

Serum Thyroid Hormone Levels in Wild and Captive Sea Turtles

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Blood samples collected from green (*Chelonia mydas*), loggerhead (*Caretta caretta*), and Kemp's ridley (*Lepidochelys kempii*) sea turtles were analyzed by radioimmunoassay to detect seasonal variations in thyroid hormones and compare levels in wild and captive individuals. Sexual dimorphism in the annual cycle of thyroxine was observed in adult Kemp's ridley. No differences were observed between immature male and female green and loggerhead sea turtles. The level of triiodothyronine was consistently low relative to thyroxine in all species investigated, and captive sea turtles exhibit higher plasma thyroid levels than do wild ones.

In vertebrates, especially in mammals, it has been proposed that thyroid hormones regulate energy demanding processes such as growth, metabolism, development, and reproduction (Norris, 1985). The function of thyroid hormones in reptiles is less clear than in mammals.

The active thyroid state in reptiles appears to facilitate reproduction (Haldar-Misra and Thapliyal, 1981), increase metabolic rate (Thapliyal, 1984; John-Alder, 1984a, 1984b, and 1990), and promote molting (Lynn, 1970). Most of these observations on thyroid function in reptiles was based on histological and radioisotopic methods. Additional research has been initiated on serum or plasma thyroid hormone levels in concert with behavior and physiology in snakes and lizards (Bona-Gallo et al., 1980; John-Alder, 1984a; Kar and Chandola-Saklani, 1985; Licht et al., 1985a and 1985b; Naulleau et al., 1987). Most of this work has shown a peak of T₄ in summer and depressed T₄ during winter months when environmental temperature drops, indicating that plasma T₄ may support energy consuming activities, such as nutrient assimilation, activity, and reproduction. Although many experiments have been conducted to describe thyroid function and its relationship to temperature and metabolism in squamates, the function of thyroid hormones in other reptilian species, such as freshwater and sea turtles, has rarely been investigated. Only Licht et al. (1985b) measured thyroid hormone levels in sea turtles. Since there are inherent difficulties in working with wild turtles, they measured annual thyroxine levels in captive green sea turtles in Grand Cayman and found that captive male greens showed very uniform serum thyroxine levels throughout the year. However, other sea turtle species, including wild populations, need to be examined since

most sea turtle species are endangered, many potentially useful conservation strategies have been proposed which are dependent upon an improved understanding of the basic biology of the animals. The study of thyroid hormones in sea turtles may help us understand their basic metabolic physiology, and may thus contribute to the design of conservation strategies.

Therefore, the purpose of the present study was to describe the serum thyroid hormone levels in wild sea turtle species and compare to levels in captive animals. This may enable us to relate thyroid hormone levels to periods of dynamic metabolic activation induced by temperature, feeding, or reproductive activity.

Materials and Methods

Wild adult and immature green (*Chelonia mydas*) and immature loggerhead (*Caretta caretta*) sea turtles captured in the water off coral reefs at Heron Island, Australia, were sampled monthly from July to November 1985 (n=6 for females and n=7 for males). Since the water was clear and shallow, the "turtle rodeo" technique described by Limpus (1978) was used to capture turtles. Researchers dove onto the turtles from a small boat, grabbed the carapace and swam the animals to the surface. Colleagues in the boat then pulled the turtles aboard by roping the front flippers. Immediately, blood samples were taken from the dorsal cervical sinus as described by Owens and Ruiz (1980) using 21G 1½ inch needles and vacutainer tubes. Nesting green turtles were also bled using the same technique. Blood samples were kept on ice until centrifuged and the serum was frozen in liquid nitrogen thereafter. The water temperature during the study period ranged from approximately 19°C in July to 25°C in November. Captive adult Kemp's ridley (*Lepidochelys kempii*) sea turtles at Sea Arama Marineworld at Galveston, Texas, were sampled monthly from March

