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Seasonal Reproductive Biology of the Kemp's Ridley Sea Turtle (*Lepidochelys kempii*): Comparison of Captive and Wild Populations

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ABSTRACT. – **The wild population of Kemp's ridley sea turtle (*Lepidochelys kempii*) nesting at Rancho Nuevo was studied from 1988 to 1990. Based on tagging results, *L. kempii* was reported to nest 1.3 to 1.5 times per season. These estimates are significantly less than any other sea turtle species. Serum testosterone levels were observed to decline over the course of the nesting season in a manner similar to that observed in *Chelonia mydas* and *Caretta caretta*. Ultrasonography was used to monitor ovarian condition and correlate reproductive status with plasma testosterone levels. The results of these studies confirmed that *L. kempii* actually nests approximately 3.0 times per season and demonstrates equally high fecundity to other sea turtle species. The reproductive cycle of captive *L. kempii* living under semi-natural conditions was also studied. Captive male *L. kempii* displayed a prenuptial rise in serum testosterone four to five months prior to the mating period (March) during which testicular recrudesence and spermatogenesis occurs. Male testosterone then declined sharply during the mating period. Captive female testosterone, estradiol, and total calcium rose four to six months prior to the mating period during which ovarian maturation and follicular growth were observed. Female testosterone and estradiol levels then declined during the nesting period (April to July) as ovarian follicles were ovulated and eggs were produced. Female estradiol is involved in stimulating vitellogensis. Total calcium was correlated with the period of vitellogenesis as determined by gel electrophoresis and ultrasonography. Serum thyroxine fluctuated seasonally with elevated levels observed in females associated with the period of vitellogenesis. Nesting in the captive study group corresponded with nesting in the wild population at Rancho Nuevo (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 kempii*; sea turtle; reproduction; ultrasonography; endocrinology; gonadal steroids; vitellogenesis; fecundity; Mexico**

The reproductive biology of the Kemp's ridley sea turtle (*Lepidochelys kempii*) was studied as part of the overall recovery goals for this critically endangered species. Captive breeding programs were initiated as part of these recovery goals as well. These captive breeding programs provided the opportunity to further study the reproductive biology of the Kemp's ridley throughout the complete seasonal cycle and apply this understanding to the wild population nesting at Rancho Nuevo, Tamaulipas, Mexico.

A complete understanding of the nesting physiology of *L. kempii* was critical to determining the true nesting fecundity of the species. Previous tagging studies involving several species of marine turtles had proven problematic regarding tag loss (Green, 1979; Balazs, 1982; Mrosovsky, 1983; Alvarado and Figuero, 1992). Serum testosterone, however, had proven a reliable index of the reproductive status of captive nesting *L. kempii* (Rostal, 1991), as well as wild nesting *C. caretta* (Wibbels et al., 1990). Although serum testosterone could not be used to identify specific individuals, it could provide information as to whether a particular female had nested previously that season or was a new arrival. Rostal et al (1990) validated the application of ultrasonography to studying the reproductive biology of female *L. kempii*. Ultrasonography is a non-invasive technique by which the ovarian status of a female can be monitored directly. The combination of ultrasonography and serum testosterone levels provides an accurate evaluation of the reproductive status of the female.

The fecundity of sea turtles is the highest among the amniotes. *Lepidochelys kempii*, however, has been reported to nest only 1.3 to 1.5 times per season, unlike other sea turtles (Márquez et al., 1982; Márquez, 1990). Adult population estimates were based solely on incomplete nesting data from Rancho Nuevo, Tamaulipas, Mexico. Better reproductive information was critical to the conservation of this species as well as to the estimation of the adult population size.

From 1987 to 1988, captive breeding programs provided the opportunity to study reproductively mature *L. kempii*. Investigations included documenting the seasonal reproductive pattern, as well as interactions of gonadal hormones and behavior. The wild nesting population at Rancho Nuevo, Mexico, was studied between 1988 and 1990 in collaboration with the Mexican government and the U.S. Fish and Wildlife Service. The objective was to collect baseline data on the nesting physiology of wild *L. kempii* and to evaluate the current estimates of nesting fecundity. The results of these studies (both published and unpublished) will be reviewed here and the relevance of these results to female population estimates will be discussed.

