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Reproductive Physiology of Nesting Leatherback Turtles (*Dermochelys coriacea*) at Las Baulas National Park, Costa Rica

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ABSTRACT. – **The reproductive physiology of nesting leatherback turtles (*Dermochelys coriacea*) was studied at Playa Grande, Costa Rica, from 1992 to 1994 during November, December, and January. Ultrasonography indicated that 82% of nesting females had mature preovulatory ovaries in November, 40% in December, and 23% in January. Mean follicular diameter was 3.33 ± 0.02 cm and did not vary significantly through the nesting season. Plasma testosterone and estradiol levels measured by radioimmunoassay correlated strongly with reproductive condition. No correlation, however, was observed between reproductive condition and plasma progesterone levels. Testosterone declined from 2245 ± 280 pg/ml at the beginning of the nesting cycle to 318 ± 89 pg/ml at the end of the nesting cycle. Estradiol declined in a similar manner. Plasma calcium levels were constant throughout the nesting cycle. Vitellogenesis appeared complete prior to the arrival of the female at the nesting beach. *Dermochelys coriacea* is a seasonal nester displaying unique variations (e.g., 9 to 10 day internesting interval, yolkless eggs) on the basic chelonian pattern. Endocrine and ovarian patterns were similar to sea turtle species of the family Cheloniidae.**

KEY WORDS. – **Reptilia; Testudines; Dermochelyidae; *Dermochelys coriacea*; sea turtle; reproduction; ultrasonography; endocrinology; gonadal hormones; plasma calcium; vitellogenesis; Costa Rica**

The leatherback turtle (*Dermochelys coriacea*) is the largest extant turtle species and belongs to the monophyletic family Dermochelyidae. *Dermochelys coriacea* displays many unique characteristics relative to other sea turtle species, both with respect to its gross anatomy (Carr, 1952), diving behavior (Eckert and Eckert, 1989), thermal biology (Frair et al., 1972; Greer et al., 1973; Mrosovsky, 1980), and nesting ecology (Tucker and Frazer, 1991, 1994). The nesting ecology of *D. coriacea* is unusual in that females have been observed to nest up to ten times in a single nesting season, display the shortest internesting interval of any sea turtle (mean 9 to 10 days), and produce small clutches relative to their large body size (Hirth, 1980; Tucker and Frazer, 1991). In addition to the above, it is unique in producing numerous yolkless eggs with each clutch. Various functions for yolkless eggs have been proposed, such as thermal buffering (Frazier and Salas, 1984), satiation of nest predators (Hirth, 1980), and increased gas exchange in the nest (Pritchard and Trebbau, 1984), however, their function still remains a mystery.

The reproductive physiology of several cheloniid sea turtle species has been studied (*Chelonia mydas*, Owens, 1976, Licht et al., 1979, Wibbels et al., 1992; *Lepidochelys olivacea*, Licht et al., 1982; *Caretta caretta*, Wibbels et al., 1990, Wibbels et al., 1992; and *Lepidochelys kempi*, Rostal, 1991). Seasonal and nesting endocrine cycles for these species display similar patterns. The reproductive functions of luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, estradiol, progesterone, and plasma calcium are relatively well understood for most members of the family Cheloniidae. The present study was undertaken to collect baseline data on the reproductive physiology of *D. coriacea*, as well as to compare the reproductive physiology of *D. coriacea* with that of other sea turtle species studied to date.

MATERIALS AND METHODS

Study Area. — We studied adult nesting *D. coriacea* during the 1992–93 and 1993–94 nesting seasons at Las Baulas National Park, Costa Rica. The national park encompasses three beaches utilized by the nesting turtles: Playa Grande (the primary beach), Playa Ventanas, and Playa Langosta. Movement between the three beaches by nesting females has been documented by flipper tag studies from 1991 to 1994 (Steyermark et al., 1996), so we sampled turtles at all three beaches during the course of the study and considered these turtles as members of the same nesting population. We used ultrasonography as a non-invasive procedure to determine the reproductive condition and ovarian state of the female at the time of nesting. Blood samples were collected for hormonal and plasma calcium analyses.