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1987 to February 1988 (n=5 for females and n=3 for males) (Rostal, 1991). Animals were maintained individually in indoor tanks and were fed a diet of squid, capeline, herring and smelt twice a week. The turtles were maintained in filtered seawater from the Gulf of Mexico. The water temperature during sampling time ranged from 21 °C in winter to 29 °C in summer. Blood samples were taken from each turtle using the same technique described above. Separated sera were frozen and kept at -70 to -80 °C until assayed for T₃ and T₄. To measure the baseline for plasma thyroid hormones in other species, black turtles (*Chelonia agassizi*) (Rostal, 1991) and olive ridley turtles (*Lepidochelys olivacea*) (Wibbles et al., 1988) were captured in the water off Michoacan, 1989, and Oaxaca, Mexico, 1985, respectively. Blood samples were also collected from these species during nesting on the beaches. For captive turtles, immature green and Kemp's ridley turtles were used for this experiment (Moon, 1992).

Thyroid hormone radioimmunoassay

A thyroid hormone radioimmunoassay (RIA) (MacKenzie et al., 1977), with modifications described by Denver and Licht (1988), was used for quantifying plasma T₃ and T₄. To validate the assay, parallelism and recovery tests were conducted. To ten ng/ml of carrier hormone in basic methanol was added plasma samples. The tubes were assayed for total thyroid hormone concentration as described above. Supplemented and non-supplemented samples appeared to parallel to the standard. The average recovery rate was 94.5%. The assay sensitivity was 0.5 ng/ml in T₃ and T₄ levels and below this level was considered to be non-detectable.

Statistical analyses

One way analysis of variance with repeated measures was used to see if there were significant changes in hormone levels of captive animals sampled monthly. Duncan's multiple range test was used to test for the significant differences in hormone levels between groups caught and sampled monthly in the wild.

Results

T₄ levels of wild immature green sea turtles over the 5-month sampling period in Australia were ranged from 1.6 ng/ml to 3.1 ng/ml in females and 1.9 ng/ml to 3.0 ng/ml in males (Fig. 1). Wild immature loggerhead sea turtles showed low thyroxine levels ranging from about 0.8 ng/ml to 1.5 ng/ml in females and 0.9 ng/ml to 1.4 ng/ml in males (Fig. 2). Statistical analysis revealed significant changes in thyroxine levels only in immature female green turtles, which were fairly constant from July to October followed by a significant increase in November (Fig. 1). While no significant changes were observed during the sampling periods, there was a

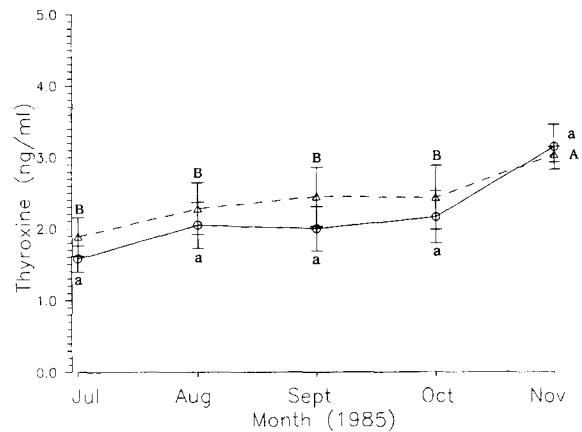


Fig. 1. The thyroxine (T₄) levels of females (○) and males (△) of wild immature green sea turtles, *Chelonia mydas*, at Heron Island, Australia through 5 months period from July to November. Vertical bars represent standard errors of the mean. Months with the same letters are not significantly different by Duncan's multiple range test.