METHODS

Captive Population Study

Subjects. — Reproductively mature *L. kempii* were studied at Sea Arama Marineworld (SAM), Galveston, Texas and at the Cayman Turtle Farm (CTF), Grand Cayman, Cayman Islands. At SAM 6 animals were maintained in individual tanks on a semi-natural photoperiod with water temperature fluctuating with the season. The group contained 2 males (mean CCL = 56.5 ± 0.8 cm, mean body weight = 29.4 ± 0.4 kg) and 4 females (mean CCL = 59.6 ± 1.3 cm, mean body weight = 31.4 ± 1.4 kg) and all turtles were sexually mature. Animals were fed a diet of fish and squid.

A captive group of 35 *L. kempii* was maintained at CTF under semi-natural conditions. Twenty-eight turtles (18 males and 10 females) were 8 years old, three turtles (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 : 1.00 female, mean body weight was 24.9 ± 0.6 kg (February 1987) 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 to a maximum 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 x 10^6 l of sea water and fresh sea water was circulated through the pond at a rate of 34,000 l per hr. 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 hrs).

Behavioral Observations. — Reproductive behavioral data were collected at CTF 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 behaviors was recorded. The sex and individual identification numbers of interactants (initiator/receiver) were also recorded. Individual turtles were identified using hindflipper tags and individual characteristic markings (shell deformities and flipper scars, etc.) when possible. The percent 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 (0800–1100 hrs) and evening (1600–1900 hrs) following feeding periods. Observation periods were based on a pilot study during March 1987 to determine periods of peak diurnal activity. Observations were conducted for 60 hours 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 and Hormone Analysis. — Non-heparinized 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). Turtles were sampled between 0800 and 1200 hrs. 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 min at 2–3000 rpm and serum was frozen at -20°C. Samples were stored at -80°C at Texas A&M University until assayed. Serum samples were analyzed using radioimmunoassay (RIA; Rostal et al., 1998).

Serum testosterone and progesterone were measured by radioimmunoassay as described in Wibbels et al. (1990). For male testosterone, 10 and 100 µl of serum was extracted using anhydrous ether. For female testosterone and progesterone, 250 and 500 µl of serum, respectively, was extracted using anhydrous ether. Samples were run in duplicate. Extraction efficiencies for testosterone and progesterone averaged 78.7% and 64.7%. Sensitivity of the testosterone and progesterone assays were 2.3 and 21 pg/tube. Intra-assay 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 (Rostal et al., 1998).

Serum estradiol was measured using a ^{125}I kit provided by Diagnostic Products Corporation, Los Angeles, CA. For estradiol, 100 µl 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. Intra-assay coefficient of variation for the estradiol assay was 5.1% and interassay coefficients of variation for the estradiol assay was 13.6% (Rostal et al., 1998).

Serum thyroxine (T_4) was measured by radioimmunoassay according to the method of MacKenzie et al.(1978) as modified by Denver and Licht (1988). *Lepidochelys kempii* serum samples were diluted parallel to T_4 standards in this assay. Average recovery of T_4 from supplemented samples was 94.0%. Blood samples were analyzed at 10-50 µl volume in the T_4 assay. Preliminary attempts to measure triiodothyronine (T_3) in a similar assay found no detectable levels of T_3 (Moon, 1992).

Laparoscopy and Ultrasonography. — Reproductive condition of both males and females at SAM were monitored using laparoscopy. The procedure is outlined in Rostal et al. (1990). Females were examined three times and males were examined twice during the annual cycle. Testicular biopsies were collected from the males and fixed in 10% formalin. Histological sections were made at the Texas A&M University Veterinary Pathology laboratory according to standard paraffin techniques.

Females at CTF were examined by ultrasound during March 1988 (mating) and July 1988 (post-nesting) to determine the reproductive status. The procedure was outlined in Rostal et al. (1990). Oviductal eggs, vitellogenic follicles, and atretic follicles were identified. Ovaries were classified

as preovulatory versus postovulatory based on the overall image of the ovary. Preovulatory ovaries contained multiple large vitellogenic follicles (> 1.5 cm diameter). Postovulatory ovaries contained small previtellogenic (1.0–1.5 cm diameter) and atretic follicles. For further details see Rostal et al. (1990).

Serum Calcium and Vitellogenesis. — Total calcium was monitored as an indicator of vitellogenesis. Serum total calcium was highly correlated with increased blood vitellogenin in captive *L. kempii* (Heck et al., 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 (La_2O_3) solution at room temperature. Total calcium (µg/ml) was compared to a standard curve (1, 5, 10, 20 µg/ml, Fisher Scientific AA Standard) at the time of assay.

