Size-Dependent, Sex-Dependent, and Seasonal Changes in Insulin-like Growth Factor I in the Loggerhead Sea Turtle (Caretta caretta)

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This study examines size-dependent, sex-dependent, and seasonal fluctuations in plasma insulin-like growth factor-I (IGF-I) concentrations in loggerhead sea turtles (*Caretta caretta*). Loggerhead turtles ($n = 158$) were captured in shrimp trawler nets during a 12-month survey in Cape Canaveral Channel, Florida. Plasma samples were analyzed using a validated heterologous radioimmunoassay. Large turtles (>75 cm straight-line carapace length) had significantly higher plasma IGF-I concentrations than small turtles (≤75 cm; $P < 0.0001$). Plasma IGF-I concentrations did not vary seasonally in small turtles, but large turtles had significantly higher plasma IGF-I concentrations during the spring and summer months ($P < 0.005$). Within the large turtles, adult males had significantly lower IGF-I concentrations than females and subadult males ($P < 0.05$). These results and a review of loggerhead turtle natural history suggest that the seasonal fluctuations in plasma IGF-I of adult turtles are due to elevated IGF-I levels in reproductively active female turtles. Further research is needed to examine correlations between reproductive activities and plasma IGF-I concentrations in reptiles. © 1995 Academic Press, Inc.

Sea turtles have been a focus of extensive endocrinological research because of their phylogenetic position as a primitively diverged reptilian lineage and their endangered status. Most studies have concentrated on reproductive cyclicity, noting hormonal changes associated with breeding (Licht *et al.*, 1979, 1980; Wibbels *et al.*, 1990), migration (Wibbels *et al.*, 1990), and nesting (Figler *et al.*, 1989; Wibbels *et al.*, 1990; Guillette *et al.*, 1991). Very little attention, however, has been given to the hormones involved in the regulation of chelonian growth. Studies comparing growth rates among sea turtle species (Frazer and

Ehrhart, 1985; Bjorndal and Bolten, 1988) and within populations (Bolten *et al.*, 1992; Collazo *et al.*, 1992; Klinger and Musick, 1992) have contributed much to the current understanding of repitilian growth, which is imperative for valid demographic modeling and adequate management. The study of growth-related hormones promises to add another level to our understanding of sea turtle growth by focusing on a hormonal response and the factors that influence this response.

Several hormones could potentially contribute to the regulation of sea turtle growth. Thyroid hormones are necessary for normal turtle growth (Denver and Licht, 1991), possibly by indirectly affecting growth hormone (GH) secretion (Denver and Licht, 1988). Injections of mammalian

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GH and prolactin enhance growth in the lizard *Lacerta s. sicula* (Licht and Hoyer, 1968), the freshwater turtle *Chelydra serpentina* (Nichols, 1973), and the marine turtle *Chelonia mydas* (Owens *et al.*, 1979). Mechanisms of GH regulatory action in reptiles are unknown, but GH directly induces insulin-like growth factor-I (IGF-I) secretion from the liver of mammals (Sara and Hall, 1990). IGF-I has been detected in the plasma of freshwater turtles (Wilson and Hintz, 1982; Crain *et al.*, 1995), lizards (*Gecko* sp.; Bautista *et al.*, 1990), and alligators (*Alligator mississippiensis;* Crain *et al.*, 1995), but no studies have examined patterns or cycles associated with plasma IGF-I concentrations in a reptile. IGF-I has been a primary focus of mammalian endocrinological research because it elicits growth-promoting effects on almost all tissues studied and because of its potential to regulate the mitogenic effects of other growth-related hormones.

