



Sex Ratio and Sex-Specific Growth Rates of Immature Green Turtles, *Chelonia mydas*, in the Southern Bahamas

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and still are not clearly understood (Ford and Seigel, 1989a). A potential confounding factor in these analyses that has not been considered by most researchers is the possible placental enhancement of neonate size over and above that provided by vitellogenesis (Stewart, 1989). However, such enhancement does not occur in *N. rhombifer* (Stewart and Castillo, 1984; Stewart, 1989). In Lonoke *N. rhombifer*, it appears that large females may have greater RCMs which is allocated to both larger litters and larger neonates. There does not appear to be a trade-off between litter size and neonate size as predicted by optimal offspring size models (e.g., Smith and Fretwell, 1974; Brockelman, 1975). Furthermore, when maternal SVL is held constant, partial correlation coefficients reveal that greater maternal mass yields longer ( $r = 0.49, P < 0.05, n = 21$ ) and heavier ( $r = 0.41, P < 0.05, n = 21$ ) neonates, suggesting possible phenotypic plasticity in these characteristics (Ford and Seigel, 1989b).

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sex ratios in sea turtles is important for both theoretical and management purposes. Of the five species of sea turtles examined, all have been found to exhibit environmental sex determination (Yntema and Mrosovsky, 1980; Morreale et al., 1982; McCoy et al., 1983; Mrosovsky et al., 1984a; Shaver et al., 1988). Sex ratios of sea turtle hatchlings from a given nesting beach are known to vary seasonally (Mrosovsky et al., 1984a, 1984b) and annually (Mrosovsky et al., 1984a; Horikoshi, 1992). Sea turtles reach sexual maturity only after many years [estimated from 20–50 years in green turtles (Balazs, 1982; Frazer and Ehrhart, 1985)], and, under natural conditions, annual survivorship of mature turtles probably exceeds 90% (Frazer et al., 1990). Thus, sex ratios of immature and/or adult populations represent a condensation of many cohorts. Does this averaging of variable hatchling sex ratios tend to result in 1:1 sex ratios in immature and/or adult populations, or do populations maintain biased sex ratios?

One option now available to those interested in conserving and managing sea turtle populations is the ability to produce hatchlings of a chosen sex ratio by controlling incubation temperatures. Current advice to managers is to mimic the natural thermal regime of the beach in artificial hatcheries until data from wild populations establish appropriate sex ratios (Richardson, 1989). The need for data on sex ratios of wild populations has been identified as a conservation priority [Magnuson et al., 1990; National Marine Fisheries Service and United States Fish and Wildlife Service (NMFS and USFWS), unpubl.].

Individual green turtles, *Chelonia mydas*, exhibit great variation in growth rates (Bjorndal and Bolten, 1988), as is characteristic of many reptiles (Andrews, 1982; Dunham and Gibbons, 1990). Because growth affects virtually all aspects of the ecology and life-history pattern of an animal, apportioning the variation in growth among the potential intrinsic and extrinsic factors can yield valuable insights into the evolution of life-history patterns (Wilbur, 1975; Dunham and Gibbons, 1990). A potential source of variation is sexually dimorphic growth rates. In most species of turtles, juveniles of both sexes grow at the same rate, even in those species that have sexually dimorphic adult body sizes (Andrews, 1982; Dunham and Gibbons, 1990). However, some species of turtle with sexual dimorphism in adult body size apparently exhibit different sex-specific growth rates in juveniles (references in Iverson, 1988). For green turtles,

there are conflicting reports of no sexual dimorphism in adult body size (Pritchard and Trebbau, 1984) or larger females (references in Berry and Shine, 1980).

We present the sex ratio for a one-time sample of immature green turtles on their feeding grounds in the southern Bahamas. We also evaluate sex-specific growth rates in those turtles for which we had data from multiple captures.

*Methods.*—As described in greater detail elsewhere (Bjorndal and Bolten, 1988), Union Creek is an impounded, 20-km<sup>2</sup> tidal bay on the north coast of Great Inagua, Bahamas, that is protected as a wildlife refuge by the Bahamas National Trust. Throughout the Bahamas, small green turtles of about 25 cm carapace length take up residence in tidal bays (termed “creeks” in the Bahamas) to feed on pastures of the sea-grass *Thalassia testudinum* (Bolten and Bjorndal, unpubl. data). We estimate that 20–40 green turtles enter Union Creek each year.