Vitellogenesis was monitored by 7.5% SDS-polyacrylamide gel electrophoresis as described by Laemmli (1970). A female-specific estradiol-inducible band with a molecular weight of approximately 205 kDa was identified for *L. kempii* (Heck et al., 1997). Ten µl of serum was diluted 1:10 in 90 µl 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 Biorad High Range molecular weight standards (Richmond, CA).

Wild Population Study

Study Site. — The nesting beach at Rancho Nuevo, Tamaulipas, Mexico, has been patrolled by a bi-national Mexican-US team of biologists annually from April to August since 1978. A total of 30 km of beach was patrolled from Barro del Tordo to Barro de Aparejo (Burchfield et al., 1989). In 1990, an additional camp was established to patrol an additional 15 km to the north of the sanctuary. The beach is composed of light colored sand with areas of rocks and crushed shells. The average width of the beach is approximately 43 m to the primary dune structure. The dune is predominately low (1 to 2 m above sea level) and covered with vegetation. Seasonal brackish estuaries are common behind the dune. During September 1988, hurricane Gilbert struck the sanctuary and eroded some of the dune and exposed major areas of rock. By 1990, much of the beach had been replenished by natural means (*pers. obs.*). Beach patrols were conducted three times daily (approximately 0600, 1100 and 1500 hrs) using four wheel all-terrain vehicles. The camp was located at the approximate mid-point of the sanctuary at Barra Coma. One person patrolled north and another patrolled south. A beach patrol would take approximately 1.5 to 2 hours to complete.

When a nesting female was encountered, she was tagged using a monel flipper tag and her curved carapace length (CCL) was measured from the nuchal midline to the tip of the caudal maginal scutes. Other information was also collected regarding orientation during nesting, location on the dune, and specific location along the beach. Following completion of nesting, the eggs were collected and transferred to a protected corral for incubation. Approximately 40% of actual nesting events were not observed, however, the majority of nests were located by identification of fresh turtle tracks on the beach. These nests were also transferred to the protected corral.

Testosterone Study 1988-1989. — Females were randomly sampled when encountered during nesting emergence. Blood samples (15 ml) were collected following completion of nesting. Both solitary nesters and *arribada* nesters were sampled. Solitary nesters were classified as turtles nesting randomly throughout the day. *Arribada* nesters were classified as turtles nesting synchronously on a particular day during a limited time period (generally greater than 20 turtles). Twenty blood samples were collected from 7 solitary and 14 *arribada* nesters between 14 and 28 May 1988. Twenty-six blood samples were collected from 18 solitary and 8 *arribada* nesters between 22 May and 16 July 1989. Blood samples were centrifuged at 3000 rpm following collection, and serum was removed and frozen in liquid nitrogen until analysis. Gonadal hormones were measured using RIA as described above (Wibbels et al., 1990; Rostal et al., 1998).

Ultrasonography / Testosterone Study 1990. — A total of 54 blood samples were collected from 47 turtles following completion of nesting during the 1990 nesting season (21 in April, 18 in May, and 15 in June). Animals with known nesting histories were sampled preferentially when possible during May and June. Blood samples were collected and handled as described above.

Following blood sampling, the female was moved over the dune and examined using ultrasonography. An Aloka 500V ultrasound scanner with 5.0 MHz convex linear probe (Corometrics Inc., Connecticut) powered by a Honda EX1000 portable generator (American Honda Motor Co., California) via a Stabiline Uninterruptible Power Supply with battery backups (Superior Electric, Connecticut) was used as described in Rostal et al. (1990, 1997). A permanent record of all observations was made using a Sony UP-850 video graphic printer (Classic Medical Supply, Florida). The ultrasound procedure required approximately 20 minutes per animal. Each ovary was scanned independently.

