GEOGRAPHIC VARIATION IN REPRODUCTION IN A FRESHWATER TURTLE (CLEMMSY GUTTATA)

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ABSTRACT: We examined reproduction over 3 yr using radio telemetry and X-rays in a South Carolina population of spotted turtles (Clemmys guttata). Spring courtship (March–April) coincided with aggregations of turtles; however, a second peak in courtship in the fall (September–October) did not coincide with such aggregations. Over 75% of the radio-tagged females were gravid in each year of the study. The nesting season lasted approximately two months (mid-May through mid-July). Nesting was primarily nocturnal, and most nests (17/21, 81%) were made in the woody debris of decaying logs and stumps. Incubation time (mean = 79 d) decreased as nest temperature increased. Clutch size was not correlated to maternal body size, and we found no evidence for a tradeoff between clutch size and egg size. However, all measures of egg size and most measures of hatching size were independent of maternal body size. We also compared reproduction among three widely separated populations of spotted turtles. Clutch size varied with latitude: clutch size was largest in the north (Ontario, mean = 5.3 eggs), mid-sized in the central population (Pennsylvania, mean = 3.9), and smallest in the south (South Carolina, mean = 2.8). Variation in reproductive output among populations was attributable to both variation in number of eggs and to egg size. Most of this variation in clutch size and egg size was explained by differences in body size of females among populations. Because the spotted turtle is considered to be a Species at Risk throughout its range, the data provided in the current study will be useful in conservation planning and for directing future research on the reproductive ecology of freshwater turtles.

Key words: Clutch size; Egg size; Geographic variation; Incubation; Nesting behavior; Turtles

GEOGRAPHIC variation in clutch size and egg size among conspecific populations has been reported for many vertebrate species. Typically, clutch size and latitude are positively associated (e.g., Bohning-Gaese et al., 2000; Cardillo, 2002; Morrison and Hero, 2003). Less is known about geographic variation in egg size, and research to date has documented both decreases (Iverson et al., 1993; Johnston and Leggett, 2002) and increases (Armbruster et al., 2001; Encabo et al., 2002; Morrison and Hero, 2003) in egg size with latitude, with no clear overall taxonomic pattern. Intraspecific variation in clutch size and egg size in several turtle species has been well documented (e.g., Iverson, 1992; Rowe, 1994; Tinkle, 1961). Turtles at northern latitudes experience shorter nesting seasons and have larger clutch sizes relative to southern conspecifics (e.g., Iverson et al., 1993, 1997; Moll, 1973). Turtles at southern latitudes experience longer nesting seasons and therefore have the ability to increase the number of clutches produced per year (Gibbons, 1982). In some turtle species, both clutch and egg size increase with maternal body size (e.g., Congdon and Gibbons, 1987; Rowe et al., 2003; Tucker et al., 1998). Geographic variation in clutch and egg size may result from local selection on egg size (Tinkle, 1961) and environmental influences on clutch size (Iverson and Smith, 1993). However, such latitudinal patterns are confounded by correlations with female body size (Iverson, 1992; Iverson et al., 1993, 1997), especially because body size increases with latitude in some species of turtles (Ashton and Feldman, 2003; Litzgus et al., 2004).

Life-history and natural-history data inform conservation and management strategies. From a conservation perspective, turtles are an especially important group because about two thirds of the almost 300 species worldwide are threatened by human activities such as habitat destruction, and collection for the pet and traditional medicine trades (Klemens, 2000). Information on courtship and nesting behavior, nest location and incubation temperatures, and nest success are important for assessments of population viability of Species at Risk. The objectives of our study were: (1) to describe the reproductive behavior (courtship and nesting), nest site parameters (including substrate type and incubation temperature),
and nest success in a southern population of the spotted turtle (*Clemmys guttata*), a location from where little is known of the species' ecology; and (2) to examine geographic variation in reproductive variables among three widely separated populations of the spotted turtle (Ontario, ON; Pennsylvania, PA; and South Carolina, SC). We tested the hypothesis that variation in reproductive output among populations of spotted turtles is attributable to variation in clutch size. In addition, we examined the relationship between body size and clutch size among populations. We also tested an alternative hypothesis: that variation in reproductive output among populations is attributable to variation in egg size. Because the spotted turtle is considered to be a Species at Risk throughout its range (Ernst et al., 1994), the data provided in the current study will be useful in conservation planning and for directing future research on the reproductive ecology of freshwater turtles.