Ultrasonography. — We used a portable Aloka 500V B-mode real-time ultrasound scanner with 3.5 MHz convex linear transducer (Corometrics Inc., Connecticut). A portable Coleman 1500 watt generator provided power. A permanent record of all observations was made using a Sony video graphic printer (Classic Medical Supply, Florida). When we located a female either emerging from the ocean

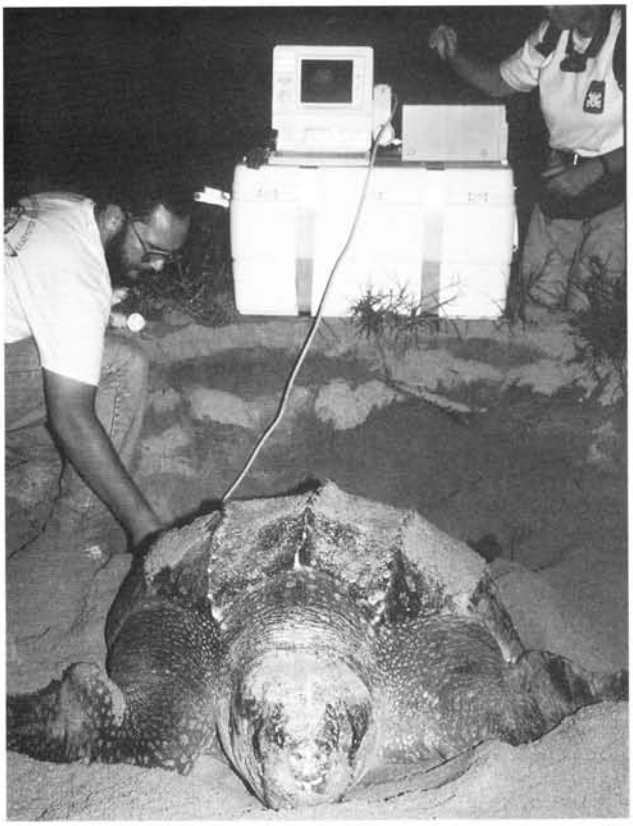

Figure 1. Photo of non-invasive ultrasound scanning procedure on a nesting female leatherback turtle (*Dermochelys coriacea*) at Las Baulas National Park, Costa Rica. The ultrasound equipment was placed behind the female and the 3.5 MHz convex linear transducer was positioned in the inguinal region.

or in an early stage of nesting (either digging a body pit or beginning to dig an egg chamber), we quietly moved the ultrasound scanner and thermal printer into position directly behind the female (Fig. 1). We conducted the scan while the female was depositing eggs into the egg chamber and the flippers were not moving. There were approximately 15 minutes during which a female could be successfully scanned. Each ovary and oviduct was scanned individually. Water-soluble coupling gel provided good contact in the inguinal region cranial to the hindflipper and enhanced imaging. Since osteoderms of the carapace block ultrasound waves, the "acoustic window" was limited to the inguinal area. We were able to identify oviductal eggs (both yolked and yolkless), vitellogenic ovarian follicles, and atretic follicles (Fig. 2). We scanned and measured oviductal eggs and ovarian follicles using the internal electronic calipers (accurate to ± 0.1 cm) as described in Rostal et al. (1990). The ovarian state or reproductive condition of the female was classified according to the following criteria:

Mature Ovary (Early Preovulatory): multiple, tightly grouped, large preovulatory vitellogenic follicles measuring > 3.0 cm in diameter; no atretic follicles observed.

Intermediate Ovary (Late Preovulatory): multiple, loosely grouped, large preovulatory vitellogenic follicles measuring > 3.0 cm in diameter; atretic follicles observed in some females; coelomic space also observed at the apex of the ovary.

Depleted Ovary (Postovulatory): fewer than ten large, preovulatory vitellogenic follicles observed per ovary; multiple atretic follicles observed in most females.

We scanned a total of 35 females using ultrasonography. Up to 8 randomly chosen follicles (3 to 4 per ovary) were measured in females with mature or intermediate ovaries.

Blood Collection and Hormonal Analysis. — We collected blood samples following the completion of nesting. After the female completed covering and camouflaging the nest site, we either manually restrained her or captured her in a cargo net as she returned to the sea. Samples were obtained within five minutes of capture and females were held for no longer than a total of ten minutes. Blood samples were obtained from the cervical sinus using a procedure similar to that of Owens and Ruiz (1980) with a 15.24 cm 18 gauge spinal tap needle and a syringe. Blood samples were stored in lithium heparin vacutainers on ice until they were centrifuged. We removed plasma from the red blood cells after centrifugation and stored it in liquid nitrogen. Altogether, 31 blood samples were used in the analysis. Blood samples which were diluted with lymphatic fluid (as determined by lowered hematocrits, i.e., below 20%) were not used.

