



SEASONAL REPRODUCTIVE CYCLE OF THE

KEMP'S RIDLEYS SEA TURTLE (*Lepidochelys kempfi*)

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MATERIALS AND METHODS

RESULTS

DISCUSSION

ACKNOWLEDGMENTS

REFERENCES

The seasonal reproductive cycle of the Kemp's ridley sea turtle (*Lepidochelys kernpi*) was studied under semi-natural conditions at the Cayman Turtle Farm, Grand Cayman, British West Indies, from June 1987 to July 1988. Male *L. kempfi* displayed a prenuptial rise in serum testosterone 4 to 5 months prior to the mating period (March). Male testosterone then declined sharply during the mating period. Female *L. kempfi* also displayed a prenuptial rise in serum testosterone, estradiol, and total calcium 4 to 6 months prior to the mating period (March). Female testosterone and estradiol declined during the nesting period (April to July) immediately following the mating period (March). Elevated levels in female estradiol and total calcium corresponded with the period of vitellogenesis as determined from gel electrophoresis and ultrasonography. Serum thyroxine also fluctuated seasonally with elevated levels observed in females associated with the period of vitellogenesis. *L. kempfi* displayed a distinct seasonal reproductive cycle in captivity. Nesting in the captive study group corresponded with nesting in the wild population at Rancho Nuevo, Mexico (April to July). Female endocrine cycles during the nesting period were similar to those observed in the wild population.

Key Words: Reptilia; Testudines; Cheloniidae; *Lepidochelys kempfi*; reproduction; ultrasonography; endocrinology; gonadal steroids; vitellogenesis.

Among the sea turtles, the reproductive physiology of four species (the green turtle *Chelonia mydas*; the olive ridley, *Lepidochelys olivacea*; the loggerhead *Caretta caretta* and the leatherback turtle, is partially understood (Owens, 1980; Licht *et al* 1982 . Owens and Morris, 1985; Wibbels *et al.*, 1990 Rostal *et al* 1996; Whittier *et al.*, 1997). The best model available to date for reproductive endocrinology is for captive female *C. mydas* from the Cayman Turtle Farm, Grand Cayman, B. W. I. (Licht *et al.*, 1979) The functions of androgens and progestins in the female sea turtle are only partially understood. As well, circannual endocrine patterns are known for only two species (Licht *et al.*, 1979) and *C. caretta* (Wibbles *et al* 1990) and the reproductive behaviour other than nesting activity is largely unstudied.

The reproductive behaviour of *C. Mydas* has received the most attention. The most detailed account is that of Booth and Peters (1972) in which wild adult *C. mydas* were observed during the mating in the waters off Australia and reproductive behaviours were described and documented. Most studies, including Booth and Peters (1972), however, lacked quantitative measurements. Captive female *C. Mydas* at the Cayman Turtle Farm appear to display a "heat" period of 2-4 days when the majority of mating occurs for a particular female (Wood and Wood, 1980) Commuzie and Owens (1990) further studied *C. Mydas* at the, Cayman Turtle Farm during the mating season and observed that male *C.mydas* were capable of distinguishing receptive females from nonreceptive females but the mechanism was unclear.

The physiological and behavioral role of testosterone in male and female sea turtles has been studied in *C. mydas*

(Licht *et al.*, 1979, 1985b). Questions, however, still remain regarding the seasonal interaction of these systems (i.e., does testosterone have a behavioral role in the female, what is the relationship between testosterone and thyroxine in both males and females, and how may testosterone influence male reproductive behavior). The behavioural data of Comuzzie and Owens (1990) was not analyzed in relation to testosterone levels. Thus, a model for the interaction of testosterone and behaviour has not been well developed for the sea turtle.

Thyroxine has been implicated in reptilian reproduction with increased thyroid activity associated with yolk deposition, mating, and/or ovulation (Leatherland, 1987). Evidence from turtles, particularly sea turtles, is largely lacking. Licht *et al* (1985b) measured plasma thyroxine (T₄) in captive male *C.mydas* and noted that it remained uniform throughout the year. In contrast, Wibbels *et al* (1986) reported a rise in serum T₄ of female *C. caretta* prior to migration although the results were preliminary. Male freshwater painted turtles (*Chrysemys picta*) are reported to display a rise in T₄ following mating and appears inversely correlated with testosterone (Licht *et al.*, 1985a). The presence of a group of reproductively mature Kemp's ridley sea turtles (*Lepidochelys kempi*) in captivity provided the opportunity to investigate the seasonal reproductive pattern of a third genus and species as well as study the interaction of gonadal hormones and behavior. *L. kempi* is also the most endangered species of sea turtle; however, virtually nothing is known regarding its reproductive biology.