**MATERIALS AND METHODS**

The spotted turtle is a relatively small species of freshwater turtle (<12 cm adult shell length) whose distribution is restricted to disjunct populations in eastern North America (Ernst et al., 1994). Throughout its range, the spotted turtle is considered vulnerable, threatened, or endangered as a result of habitat destruction and over-collection for the pet trade (Ernst et al., 1994; Levell, 1997). The field site, Francis Beidler Forest, is an approximately 4500-ha National Audubon and Nature Conservancy Sanctuary located in Four Holes Swamp on the Atlantic Coastal Plain of southeastern South Carolina, USA (33° N). The site includes upland pine and mesophytic hardwood forest, seasonally flooded hardwood bottom swamp forest, and cypress-tupelo blackwater swamp forest (see Porcher, 1981 for a detailed description of the plant communities).

Reproduction of spotted turtles at Beidler Forest, SC was monitored during the summer months for 3 yr using radio telemetry. Four females were followed in 2000 (AVM Instruments, Livermore, CA), 11 in 2001 (two AVM units, nine Holohil units, Holohil Systems Inc., Carp, ON), and 11 in 2002 (one AVM unit, 10 Holohil units). Three females were radio-tracked in all 3 yr, seven were tracked in 2 of the 3 yr, and three were tracked for one nesting season; thus, a total of 13 different females were monitored over the 3-yr study. The nesting season in each year was demarcated based on the day the first known female was gravid and ended the day the last known gravid female oviposited. Gravid females were radio-located 2–3 times/d (during the day and at sundown) until they oviposited. From late March through July, the reproductive condition of female turtles was determined by palpation, change in body mass, and X-ray photographs (Gibbons and Greene, 1979). Turtles were X-rayed at 52 kV peak and 2.5 mA·s at a local veterinary clinic. Clutch size was determined from X-rays and egg counts at oviposition sites. Information on the duration of nesting behavior was not obtained because female spotted turtles abandon nesting when disturbed (J. D. Litzgus, personal observations); therefore, once a female was observed in the evening on a potential nest site, the location was flagged, and researchers departed. The putative nest site was examined the following morning for the presence of eggs.

Nests were excavated and eggs were processed (measured and weighed) and then reburied. The nesting substrate (e.g., soil, woody debris, leaf litter) was described, based on visual inspection, at the time the eggs were processed. Maximum egg length and width (mm) were measured with vernier calipers, and egg mass (g) was measured using a 10-g Pesola™ spring scale. Egg volume (cm³) was calculated using the formula for an ellipsoid: \(V = \frac{QrT}{6000} \cdot LW^2\) (Iverson and Ewert, 1991). After processing, each nest was covered with a hardware cloth cage (~1.27 cm mesh; ~20 cm × 15 cm × 4 cm; L × W × H) anchored with sticks. The cages allowed researchers to relocate the exact position of nests and retain hatchlings, but did not deter predators (J. D. Litzgus, personal observations). Nests were checked 2–3 times/wk until hatching. Hatchlings were measured (straight-line carapace length, CL; maximum carapace height, CH; straight-line plastron length, PL) with calipers, weighed using a 10-g Pesola™ spring scale and given a clutch-specific identification notch in a marginal scute. Many were photographed and all were released at or near their natal nests. Mothers were radio-located and weighed after oviposition to obtain non-gravid body mass.
Maternal body size measures (CL, CH, PL) were obtained at the female’s initial capture of the field season. We used regression analyses to examine the relationships between maternal body size (PL) and hatchling morphometrics (CL; CH; PL; body mass, BM).

Nests were outfitted with temperature data loggers to monitor incubation temperature. Nest temperatures were recorded every 2 h with Hobo™ data loggers (±0.01 C; Onset Computer Corp., Pocasset, MA) in summer 2000, and with iButton™ data loggers (±0.5 C; Dallas Semiconductor, Dallas, TX) in summer 2001 and summer 2002. Data loggers were usually placed in nests the day following oviposition and were recovered upon hatching, or estimated date of hatching for nests apparently subjected to predation when hatchlings were pipping from eggs. We used regression analysis to examine the relationship between nest temperature and length of incubation.