We measured plasma testosterone and progesterone levels using an H^3 radioimmunoassay test as described by Wibbels et al. (1990). For testosterone and progesterone, 10 and 100 μl, respectively, of plasma was extracted using anhydrous ether. Samples were run in duplicate. Extraction efficiencies for testosterone and progesterone averaged 93.2% and 64.7%, respectively. Sensitivity of the testosterone and progesterone assays were 1.5 and 21 pg/tube, respectively. Intra-assay coefficients of variation for testosterone and progesterone assays were 3.3% and 2.4%, respectively, and inter-assay coefficients of variation were 15.2% and 19.6%, respectively.

We measured plasma estradiol levels using an iodine kit (Diagnostic Products Corp., Los Angeles, California). For estradiol, 100 μl of plasma was extracted using anhydrous ether. Samples were run in duplicate. Extraction efficiencies for estradiol averaged 99.1%. Sensitivity of the estradiol assay was 0.1 pg/ml. Intra-assay coefficient of variation for the estradiol assay was 5.1% and inter-assay coefficient of variation was 13.6%.

We measured total plasma calcium (an indicator of vitellogenesis) to determine if follicular maturation was occurring during the nesting season. Calcium levels were measured using a VetTest 8008 Blood Chemistry Analyzer (IDEXX Laboratories, Inc., Maine).

Statistical Analyses. — We identified significant changes in plasma testosterone, progesterone, estradiol, and calcium levels using a one-way ANOVA followed by a Student-Newman-Keuls test ($P \leq 0.05$). Linear regression analysis was used to test the relationship of mean follicle diameter vs. female carapace length ($P \leq 0.05$).