MATERIALS AND METHODS

Subjects

A captive group of 35 *L. Kempii* was maintained at the Cayman Turtle Farm (CTF), Grand Cayman, Cayman Islands, B. W. I., under semi-natural conditions. Twenty-eight turtles (18 males and 10 females) were 8 years old, three turtles (3 females) were 7 years old and four turtles (2 males and 2 females) were 5 years old. The sex ratio of the group was 1.33 males to 1.00 females, mean body weight was 24.9 ± 0.6 kg (February 1997) and all turtles were sexually mature. The turtles were maintained in a 9 x 21 m section of the main CTF breeding pond. Depth ranged from 0.0 m at the nesting beach to a maximum depth of 2.8 m (Wood and Wood, 1988). The nesting beach was available year round and turtles were exposed to natural photoperiod and weather conditions. The main CTF breeding pond holds approximately 3.7×10^6 litres of sea water and fresh sea water was circulated through the pond at a rate of 34,000 liters per hour. Temperature is reported to range from 26°C in January to 31°C in August (Licht *et al*, 1979). Natural photoperiod varies ; from 11L: 13D in January/February to 14L:10D in July/August (Licht *et al.*, 1985b). The group of Turtles was fed approximately 5 kg of modified Purina Trout Chow pellets twice daily (0730 and 1530 h)

Observations

Reproductive behavioral data were collected using continuous recording observation techniques (Martin and Bateson, 1986). Total frequency of reproductive behaviors (courtships and mounts) was recorded on a checksheet and the time of occurrence of the behaviours was recorded. The sex and individual identification numbers of interactants (initiator/receiver) were also recorded. Individual turtles were identified using hind- flipper tags and individual characteristic markings (shell deformities and flipper scars etc). when possible. The percentage of active males and females (swimming, moving, and/or feeding versus stationary on the bottom of the pond) was also recorded as an index of overall activity levels during each observation period.

Observations were conducted during morning (0800h - 1100 h) and evening (1600-1900 h) periods following feeding periods. Observation periods were based on a pilot study during March 1987 to determine periods of peak diurnal activity. Observations werer conducted for 60 h per sampling month (June 1987, September 1987, December 1987, March 1988, May 1988 and July 1988) for a total of 360 hours observation time.

Blood Sampling

Nonheparinized blood samples (15 ml) were collected from the cervical sinus using a 3.8-cm 21 gauge needle, needle holder, and a sterile vacuum tube (Owens and Ruiz, 1980). Twenty eight-year-old turtles (10 males and 10 females) were sampled between 0800 and 1200 h during the months listed above. Blood samples were also collected during the general farm censusing during February 1988. The same turtles were sampled each time. Blood samples were also collected from nesting females during April, May, and June. Samples were centrifuged for 15 mm at 2-3000 rpm and serum was frozen. Serum samples were analyzed using radioimmunoassay (see below).

Ultrasonography

Ten 8-year-old females were scanned using ultrasonography during March 1988 (mating) and July 1988 (postnesting) to determine the reproductive status. The procedure was outlined in Rostal *et al.* (1990). Two mechanical sector scanners (Microimager 1000, Ausonics Corp., WI, and an ATL 4600, Advanced Technology Laboratories, Inc., WA) with 5.0 MHZ transducers were used in the study. The turtle was placed in dorsal

recumbency, and the transducer was positioned in the inguinal regional cranial to the hindlimb. Ultrasound waves do not penetrate bone or the heavily keratinized shell, limiting the available acoustic windows. Both sides were scanned because both ovaries could not be imaged simultaneously. K-Y Jelly (Johnson & Johnson Products, Inc., NJ) or other suitable material was used as a coupling gel. No anesthesia was required, and the turtle required minimal restraint. Imaged structures were electronically measured with built-in electronic calipers.

Oviductal eggs, vitellogenic follicles, and atretic follicles were identified using ultrasonography. Oviductal eggs were identifiable by an echoic ring (shell) around an anechoic ring (albumen) and an echoic yolk (Rostal *et al.*, 1990). Vitellogenic follicles were identified based on size (>1.5 cm diameter) and echoic image (Rostal *et al.*, 1990). Atretic follicles were identifiable by an echoic cortex and an anechoic core (Rostal *et al.*, 1990). Ovaries were classified as preovulatory versus postovulatory based on the overall image of the ovary. In a preovulatory ovary, vitellogenic follicles were readily scanned and four to six follicles per female were measured as an estimate of follicle size. In a postovulatory ovary, vitellogenic follicles were not observed, small previtellogenic (1.0-1.5 cm diameter) and atretic follicles were present, and intestinal loops were readily imaged. The oviduct is only discernible from intestine when it contains shelled egg.

Steroid Analysis

Serum testosterone and progesterone were measured using a H₃ radioimmunoassay as described in Wibbels *et al.* (1990). For male testosterone 10 and 100 μ l of serum were extracted using female testosterone and progesterone, 259 and 500 μ l of serum was extracted using anhydrous ether respectively. Samples were run in duplicate. Extraction efficiencies for testosterone and progesterone averaged 78.7 and 64.7%, respectively. Sensitivity of the testosterone and progesterone assays were 2.3 and 21 pg/tube, respectively. Intraassay coefficients of variation for testosterone and progesterone assays were 4.8 and 2.4%, respectively, and interassay coefficients of variation for testosterone and progesterone assays were 15.0 and 19.6% respectively.