From 11–22 April 1988, 120 immature green turtles were captured in Union Creek. We measured, sampled blood, and tagged—or recorded the old tags on—each turtle. Straight-line carapace length (SCL) was measured from anterior to posterior point of midline (nuchal notch to posterior notch) to the nearest 0.1 cm with anthropometer calipers (GPM model 101). Every measurement was made by one of us (ABB) to decrease measurement error due to individual variation in technique. Blood samples were collected from the dorsal cervical sinus by a noninjurious technique (Owens and Ruiz, 1980) using 22-gauge needles and vacutainers treated with sodium heparin as an anticoagulant. The whole blood was centrifuged for 5 min, and the plasma was then pipetted into cryogenic vials and stored in liquid nitrogen for shipment back to the United States. No more than 15 min elapsed from the time that the blood was drawn until storage of the plasma in liquid nitrogen. After return to the United States, samples were stored at –70 C until analyzed.

The sex of immature green turtles cannot be determined from external characteristics. Circulating testosterone titers have been used successfully to determine the sex of immature green turtles (Owens et al., 1978; Wibbels, 1988). Testosterone concentrations were measured according to a radioimmunoassay method developed for sea turtles (Wibbels, 1988; Wibbels et al., 1990). The sensitivity of the testosterone assay (at 80% B/B<sub>0</sub>) was 2.5 picograms/tube. The intra-assay coefficient of variation was

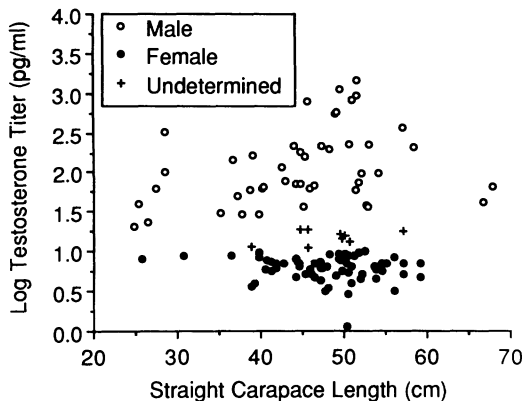


Fig. 1. Log testosterone concentration (pg/ml) against straight carapace length (cm) for 120 immature green turtles in Union Creek, Inagua. There is a significant correlation between carapace length and testosterone titer for males (see text).

12.5% and the inter-assay coefficient of variation was 18.4%. Extraction efficiency was 80%. Turtles with testosterone titers less than 10 picograms/ml (pg/ml) were classified as females; turtles with titers greater than 20 pg/ml were classified as males; turtles with values between 10 and 20 were classified as undetermined. We used this conservative classification to ensure accurate assignment of sex.

We have measured growth of SCL (cm/yr) in 88 of the turtles of known sex from data recorded at captures either before or after the April 1988 capture. Individual turtles were used once in the regression of growth rate against mean SCL (mean of initial and recapture SCL). Simple linear regressions were used with SCL as the independent variable and growth rates as the dependent variables. All regressions met the assumptions of homogeneous variance about the regression line and normal distribution of the residuals about the line. Unless otherwise stated,  $\alpha = 0.05$ .

*Results and discussion.*—There was considerable variation in plasma testosterone titers (Fig. 1). There is no significant correlation between SCL and testosterone titers for females (Spearman  $r = -0.140$ ,  $P = 0.265$ ,  $n = 65$ ). However, there is a significant correlation between SCL and testosterone concentration for males (Spearman  $r = 0.376$ ,  $P = 0.0099$ ,  $n = 46$ ), although SCL accounts for only 14% of the variation in testosterone titers. In contrast, Wibbels (1988) found no significant correlation between carapace

length and serum testosterone titer for immature, male green turtles, loggerheads (*Caretta caretta*), or hawksbills (*Eretmochelys imbricata*).

Of the 120 green turtles, 65 were females, 46 males, and nine undetermined. The resulting sex ratio of 1.4F:1.0M is not significantly different from a 1:1 ratio (chi-square = 3.25,  $df = 1$ ).

Wibbels (1988) discusses the sampling problems inherent in estimating hatchling and adult sex ratios in sea turtles and concludes that immature populations may be the most amenable to sex ratio studies. There are few estimates of sex ratios for sea turtle populations (Gibbons, 1990), and many of these are flawed because of sexual bias in method of capture, because of different movement patterns between sexes, or because external characteristics were used to assign sex above a given carapace length. In many areas, turtle fishermen prefer female sea turtles because they are believed to have fattier, sweeter meat; male turtles are released to save space in the boats for female turtles (Ross, 1984). Adult males and females appear to have different movement patterns that would bias estimates of sex ratios in certain areas in different seasons (Wibbels et al., 1987). Assigning sex based on external characteristics for turtles longer than a given minimum shell length is not reliable and may assign large males that have not yet developed secondary sex characteristics to the female category (Limpus and Reed, 1985).