Oviductal eggs, vitellogenic follicles, and atretic follicles were identified using ultrasonography as described in Rostal et al. (1990). Oviductal eggs were identified by the presence of a thin echoic shell and non-echoic albumin layer surrounding a highly echoic yolk (Rostal et al., 1990, 1997). Ovaries were classified as mature versus depleted based on the overall image of the ovary. In a mature ovary, large preovulatory vitellogenic follicles were readily scanned and multiple follicles were measured (approximately 6 per female as an estimate of follicle size; Rostal et al., 1990, 1997). In a depleted ovary, vitellogenic follicles were not observed, small previtellogenic (1.0–1.5 cm diameter) and atretic follicles were present, and intestinal loops were readily

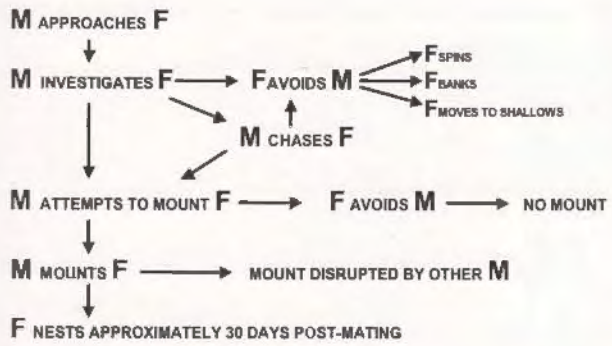

Figure 1. Chronology of reproductive behaviors observed in male (M) and female (F) *Lepidochelys kempii* maintained under semi-natural conditions at the Cayman Turtle Farm, Cayman Islands.

imaged (Rostal et al., 1990, 1997). A total of 57 ultrasound scans were conducted on a total of 50 turtles (21 in April, 21 in May, and 15 in June). Blood samples were not collected on three turtles in May. Turtles with known nesting histories for the 1990 nesting season (i.e., 1st, 2nd, or 3rd nest) were analyzed separately. For more detail, see Rostal et al. (1997).

Data Analysis. — Seasonal changes in reproductive behaviors, hormone levels, and body weight of both males and females were determined using repeated-measures analysis of variance by ranks ($p \leq 0.05$). Correlations between changes in frequency of reproductive behaviors and hormone levels were determined using Pearson product-moment correlation coefficient ($r, p \leq 0.05$; Bruning and Kintz, 1977). Changes in hormone levels during the nesting season were determined using non-parametric Kruskal-Wallis analysis of variance ($p \leq 0.05$; Bruning and Kintz, 1977). All means reported are ± standard error except where noted.

RESULTS

Captive Population Study

Reproductive Behavior. — At CTF, reproductive behaviors were identified (courtships, mounts, and male avoidance by females) and varied seasonally. Chronology of reproductive behavior is outlined in Fig. 1. Reproductive behavior increased significantly (courtships: $F = 10.03$, $df = 5, 54$, $p < 0.001$; mounts: $F = 2.64$, $df = 5, 54$, $p < 0.05$) during mating season (March) just prior to the nesting season (April to June). The frequency of male-female courtship and mounts remained low during the remainder of the year: June (late nesting), September (post-nesting), and December (pre-mating). There was also an overall increase (males: $F = 98.97, 114$, $p < 0.001$; females: $F = 8.00$, $df = 5, 114$, $p <$