Serum estradiol was measured using a Iodine kit provided by Diagnostic Products Corp. Los Angeles, CA. For estradiol, 100 μ l of serum 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/tube. Interassay coefficient of variation for the estradiol assay was 5.1% and interassay coefficients of variation for estradiol assay was 13.6%.

Serum Calcium

Total calcium was monitored as an indicator of vitellogenesis. Serum total calcium was highly correlated with increased blood vitellogenin in captive *L. kempfi* (Heck *et al.*, 1990, 1997). Serum total calcium was measured by flame atomic absorption spectrophotometry using a SpectrAA-20 atomic absorption spectrophotometer (Varian Techtron Pty. Ltd. Australia) Serum was diluted (1:26) in a 1% lanthanum oxide 1 (La₂O₃) solution at room temperature. Total calcium (μ g/ml) was compared to a stand curve (1,5,10,20, μ g /ml, Fisher Scientific AA Standard) at the time of assay.

Thyroxine Radioimmunoassay

Serum T₄ was measured by radioimmunoassay according to the method of MacKenzie *et al.* (1978) as modified by Denver and Licht (1988). *L. kempfi* serum samples were diluted parallel to T₄ standards in this assay. Average recovery of T₄ from supplemented samples was 94.0%. Blood samples were analyzed at 10-50 μ l volume in the T₄ assay. Preliminary attempts to measure triiodothyronine (T₃) in a similar assay found no detectable levels of T₃ (Moon, 1992)

Vitellogenesis

Vitellogenesis was monitored by 7.5% SDS-polyacrylamide gel electrophoresis as described by Laemml (1970). A female-specific estradiol-inducible band with a molecular weight of approximately 205 kDa was identified for *L. kempfi* (Heck *et al.*, 1990, 1997). Ten microliters of serum was diluted 1:10 in 90 μ l sample buffer with SDS. Samples were run on a 7.5% SDS-polyacrylamide gel. Gels were 17 cm x 17 cm x 1.5 mm in size. Gels were fixed and stained using 0.05% Coomassie brilliant blue stain in 25% methanol-10% acetic acid. The calibration proteins were Bio-Rad high range molecular weight standards (Richmond, CA).

Data Analysis

Seasonal changes in reproductive behaviours, hormone levels, and body weight of both males and females were determined using repeated-measures analysis of variance by ranks ($P \geq 0.05$). Correlations between changes in frequency of reproductive behaviours and hormone levels were determined using Pearson product-moment coefficient (r , $P \geq 0.05$) (Bruning and Kintz 1977). Changes in serum testosterone during the nesting season were determined using nonparametric. Kruskal-Wallis analysis of variance ($P \geq 0.05$; Bruning and Kintz, 1977).

RESULTS

Behaviour

Seasonal changes were observed in reproductive behaviours monitored (courtships and mounts), **Figure 1A**. The frequency of male-female courtship and mounts remained low during September (postnesting) and December (pre mating). During March (mating period), the frequency of behaviours increased significantly (courtships: $F = 10.03$, $df = 5, 54$, $P < 0.001$; mounts: $F = 2.64$, $df = 5, 54$, $P < 0.05$) and then declined sharply during May (nesting) and July (postnesting). Both males and females displayed significant increases in activity levels during March (mating) (males: $F = 98.97$, $df = 5, 114$, $P < 0.001$; females: $F = 8.00$, $df = 5, 114$, $P < 0.001$); males were particularly more active during this period. (**Figure 1B**). Reproductive behaviours and increased activity levels coincided seasonally as would be behaviours.

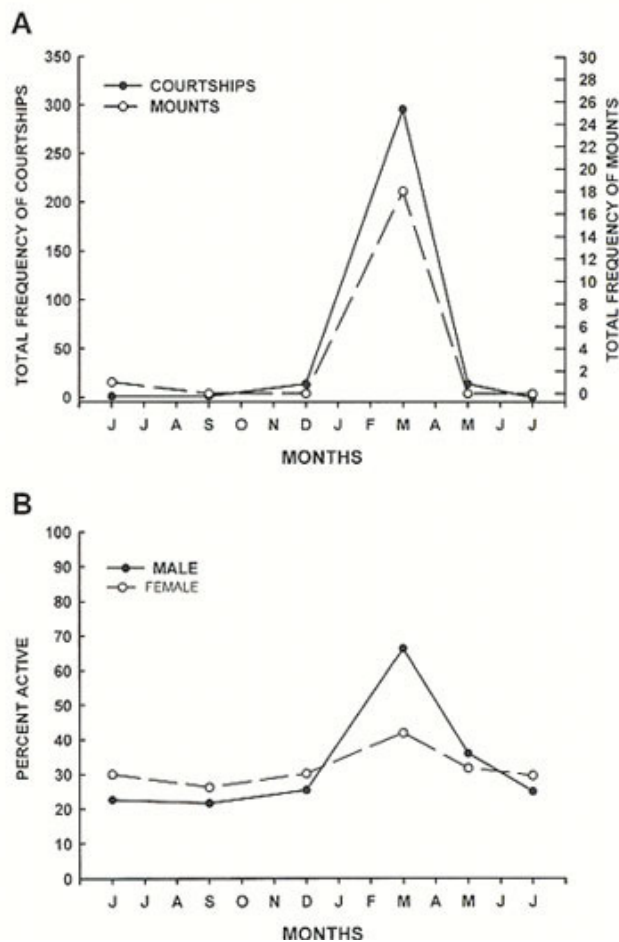


FIG. 1 Seasonal behaviour patterns of Kemp's Ridley sea turtles (*Lepidochelys kempfi*) maintained under semi natural conditions.