No clear pattern has emerged for sex ratios in immature sea turtle populations. A sample of 145 immature green turtles on Heron Reef, Australia, over a period of six months, gave a ratio of 1.2F:1.0M, which is not significantly different from an unbiased sex ratio (Limpus and Reed, 1985). For the loggerhead turtle, the pooled sex ratio (1.9F:1.0M) for immature turtles captured over a 2.5-yr period ( $n = 256$ ) at four sites along the Atlantic coast of the southeastern United States was significantly female biased (Wibbels et al., 1987). There was no significant seasonal effect or site-of-capture effect on sex ratios (Wibbels et al., 1987). A larger sample ( $n = 218$ ) of immature loggerheads from one of these sites—Hutchinson Island, Florida—over a period of 2 yr again yielded a significantly female-biased sex ratio of 2.1F:1.0M and no significant seasonal effect (Wibbels, 1988). In a resident population of loggerheads—including both immature and adult turtles—on the Capricornia reefs, Australia, there

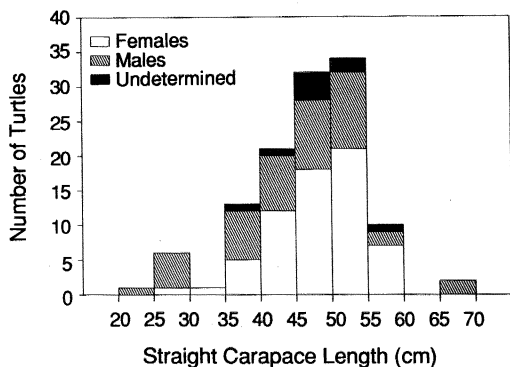


Fig. 2. Distribution of immature green turtles by sex category among 5-cm size classes ( $n = 120$ ). See text for statistical analyses.

was a significant male-biased ratio for a pooled sample from three sites, each sampled over a four-month period (Limpus, 1985).

For the Inagua population, sex ratios did vary among the size classes (Fig. 2). For statistical analyses, size classes were grouped and the sex ratios in the three size classes with adequate sample size were tested: 30.0–39.9 cm and 40.0–49.9 cm had unbiased sex ratios (chi-square = 0.08 and 3.00, respectively,  $df = 1$ ), but 50.0–59.9 cm turtles had a female-biased sex ratio of 2.2F:1.0M (chi-square = 5.49,  $df = 1$ ,  $P < 0.025$ ). Wibbels (1988) also found that sex ratios varied among size classes in loggerheads from Hutchinson Island, Florida. Two size classes had unbiased sex ratios, whereas two had female-biased sex ratios. However, Limpus and Reed (1985) found no difference in sex ratios for 10-cm size classes of green turtles on Heron Reef. Variation in sex ratios among size classes may reflect variable hatching sex ratios in previous years due to environmental effects, although size classes cannot be definitely assigned to different age classes because of the great variation in individual growth rates (Bjorndal and Bolten, 1988).

The relationship between growth rate of SCL and mean SCL (Fig. 3) was linear (test for linearity,  $F_{4,82} = 0.78$ ,  $P > 0.25$ ; Zar, 1984). The regression equation for males is

$$Y = 13.4 - 0.184X$$

and for females is

$$Y = 12.5 - 0.186X$$

where Y is growth rate of SCL and X is mean SCL. These regression equations do not differ

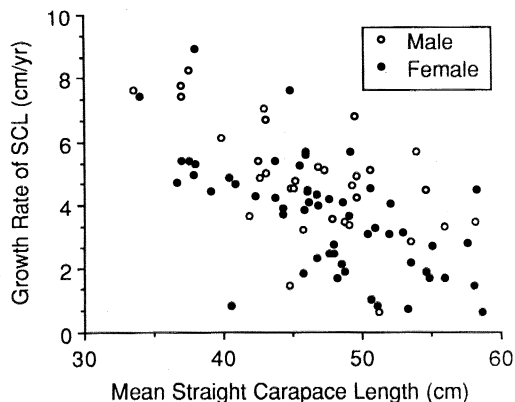


Fig. 3. Growth rates of straight carapace length (cm/yr) against mean straight carapace length (cm) for 88 immature green turtles in Union Creek, Inagua. See text for regression analyses.

in slope (t-test for comparison of two slopes,  $t = 0.035$ ,  $df = 84$ ; Zar, 1984) or elevation (t-test for comparison of two elevations when the Y-intercepts are far from the mean values of X,  $t = 0.36$ ,  $df = 84$ ; Zar, 1984). Therefore, immature green turtles do not exhibit sexually dimorphic growth rates, and the sex of individuals does not contribute significantly to the great variation in growth rates measured in these turtles.