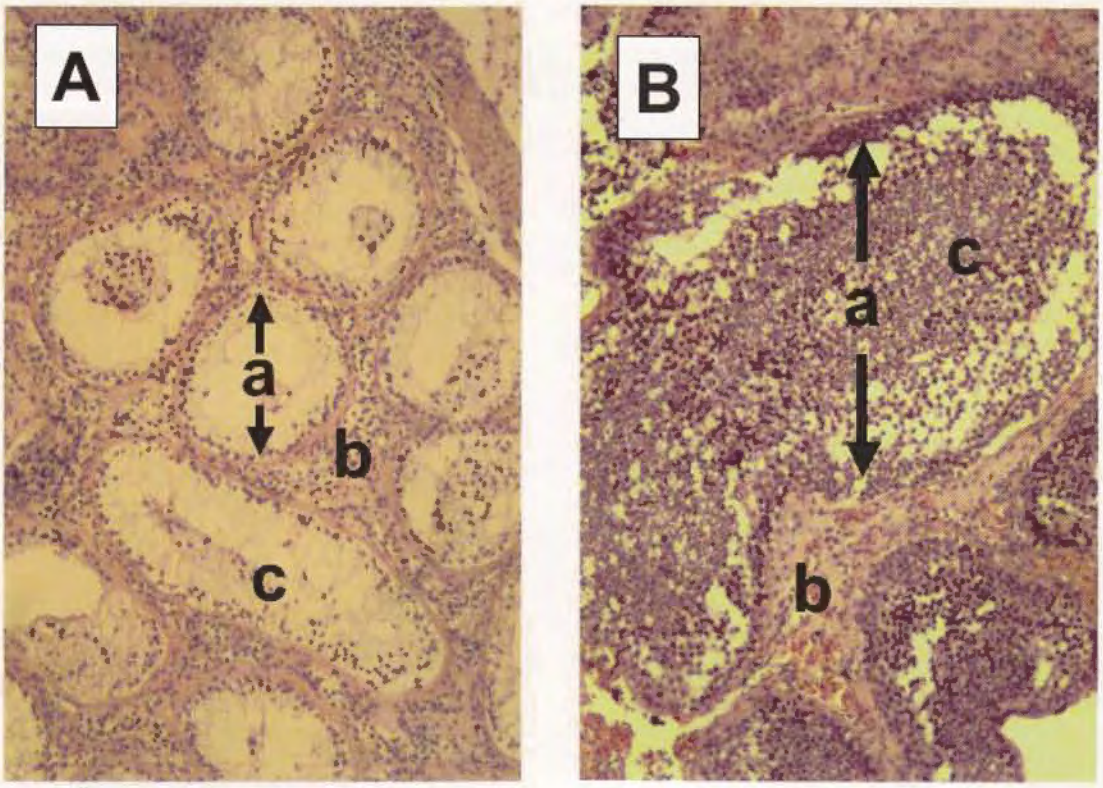

Figure 2. Testis biopsies collected from captive male *Lepidochelys kempii* maintained at Sea Arama Marineworld. **A.** Testis biopsy from an animal in May (post-mating). Seminiferous tubules (a) were regressed and the lumens were filled with debris (c) from the previous spermatogenesis cycle. **B.** Testis biopsy from an animal in November (pre-mating). Spermatogenesis had progressed to stage 4-5 McPherson et al. (1982). Seminiferous tubules are enlarged (a); spermatids and spermatocytes (c) are abundant in the lumen of the seminiferous tubules; interstitial cells (b) located between the seminiferous tubules are the primary site of testosterone synthesis. Micrographs at 400X magnification.

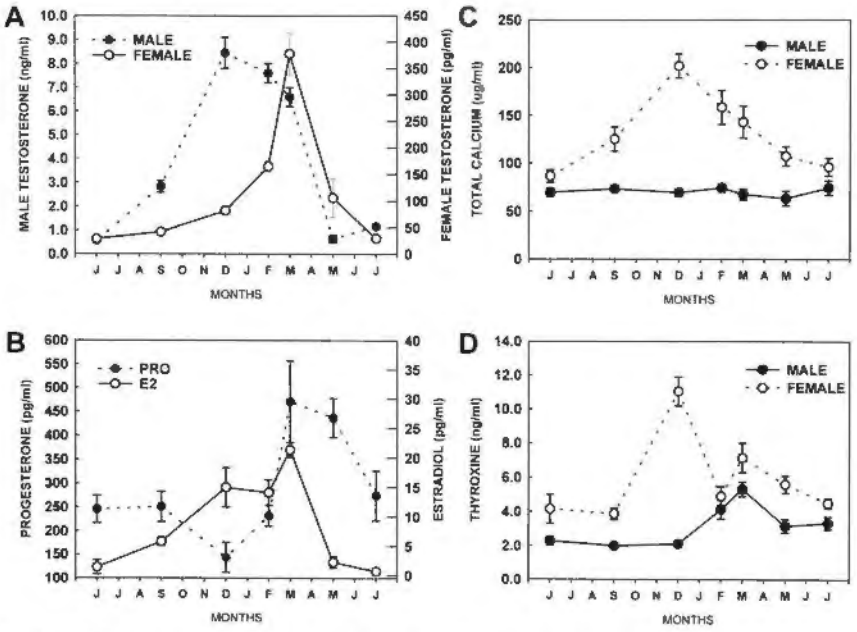

Figure 3. Seasonal cycles for adult male and female *Lepidochelys kempii* maintained under semi-natural conditions. **A.** Male and female testosterone levels, **B.** female progesterone (PRO) and estradiol (E2) levels, **C.** male and female total serum calcium levels, and **D.** male and female thyroxine levels. Values are means ± SE (n = 10). (From Rostal et al., 1998).

0.001) in male and female activity levels during the mating season (March); males were particularly more active during this period. Reproductive behaviors and increased activity levels coincided seasonally as would be expected for mating behaviors.