A Total frequency of observed courtship and mounting behaviour, and

B Percentage of male female turtles active during months observed.

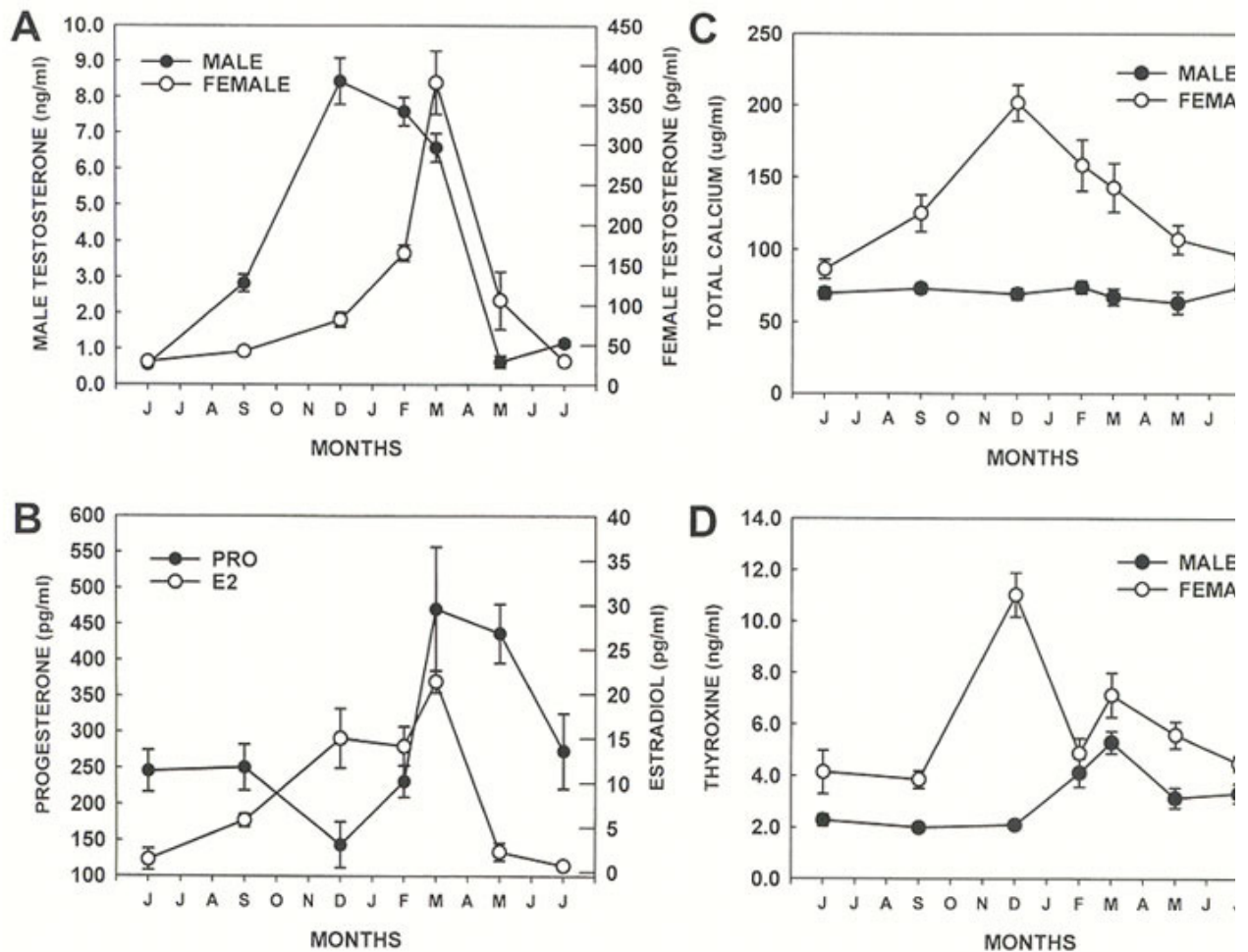
Serum Testosterone, Estradiol, and Progesterone

Seasonal changes were observed in serum testosterone levels of both male and female *L. kempfi*. Male testosterone rose significantly ($F = 99.39$, $df = 6, 63$, $P < 0.001$;) during September (postnesting) to its maximum (mean \pm SE = 8.44 ± 0.65 ng/ml; $n = 10$) during December (pre mating) and remained elevated until the onset of mating activity during March and then declined sharply during May (nesting) to its nadir (mean \pm SE = 0.65 ± 0.16 ng/ml; $n = 10$) (**Fig. 2A**).

