since sex steroid patterns and reproductive cycles in turtles were reviewed (Licht, 1982). Since that time there have been many additional studies (Tables 1, 2), and our picture of the variation and similarities of these cycles has become clearer, which should allow information about *G. gibbonsi* to be placed in a more robust context. Testosterone (T) secretion is linked to both spermatogenesis and male sexual behavior in most vertebrates and shows seasonal patterns associated with these activities in male turtles (Norris, 2006; Moore and Lindzey, 1992). Similarly, in females, estradiol (E₂) secretion shows seasonal peaks associated with vitellogenesis and ovulation (Altland, 1951; Callard et al., 1978). To contribute to our

understanding of the reproductive biology of this threatened turtle species, we documented reproductive characteristics of *G. gibbonsi* from two rivers in Mississippi. We provide a quantitative analysis of sexual size dimorphism in this species and document seasonal patterns of hormone secretion in males and females.

MATERIALS AND METHODS

Study area.—*Graptemys gibbonsi* were captured at sites located in Greene County, Mississippi, near the town of Leaksville on a 3 km section of the Chickasawhay River (North end = 31.14861, -88.54816, South end = 31.04143, -88.65727) and in Forrest County, Mississippi, near the town of Hattiesburg on a 5 km section of the Leaf River (North end = 31.32642, -89.26703, South end = 31.30026, -89.25297; Fig. 1), in conjunction with a study on Yellow-Blotched Map Turtles (*Graptemys flavimaculata*; Shelby et al., 2000).

Field methods.—Turtles were caught using handmade basking traps. Traps consisted of an open wire basket attached to

Table 2. Reproductive cycles of female nonmarine, southeastern U.S. turtles. Bimodal estradiol cycles are associated with ovulatory cycles initiated in late summer/fall that conclude the following spring. Unimodal estradiol cycles typify turtles that complete vitellogenesis, ovulation, and nesting during a single year (spring-summer).

| Species | Follicular enlargement initiated | Ovulation and nesting season | Clutch frequency | Estradiol cycle | According to |
|------------------------------------|--|------------------------------------|----------------------------|---------------------------------|--|
| <i>Chelydra serpentina</i> | late summer/fall | spring summer | single, sometimes two | bimodal | White and Murphy, 1973; Lewis et al., 1979; Mahmoud and Licht, 1997 |
| <i>Chrysemys picta</i> | late summer/fall | spring summer | multiple, sometimes single | bimodal | Callard et al., 1978 |
| <i>Chrysemys picta dorsalis</i> | late summer | spring summer | multiple | | Moll, 1973 |
| <i>Deirochelys reticularia</i> | | winter; egg retention | multiple | | Jackson, 1988; Buhlmann et al., 1995 |
| <i>Graptemys barbouri</i> | | spring summer | multiple | | Ewert et al., 2006 |
| <i>Graptemys ernsti</i> | | spring summer | multiple | | Shealy, 1976 |
| <i>Graptemys flavimaculata</i> | | spring summer | single, sometimes two | unimodal | Shelby et al., 2000; Shelby and Mendonça, 2001; Horne et al., 2003 |
| <i>Graptemys geographica</i> | | | single, sometimes two | | Vogt, 1980 |
| <i>Graptemys gibbonsi</i> | | | | | this study |
| <i>Graptemys nigrinoda</i> | late summer/fall | spring summer | multiple | none; low | Lahanas, 1982 |
| <i>Graptemys oculifera</i> | | spring summer | multiple | | Kofron, 1991 |
| <i>Graptemys ouachitensis</i> | late summer | spring summer | multiple | | Vogt, 1980 |
| <i>Graptemys pseudogeographica</i> | late summer | spring summer | multiple | | Vogt, 1980 |
| <i>Graptemys pulchra</i> | | spring summer | multiple | | Shealy, 1976 |
| <i>Malaclemys terrapin</i> | | spring summer | multiple | | Feinberg and Burke, 2003 |
| <i>Pseudemys nelsoni</i> | continuous, slightly quiescent during winter | | | | Jackson, 2006 |
| <i>Terrapene carolina</i> | late summer | spring summer | multiple | unimodal | Altland, 1951; Currylow et al., 2013 |
| <i>Trachemys scripta</i> | | spring summer | multiple | | Gibbons and Lovich, 1990 |
| <i>Kinostemon baurii</i> | continuous, excluding late spring | late summer fall | multiple | | Iverson, 1979; Wilson et al., 1999 |
| <i>Kinostemon subrubrum</i> | spring | spring summer | multiple | | Mahmoud and Klicka, 1972 |
| <i>Sternotherus carinatus</i> | late summer | spring summer | multiple | | Mahmoud and Klicka, 1972 |
| <i>Sternotherus minor</i> | late summer | bimodal, spring and fall ovulation | multiple | | Iverson, 1978; Etchberger and Ehrhart, 1987 |
| <i>Sternotherus odoratus</i> | spring | spring summer | multiple | unimodal; peaks for each clutch | McPherson and Marion, 1981; McPherson et al., 1982; Mendonça and Licht, 1986 |
| <i>Gopherus polyphemus</i> | late summer | spring summer | single | unimodal | Palmer and Guillette, 1988, 1990; Ott et al., 2000 |
| <i>Apalone ferox</i> | | spring summer | multiple | | Iverson and Moler, 1997 |
| <i>Apalone mutica</i> | late summer | spring summer | multiple | | Nagle et al., 2003 |
| <i>Apalone spinifer</i> | late summer | spring summer | multiple | | Robinson and Murphy, 1978 |