The purposes of this study are to establish the existence of IGF-I in plasma of loggerhead sea turtles (*Caretta caretta*) and determine if IGF-I concentrations vary among seasons, among turtles of different sizes, and between turtles of different sexes. If we assume that IGF-I promotes growth in reptiles as it does in mammals, then the following predictions can be made. Decreased activity during winter months is expected to decrease feeding intensity and correspondingly reduce concentrations of plasma IGF-I. Small turtles are expected to have higher IGF-I concentrations than large turtles based on the accelerated growth rates of juvenile sea turtles (Frazer and Ehrhart, 1985; Bjorndal and Bolten, 1988) and data from studies on mammals. Additionally, female turtles are expected to have high plasma IGF-I concentrations during egg formation because maternal IGF-I must be synthesized and placed in the egg at this time (Guillette and Williams, 1991; Cox and Guillette, 1993). Loggerhead plasma samples from a 12-month study in

Cape Canaveral Channel, Florida, were analyzed to determine if such patterns occur. These data are necessary (1) to examine whether a conserved growth regulatory mechanism exists in vertebrates, (2) to establish size-dependent, sex-dependent, and seasonal trends in IGF-I for a turtle, and (3) to provide a more complete understanding of sea turtle growth.

MATERIALS AND METHODS

Samples

Turtles were trawled from Cape Canaveral Channel, Florida, as part of a study to determine seasonal densities of turtles in the channel (Bolten *et al.*, 1994). Three-day surveys were conducted during each month from March 1992 to February 1993. Turtles were measured to obtain straight-line carapace length (nuchal notch to posterior notch) and tail length (posterior tip of plastron to tip of tail). Blood was obtained from the dorsal cervical sinus with a heparinized Vacutainer tube and a 20-gauge needle. Samples (5–10 ml) were immediately centrifuged and resultant plasma was frozen in liquid nitrogen. Samples were transported to a −72° freezer and thawed twice prior to analysis.

Size and Sex Designations

For data analyses, turtles were categorized by size and sex based on *a posteriori* observations. The bimodality in size frequency of turtles caught (Fig. 1) led to
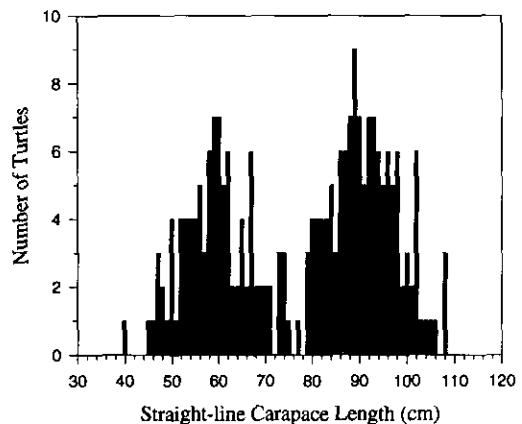

FIG. 1. Numbers of turtles in 1-cm categories that were captured in Cape Canaveral Channel. Note the bimodal distribution justifying separation of large and small turtles.

the designation of two size classes of turtles: large and small. Turtles greater than 75 cm were designated "large," whereas turtles less than or equal to 75 cm were designated "small." These designations agree with those of another study of Canaveral Channel (Henwood, 1987) and roughly correspond to adult and subadult designations (see Discussion). Designation of sex was based on tail length. Males were defined as having a tail length greater than 40 cm, and any turtle with tail length less than or equal to 40 cm was designated "other." This conservative designation fails to identify many subadult males as males, but avoids misidentifying females as males.

Radioimmunoassay Procedures

Human IGF-I antisera (UB3-189) was a gift from Drs. Louis Underwood and Judson J. Van Wyk and distributed through the National Hormone and Pituitary Program. Human recombinant IGF-I standard, iodinated IGF-I label, and Amerlex-M goat anti-rabbit secondary antibody were purchased from Amersham International (Arlington Heights, IL). Buffer reagents were purchased from Fisher Chemical Company (Pittsburgh, PA).