Male Reproductive Cycle. — Seasonal changes were observed in the male testis, serum testosterone, and thyroxine. Testis biopsy tissue collected from two males maintained at SAM displayed seasonal patterns of spermatogenesis. Seminiferous tubules were regressed in May (post-mating) and the lumens were filled with debris from the previous spermatogenesis cycle (Fig. 2A). Conversely, spermatogenesis had progressed to stage 4 and 5 by November with enlarged seminiferous tubules and abundant spermatocytes and spermatids present (McPherson et al., 1982) (Fig. 2B). Male testosterone rose significantly during September (post-nesting) to its maximum (mean = 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 = 0.65 ± 0.16 ng/ml; n = 10) ($F = 99.39$, df = 6, 63, $p < 0.001$) in CTF turtles (Fig. 3A). Male turtles at SAM displayed a similar testosterone cycle (Rostal, 1991). Male thyroxine levels in CTF turtles also increased significantly ($F = 11.724$, df = 6, 63, $p < 0.01$) 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, $p < 0.01$; Fig. 3D).

Female Reproductive Cycle. — Seasonal changes were observed in serum testosterone, progesterone, estradiol, and thyroxine levels of CTF female *L. kempii*. Female testosterone remained near basal levels during late nesting (June) and post-nesting (September), gradually began to rise during pre-mating season (December and February), then increased significantly to its maximum (mean = 378 ± 40 pg/ml; n = 10) during the mating season (March), and then declined during the nesting season (May) to its nadir (mean = 30 ± 4 pg/ml; n = 10) in the post-nesting season (July) ($F = 90.15$, df = 6, 63, $p < 0.001$; Fig. 3A). Female turtles at SAM displayed a similar testosterone cycle (Rostal, 1991). Female progesterone remained low during the late nesting (June) and post-nesting seasons (September), declined to the lowest levels observed (mean = 143.6 ± 31.6 pg/ml; n = 10) during the pre-mating season (December), then increased significantly ($F = 90.15$, df = 6, 63, $p < 0.001$) to its

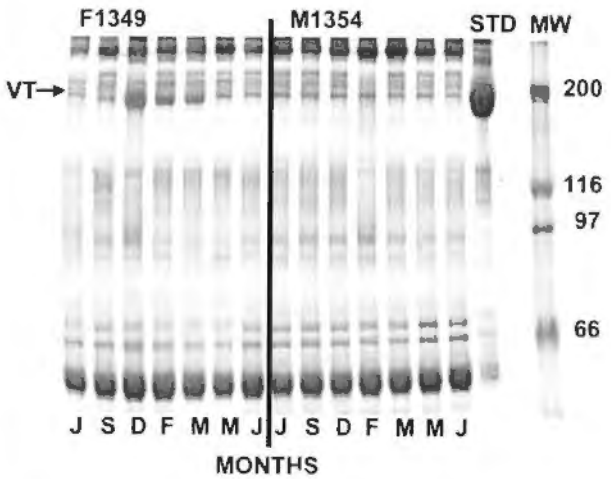

Figure 4. SDS-polyacrylamide gel displaying a marked increase in the E_2-inducible vitellogenin protein band (EIB) from September (post-nesting) and December (pre-mating) until March (mating) in adult female *Lepidochelys kempii* (#1349) maintained under semi-natural conditions. Note that no increase in the EIB was observed during any month sampled in adult male *L. kempii* (#1354). The two right hand columns are (V) standard purified vitellogenin molecule (~200 kDa) and (MW) molecular weight markers in kDa.

Table 1. Ultrasonography results from captive female Kemp's ridleys maintained at Cayman Turtle Farm, Cayman Islands.