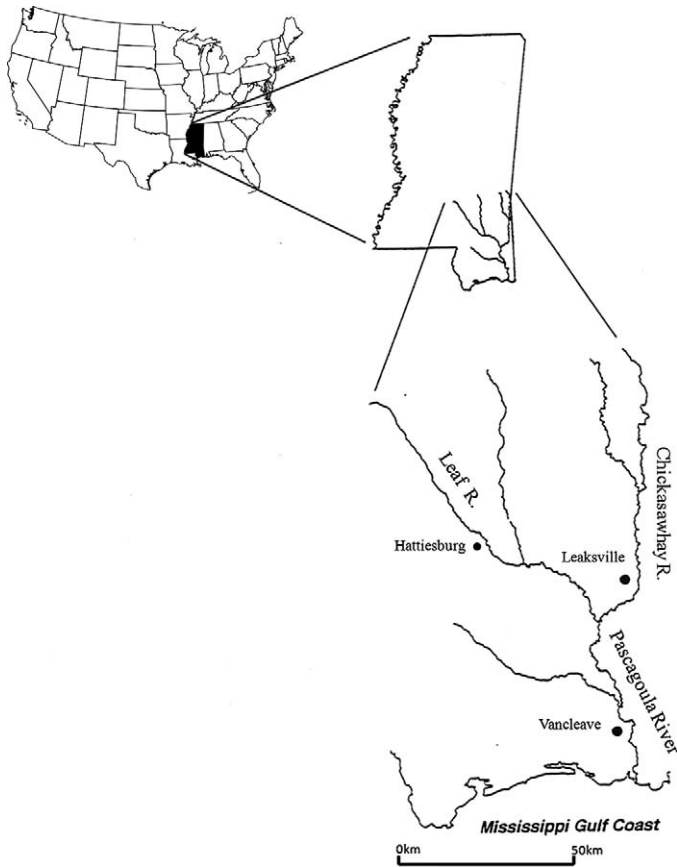


Fig. 1. Map of the Pascagoula River drainage and the study area including the Chickasawhay and lower Pascagoula rivers.

wood snags at water level along the banks of the river where turtles were observed basking (Carr, 1952). Turtles were scared into traps by an approaching boat. All turtles were removed from traps immediately, and 1 mL of blood was collected from the caudal sinus within 2 min of capture using a heparinized 1 mL syringe and a 26.5-gauge needle. Turtles that could not be sampled within two minutes were measured, marked, and released. Blood samples were stored on ice for less than 6 h then centrifuged 10 min at 1000 rpm. Plasma was removed by pipette and placed into cryovials and frozen in liquid nitrogen for transport. All plasma samples were stored at -20°C until processing (Shelby and et al., 2000).