Radioimmunoassay techniques were performed as described in Crain et $al.$ (1995). Briefly, samples (100 μl) were extracted with 400 μl acid–ethanol (12.5% 2 N HCl, 87.5% ethanol). The supernatant (100 μl) was pipetted into fresh polypropylene tubes and dried under a constant air stream prior to assay. RIA buffer (400 μl; 200 mg/liter protamine sulfate, 4.14 g/liter sodium phosphate monobasic, 0.05% Tween 20, 0.02% sodium azide, 3.72 g/liter EDTA) was added to reconstitute the dried samples. After vortexing to resuspend the samples, 50 μl antibody was added (1:10,000 final dilution) followed by 50 μl ^{125}I-IGF-I (approximately 20,000 cpm). Tubes were vortexed and incubated overnight at 4°. Separation of bound and free IGF-I was accomplished by adding 300 μl of the metallic-bound secondary antibody to each tube. Secondary antibody was incubated 10 min followed by a 15-min magnetic separation. Supernatant was discarded, tubes were drained, and pellets were counted on a Beckman 5500B gamma counter. Interassay variability was 9.26%, and intraassay variability averaged 3.83%. Extraction efficiency was determined by spiking 100 μl of pooled alligator plasma with 20,000 cpm ^{125}I-IGF-I, extracting the plasma with 400 μl acid–ethanol, and counting the amount of label in the supernatant after extraction. Extraction efficiency was 75%, and all samples were adjusted for this 25% loss.

Radioimmunoassay Validation

Validation plasma was obtained by pooling 100 μl of each sample. Two methods were utilized to validate the RIA for $C.$ $caretta$ plasma: internal standards and plasma dilutions. Plasma (100 μl) for internal standards was spiked with 0, 39, 156, 313, 625, and 1000 pg prior to extraction with 400 μl of acid–ethanol. Supernatant (100 μl) was aliquanted, resulting in 0, 7.81, 31.25, 62.5, 125, 250, and 500 pg standards in 20 μl plasma equivalent. Plasma (200 μl) for plasma dilutions was extracted with 800 μl acid–ethanol. Supernatant was aliquanted in 10-, 25-, 50-, and 100-μl amounts to produce plasma equivalents of 2.5, 5, 10, and 20 μl, respectively. Volumes of the plasma dilutions were brought to 100 μl with acid–ethanol. Internal standards and plasma dilutions were air dried and assayed as described above.

Analyses

Concentrations of IGF-I were estimated from raw data with the commercially available Beckman EIA/RIA ImmunoFit software program (Fullerton, CA). Statistics were performed with the software packages Statview (Abacus Concepts, Inc., Berkeley, CA, 1992) and Superanova (Abacus Concepts, Inc., Berkeley, CA, 1989). For size versus IGF-I analysis, a two-sample t test was used. Because heterogeneity of variance existed among months, data for season versus IGF-I were log transformed prior to analysis by one-way ANOVA. Duncan's multiple comparison test was used to identify which months differed. For analysis of sex versus IGF-I, data were log transformed due to heterogeneity of variance prior to two-sample t test analysis. Validation curves were tested for homogeneity of slopes by ANCOVA. Means are listed ±1 SE.

RESULTS

Radioimmunoassay Validation

Results of the radioimmunoassay validation are summarized in Fig. 2. Plasma dilutions, internal standards, and human recombinant IGF-I standards gave parallel displacement curves (ANCOVA; $F = 0.74$; $P = 0.5$).

Size versus IGF-I

A significant relationship was detected between turtle size (straight-line carapace length) and IGF-I concentration ($P < 0.001$). However, the variation in IGF-I concentration was not explained by turtle size, as no correlation existed between these variables ($r^2 = 0.076$; Fig. 3). There
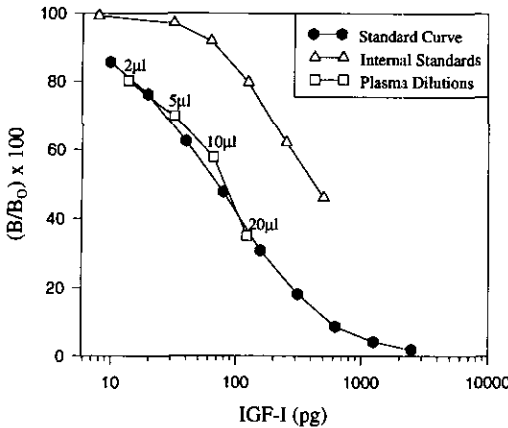
FIG. 2. Radioimmunoassay validation including internal standards and plasma dilutions. For plasma dilutions, $(B/B_o) \times 100$ was calculated with B_o from the standard curve; for internal standards, B_o was derived from binding in 20 μl plasma with no standard.