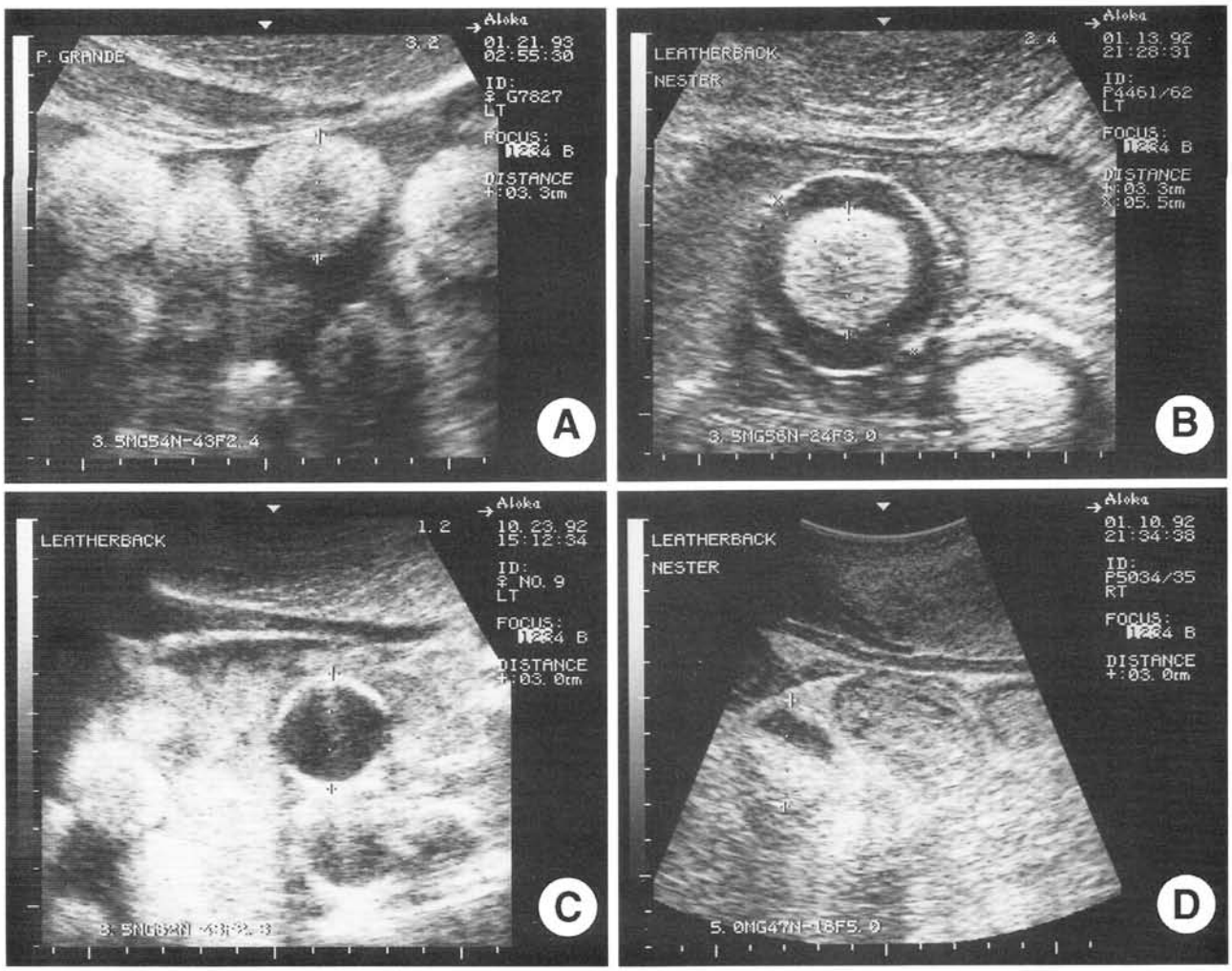

Figure 2. Ultrasonography of leatherback turtle (*Dermochelys coriacea*) reproductive structures. **A.** Ultrasound image of large preovulatory vitellogenic follicles (3.3 cm diameter) in a female with mature ovaries. **B.** Ultrasound image of a shelled oviductal egg at the time of nesting showing a well defined yolk (3.3 cm diameter) and calcified shell (5.5 cm diameter). **C.** Ultrasound image of an oviductal yolkless egg (3.0 cm diameter) showing a dark anechoic center. **D.** Ultrasound image of an atretic follicle (3.0 cm diameter) in a female with depleted ovaries. Note the absence of vitellogenic follicles in the image.

RESULTS

Ovarian Cycle. — Nesting females began arriving at Las Baulas in October and continued to nest up to March of the following year. Using ultrasonography, we were able to determine the reproductive condition of a subset of nesting females through the major portion of the season (November, December, and January; Fig. 3). The majority of females scanned in November displayed mature ovaries (82%). In December, there were fewer females displaying mature ovaries (40%) and an increase in females displaying intermediate ovaries (60%). By January, the number of females displaying either mature or intermediate ovaries (23% each) had continued to decrease while females displaying depleted ovaries at the end of their nesting cycle had increased (54%).

Preovulatory vitellogenic follicles occurred throughout the season. Mean follicular diameter did not appear to vary during the nesting season (November = 3.25 ± 0.06, SE, n = 10; December = 3.32 ± 0.02, SE, n = 9; January = 3.36 ± 0.06, SE, n = 5; F = 1.42, df = 2,21, P = 0.2648, not significant). Mean follicular diameter per female ranged from 2.95 to 3.55 cm with an overall mean diameter of 3.33 ± 0.02 cm (SE, n = 30; Fig. 4). We did not observe enlarging previtellogenic follicles in any females. We did observe atretic follicles in females with intermediate or depleted ovaries. No correlation was observed between follicle size and carapace length (F = 0.322, df = 1,28, P = 0.546, not significant; Fig. 5), however, it is interesting to note that the smallest follicles were seen in the smallest female observed (curved carapace length = 125 cm).