Sex was identified by head and tail morphology (Lovich and McCoy, 1994). Turtles were categorized as adult males if they exhibited an enlarged tail and indistinct annuli, while adult females were identified by an enlarged head and indistinct annuli. Juveniles exhibited distinct annuli and no defining secondary sexual characteristics (Shelby et al., 2000; Shelby and Mendonça, 2001; Shelby-Walker et al., 2009; Selman and Jones, 2011; Selman, 2012). Juvenile individuals were excluded from some analyses. All female turtles were transported to a local veterinary clinic for X-radiography using a free standing machine at 200 mA and 70 kV peak for 0.7 s at a distance of 1 m to determine if they were gravid (Gibbons and Greene, 1979; Shelby-Walker et al., 2009). This was necessary because initial palpating of the body cavity and ultrasonography was confounded by the large amount of mollusk shells in the gut. Body size was measured as straight-line maximum carapace length (CL) with 80 cm (± 0.1 cm) calipers (Haglöf Sweden, Madison, WI) to the nearest millimeter. The smallest turtles were

measured using 20 cm (± 0.1 mm) Vernier calipers (Grainger Scientific, Lake Forest, IL). Carapace height (to the nearest millimeter with calipers) and mass in grams (to the nearest 0.1 g) using a 2000 g (± 0.1 g) digital balance (Ohaus, Parsippany, NJ) were also recorded. All animals were released at the sites of capture (Shelby et al., 2000). A small subset of turtles was recaptured during the study. These turtles were not included in the morphological analysis, but a blood sample was taken when possible and included in the hormonal cycle assuming that the sample was in a new sampling period (i.e., a different month or year).

Hormone analysis.—Plasma testosterone (T) and 17- β estradiol (E_2) were measured via radioimmunoassay (Shelby et al., 2000). Plasma volumes of 75 μL for T and 125 μL for E_2 were extracted with 3 mL of anhydrous diethyl ether. T and E_2 antibodies were obtained from Endocrine Sciences (Tarzana, CA). The efficiency of hormone ether extraction techniques from plasma samples (Mendonça et al., 1996) averaged 96.4% and 87.8%, and interassay variations with respect to spiked controls were 8.0% and 8.6%, respectively. The intra-assay variation averaged 7.1%. Sensitivity of the assays averaged 9 pg/mL for T and 12 pg/mL for E_2 .

Data analysis.—We log-transformed hormone data to satisfy assumptions of normality; however, untransformed data are presented in figures to assist interpretation. We tested for deviance from a 1:1 sex ratio in our sample of adult *G. gibbonsi* using a Chi-square test. We tested for differences between the sexes in carapace length (CL) using ANOVA. Seasonal differences in testosterone (T) and estradiol (E_2) levels in males and females, respectively, were tested using general linear models. For males, we used ANCOVA to test for variation in log T with location (Leaf vs. Chickasawhay River), year, and month as main effects and CL as covariate. For females, our sample size was too low to attempt a similar analysis; instead, we tested for seasonal variation in log E_2 using an ANCOVA with month as main effect and CL as covariate. All statistics were calculated using JMP statistical software (SAS, Cary, NC).

RESULTS

During 1997–2000, we captured 117 *G. gibbonsi* from our study sites. Of these, 105 were adults, and these exhibited a 1:3.2 sex ratio (25 females to 80 males), which deviates significantly from 1:1 ($\chi^2 = 30.30$, $\text{df} = 1$; $P < 0.0001$). However, this trend was driven mostly by the heavily skewed sex ratio exhibited by turtles collected from the Chickasawhay River (11 females to 58 males; 1:5.2; $\chi^2 = 35.11$, $\text{df} = 1$; $P < 0.0001$). Turtles from the Leaf River did not show a significantly skewed sex ratio (14 females to 22 males; 1:1.58; $\chi^2 = 1.79$, $\text{df} = 1$; $P = 0.18$). On average, female *G. gibbonsi* had a mean CL of 18.9 cm (± 1.07 cm) versus a CL of 11.02 cm (± 0.16 cm) in males (Fig. 2). This difference was statistically significant ($F_{1,77} = 145.71$, $P < 0.0001$). Adult turtles from the different collecting sites differed significantly in CL (females: $F_{1,17} = 11.44$, $P = 0.0035$; males: $F_{1,58} = 7.99$, $P < 0.0064$); however, juveniles did not ($F_{1,30} = 0.66$, $P > 0.05$; Table 3).