We examined correlations among clutch variables and maternal morphometrics. For the SC data set, clutch variables (clutch size, CS; clutch mass, CM; mean egg length, EL; mean egg width, EW; mean egg volume, EV; mean egg mass, EM) were compared to maternal body size measures (ngBM, CL, CH, PL) using Pearson correlation analyses. The potential tradeoff between egg size (EL, EW, EV, EM) and clutch size was tested using Pearson partial correlation analyses adjusted for female body size (ngBM and PL). Statistical significance of Pearson correlations were assessed with a Bonferroni adjustment for the number of comparisons. Using the current field data and data published in the literature, we compared reproductive variables among three relatively well-studied populations of spotted turtles using ANOVA followed by Bonferroni-adjusted t-tests when significant differences were detected. A relative egg size ratio (RES = EW/maternal PL) was calculated to allow comparison to the other studies in PA (Ernst, 1970) and ON (Litzgus and Brooks, 1998). We examined variation in egg size (EW) among populations using ANCOVA, with maternal body size (PL) as the covariate. Values for egg measurements (EL, EW, EV, EM) are means of clutch means. We treated each clutch as independent for the calculation of clutch size, thus for a female that produced two or more clutches (whether in the same year or in different years), each clutch was considered an independent datum. All statistical analyses were performed on SAS (Version 8.2 for Windows, 2001) or Microsoft Excel (1997).

RESULTS

Over the 3-yr study, we made several observations of behavior related to courtship, although mating was not directly observed. We observed 27 male-female pairs in which the male was on top of the female, the male was chasing the female, or the male and female were within a few cm of each other. We also observed aggressive encounters (chasing and biting) between two male-male pairs: one pair on 23 January, one pair on 16 March. The sexes of turtles involved in courtship activities were confirmed based on secondary sex characteristics (Ernst et al., 1994). Courtship peaked in spring (17 of 27 observations; 63%) between 2 February and 10 April, and there was a secondary period of courtship activity in fall (10 of 27 observations; 37%) between 9 September and 16 December. Observations of courtship indicated that spotted turtles courted and were courted by multiple individuals. Two males each were observed courting three different females, and three females were each observed with three different males.

In early spring, spotted turtles aggregated. The gathering occurred within a relatively narrow window of time (~2 wk) at approximately the same time each year, and coincided with the spring peak in courtship behavior. Aggregations were observed on 1 April 2000 (5 females, 4 males), 1 April 2001 (4 females, 2 males), 4 April 2001 (5 females, 3 males), and 31 March 2002 (3 females, 3 males). Aggregations occurred in shallow water (range of depth = ~5–50 cm) at the “shore” of the cypress-hardwood-palmetto swamp forest in an area no larger than ~10 m X 20 m. The gathering site in 2002 was ~100 m from the site used in both 2000 and 2001 which was dry as a result of drought in 2002. Turtles showed fidelity to aggregations; for example, one male and one female were captured from an aggregation in all 3 yr and three other females were captured at an aggregation in 2 of the 3 yr.

A high proportion of females were gravid in each year of the study. In the summer of 2000, three of the four females (75%) fitted with...
transmitters were gravid. In summer 2001, nine of the 11 females (82%) with transmitters were gravid, and in summer 2002, all 11 females (100%) with transmitters were gravid. Of the three females tracked through three nesting seasons, two were gravid in all years and one was gravid in one year. Of the seven females tracked for two nesting seasons, six were gravid in both years and one was gravid in one year. In addition, females produced multiple nests within a season in this population (Litzgus and Mousseau, 2003).

Nesting was primarily nocturnal and nest success was moderate. The nesting season lasted approximately two months, from early-May through mid-July. Of the 21 nests documented over the 3-yr study, only one was constructed during the daytime, in the late afternoon following a heavy morning rain. All other nests (n = 20) were constructed starting at about, or shortly after, sundown. Most nests (17/21, 81%) were constructed in woody debris (and sometimes leaf litter) of decaying logs and stumps; five of these nests were deposited under moss on the upper surface of logs (5/17, 29%). The remaining nests (4/21, 19%) were dug in small mounds of soil (~20–40 cm diameter, ~30–60 cm height) covered with herbaceous vegetation. Seventeen nests (81%) were constructed under forest canopy, whereas four (19%) were constructed in overgrown clearcuts (including the edge of a powerline right-of-way) under shrub and herbaceous cover.