was a significant difference in mean IGF-I concentration between small (≤ 75 cm carapace length) and large (>75 cm) turtles ($t = -4.168$; df = 156; $P < 0.0001$). Large turtles had higher IGF-I concentrations ($\mu = 6.307$ ng/ml ± 0.337; $n = 92$) compared to IGF-I concentrations in small turtles ($\mu = 4.372$ ng/ml $+ 0.283$; $n = 66$).

Season versus IGF-I

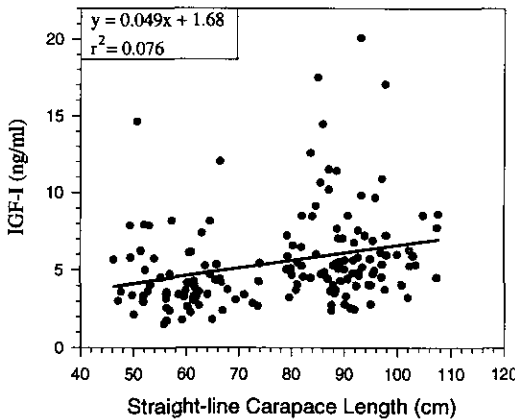Seasonal variation in plasma IGF-I was evaluated in both small and large turtles

FIG. 3. Plasma IGF-I concentrations for loggerhead turtles of different sizes.

based on the observation that a significant difference in plasma IGF-I existed between size classes. For small turtles, one-way ANOVA detected no differences in IGF-I concentrations among the months ($F = 0.322$; df = 11, 54; $P = 0.978$; Fig. 4). For large turtles, however, significant differences were detected ($F = 2.913$; df = 9, 82; $P < 0.005$; Fig. 5). The mean IGF-I concentrations in April, May, and June were significantly higher than those in February, September, and November ($P < 0.05$). Additionally, the mean in March was significantly higher than that in November ($P < 0.05$).

Sex versus IGF-I

Comparison of adult male turtles (tail >40 cm) to other large turtles (tail ≤ 40 cm; includes females and subadult males) revealed that adult males had a significantly lower mean IGF-I concentration ($t = 2.240$; df = 90; $P < 0.05$). The mean IGF-I concentration for adult males was 5.275 ng/ml ± 0.486 ($n = 24$), whereas the mean for other large turtles was 6.671 ng/ml $\pm .0.415$ ($n = 68$).
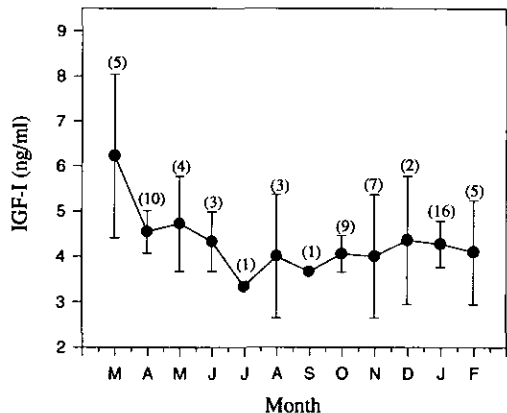
DISCUSSION

Results of this study indicate that IGF-I

FIG. 4. Mean plasma IGF-I concentrations (± 1 SE) for small loggerhead turtles (≤ 75 cm carapace length) captured in different months. Numbers above SE bars are samples sizes.
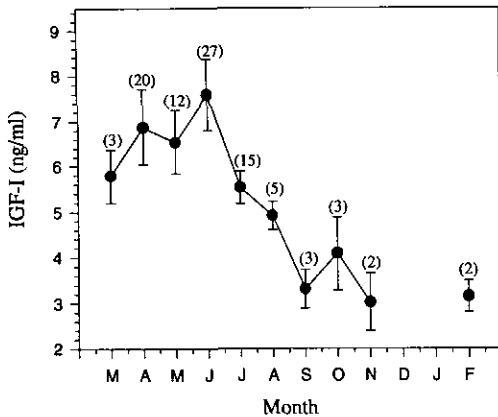
FIG. 5. Mean plasma IGF-I concentrations (± 1 SE) for large loggerhead turtles (>75 cm carapace length) captured in different months. Numbers above SE bars are sample sizes. No samples were obtained from large turtles during December 1992 or January 1993.