Testosterone varied seasonally in males (month: $F_{7,53} = 2.80$, $P = 0.02$) and covaried with carapace length (CL: $F_{1,53} = 7.91$, $P = 0.009$; all other effects $P > 0.05$). A Student's *t* post hoc test revealed some months grouped with others with similar levels of testosterone: April and August–November form one group, July a second, and May–June form a third

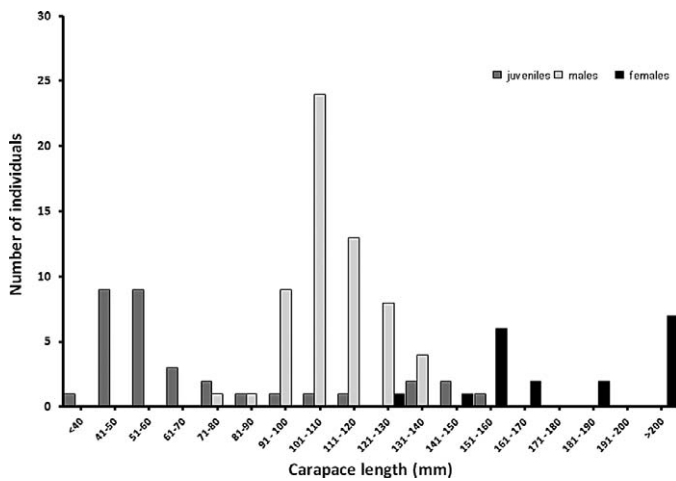


Fig. 2. Size frequency distribution of 117 *G. gibbonsi* from the Pascagoula River system of Mississippi. Males are indicated by black bars, juveniles by medium gray, and females by light gray.

(Fig. 3). Estradiol showed no significant seasonal variation among months in our sample of females ($F_{5,18} = 1.00$, $P = 0.46$; Fig. 4).

DISCUSSION

Our sample of *G. gibbonsi* shows a skewed sex ratio favoring males. This is a commonly observed characteristic of turtle populations attributable both to intrinsic biological process (e.g., differential survivorship, non-even hatchling sex ratios, etc.) and sampling biases (differential habitat preferences or capture probabilities; Gibbons and Lovich, 1990). It is possible that our sampling technique (basking trap) led to this pattern, as it is often observed that male map turtles bask more frequently than females (Boyer, 1965; Don, 1965; Coleman and Gutberlet, 2008). Our study population also exhibits significant sexual size dimorphism, in which female size is greater than that in males. Female-biased sexual size dimorphism is usually attributed to size-fecundity selection (Berry and Shine, 1980; Gibbons and Lovich, 1990), and the extreme differences between male and female *Graptemys* are well known and covary with dietary preferences (i.e., females are molluscivores and males insectivores; Lindeman, 2008). Lastly, the size of *G. gibbonsi* significantly differed between our two sampling sites for both males and females. This is consistent with measurements taken at the

same sites for *Graptemys flavimaculata* (Shelby and Mendonça, 2001; Shelby-Walker et al., 2009; Selman, 2012) and may indicate a difference in resource availability, especially given that juvenile size did not differ.

In most North American turtles, testicular recrudescence typically begins during spring, continues through the summer, and peak spermatogenesis and testis size occurs during late summer and fall (Table 1). This pattern has been documented in other species of *Graptemys* (e.g., *G. nigrinoda*, *G. oculifera*, *G. pseudogeographica*, and *G. ouachitensis*; Table 1). Spermiation and movement of spermatozoa into the epididymes begins in late summer-autumn, and active spermatozoa either persist in the epididymes in spring when testicular activity is quiescent (*G. nigrinoda*; Table 1), or active spermatozoa are maintained in the epididymes and vas deferens year round (e.g., *Terrapene carolina*, *Trachemys scripta*, *Graptemys ernsti*; Table 1).

The annual pattern of T appears to track the presence of sperm in the epididymes, i.e., there is a bimodal peak of T in spring and late summer-autumn (shown in *G. flavimaculata* and other turtles; Table 1). Our results are consistent with this pattern; *G. gibbonsi* shows a clear bimodal peak of T. Although the precise timing of mating in the wild is unknown in *G. gibbonsi*, as well as in many other turtle species, it seems likely that mating occurs during spring and also during the fall, as has been observed in *G. geographica*, *G. pseudogeographica*, *G. ouachitensis*, *Trachemys scripta*, and *Sternotherus odoratus* (Ernst and Lovich, 2009). Thus, the pattern of hormone secretion, spermatogenesis, and male sexual behavior appears to be generally conserved in most North American turtles, including *Graptemys* (Table 1).