Female testosterone remained near basal levels during June (late nesting) and September (postnesting), gradually began to rise during December and February (pre mating), then increased significantly ($F = 90.15$, $df = 6, 63$, $P < 0.001$) to its maximum (mean \pm SE = 378 ± 40 pg/ml; $n = 10$) during March (mating) and then declined during May (nesting) to its nadir (mean \pm SE = 30 ± 4 pg/ml; $n = 10$) in July (postnesting; **Fig. 2A**). Female progesterone remained low during June (late nesting) and September (post-nesting), declined to the lowest levels observed (mean \pm SE = 143.6 ± 31.6 pg/ml; $n = 10$) during December (pre mating), then increased significantly, ($F = 90.15$, $df = 6, 63$, $P < 0.001$) to its maximum (mean \pm SE = 471.3 ± 86.2 pg/ml; $n = 10$) during March (mating), and then declined slowly during May (nesting) into July (postnesting; **Fig 2B**). Female estradiol also remained near basal levels during June (late nesting), then began to rise during September (postnesting) into December and February (pre mating), then increased significantly ($F = 90.15$, $df = 6, 63$, $P < 0.001$) to its maximum (mean \pm SE = 21.4 ± 1.2 pg/ml; $n = 10$)

during March (mating), and then declined during May (nesting) to its nadir (mean \pm SE = 0.7 ± 0.4 pg/ml; $n = 10$) in . (postnesting; Fig. 2B).

FIG. 2. Seasonal cycles for adult male and female Kemp's ridley' sea turtles (*Lepidochelys kempi*) maintained u semi natural conditions (A) Male and female testosterone levels, (B) female progesterone (PRO) and estradiol (levels,(C) male and female total serum calcium levels and (D) male and female thyroxine levels. Values are me SE ($n= 10$).



Serum Calcium

Female serum calcium levels (indicative of vitellogenin) increased significantly ($F = 9.95$, $df = 6, 63$, $P < 0.001$) from June (postnesting, mean \pm SE = 86.42 ± 6.5 μ g/ml, $n = 10$) to December (pre mating, mean \pm SE = 201.79 ± 12.74 μ g/ml, $n=10$) and then declined during March (mating) and May (nesting; Fig. 2C). Female calcium followed a similar pattern of vitellogenesis. Male serum calcium levels remained low and constant ($F = 0.54$, $df = 6, 63$, NS) throughout the year (it ranged from 67.55 to 74.39 μ g/ml, $n = 10$; Fig. 2C).

Serum Thyroxine

Male and female thyroxine levels displayed seasonal changes that coincided with changes in reproductive behaviour and ovarian physiology, respectively (Fig. 2D). Male thyroxine levels increased significantly ($F = 11.724$, $df = 6, 63$, $P < 0.001$) during February and March correlating with the onset of mating activity (mounts: $r = 0.835$, $df = 4$, $P < 0.05$) and increased activity of males in March ($r = 0.938$, $df = 4$, $P < 0.01$) Female thyroxine levels increased significantly ($F = 14.368$, $df = 6, 63$, $P < 0.01$) during December and correlated with increased serum calcium levels ($r = 0.910$, $df = 4$, $P < 0.05$; Fig. 2D) during vitellogenesis. The second smaller increase in female thyroxine coincides with the onset of mating activity in March.

Vitellogenesis

Vitellogenesis was monitored throughout the year using SDS-polyacrylamide gel electrophoresis and was seasonal in females. Females displayed a marked increase in the E₂-inducible vitellogenin protein band during September (postnesting) and December (prematuring) which persisted until March (mating; Fig. 3). The E₂-inducible band was barely detectable in males throughout the year.



FIG 3. SDS -polyacrylamide gel displaying a marked increase in the E₂-inducible vitellogenin protein band (EIB) from, September (postnesting) and December (prematuring) until March (mating)in two adult female Kemp's ridley sea turtles (*Lepidochelys kempi*) maintained under semi-natural conditions the two right columns are (V) standard purified vitellogenin miosule (~200kDa) and (MW) molecular weight markers in kilodaltons.

Nesting Cycle

During March 1988, the ovarian condition of the 10 study females was determined using ultrasonography. All 10 females displayed preovulatory ovaries with a mean follicular diameter of 2.12 ± 0.05 cm (SE). Vitellogenic follicles were randomly scanned and were homogeneous in size. Following the completion of the nesting season (April to July), the females were reexamined using ultrasonography in July 1988. Seven females displayed either postovulatory or late preovulatory ovaries, 2 females displayed oviductal egg and one female was still preovulatory. The mean vitellogenic follicle diameter (2.03 ± 0.06 cm, SE, $n=8$) had not changed significantly. Females No 0349 and No 1353 appeared to have been retaining oviductal eggs for greater than 66 days based on the time interval between their last nesting and the date of ultrasonography conducted in July 1988. These females were induced to drop these eggs using oxytocin during July 1988. These females did not display normal nesting following oxytocin injection, but instead, dropped the eggs in the pond.