tendency that T₄ levels increased towards summer (November) as environmental temperature increases (water temperature increased from 19 °C in July to 25 °C in November) in all immature green sea turtles. Circulating T₄ in adult green sea turtles was constant during 4 months, ranging from 1.41 to 1.61 ng/ml (Fig. 3). Captive adult Kemp's ridley turtles at Sea Arama Marineworld, Texas, showed a seasonal cycle of thyroxine in females but not in males (Fig. 4). One way repeated measures (ANOVA) revealed that female thyroxine level changed significantly over 12 month period. Females displayed higher levels of thyroxine in November and December (9.5 to 11 ng/ml) followed by a significant decrease in January. Another higher phase of T₄ began in February through May (12 to 13 ng/ml). Thereafter, plasma T₄ concentration remained at relatively low levels from June to October (4.5 to 7.0 ng/ml). There were no significant changes in plasma

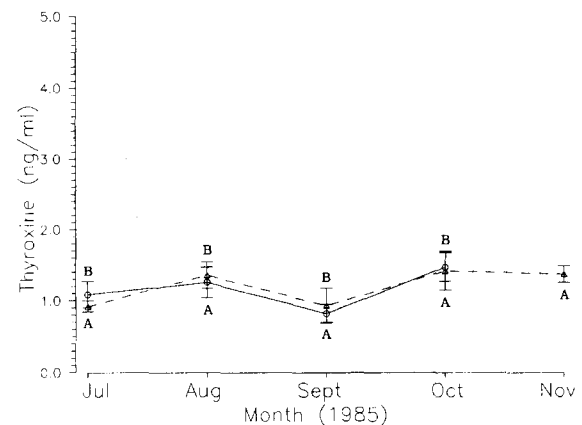


Fig. 2. The thyroxine (T₄) levels of females (○) and males (△) of wild immature loggerhead sea turtles, *Caretta caretta*, at Heron Island, Australia through 5 months period from July to November. Vertical bars represent standard errors of the mean. Months with the same letters are not significantly different by Duncan's multiple range test.

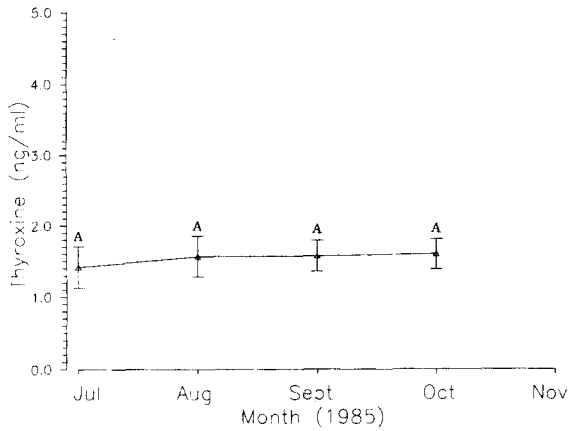


Fig. 3. The T_4 levels of wild adult green sea turtles, *Chelonia mydas*, at Heron Island, Australia through 4 months period from July to October. Vertical bars represent standard errors of the mean. There were no significant changes in T_4 levels by Duncan's multiple range test.

thyroid hormone levels of male Kemp's ridleys over the 12 month period although there was a trend downward (ANOVA table not shown). Ridley males showed relatively lower levels of thyroxine than females during the study periods, which ranged from 3 to 6.5 ng/ml. Unlike immature green and loggerhead turtles, plasma thyroid hormone levels in adult Kemp's ridley turtles seemed unrelated to environmental temperature since male ridley turtles showed fairly constant T_4 levels although these gradually increasing with varying water temperature from 21°C in December to 29°C in August. Additionally, female ridley turtles showed increased T_4 levels in spring and winter, which were coincident with the low temperature of about 21°C.