In females of many southeastern turtle species, there is considerable variation in the timing of follicular enlargement prior to ovulation (Table 2). However, two major patterns are exhibited by southeastern U.S. turtles: 1) vitellogenesis is initiated during the late summer-fall prior to nesting, continues or pauses over the winter, and concludes during the spring prior to ovulation and nesting, or 2) vitellogenesis is initiated during the late winter or spring prior to and in the same year of ovulation and nesting (Table 2). The ovulation and nesting period in most North American turtles is during spring and summer, respectively (Table 2). In addition to these patterns, females can either have a single (e.g., *Chelydra serpentina*) or multiple clutches (e.g., *Sternotherus odoratus*), and this influences their sex hormone secretion patterns (Table 2). In those turtles with multiple clutches, estradiol (E_2) peaks numerous times

Table 3. Size ranges and combined measurements for *Graptemys flavimaculata* captured in our two sampling locations: Chickasawhay River (North end = 31.14861, -88.54816, South end = 31.04143, -88.65727) and the Leaf River (North end = 31.32642, -89.26703, South end = 31.30026, -89.25297). Measurements were taken as straight-line maximum carapace length to the nearest mm. Adult turtles were classified as those expressing secondary sexual characteristics (large heads for female and elongated tail for male) and the absence of annuli.

| Sex | Drainage | n | Mean | Min | Max | Std err |
|----------|--------------|----|------|-----|-----|---------|
| Juvenile | Leaf | 20 | 67 | 40 | 158 | 7 |
| Male | Leaf | 22 | 104 | 85 | 130 | 2 |
| Female | Leaf | 14 | 167 | 125 | 227 | 8 |
| Juvenile | Chickasawhay | 12 | 77 | 41 | 150 | 11 |
| Male | Chickasawhay | 58 | 113 | 71 | 135 | 2 |
| Female | Chickasawhay | 11 | 227 | 154 | 267 | 18 |
| Juvenile | Combined | 32 | 70 | 40 | 158 | 6 |
| Male | Combined | 80 | 110 | 71 | 135 | 2 |
| Female | Combined | 25 | 189 | 125 | 267 | 11 |

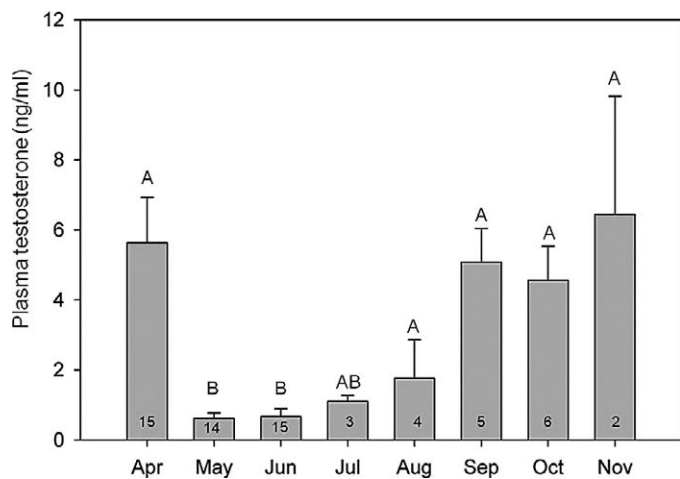


Fig. 3. Seasonal variation in plasma testosterone in male *G. gibbonsi*. *Post hoc* tests revealed that some months grouped together; months indicated with the same letter did not differ significantly. Sample sizes indicated within or adjacent to bars. Error bars indicate standard error.

during the summer nesting season and individual-level variation peaks coincide with ovulation prior to nesting (McPherson et al., 1982; Mendonça and Licht, 1986).