A total of 21 nests were laid by 11 of the 15 females maintained in the pond during April, May, and June 1988. Eight of the 10 study females nested with a mean of 2.0 nests per female ($n = 17$, including oviductal clutches observed by ultrasonography). Mean clutch size was 78.1 ± 3.2 eggs (SE, $n = 15$). Blood samples were collected for 17 of the total 21 nesting events observed. Female testosterone and progesterone levels declined significantly (Testosterone - $H = 11.9$, $df = 2$, $P < 0.0026$; Progesterone - $H = 7.64$, $df=2$, $P < 0.0219$) from April to June. Testosterone levels declined from elevated levels observed during their first nest (mean \pm SE 314.7 ± 23.2 pg/ml; $n = 9$) to intermediate levels at their second nest (mean \pm SE 161.5 ± 41.9 pg/ml; $n = 4$) to basal levels in postovulatory females laying their third or final nest (mean \pm SE = 22.3 ± 1.6 pg/ml; $n = 4$) (Fig. 4). Plasma progesterone levels declined in a similar manner with elevated levels at their first nest (mean \pm SE = 679.6 ± 94.4 pg/ml; $n = 8$) to intermediate levels at their second nest (mean \pm SE = 511.3 ± 33.6 pg/ml; $n = 3$) to basal levels in postovulatory females laying their third or final nest (mean \pm SE = 272.0 ± 42.3 pg/ml; $n = 4$; Fig. 4). Plasma estradiol levels did not vary significantly over the course of the nesting cycle ($H = 4.49$, $df = 2$, NS) although a slight decline was observed (Fig. 4).

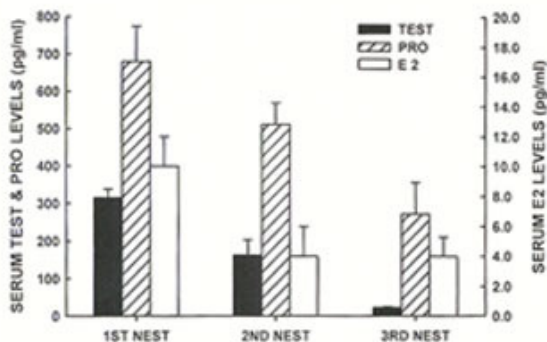


FIG. 4. Nesting levels of serum testosterone (TEST), progesterone (PRO), and estradiol (F2) for adult female Kemp's ridley sea turtles (*Lepidochelys kempi*) maintained under semi-natural conditions. Values are means \pm SE.

DISCUSSION

The Kemp's ridley sea turtle (*L. Kempi*) displays aseasonal reproductive cycle With a distinct spring mating period (March) followed by a 3- month nesting period (mid-April to mid-July). Seasonal reproductive cycles have been reported in two other species of sea turtle, *C. mydas* (Licht *et al.*, 1979) and *C. caretta*, (Wibbels *et al.*, 1990).

Male Reproductive Cycle

Male *L. kempi* displayed a prenuptial rise in testosterone one 4 to 5 months prior to mating during which time testicular recrudescence and spermatogenesis occurred. Serum testosterone rose during the fall and winter when water temperatures began to decline During this period, males did not display reproductive behaviour (courtship or mounts) or increased activity. As water temperatures increased in the spring, we observed a decline in serum testosterone during the mating and nesting period. Water temperatures range from 26°C in January to 31°C in August at the Cayman Turtle Farm (Licht *et al.* 1979). Similar seasonal reproductive patterns have been observed in captive *C. mydas* (Licht *et al.*, 1985b) and wild *C. caretta* (W Wibbels *et al.*, 1990). Spermatogenesis was confirmed to be seasonal during a pilot study with male *L. Kempi* using laparoscopy (Rostal, 1991). In November, spermatogenesis had progressed to stage 4 and 5 of the classification of McPherson *et al.*(1982). Seminiferous tubules were enlarged with abundant spermatocytes and spermatids. By May, seminiferous tubules had regressed and the lumens were filled with debris from the previous cycle. A similar pattern of spermatogenesis was observed in wild male *C. caretta* (Wibbels et al 1990).

Female Reproductive Cycle

Female *L. kempi* also displayed distinct seasonal cycles in serum testosterone, estradiol, progesterone total calcium, and vitellogenin. The ovary appears to be the primary source of testosterone and estradiol in the female sea turtle (Owens, 1997);