is present in the plasma of loggerhead sea turtles. Although the slope of turtle size vs IGF-I was significantly greater than zero, only 7.6% of the variation in IGF-I concentrations could be accounted for by turtle size. However, when large turtles (>75 cm carapace length) and small turtles (≤ 75 cm carapace length) were compared, large turtles had a significantly higher mean plasma IGF-I concentration. The designation of 75 cm as the distinction between small and large turtles is based on bimodality in size frequency of turtles caught in this study and sea turtle natural history. Henwood (1987) found a similar bimodal distribution in Cape Canaveral Channel, which he used to distinguish between adults and juveniles. Care must be taken when designating adult status based only on size of the turtle because turtles mature at various sizes dependent upon environmental conditions (Carr and Goodman, 1970; Gailbraith et al., 1989). However, Henwood's distinction between adults and juveniles is supported by the size of the smallest documented nesting female in the area (Witherington, 1986). For clarity, we use the terms large and small instead of adult and subadult to categorize turtles. Based on the use of 75 cm as the distinction

between large and small turtles, it is assumed that most large turtles are adults.

Plasma IGF-I concentrations in small turtles did not vary seasonally. Mean plasma IGF-I varied between 3.7 and 6.2 ng/ml throughout the year, with no apparent pattern. However, plasma IGF-I concentrations did vary seasonally in large turtles, with spring and summer months having the highest mean concentrations. This seasonal influence on large but not small turtles was unexpected, but can be explained by the population dynamics at the sampling location, Cape Canaveral Channel. In a 7-year survey of turtles in the Channel, Henwood (1987) noted that although turtles of different sizes and sexes are present throughout the year, trends in population structure are evident. In general, subadults are present throughout the year; breeding adult males become concentrated in the Channel in March and disperse in May; breeding adult females begin arriving in April and emigrate in August. The increase in mean IGF-I concentration in large turtles coincides with the arrival of reproductively active turtles. Thus, it is possible that the elevated IGF-I concentrations in large turtles are due to the turtles' reproductive activity.

The relationship between reproductive status and IGF-I concentration has gained much attention in human and laboratory animal research, with most research focusing on the female reproductive system. Both local (for review see Giudice, 1992) and systemic IGF-I are increased at various reproductive stages in females. Circulating levels of IGF-I increase progressively during human pregnancy (Furlanetto et al., 1978; Wilson et al., 1982) and increase from early to midpregnancy in rats (Gargosky et al., 1993). Such increased circulating concentrations are of obvious importance to viviparous species that can directly pass growth-promoting factors to developing offspring via a placenta. In oviparous species, factors associated with embryonic growth must be placed in the egg prior to

oviposition (Palmer and Guillette, 1991). If IGF-I from the blood is transported into the egg via albumen or yolk, the benefits of elevated circulating IGF-I prior to, during, and after ovulation are obvious. IGF-I has been identified in the yolk of chicken eggs (Scavo *et al.*, 1989) and the albumen of alligator eggs (Guillette and Williams 1991). Additionally, IGF-I is present in oviducal glands of vitellogenic alligators (Cox and Guillette, 1993). The source of IGF-I in the yolk and albumen of eggs is unknown; it may be derived from the blood or synthesized directly in the ovary or uterus (Cox, 1994).