		March 1987 (Mating)		July 1987 (Post-Nesting)	
ID #	Nests Laid	Ovary	Follicles (dia.)	Ovary	Follicles (dia.)
1318	2	Preovulatory	2.3 cm	Postovulatory	2.0 cm
1324	3	Preovulatory	2.4 cm	Postovulatory	1.9 cm
1329	2	Preovulatory	2.2 cm	Postovulatory	1.8 cm
1335	2	Preovulatory	2.0 cm	Late Preovulatory	2.2 cm
1336	2	Preovulatory	2.0 cm	Late Preovulatory	2.3 cm
1344	0	Preovulatory	1.9 cm	Preovulatory	1.9 cm
1349	3*	Preovulatory	2.2 cm	Oviductal eggs	Yolk = 2.9 cm / Shell = 3.8 cm
1351	0	Preovulatory	2.0 cm	Preovulatory	2.0 cm
1353	2*	Preovulatory	2.2 cm	Oviductal eggs	Yolk = 2.4 cm / Shell = 3.8 cm
1355	1	Preovulatory	2.0 cm	Late Preovulatory	2.1 cm
		Mean Dia. = 2.12 cm SE = 0.05; n = 10			Mean Dia. = 2.03 cm SE = 0.06; n = 8

* Nest values include clutches observed as oviductal eggs using ultrasonography during July 1988.

maximum (mean = 471.3 ± 86.2 pg/ml; n = 10) during the mating season (March), and then declined slowly during the nesting season (May) into the post-nesting season (July; Fig. 3B). Female estradiol also remained near basal levels during the late nesting season (June), then began to rise during the post-nesting season (September) into the pre-mating season (December and February), then increased significantly (F = 90.15, df = 6, 63, $p < 0.001$) to its maximum (mean = 21.4 ± 1.2 pg/ml; n = 10) during the mating season (March), and then declined during the nesting season (May) to its nadir (mean = 0.7 ± 0.4 pg/ml; n = 10) in the post-nesting season (July; Fig. 3B).

Significant seasonal changes were observed in female serum calcium levels (an indicator of vitellogenesis) (F = 9.95, df = 6, 63, $p < 0.001$). Female serum calcium increased from June (post-nesting, mean = 86.42 ± 6.5 µg/ml, n = 10) to December (pre-mating, mean = 201.79 ± 12.74 µg/ml, n = 10) and then declined during March (mating) and May (nesting; Fig. 3C). Female calcium followed a similar pattern to vitellogenesis. Male serum calcium levels remained low throughout the year (mean ranged from 67.55 to 74.39 µg/ml, n = 10) and no significant changes were observed (F = 0.54, df = 6, 63, NS; Fig. 3C).

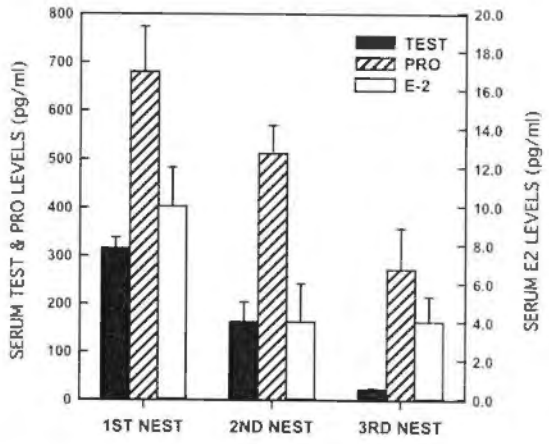

Figure 5. Nesting levels of serum testosterone (TEST), progesterone (PRO) and estradiol (E2) for adult female *Lepidochelys kempii* maintained under semi-natural conditions at the Cayman Turtle Farm. Values are means ± SE. (From Rostal et al., 1998).

Female thyroxine levels displayed seasonal changes in the CTF turtles that coincided with changes in reproductive behavior and/or ovarian physiology. Female thyroxine levels increased significantly (F = 14.368, df = 6, 63, $p < 0.01$) during the pre-mating season (December) and correlated with increased serum calcium levels (r = 0.910, df = 4, $p <$ 0.05) during vitellogenesis (Fig. 3D). The second smaller increase in female thyroxine coincides with the onset of mating activity in March (Fig. 3D).

Vitellogenesis was monitored in the CTF turtles using SDS-polyacrylamide gel electrophoresis throughout the year. Females displayed a marked seasonal increase in the E_2-inducible vitellogenin protein band during the post-nesting season (September) and pre-mating season (December) which persisted until mating season (March; Fig. 4). The E_2-inducible band was barely detectible in males throughout the year.

Nesting Cycle. — The ovarian state of the 10 CTF females was determined using ultrasonography during March 1988. Preovulatory ovaries with a mean follicular diameter of 2.12 ± 0.05 cm were observed in all 10 females (Table 1). Vitellogenic follicles were observed to be homogeneous in size. Females were re-examined using ultrasonography in July 1988 following the completion of the nesting season (April to July). Postovulatory or late preovulatory ovaries were observed in 6 females, oviductal eggs were observed in 2 females and 2 females were still preovulatory. Two females 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.