We observed both yolked and yolkless eggs in the oviduct during nesting with ultrasonography. Yolked eggs ranged from 5.0 to 5.6 cm in diameter with mean diameter of 5.31 ± 0.06 cm (SE, n = 16). Yolk diameters ranged from 3.2 to 3.8 cm with a mean diameter of 3.53 ± 0.04 (SE, n = 20). Mean yolk diameter was similar to preovulatory vitellogenic follicles (3.33 ± 0.02 cm, SE, n = 30). We observed that yolkless eggs were the last structures to be produced in the oviduct and were not interspersed between the yolked eggs while in the oviduct. Yolkless eggs are, however, inter-

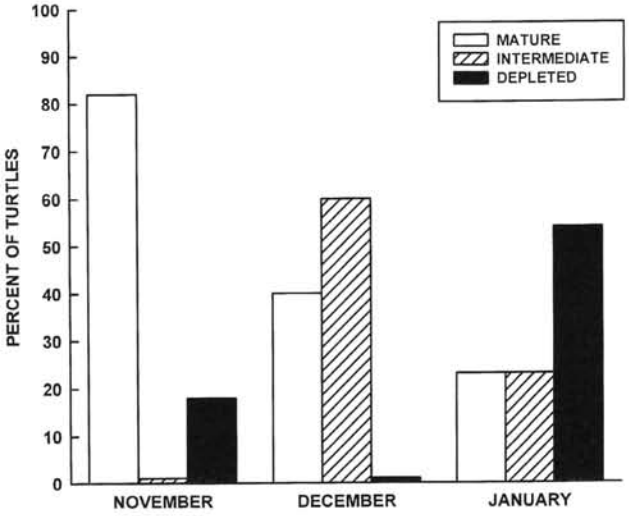

Figure 3. Reproductive condition of leatherback turtles nesting at Las Baulas National Park, Costa Rica, during the 1992–93 and 1993–94 nesting seasons. Results of ovarian scans during November (n = 11), December (n = 9), and January (n = 13).

spersed among eggs in the nest. This may be the result of one oviduct releasing its eggs prior to the completion of egg deposition by the other oviduct.

Plasma Testosterone, Progesterone, and Estradiol. — Circulating gonadal steroid levels varied over the course of the nesting season (Fig. 6). When compared with the reproductive condition of the female as determined by ultrasonography, endocrine patterns were elucidated (Fig. 7). Plasma testosterone levels declined significantly ($F = 14.9$, df = 2,23, $P < 0.0001$) over the course of the nesting cycle from elevated levels in females at the beginning of their nesting cycle when their ovaries were mature (2245 ± 280 pg/ml, SE, n = 13) to intermediate levels midway through their nesting cycle (1289 ± 166 pg/ml, SE, n = 6) to low levels in females displaying depleted ovaries at the end of their nesting cycle (318 ± 89 pg/ml, SE, n = 7). Plasma estradiol levels declined in a similar fashion over the course of the nesting cycle ($F = 9.69$, df = 2,23, $P < 0.0009$). Estradiol levels were highest in females displaying mature ovaries (53.30 ± 6.54 pg/ml, SE, n = 13), intermediate in females midway through their nesting cycle (28.70 ± 4.78 pg/ml, SE, n = 6) and lowest in females displaying depleted ovaries at the end of their nesting cycle (16.50 ± 4.11 pg/ml, SE, n = 7). Plasma progesterone levels were highly variable between individuals (ranging from < 10 to 1793 pg/ml) and were not correlated with reproductive condition ($F = 3.04$, df = 2,23, $P = 0.0675$, not significant).

Plasma Calcium. — Plasma calcium levels (an indicator of vitellogenesis) were not correlated with reproductive condition or ovarian state during the nesting season ($F = 0.0367$, df = 2,23, $P = 0.964$, not significant). Plasma calcium levels remained relatively constant (mature ovary: 8.10 ± 0.84 mg/dl, SE, n = 13; intermediate ovary: 8.18 ± 1.29 mg/dl, SE, n = 6; depleted ovary: 7.75 ± 1.32 mg/dl, SE, n = 7), while circulating plasma testosterone levels were observed to decline over the course of a female's nesting cycle (Fig. 8).

DISCUSSION

The leatherback turtle (*D. coriacea*) is a seasonal nester. Females nest up to ten times in a single nesting season (Tucker and Frazer, 1991). Although some *D. coriacea* nest during most months at Las Baulas, the main nesting season begins in September or October (Steyermark et al., 1996). At the time of arrival, the females display mature ovaries containing hundreds of large, preovulatory vitellogenic follicles. As each female repeatedly nests during the season, follicles are ovulated from the ovaries, and a gradual decrease in the number of follicles and size of the ovaries occurs. Concurrent with this decline in the number of follicles and size of the ovaries, a decline in circulating testosterone and estradiol occurs.