Our results for female *G. gibbonsi* are puzzling; we found no monthly variation in E_2 levels across their presumed nesting season. The simplest explanation is that, due to limits of our sampling technique, we were only able to capture a small number of females, and these females were all captured during their post-nesting period when E_2 levels are lowest. Consistent with this explanation, none of the females we captured had oviductal eggs in a mature stage of development. Had we been able to sample a larger number of females, it is possible we would have detected more females with oviductal eggs and higher levels of E_2 . Another possibility is that *G. gibbonsi* produces a single clutch of eggs, which were ovulated and oviposited before the beginning our sampling period (April), and we therefore missed a peak of E_2 . Although possible, this explanation seems unlikely,

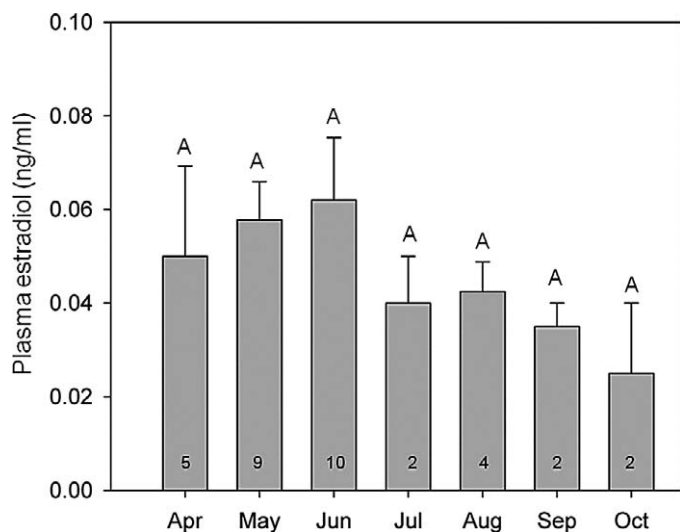


Fig. 4. Seasonal variation in plasma estradiol in female *G. gibbonsi*. We did not detect statistical variation in estradiol levels among months in our sample; months indicated with the same letter did not differ significantly in *post hoc* tests. Sample sizes indicated within bars. Error bars indicate standard error.

since most *Graptemys* sp. have a summer nesting season, are known to be gravid from May to August (Lovich et al., 2009), and produce multiple clutches (Table 2).

Another, more alarming possibility is that this population is experiencing a significant decline in reproduction, and that female *G. gibbonsi* are under such heavy stress as a result of pollutants, habitat alteration, and human persecution that only a few females are producing clutches. This explanation is purely speculative and should be confirmed with additional research. However, it is odd that even within our limited sample ($n = 25$), not one female was found to be gravid during the probable peak nesting season of this turtle (June–August, similar to *G. ernsti*, *G. pulchra*, and *G. barbouri*; Table 2). Although no gravid females were found, we did capture juvenile turtles and find nests of *G. gibbonsi* (eggs were collected and hatched as part of a study on *G. flavimaculata*) on the Chickasawhay River during the nesting season of *G. flavimaculata* (unpubl.). Given how rare this turtle has become within the Pascagoula drainage (Selman and Qualls, 2009), this hypothesis should not be dismissed lightly and deserves further investigation.

Possibility of reproductive decline in this species is also supported from previous studies in the turtle *G. flavimaculata* from the same drainage. Shelby and Mendonça (2001) found significantly lower T levels and abnormally high E_2 levels in males from sites impacted by industrial pollutants from one of our study areas. These and other pollutants can act as endocrine system disruptors, leading to abnormal hormone levels and reproductive abnormalities in reptiles (Crain and Guillelte, 1998; Crews et al., 2000; Shelby and Mendonça, 2001). Horne et al. (2003) found relatively small numbers of gravid female *G. flavimaculata* in the Pascagoula drainage, and these females had small clutch sizes, frequencies, and low nesting success, which the authors attributed to a combination of human disturbance of nesting sites and high predation pressure by fish crows (*Corvus ossifragus*). Nesting and basking behavior of *G. flavimaculata* is also interrupted by human disturbance in the Pascagoula River (Moore and Seigel, 2006), and thus the combined effects of industrial pollution, habitat modification, and human harassment appear to be contributing to the decline of *G. flavimaculata* (Shelby and Mendonça, 2001; Horne et al., 2003; Moore and Seigel, 2006). We offer evidence of possible reproductive inhibition in the sympatric turtle *G. gibbonsi*, and recommend additional studies focused on the reproductive physiology and nesting behavior of female *G. gibbonsi* to determine whether there is cause for similar concern in this species.

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