Female loggerhead turtles lay multiple nests in a single season, with each clutch resulting from a separate ovulation (Licht *et al.*, 1979, 1980). Therefore, increased mean IGF-I concentrations during April, May, and June could be associated with female reproductive activity. Determining the sex of individuals is critical for support of this hypothesis, and two methods are currently used to sex adult loggerhead turtles: sex hormone concentration and tail length. Attempts to sex the individuals in this study from plasma testosterone and estradiol-17β concentrations were unsuccessful. Tail length is commonly used to sex individuals since sexually mature males have longer tails than females (Pritchard *et al.*, 1983), but designations of maximum and minimum acceptable tail lengths are arbitrary. A very conservative estimate of male tail length (>40 cm) was used to avoid misidentifying females as males. Results indicate that definitive males had a significantly lower mean plasma IGF-I concentration than all other turtles, supporting the hypothesis that IGF-I levels are elevated in reproductively active female loggerhead turtles. Therefore, the seasonal pattern in plasma IGF-I associated with large turtles is attributed to the reproductive status of adult females.

An alternative explanation for the elevated IGF-I concentrations during spring and summer months is increased food intake due to greater activity. Diet directly influences plasma IGF-I levels in freshwater turtles (Crain, 1994; Crain *et al.*, 1995), and IGF-I may signal the availability of nutrients for cellular division and protein synthesis (Clemmons and Underwood, 1991). An increase in feeding during spring and summer months is expected to increase plasma IGF-I concentrations. If this increased feeding stimulated a rise in plasma IGF-I concentration, then both juveniles and adults would be expected to show seasonal patterns, with peaks in the spring; this was not the case. Thus, observed seasonal fluctuations are thought to be independent of nutrition, although individual differences in nutritional status may explain variation within months.

The purposes of this study were to determine if IGF-I was detectable in loggerhead turtle plasma and to examine size-dependent, sex-dependent, and seasonal changes in plasma IGF-I concentrations. Due to the use of a heterologous assay system, the concentrations of IGF-I measured in this study likely underestimate the true plasma concentrations. Moriyama *et al.* (1994) recently noted that a heterologous assay using fish plasma severely underestimated concentrations of IGF-I. Nevertheless, our heterologous RIA detected differences in plasma IGF-I concentration between large and small turtles. Within large turtles, seasonal fluctuations occurred, with turtles having significantly greater mean plasma IGF-I concentrations in spring and summer months. It is hypothesized that such fluctuations are due to the reproductive condition of individuals. Although this hypothesis could not be tested directly, the hypothesis is supported by established population dynamics within the sampling area and data analysis based on conservative sex designation.

Growth-related hormones can be used as a measure of physiological response to different environmental stimuli. Of these hor-

mones, IGF-I is of particular interest given its endocrine, paracrine, and autocrine actions in mammals. Although the role of IGF-I in growth regulation of reptiles is unknown, IGF-I is present in the plasma of loggerhead turtles and apparently varies with reproductive and, possibly, nutritional status. Further studies are needed to determine specific growth-promoting actions of IGF-I in reptiles, if any, and to examine correlations between reproductive and nutritional status and circulating IGF-I concentrations.

ACKNOWLEDGMENTS

REFERENCES

Bautista, C. M., Mohan, S., and Baylink, D. J. (1990). Insulin-like growth factors I and II are present in the skeletal tissues of ten vertebrates. *Metabolism* 39, 96–100.

Bjorndal, K. A., and Bolten, A. B. (1988). Growth rates of immature green turtles, *Chelonia mydas*, on feeding grounds in the southern Bahamas. *Copeia* 1988, 555–564.

Bolten, A. B., Bjorndal, K. A., Grumbles, J. S., and Owens, D. W. (1992). Sex ratio and sex-specific growth rates of immature green turtles, *Chelonia mydas*, in the southern Bahamas. *Copeia* 1992, 1098–1103.

Bolten, A. B., Bjorndal, K. A., Eliazar, P. J., and Gregory, L. F. (1994). Seasonal abundance, size distribution, and blood biochemical values of loggerheads (*Caretta caretta*) in Port Canaveral Ship Channel, Florida. *NOAA Technical Memorandum NMFS-SEFC-353.*

Carr, A., and Goodman, D. (1970). Ecologic implica-

tions of size and growth in *Chelonia. Copeia* 1970, 783–786.