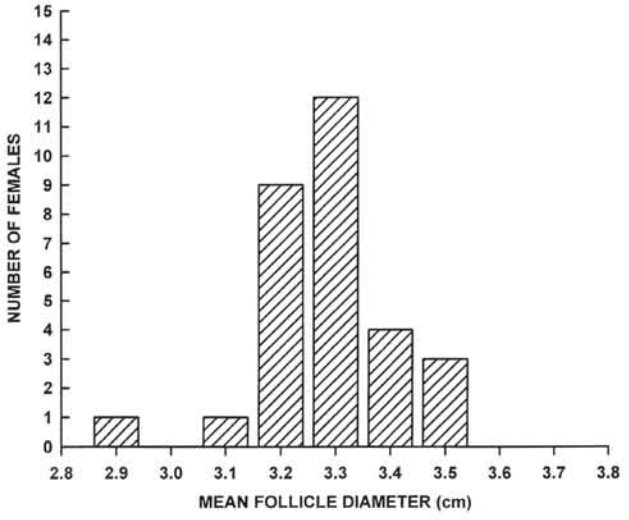

Figure 4. Histogram of mean follicle size classes (n = 30) observed for 23 female leatherback turtles at Las Baulas National Park, Costa Rica, during the 1992–93 and 1993–94 nesting seasons. Three to eight vitellogenic follicles were measured per female.

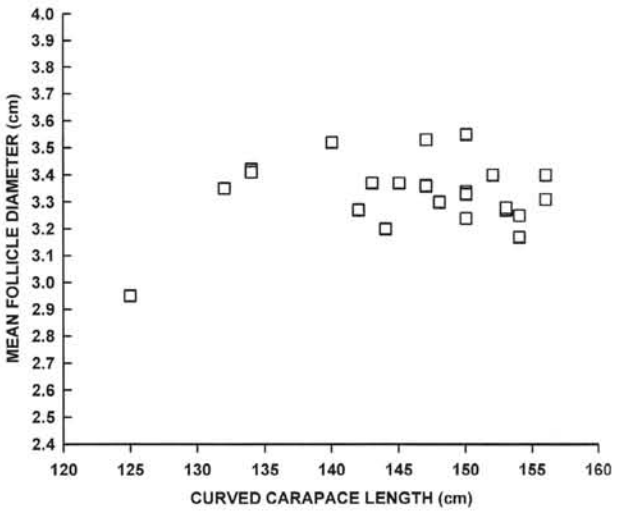

Figure 5. Mean follicle diameter plotted vs. the curved carapace length of leatherback turtles at Las Baulas National Park, Costa Rica, during the 1992–93 and 1993–94 nesting seasons. Three to eight vitellogenic follicles were measured per female.

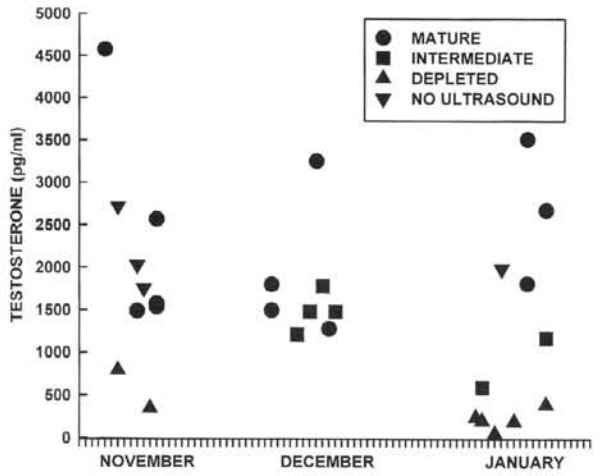

Figure 6. Plasma testosterone levels of leatherback turtles at Las Baulas National Park, Costa Rica, during the 1992–93 and 1993–94 nesting seasons.