A total of 21 nests containing 57 eggs were monitored for hatching success over the 3-yr study. Of the 57 eggs, 33 hatchlings emerged. When reviewed by year, there was 100% hatching success in 2000; 56% hatching success in 2001; and 48% hatching success in 2002. Unsuccessful eggs were eaten by fire ants, and, possibly, snakes (likely Elaphe obsoleta). We surmised predation by a snake because the nest cage cover was not removed, a smooth tunnel entered the nest cavity, there was no evidence of digging, entire eggs were consumed (no eggshells were present), and in one case, an iButton™ also was missing and presumably consumed along with the eggs.

Average incubation length for the 13 nests that produced hatchlings was 79 d (SE = 1.7, range = 72–90 d). In 2000, hatch dates were 7 and 10 August. In 2001, first clutches hatched on 13, 16, and 19 August, and a second clutch hatched on 17 September. In 2002, clutches hatched on 17 and 22 August. These hatch dates are estimates because we checked nests only 2–3 times/wk allowing the potential for hatchlings to have pipped a day or two before they were observed in the nest cage. Mean nest temperatures over the course of incubation were surprisingly cool and ranged from 22.5 C to 24.9 C for 16 nests (overall minimum = 12.0 C, overall maximum = 32.0 C). Duration of incubation was significantly shorter in warmer nests relative to cooler nests (F115 = 15.4, P < 0.01, r² = 0.52; y = -7.1x + 247.0).

Average clutch size for 21 nests was approximately three eggs (Table 1). No correlations existed between any maternal body size parameter (ngBM, CL, CH, PL) and any clutch variable (CS, CM, EL, EW, EV, EM; Bonferroni α = 0.002; P > 0.002 in all cases). Furthermore, none of the partial correlations (maternal ngBM or PL as partial correlate) between egg size (EL, EW, EV, EM) and clutch size were significant (Bonferroni α = 0.0125; P > 0.0125 in all cases). Variation in hatchling morphology was minimal, and hatchling body mass was the only morphological measure correlated with maternal body size (Table 2).

To examine geographic variation in reproduction, we compared the reproductive data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SE</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td>ngBM (g)</td>
<td>193</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>CL (mm)</td>
<td>103.7</td>
<td>0.9</td>
<td>21</td>
</tr>
<tr>
<td>CH (mm)</td>
<td>37.8</td>
<td>0.3</td>
<td>21</td>
</tr>
<tr>
<td>PL (mm)</td>
<td>91.4</td>
<td>1.0</td>
<td>21</td>
</tr>
<tr>
<td>Clutch size (no. of eggs)</td>
<td>2.7</td>
<td>0.1</td>
<td>21</td>
</tr>
<tr>
<td>Clutch mass (g)</td>
<td>18.43</td>
<td>1.15</td>
<td>20</td>
</tr>
<tr>
<td>Mean EL (mm)</td>
<td>32.8</td>
<td>0.3</td>
<td>21</td>
</tr>
<tr>
<td>Mean EW (mm)</td>
<td>18.4</td>
<td>0.2</td>
<td>21</td>
</tr>
<tr>
<td>Mean EV (cm³)</td>
<td>5.79</td>
<td>0.13</td>
<td>21</td>
</tr>
<tr>
<td>Mean EM (g)</td>
<td>6.80</td>
<td>0.16</td>
<td>20</td>
</tr>
</tbody>
</table>
TABLE 2.—Mean body size measurements for hatchling spotted turtles (Clemmys guttata) as they relate to mean maternal body size (PL = straight-line plastron length; mean ± SE, n = 13 mothers). Hatchling measurements are means ± SE of means for a clutch (n = 13 clutches). CL = carapace length, CH = carapace height. * indicates a significant relationship between maternal size and hatchling size at P < 0.05.