The sea turtle ovary undergoes prenuptial recrudescence prior to the mating period. Four to 6 months prior to the mating period (March) increased levels of serum estradiol, vitellogenin protein and total calcium were observed in the Cayman female *L. kempi* and are indicators of vitellogenesis. Vitellogenesis has been demonstrated to be estradiol-17 β (E₂)-dependent in a variety of reptiles (Ho, 1987). The source of the E₂ is thought to be the granulosa cells of the previtellogenic follicles in response to gonadotropin secretion by the pituitary. Wibbels *et al.* (1990) measured elevated levels of E₂ in wild female *C. caretta* 1 to 2 months prior to migration. As well, injections of E₂ in captive immature *C. mydas* and *L. kempi* stimulated vitellogenesis (Owens, 1976; Heck *et al.*, 1990, 1997). Follicular maturation and ovarian recrudescence were confirmed using ultrasonography in *L. kempi*. As the ovarian follicles mature and increase in size from previtellogenic to vitellogenic, the synthesis of testosterone increases. Serum testosterone increases in association with ovarian recrudescence prior to the mating period. At the time of mating, the ovary is fully developed in *L. kempi* and the entire complement of follicles for the nesting season is present. Coincident to the onset of mating, serum testosterone and estradiol are also at their maximum level. As the nesting season progresses, subsequent clutches are ovulated and serum testosterone and estradiol levels are observed to decline to their nadir in June and July (late nesting). *L. kempi* is capable of multiple nesting in captivity. It appears that as each clutch of follicles is ovulated, a proportion of the steroid source (i.e., granulosa cells of the follicles) appears to be depleted and serum testosterone, E₂, and progesterone declines. Similar decline in testosterone, E₂, and progesterone over the nesting season have been observed for wild *C. caretta* (Wibbels *et al.*, 1990; Whittier *et al.*, 1997) and *D. coriacea* (Rostal *et al.*, 1996). It was also suggested that in *C. caretta* vitellogenesis may continue into the early nesting season based on elevated E₂ levels observed during early nestings; however, other indices of vitellogenesis were not monitored. In *L. kempi*, the E₂-inducible vitellogenin protein band had decreased prior to nesting and total serum calcium was near its nadir. Serum estradiol was also near basal level by May, indicative that vitellogenesis is completed prior to mating in this species. Wibbels *et al.* (1990) suggest that in the larger sea turtle species (e.g., *C. caretta* and *C. mydas*) that lay between 4 and 10 clutches in a nesting season, vitellogenesis may continue into the early nesting season.

However, in *D. coriacea* which nests up to 10 times or more in a single nesting season vitellogenesis is complete prior to the arrival of the female at the nesting beach (Rostal *et al.*, 1996).

Progesterone appears to be primarily associated with ovulation in sea turtles. Progesterone levels are reported to increase sharply 24 - 48 hours following nesting in *L. olivacea* (Licht *et al.*, 1982), *C. mydas* and *C. caretta* (Licht *et al.*, 1979; Wibbels *et al.* 1992.) We first observed a significant increase in progesterone in March in association with mating and the probable ovulation of the first clutch of eggs. As the nesting season progressed, we observed a steady

decline in progesterone levels into July. Progesterone levels monitored at the time of nesting were also observed to decline with each subsequent clutch a female laid. Serum progesterone levels reported for *C. mydas* and *C. caretta* sampled at approximately 48 h postnesting were significantly higher than those reported here (Wibbels *et al* 1992). While we did observe an association between nesting and increased progesterone levels, our sampling protocol did not allow us to directly correlate progesterone with ovulation.

Seasonal Thyroxine Cycles

Seasonal patterns in thyroxine have been observed in both male and female reptile (Bona-Gallo *et al* 1980; Kar and Chadola-Saklani 1985; Licht *et al* 1985a; Naulleau *et al.*, 1987). Male *L. kempfi* thyroxine levels increased during March (mating) and coincided with the observed decline in male testosterone. These observations are contrary to the observations of Licht *et al* (1985b) in captive male *C. mydas* in which male thyroxine levels did not cycle. Licht *et al* (1985a) however, observed a seasonal peak in thyroxine in summer coincident with the nadir in testosterone of male *Chrysemys picta*. Similar testosterone/thyroxine interactions have been observed in other male reptiles (*Naja naja*, Bona-Gallo *et al.*, 1980; the asp viper, *Vipera aspis* ., Naulleau *et al.*, 1987; the Indian garden lizard, *Calotes versicolor*, Kar and Chadola Saklani 1995). These observations support the suggestion of a negative interaction between androgens thyroxine in male reptiles (Bona-Gallo *et al*, 1980). The lack of this effect in captive male *C. mydas* may be a species-specific difference since the captive males *L. kempfi* in this study were maintained under the same conditions. The increased thyroxine levels observed in female *L. kempfi* during December (pre-mating) correlated with the onset of vitellogenesis and ovarian recrudescence. The exact role of thyroxine during this period is not clear. Seasonal thyroxine levels have not been reported for any other female chelonian species. Preliminary results from wild female *C. caretta* suggest an elevation in serum thyroxine prior to migration (Wibbels *et al.*, 1986). Increased thyroxine levels may be related to increased metabolism during vitellogenesis or thyroxine may be deposited into the follicles coincident with vitellogenin (MacKenzie *et al.*, 1978). The female-specific increase in thyroxine in December implies that this hormone may be involved in the promotion of nutrient mobilization for the active vitellogenesis which is occurring at the time. It does not appear that this increase is due to increased thyroxine binding to plasma vitellogenin (Heck *et al.*, 1990, 1997). Interestingly, a second peak in thyroxine is observed in March in both sexes, indicating that it serves perhaps to promote the metabolic activity associated with increased mating activity in the spring. The thyroxine profiles support the suggestion that this hormone is participating in the promotion of metabolically demanding processes in this species, as has been proposed for fish (Eales, 1979).