Over the course of the nesting season, circulating testosterone, estradiol, and progesterone levels showed similar patterns to those of other sea turtle species. Testosterone levels declined as ovarian size decreased in a similar fashion to that observed in *C. mydas* (Licht et al., 1979), *C. caretta* (Wibbels et al., 1990), and *L. kempi* (Rostal, 1991). Estradiol levels also declined slightly during the nesting season and were relatively low similar to those observed in *C. mydas* and *C. caretta* during the nesting season (Licht et al., 1979; Wibbels et al., 1990). The physiological and behavioral functions of testosterone and estradiol are only partially understood in sea turtles. Testosterone may function in stimulating mating and nesting behavior in sea turtles (Licht et al., 1979; Rostal, 1991). Circulating testosterone levels were observed to peak during the mating period prior to nesting in captive female *C. mydas* (Licht et al., 1979) and *L. kempi* (Rostal, 1991). Estradiol has been demonstrated to stimulate vitellogenesis in other reptiles (Ho, 1987). Licht et al. (1979) reported elevated estradiol levels in captive *C. mydas* only during the prebreeding period when follicular maturation was occurring in the ovary. Progesterone levels did not vary greatly during the nesting season, but this could be due to our sampling of females within 30 minutes post-nesting. Progesterone is associated with ovulation and increases sharply 24–48 hours post-nesting in other sea turtles (*L. olivacea*, Licht et al., 1982; *C. mydas* and *C. caretta*, Licht et al., 1979, Wibbels et al., 1992). If we had been able to capture females at this later time, progesterone levels may have been elevated.

Circulating levels of testosterone and estradiol were higher overall in *D. coriacea* compared to those reported for other sea turtles (Licht et al., 1979; Wibbels et al., 1990; Rostal, 1991). However, some of the highest gonadal steroid levels measured in turtles have been observed in relatively small species (e.g., desert tortoise, *Gopherus agassizii*, Rostal et al., 1994; stinkpot turtle, *Sternotherus odoratus*, McPherson et al., 1982). Factors that influence overall circulating steroid levels in different taxa are unclear.

Vitellogenesis appears complete prior to the arrival of the female at the nesting beach. We did not observe evidence of multiple vitellogenic periods during the nesting season. Multiple size classes of vitellogenic follicles were not observed using ultrasonography nor did plasma calcium levels vary relative to ovarian condition. In contrast, multiple size class follicles have been observed using ultrasonography in the desert tortoise, *G. agassizii*, during vitellogenesis as well as the nesting season (Rostal et al., 1994). Increases in total calcium levels have been correlated with vitellogenesis (Ho, 1987) and ovarian follicular growth in a variety of reptiles: cobra, *Naja naja* (Lance, 1976); painted turtle, *Chrysemys picta* (Callard et al., 1978); American alligator, *Alligator mississippiensis* (Lance, 1987; Lance et al., 1983); Kemp's ridley sea turtle, *L. kempi* (Rostal, 1991); desert tortoise, *G. agassizii* (Rostal et al., 1994); and tuatara, *Sphenodon punctatus* (Cree et al., 1991). In captive *L. kempi*, plasma calcium levels rose from 8.64±0.65 mg/dl in non-vitellogenic females to 20.18 ± 1.27 mg/dl in vitellogenic females, a twofold increase (Rostal, 1991). During this study, plasma calcium levels remained relatively constant throughout the nesting season.

Clutch size has been correlated with female size in most sea turtle species (Hirth, 1980; Van Buskirk and Crowder, 1994), but Hirth (1980) noted that mean nesting female size