| Mother’s Mean hatchling Mean hatchling Mean hatchling Mean hatchling | Mean hatchling |
|----------------------|----------------|-----------------|-----------------|----------------|
| Mother’s Mean Maternal PL (mm) | CL (mm) | CH (mm) | PL (mm) | body mass (g) |
| 90.6 ± 1.4 | 29.5 ± 0.4 | 12.6 ± 0.1 | 24.4 ± 0.4 | 5.28 ± 0.10 |
| F1,12 = 4.20 | F1,12 = 1.48 | F1,12 = 2.60 | F1,11 = 6.92 |
| P = 0.065 | P = 0.25 | P = 0.14 | P = 0.025* |
| r2 = 0.28 | r2 = 0.12 | r2 = 0.19 | r2 = 0.41 |

collected in the current study (SC, 33° N) to published data collected from two other populations, one in Ontario (ON, 45° N) at the northern extreme of the species’ distribution (Litzgus and Brooks, 1998), and one in Pennsylvania (PA, 40° N) in the central part of the species’ range (Ernst, 1967, 1970). Most of the variation in clutch size and egg size among populations was explained by differences in female body size (PL). ON turtles were larger than PA and SC turtles. Because homogeneity of the slopes was confirmed (Type III SS F2,62 = 0.75, P = 0.47), we could determine that clutch size was affected by population of origin (F2,62 = 22.49, P < 0.0001) and body size (F1,62 = 12.99, P < 0.001). Least squares means analysis indicated that, when body size (PL) was treated as a covariate, clutch size did not differ between ON and PA (P = 0.30), but clutch size did differ between ON and SC (P < 0.0001), and between PA and SC (P < 0.0001). In contrast, when maternal body size (PL) differences were not accounted for, absolute clutch size differed among the three populations (Table 3; Litzgus and Mousseau, 2003). Variation in egg size was affected by both population (F2,39 = 18.82, P < 0.0001) and body size (F1,39 = 5.66, P < 0.05); homogeneity of the slopes was confirmed [Type III SS F2,39 = 0.61, P = 0.55]. Least squares means analysis indicated that when body size (PL) was treated as a covariate, egg size did not differ between ON and PA (P = 0.90), whereas egg size differed between ON and SC (P < 0.01), and between PA and SC (P < 0.0001). The RES ratio differed among the three populations (Table 3). Absolute egg size (EL, EW, EM; body size variation ignored) varied among populations, although the variation was not consistent (Table 3). For example, ON females produced the shortest eggs relative to the other two populations, PA females produced the narrowest eggs, and SC females produced the heaviest eggs (Table 3).

TABLE 3.—Reproductive variables for gravid female spotted turtles (Clemmys guttata) compared among three populations. Values are means ± SE. Egg size variables are means of clutch means. RES = mean egg width/maternal plastron length. Values within rows with different superscripts were significantly different by a Bonferroni-adjusted t-test (α = 0.017). Note that inter-population comparisons shown here are not adjusted for female body size. n = number of females for plastron length; n = number of clutches for clutch and egg size data. Sources: Ontario (Litzgus and Brooks, 1998), Pennsylvania (Ernst, 1970), South Carolina (current study).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ontario (45° N)</th>
<th>Pennsylvania (40° N)</th>
<th>South Carolina (33° N)</th>
<th>Statistical results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plastron length (mm)</td>
<td>103.4 ± 0.9a</td>
<td>89.2 ± 1.7b</td>
<td>91.2 ± 1.2b</td>
<td>F2,39 = 48.2</td>
</tr>
<tr>
<td>n = 19</td>
<td>n = 8</td>
<td>n = 13</td>
<td>P &lt; 0.0001</td>
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<tr>
<td>Clutch size (no. of eggs)</td>
<td>5.3 ± 0.04a</td>
<td>3.9 ± 0.1b</td>
<td>2.8 ± 0.02c</td>
<td>F2,64 = 72.3</td>
</tr>
<tr>
<td>n = 24</td>
<td>n = 8</td>
<td>n = 33</td>
<td>P &lt; 0.0001</td>
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<tr>
<td>Mean egg length (mm)</td>
<td>31.0 ± 0.4a</td>
<td>33.0 ± 0.1b</td>
<td>32.8 ± 0.3b</td>
<td>F2,39 = 10.2</td>
</tr>
<tr>
<td>n = 11</td>
<td>n = 8</td>
<td>n = 21</td>
<td>P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Mean egg width (mm)</td>
<td>17.5 ± 0.09a</td>
<td>16.7 ± 0.06b</td>
<td>18.4 ± 0.04a</td>
<td>F2,39 = 15.0</td>
</tr>
<tr>
<td>n = 11</td>
<td>n = 8</td>
<td>n = 21</td>
<td>P &lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>Mean egg mass (g)</td>
<td>5.9 ± 0.3a</td>
<td>—</td>
<td>6.8 ± 0.2b</td>
<td>F1,25 = 7.1</td>
</tr>
<tr>
<td>n = 6</td>
<td>n = 20</td>
<td>n = 20</td>
<td>P &lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>Relative Egg Size (RES)</td>
<td>0.17 ± 0.009a</td>
<td>0.19 ± 0.01b</td>
<td>0.20 ± 0.005c</td>
<td>F2,39 = 40.7</td>
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<tr>
<td>n = 11</td>
<td>n = 8</td>
<td>n = 21</td>
<td>P &lt; 0.0001</td>
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</table>
DISCUSSION