Clemmons, D. R., and Underwood, L. E. (1991). Nutritional regulation of IGF-I and IGF binding proteins. *Annu. Rev. Nutr.* 11, 393–412.

Collazo, J. A., Boulon, R., Jr., and Tallevast, T. L. (1992). Abundance and growth patterns of *Chelonia mydas* in Culebra, Puerto Rico. *J. Herpetol.* 26, 293–300.

Cox, C., and Guillette, L. J., Jr. (1993). Localization of insulin-like growth factor-I-like immunoreactivity in the reproductive tract of the vitellogenic female American alligator, *Alligator missisippiensis. Anat. Rec.* 236, 635–640.

Cox, M. C. (1994). Presence and functional role of the polypeptide growth factors insulin-like growth factor-I (IGF-I) and epidermal growth factor (EGF) in the reptilian oviduct. Ph.D. Dissertation. University of Florida, Gainesville.

Crain, D. A. (1994). Insulin-like growth factor-I in the plasma of two chelonians: *Caretta caretta* and *Trachemys scripta elegans*. M.S. Thesis. University of Florida, Gainesville.

Crain, D. A., Gross, T. S., Cox, M. C., and Guillette, L. J., Jr. (1995). Insulin-like growth factor-I in the plasma of two reptiles: Assay development and validations. *Gen. Comp. Endocrinol.* 98, 26–34.

Denver, R. J., and Licht, P. (1988). Thyroid status influences in vitro thyrotropin and growth hormone responses to thyrotropin-releasing hormone by pituitary glands of hatchling slider turtles (*Pseudemys scripta elegans*). *J. Exp. Zool.* 246, 293–304.

Denver, R. J., and Licht, P. (1991). Dependence of body growth on thyroid activity in turtles. *J. Exp. Zool.* 258, 48–59.

Figler, R. A., MacKenzie, D. S., Owens, D. W., Licht, P., and Amoss, M. S. (1989). Increased levels of arginine vasotocin and neurophysin during nesting in sea turtles. *Gen. Comp. Endocrinol.* 73, 223–232.

Frazer, N. B., and Ehrhart, L. M. (1985). Preliminary growth models for green, *Chelonia mydas*, and loggerhead, *Caretta caretta*, turtles in the wild. *Copeia* 1985, 73–79.

Furlanetto, R. W., Underwood, L. E., Van Wyk, J. J., and Handwerger, S. (1978). Serum immunoreactive somatomedin-C is elevated late in pregnancy. *J. Clin. Endocrinol. Metab.* 41, 695–698.

Galbraith, D. A., Brooks, R. J., and Obbard, M. E. (1989). The influence of growth rate on age and body size at maturity in female snapping turtles (*Chelydra serpentina*). *Copeia* 1989, 896–904.

Gargosky, S. E., Nanto-Salonen, K., Tapanainen, P., and Rosenfeld, R. G. (1993). Pregnancy in growth hormone-deficient rats: Assessment of insulin-like growth factors (IGFs), IGF-binding proteins

(IGFBPs) and IGFBP protease activity. *J. Endocrinol.* **136**, 479–489.

Giudice, L. C. (1992). Insulin-like growth factors and ovarian follicular development. *Endocr. Rev.* **13**, 641–669.

Guillette, L. J., Jr., and Williams, P. E. (1991). Detection of insulin-like growth factors I and II in the egg albumen of the American alligator (*Alligator mississippiensis*) using microcapillary electrophoresis. *Biol. Reprod.* **44**(Suppl. 1), 58.

Guillette, L. J., Jr., Bjorndal, K. A., Bolten, A. B., Gross, T. S., Palmer, B. D., Witherington, B. E., and Matter, J. M. (1991). Plasma estradiol-17β, progesterone, prostaglandin F, and prostaglandin E₂ concentrations during natural oviposition in the loggerhead turtle (*Caretta caretta*). *Gen. Comp. Endocrinol.* **82**, 121–130.