*Conclusions.*—Based on a sample of 111 turtles, the population of immature green turtles in Union Creek has an unbiased sex ratio. Additional estimates of sex ratios from other populations are needed for both theoretical analyses and practical management applications. Juvenile male and female green turtles do not grow at different rates.

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**OVIDUCTAL ANATOMY AND SEASONAL SPERM STORAGE IN THE SOUTHEASTERN CROWNED SNAKE (*TANTILLA CORONATA*).**—In snakes, many aspects of the male reproductive cycle occur independently of the female cycle. In most temperate zone snakes, ovulation and fertilization occur in the spring, whereas spermatogenesis occurs in the summer (Saint Girons, 1982). In species that mate in the spring, the sperm are stored in the vas deferens over the winter, whereas in species that mate in the summer or fall, sperm are stored in the oviduct of the female until ovulation in the spring. The purpose of the present paper is to describe seasonal changes in the oviduct and the temporal pattern of sperm storage in the southeastern crowned snake (*Tantilla coronata*). This species is a small semifossorial colubrid inhabiting the southeastern United States. Among the family Colubridae, the genus *Tantilla* is unique in that some species, including *T. coronata*, have a vestigial left oviduct (Clark, 1970), yet have functional left and right ovaries.

Snakes were collected on the United States Department of Energy's Savannah River Site near Aiken, South Carolina. See Semlitsch et al. (1981) for description of habitats, population structure, and seasonal activity of this population. Oviducts of 27 adult females (snout–vent length 156–213 mm), collected from May through late Oct., were examined histologically

to determine anatomical changes and the presence and distribution of sperm. Specimens were preserved in 10% formalin. Snout–vent lengths (SVL) were measured on preserved individuals. Oviducts were prepared using standard paraffin infiltration technique, sectioned at 8  $\mu$ m and stained with hematoxylin, Biebrich scarlet, orange II, and fast green.

Female *T. coronata* reach sexual maturity at approximately 150 mm SVL. Vitellogenesis occurs in the spring, ovulation in June, and oviposition in June to early July. Copulation, based on the temporal distribution of sperm in the oviduct, occurs in the summer (and fall) and in the following spring (Aldridge, unpubl. data).

The left oviduct of *T. coronata* terminates 2–3 mm anterior to the vagina. All the histologically distinct regions of the right oviduct, the seasonal changes in glands, and the presence and distribution of sperm also occur in the vestigial left oviduct.

The oviduct of *T. coronata* has three morphologically distinct regions, vagina, uterus, and infundibulum. A specialized portion of the oviduct, termed the seminal receptacles by Fox (1956), occurs at the junction of the uterus and infundibulum.

The vagina is thick walled and lined with ciliated and nonciliated columnar epithelial cells (Fig. 1A). The mass of the wall of the vagina consists of connective tissue surrounded by a thin layer of smooth muscle. The left vaginal pouch is similar histologically to the right; however, it is approximately 20% smaller in diameter and length than the right vaginal pouch.

The posterior uterus is cephalad to the vagina. It is approximately 20 mm in length and is thin walled and lined with a ciliated pseudostratified columnar epithelium. Approximately 24 longitudinal ridges project into the lumen (Fig. 1B–C). This region was termed the anterior vagina by Ludwig and Rahn (1943) because the lining is histologically similar to the vagina. This term, however, appears inappropriate both morphologically and functionally. This portion is different from the vagina in that it lacks the thick connective tissue and muscle layers and, during copulation, the hemipenis (length  $5.74 \pm 0.31$  mm,  $n = 8$ ) does not appear to penetrate beyond the vagina proper (length  $8.02 \pm 0.22$  mm,  $n = 20$ ). The segment was termed furrowed portion by Halpert et al. (1982) because of the longitudinal folds projecting into the lumen.

The posterior uterine segment ends abruptly