Spotted turtles at Beidler Forest, SC potentially have a promiscuous mating system with two peaks in courtship in the spring and fall. The greater peak in spring breeding coincided with a predictable annual aggregation of turtles to which individuals showed fidelity. Spring aggregations have been reported in other populations of spotted turtles, and the gatherings have been attributed to exploitation of localized food abundance (Graham, 1995; Perillo, 1997) and breeding (Ernst, 1967; Litzgus and Brooks, 1998, 2000). Aggregations may allow males to increase their reproductive success by facilitating mating with several females. Likewise, females could mate with several males, and thus there is the potential for multiple paternity, as has been shown in some other species of turtles (e.g., Bollmer et al., 1999; Galbraith, 1993; Galbraith et al., 1993; Hoekert et al., 2002; Kaufmann, 1992; Moore and Ball, 2002). Future work should examine the possibility of multiple paternity in spotted turtles as such information may have implications for successful captive breeding endeavors. Another conservation-related implication of predictable spring aggregations is that local breeding populations can be easily extirpated by habitat alterations that destroy aggregation sites, and by collectors who can collect large numbers of spotted turtles from localized aggregation areas in a short time.

More than 75% of female spotted turtles fitted with radio transmitters were gravid in each year at the SC site. In contrast, only 58% of the females examined were gravid in any given year, and only 37% were gravid in all three consecutive years in the ON population of spotted turtles (Litzgus and Brooks, 1998). The regularity of reproductive cycles at southern latitudes may reflect a constant and/or predictable supply of energy (Congdon and Tinkle, 1982; Gibbons, 1982) concomitant with higher productivity and resource abundance (Dunn et al., 2000).

Spotted turtles at Beidler Forest, SC typically initiated nesting after sundown. Most nests were constructed in the shade of the forest canopy in decayed logs and stumps. Nest location conditions likely mediated temperature and humidity which prevented the overheating and desiccation of eggs during incubation. At the northern extreme of the species’ distribution (ON), nocturnal nesting predominates and nests were constructed in shallow depressions containing soil on exposed granite rock outcrops (Litzgus and Brooks, 1998, 2000). Rock outcrops presumably provided the thermal characteristics necessary for egg incubation through the short, cool northern summer (Litzgus and Brooks, 1998). In the central part of their range (PA), spotted turtles nested in the evening in open-canopy marshy pastures (Ernst, 1970). Nest site choice may reflect selection for optimal incubation temperatures rather than for a particular nest substrate or habitat type. Incubation length of spotted turtle eggs in SC was approximately 79 d, and the earliest hatching occurred on 7 August. Similarly, in PA, incubation lasted 70–83 d, and the earliest hatching occurred a little later, on 18 August (Ernst, 1970). The nesting season was longer in SC (~2 months) relative to that in PA (~1 month; Ernst, 1970), which in turn was longer than that in ON (~2 wk; Litzgus and Brooks, 1998). Geographic variation in length of the nesting season could be related to temperatures conducive for successful egg incubation.