Behavioral Role of Testosterone

The behavioral role of testosterone in female reptiles can only be speculated on at this time. The fact that both female chelonians (freshwater and marine) and alligators display seasonal changes in testosterone coincident with reproductive receptivity suggests a behavioral role (Callard *et al*, 1978; Licht *et al* 1979; McPherson *et al*, 1982; Lance, 1987; Wibbels *et al.*, 1990). Callard and Kleis (1987) review that a variety of female squamates also produce substantial quantities of testosterone. In the oviparous snake (*N. naja*), a similar increase in female testosterone has been observed during the mating period (Bona-Gallo *et al.*, 1980). The observed increase in female testosterone is associated with maximum ovarian growth and reproductive receptivity. In sea turtles, female receptivity was correlated with testosterone levels. Following ovulation both female receptivity and testosterone levels declined. Based on these observations, testosterone appears to have a behavioural role which, maybe conserved throughout the Class Reptilia. Serum testosterone appears to function in regulating seasonal reproduction in both male and female sea turtles. The long term elevation of testosterone in males appears to primarily have a physiological role but may have a behavioural role in priming specific regions of the brain also. In female *L. Kempe*, however, testosterone would appear to be directly involved in triggering receptivity and the onset of mating. The onset of mating activity occurred following the increase in female testosterone from December (mean \pm SE = 82.0 \pm 9.0 pg/ml; n = 10) to March (mean \pm SF = 378 \pm 40 pg/ml; n = 10) Mating activity was positively correlated with female testosterone levels. Mating occurred in captivity during a 3- to 4-week period prior to nesting during which certain females appeared to be receptive. In female *C. mydas*. A distinct "heat" period of 10 to 15 days has been observed during which a particular female is receptive (Comuzzie and Owens, 1990; Wood and Wood 1980) Licht *et al.* (1979) noted that testosterone levels were high during mating in captive female *C. mydas*. Following this period, the female is no longer receptive and will avoid interactions with males. A similar pattern was observed for captive female *L. Kempfi*. Females appeared to be receptive for only part of this mating period. The mating period for female *L. kempfi* was too short to discern a heat period; however, the fact that certain females did avoid males later in the mating period suggests a similar period of receptivity. Following mating female *L. kempfi* were observed to avoid males moving into the shallow portion of the enclosure. Booth and Peters (1972) observed that wild female *C. mydas* that were unreceptive moved into the shallow areas to avoid males. During May (midnesting), virtually no mating behaviour (courtship or mounts) by males was recorded. Nesting in captivity occurred from mid April to late June. In the wild

population of *L. kemp* at Rancho Nuevo, Tamaulipas Mexico, the nesting season begins in mid April and continues into July (Pritchard and Marquez 1973) The parallelism in the onset of nesting in both captivity and the wild suggests that these animals in captivity are cycling similarly to the wild population. In addition the nesting behavior of captive *L. Kemp* is similar to that of the wild females.

Callard *et al.* (1978) noted that the presence of significant quantities of testosterone in the plasma of female *C. picta* may be of importance regarding testosterone as a substrate for estrogen formation in other tissues, such as the brain. Certain areas of the brain in *Chrysemys* are more active in aromatizing androgens to estrogen than the ovary. The behavioural importance of aromatization of testosterone to estrogen has been demonstrated in the Japanese quail (Schlinger and Callard, 1990). It is plausible that testosterone may be aromatized to estradiol in the brain of *L. kemp* prior to mating and may function in triggering receptivity.

In *L. kemp* as well as *C. caretta* (Wibbels *et al.*, 1990), circulating estradiol levels are significantly lower than testosterone levels during the mating period. The secretion of testosterone in the female would provide a substrate for aromatization of estradiol in specific nuclei of the brain without further stimulation of vitellogenesis by estradiol in circulation. This would negate the need of down regulation or inhibition of the liver to estrogens. A dual hormone system (estradiol and testosterone) in female reptiles (chelonians, alligators, and squamates) may represent a conserved system. We suggest based on our observations that chelonians display a conserved reproductive system and are a valuable model for the comparative study of hormonal and behavioral interactions.

CONCLUSIONS

Male and female *L. kemp* both display distinct seasonal reproductive cycles. These cycles appear adapted to the environmental conditions that marine sea turtles encounter throughout their ranges. In *L. kemp* both testicular and ovarian maturation are pre-nuptial occurring in the fall and winter months when resources should be available while mating and nesting occur in the spring prior to optimal incubation conditions at the primary nesting beach at Rancho Nuevo, Tamaulipas, Mexico. The proximate environmental cues that influence reproductive cycles in reptiles appear to be temperature, light, and moisture (Moll, 1979).

Temperature has been linked to the reproductive cycles of other chelonian species (Licht, 1984; Owens and Morris, 1985; Whittier and Crews, 1987; Sarkar *et al* 1996; Owens, 1997). Temperature may play an important role in regulating reproductive cycles of tropical ranging sea turtles where daylength does not vary greatly. Other comparative studies on long-lived species such as sea turtles are needed to elucidate the environmental and physiological factors regulating seasonal reproductive cycles.

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