Plasma T_4 levels were higher in nesting olive ridley turtles (6.67 ng/ml) (n=41) at Oaxaca, Mexico, than they were in swimming turtles (3.3 ng/ml) (n=9). A similar contrast occurred in black turtles at Michoacan, Mexico; 5.6 ng/ml in nesters (n=20) and 4.3 ng/ml in swimmers (n=5). Plasma T_4 in nesting green turtles at Heron Island, Australia, was 3.7 ng/ml, but was deter-

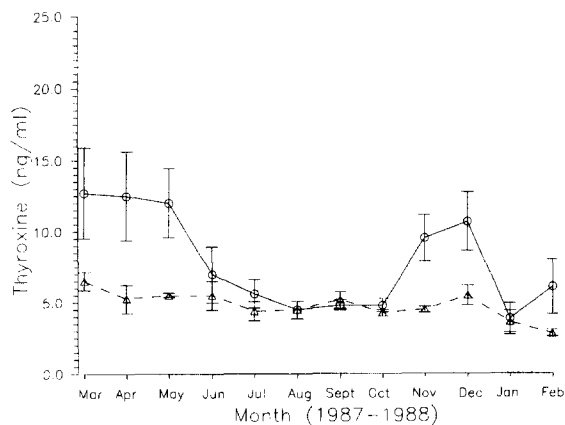


Fig. 4. The T_4 levels of females (○) and males (△) of captive adult Kemp's ridley sea turtles, *Lepidochelys kempi*, at Sea Arama through 12 months. Vertical bars represent standard errors of the mean.

Table 1. Summary of serum T_3 levels (ng/ml) in wild and captive sea turtles.

Species		Wild	Captive
green	immature	0.0 - 0.3	0.5 - 3.9
	adult	0.0 - 0.3	NA
loggerhead	immature	0.0 - 0.4	NA
	adult	NA	NA
olive ridley	adult	0.0 - 0.9	NA
black	adult	0.1 - 1.7	NA
Kemp's ridley	immature	NA	0.0 - 0.6
	adult	NA	0.0 - 0.4

* NA: data not available

mined for only two animals.

Plasma T_3 was non-detectable (lower than 0.5 ng/ml) throughout the study periods in immature green and loggerhead turtles (Table 1). However, wild olive ridleys (up to 0.9 ng/ml) at Oaxaca and black turtles (up to 1.7 ng/ml) at Michoacan, Mexico, showed slightly higher T_3 levels than immature green and loggerhead turtles. While captive immature greens maintained at constant water temperature at around 26°C with artificial diet showed higher T_3 levels (up to 3.9 ng/ml) than wild turtles, captive Kemp's ridley turtles had plasma T_3 levels lower than 0.5 ng/ml.

Discussion

Seasonal or annual cycles in serum thyroid hormone levels have been examined in various reptilian species (Bona-Gallo et al., 1980; John-Alder, 1984a; Kar and Chandola-Saklani, 1985; Licht et al., 1985a and 1985b; Naulleau et al., 1987). From many studies of seasonal endocrine cycles of reptiles, it is indicated that the cycles in plasma thyroid hormones may be related to complex causes (Bona-Gallo et al., 1980; John-Alder, 1984a; Kar and Chandola-Saklani, 1985; Licht et al., 1985a; Naulleau et al., 1987). One hypothesis to explain these cycles is that temperature drives the seasonal cycle in plasma thyroid hormone levels. Plasma T_4 levels in captive male green sea turtles showed constant levels throughout the year possibly due to uniform water temperature (Licht et al., 1985b), but in fresh water turtles plasma T_4 increased after emergence from hibernation in response to a rapid rise in temperature (Licht et al., 1985a). Plasma T_4 increased in summer and decreased in winter as squamates hibernate (Kar and Chandola-Saklani, 1985; Naulleau et al., 1987). However, as Sellers et al. (1982) suggested, plasma T_4 levels did not exactly match to environmental temperatures. A simple relationship between environmental temperature and plasma T_4 was also not observed in the present study. Wild immature green turtles exhibited a tendency to increase T_4 as water temperature increased, whereas wild immature loggerhead and captive adult male and female Kemp's ridley turtles did not. Changes in thyroxine levels were

generally low in magnitude (except in captive Kemp's ridleys), indicating that control systems may function to regulate plasma thyroxine within relatively narrow limits. The lack of simple relationship with temperature indicates that other factors may influence thyroxine secretion and utilization.