Mean incubation temperatures in spotted turtle nests in SC were surprisingly cool with an average of approximately 24 C; and maximum nest temperatures were only around 30 C. These findings may have important implications for offspring sex ratios because spotted turtles, like many reptiles (Bull, 1985; Shine, 1999), have temperature-dependent sex determination (TSD; Ewert and Nelson, 1991). Many turtle species with TSD have a pivotal temperature (temperature at which hatching sex ratio is 1:1) around 29 C, with males produced below 29 C and females produced at temperatures higher than 29 C (Ewert and Nelson, 1991; Ewert et al., 1994; Janzen, 1995). If the pivotal temperature for spotted turtles is 29 C, then our field data imply that almost exclusively males would be produced from nests monitored in SC, which is unlikely because the adult sex ratio is 1:1 (Litzgus and Mousseau, 2004). Our data suggest that future lab studies of TSD in spotted turtles should explore the variability and lower range of incubation temperatures observed in the wild. The variation in reproductive output among populations of spotted turtles was attributable
to both variation in number of eggs and egg size. Clutch size differed among the three populations such that at the most northern latitude (ON) clutch size was largest, at the central latitude (PA) clutch size was intermediate, and at the most southern latitude (SC) clutch size was smallest. Interestingly, despite these differences in clutch size, spotted turtles have similar annual reproductive output in ON, PA and SC that is achieved through variation in clutch frequency (Litzgus and Mousseau, 2003). Egg size variation among populations had a less consistent pattern with latitude; whereas egg length differed between ON and PA and between ON and SC, egg width differed between ON and PA and between PA and SC. Egg size differed among populations, and this difference can be explained by the intraspecific variation in body size. These analyses did not account for differences in body size among populations so that absolute differences in clutch and egg size could be examined. The geographic patterns in reproductive variables are different when body size is included as a covariate; for example, clutch size and egg width did not differ between ON and PA. Thus, interpretations of intraspecific patterns in reproductive output need to be clarified in terms of relationships to maternal body size (Iverson, 1992).

Intraspecific variation in reproductive output has also been examined in the context of Optimal Egg Size (OES) Theory. OES Theory assumes parents have limited, finite energy for reproduction at any given time (Brockelman, 1975; Smith and Fretwell, 1974). OES theory also assumes a tradeoff between offspring size and number, and that offspring fitness increases with increasing parental investment (Brockelman, 1975; Smith and Fretwell, 1974). However, the cost of investing in individual offspring will be balanced by parental fitness (Smith and Fretwell, 1974). Therefore, as maternal body size increases and/or as resource acquisition increases, variation in reproductive output in a given reproductive bout should come from changes in clutch size rather than from changes in egg size (Hendry et al., 2001), resulting in an optimal egg size that is independent of maternal body size.

Spotted turtles in SC conformed to some of the assumptions of OES Theory but not others. Although hatching mass was correlated with maternal body size (PL), all other egg and hatching measures were independent of maternal body size, providing some support for the idea of an optimally-sized egg. However, clutch size was not related to maternal body size. The lack of significant correlations between some body size and clutch variables may be a result of small clutch size. Because clutch size is so small, there was little statistical power (i.e., little variation) to detect significant correlations. Studies of other turtle species that have larger clutch sizes than spotted turtles found a positive relationship between clutch size and maternal body size (e.g., Iverson and Moler, 1997; Nieuwolt-Ducanay, 1997; Tucker et al., 1998). In ON, where spotted turtles are larger, all measures of egg size were independent of maternal body size, and clutch size increased with body size, supporting OES Theory (Litzgus and Brooks, 1998). In contrast to the assumptions of OES Theory, we found no evidence for a tradeoff between clutch size and egg size in the SC population of spotted turtles. At the geographic scale, some evidence for a tradeoff between clutch size and egg size is provided by the finding that ON females produce larger clutches of lower mass eggs relative to SC females who produce fewer heavier eggs.

Our study provides new data on the reproductive ecology of a southern population of spotted turtles, and we found that reproduction varies across widely-separated populations of this declining species. The differences among populations in nesting season, nest site location, clutch size, and egg size have important implications for conservation. Before management recommendations can be made, the life history of the specific target population needs to be studied as findings from one population will not necessarily apply to other populations, and this is especially true for populations at distant locales where seasonality and climate will be substantially different.

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