Henwood, T. A. (1987). Movements and seasonal changes in loggerhead turtle *Caretta caretta* aggregations in the vicinity of Cape Canaveral, Florida (1978–84). *Biol. Conserv.* **40**, 191–202.

Klinger, R. C., and Musick, J. A. (1992). Annular growth layers in juvenile loggerhead turtles (*Caretta caretta*). *Bull. Mar. Sci.* **51**, 224–230.

Licht, P., and Hoyer, H. (1968). Somatotropic effects of exogenous prolactin and growth hormone in juvenile lizards (*Lacerta s. sicula*). *Gen. Comp. Endocrinol.* **11**, 338–346.

Licht, P., Wood, J., Owens, D. W., and Wood, F. (1979). Serum gonadotropins and steroids associated with breeding activities in the green sea turtle, *Chelonia mydas*. I. Captive animals. *Gen. Comp. Endocrinol.* **39**, 274–289.

Licht, P., Rainey, W., and Clifton, K. (1980). Serum gonadotropin and steroids associated with breeding activities in the green sea turtle, *Chelonia mydas*. II. Mating and nesting in natural populations. *Gen. Comp. Endocrinol.* **40**, 116–122.

Moriyama, S., Swanson, P., Nishii, M., Takahashi, A., Kawauchi, H., Dickhoff, W. W., and Plisetskaya, E. M. (1994). Development of a homologous radioimmunoassay for coho salmon insulin-like growth factor-I. *Gen. Comp. Endocrinol.* **96**, 149–161.

Nichols, C. W. (1973). Somatotropic effects of prolactin and growth hormone in juvenile snapping turtles (*Chelydra serpentina*). *Gen. Comp. Endocrinol.* **21**, 219–224.

Owens, D. W., Hendrickson, J. R., and Endres, D. B. (1979). Somatic and immune responses to bovine growth hormone, bovine prolactin, and diethylstilbesterol in the green sea turtle. *Gen. Comp. Endocrinol.* **38**, 53–61.

Palmer, B. D., and Guillette, L. J., Jr. (1990). Oviducal proteins and their influence on embryonic development in birds and reptiles. *In* "Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles," (D. C. Demming and M. W. J. Ferguson, Eds.), pp. 29–46. Cambridge Univ. Press, Cambridge.

Pritchard, P., Bacon, P., Berry, F., Carr, A., Fletemeyer, J., Gallagher, R., Hopkins, S., Lankford, R., Marquez, R., Ogren, L., Pringle, W., Reichart, H., and Witham, R. (1983). "Manual of Sea Turtle Research and Conservation Techniques," second ed. Center for Environmental Education, Washington, DC.

Sara, V. I., and Hall, K. (1990). Insulin-like growth factors and their binding proteins. *Phys. Rev.* **70**, 591–613.

Scavo, L., Alemany, J., Roth, J., and de Pablo, F. (1989). Insulin-like growth factor 1 activity is stored in the yolk of the avian egg. *Biochem. Biophys. Res. Commun.* **162**, 1167–1173.

Wibbels, T., Owens, D. W., Limpus, C. J., Reed, P. C., and Amoss, M. S., Jr. (1990). Seasonal changes in serum gonadal steroids associated with migration, mating, and nesting in the loggerhead sea turtle (*Caretta caretta*). *Gen. Comp. Endocrinol.* **79**, 154–164.

Wilson, D. M., and Hintz, R. L. (1982). Inter-species comparison of somatomedin structure using immunological probes. *J. Endocrinol.* **95**, 59–64.

Wilson, D. M., Bennett, A., Adamson, G. D., Nagashima, R. L., Liu, F., De Natale, M. L., Hintz, R. L., and Rosenfeld, R. G. (1982). Somatomedins in pregnancy: A cross-sectional study of insulin-like growth factors I and II and somatomedin peptide content in normal human pregnancies. *J. Clin. Endocrinol. Metab.* **55**, 858–861.

Witherington, B. E. (1986). Human and natural causes of marine turtle clutch and hatchling mortality and their relationship to hatchling production on an important Florida nesting beach. M.S. Thesis. University of Central Florida, Orlando.