Another hypothesis to explain the seasonality of serum thyroid hormone levels is that they may be influenced by reproductive state of the animals. In snakes and lizards thyroid hormones appeared to be antigonadotropic in males (Bona-Gallo et al., 1980; Naulleau et al., 1987). In females, thyroid activity was highest during reproduction including vitellogenesis and ovulation (Nilson, 1982). Therefore, it is assumed that thyroid hormones participate in reproduction by interacting with other hormones such as gonadotropic hormones, regulating body metabolism, and modifying sensitivity of tissues to hormones (Haldar-Misra and Thapliyal, 1981; Leatherland, 1985). The possible role of thyroid stimulation in secretion of pituitary hormones involving reproduction was suggested by Haldar-Misra and Thapliyal (1981). In the present study, as seen from the population of Kemp's ridley sea turtles at Sea Arama, Texas, thyroid hormones appear to be involved in reproduction. According to Rostal (1991), the same female Kemp's ridleys were vitellogenic and were observed by laparoscopy to have follicles yolking up during the fall and winter when T_4 increases, but no follicles were observed yolking up during the summer when T_4 levels were low. The winter peak may therefore be due to stimulation of thyroid axis by reproductive steroids in females. A similar thyroid hormone/reproduction relationship was observed in a second group of Kemp's ridley by Rostal (1991) from a different population at Cayman Turtle Farm, Grand Cayman, B.W.I.

A potentially important observation is that captive sea turtles exhibit higher plasma thyroxine levels than do wild ones. This may be ascribed to nutritional effects since as in other classes of vertebrates, such as mammals, birds, and fishes (Eales, 1988), the reptilian thyroid system may also be stimulated by food intake as well as temperature to secrete thyroid hormones. In our observations, Heron Island immature and mature green turtles displayed lower T_4 levels than Cayman greens and Sea Arama ridleys. Wild turtles at Heron Island seem to receive lower nutrients, quantitatively or qualitatively, since their annual growth rate ranged from 0.75 to 1.46 cm/year (in immatures, probably even lower in mature) which is much lower than in captivity (Limpus and Walter, 1980). In general, green turtles in the wild are grazing on marine spermatophytes such as *Thalassia* or algae (Bjondal, 1985). However, greens in captivity are fed high protein pelleted diets which are 50% higher in nitrogen than *Thalassia* (Bjondal, 1985). Cayman male adult green turtles had been fed these pelleted diets and showed higher T_4 level (Licht et al., 1985b). In addition,

our laboratory reared immature green turtles, which have been fed the same diet as that at the Cayman Turtle Farm, showed higher T_4 levels than wild greens from Heron Island (Moon, 1992). Therefore, as shown by Eales (1988), the quantity or quality of diet may influence thyroid system in sea turtles as well as in various vertebrates. Plasma T_3 was non-detectable in most sea turtles examined except some wild mature animals. This suggests that T_4 5' monodeiodinase activity may be low or T_3 clearance rate may be fast in sea turtles. The importance of lower T_3 levels in sea turtle is not certain, but lower T_3 levels may be due to lower circulating T_4 level. In conclusion, changes in plasma thyroid hormone levels in sea turtles may be influenced by temperature, reproduction, and nutritional state. Even though a full year's cycle of T_4 was not available for immature turtles, the relatively short sampling period indicated that in most cases, changes in thyroxine levels in immatures are of low magnitude. In contrast, adult female turtles showed a distinct annual change in thyroid hormones indicating that the effect of reproductive state may override that of temperature on thyroid activity. However, the reproductive state of subject animals needs to be described in detail for better elucidation of the interaction between thyroid hormones and reproduction. Therefore, more research on the relationship between temperature and thyroid function will be of interest for the future.

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