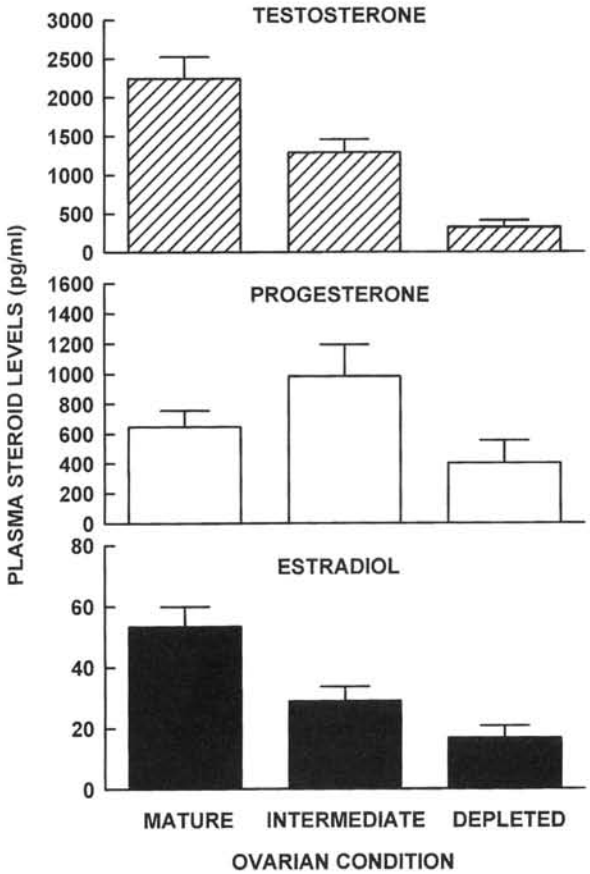

Figure 7. Mean plasma testosterone, progesterone, and estradiol levels plotted relative to ovarian condition based on ultrasonography results for leatheracks at Las Baulas National Park, Costa Rica, during the 1992–93 and 1993–94 nesting seasons. Values are means ± SE.

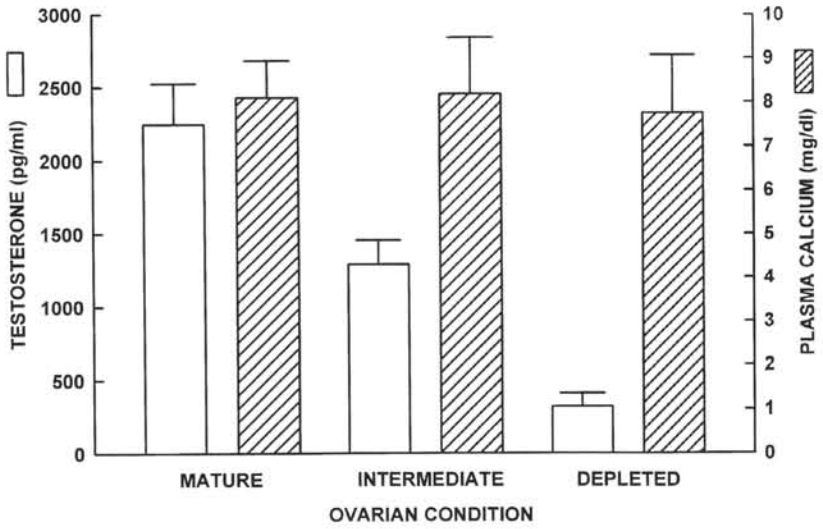

Figure 8. Mean plasma testosterone and calcium levels plotted relative to ovarian condition based on ultrasonography for leatheracks at Las Baulas National Park, Costa Rica. Values are means ± SE (mature, $n = 13$; intermediate, $n = 6$; depleted, $n = 7$).

was not correlated with mean hatchling size for *C. mydas* populations. The relationship of follicle size to female size has not been studied. The lack of correlation between follicle size and female size observed in *D. coriacea* is not unexpected. A set amount of yolk is required to produce a viable hatchling with a yolk reserve. Congdon (1989) noted that larger hatchlings do not directly equate to increased fitness. Therefore, larger females should not necessarily produce larger follicles or larger hatchlings. While *D. coriacea* may be capable of producing larger eggs, selection appears to favor larger numbers of smaller eggs as well as an increased number of nests. Interestingly, the flatback turtle, *Natator depressa*, does produce smaller clutches (fewer than 50 eggs) and larger hatchlings relative to other sea turtles (Hirth, 1980). Hirth (1980) suggests that the larger hatchling size should offset the lower number of hatchlings produced and have similar survival value. Factors influencing hatchling size and survival need further research.

A selective advantage for yolkless eggs remains to be demonstrated. It is possible that these yolkless eggs may not have a function. The size and number of yolkless eggs is highly variable in contrast to the uniform size and number of yolked eggs. Studies of nest success have not demonstrated an advantageous function for these yolkless eggs to date (Eckert, 1987; Hirth and Ogren, 1987; Eckert and Eckert, 1990; Leslie et al., 1996; Steyermark et al., 1996). Further studies on these unique eggs are needed to determine energy investment and potential cost to reproduction.

The reproductive physiology of the leatherback turtle, *D. coriacea*, is similar to that of other sea turtles with respect to overall seasonal pattern. Testosterone, progesterone, and estradiol appear to have similar circulating patterns to those observed in other sea turtles; however, further research is needed to clarify their physiological functions. While this paper has concentrated only on nesting females, there is still a need for data on leatherback turtles at sea, and in particular, male leatherback turtles.

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