Eleven of the 15 CTF females laid a total of 21 nests during April, May, and June 1988. Eight of the 10 CTF 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 (n = 15). Blood samples were collected for 17 of the total 21 nesting events observed. Both testosterone and progesterone levels were observed to decline significantly (testosterone: H = 11.9, df = 2, $p <$ 0.0026; progesterone: H = 7.64, df = 2, $p < 0.0219$) from April to June in the nesting CTF females. Testosterone levels declined from elevated levels observed during their first nest (mean = 314.7 ± 23.2 pg/ml; n = 9) to intermediate levels at

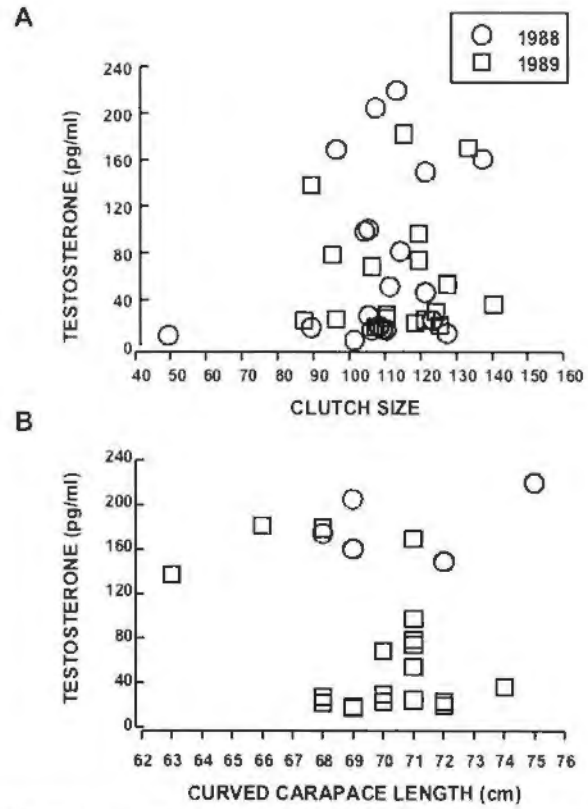

Figure 6. A. Plot of serum testosterone versus curved carapace length for wild nesting *Lepidochelys kempii* at Rancho Nuevo, Mexico (1988: $n = 20$; 1989: $n = 19$). No correlation was observed. **B.** Plot of serum testosterone versus clutch size for wild nesting *L. kempii* at Rancho Nuevo, Mexico (1988: $n = 19$; 1989: $n = 18$). No correlation was observed. (From Rostal et al., 1997).

their second nest (mean = 161.5 ± 41.9 pg/ml; $n = 4$) to basal levels in postovulatory females laying their third or final nest (mean = 22.3 ± 1.6 pg/ml; $n = 4$; Fig. 5). Plasma progesterone levels declined in a similar manner with elevated levels at their first nest (mean = 679.6 ± 94.4 pg/ml; $n = 8$) to intermediate levels at their second nest (mean = 511.3 ± 33.6 pg/ml; $n = 3$) to basal levels in postovulatory females laying

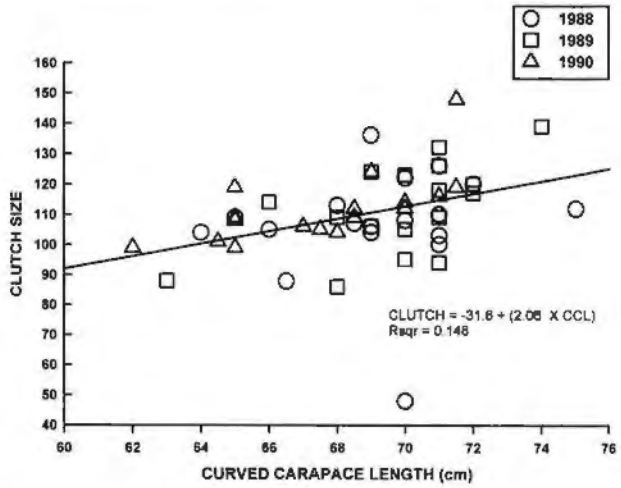

Figure 8. Plot of clutch size versus curved carapace length for wild nesting *Lepidochelys kempii* at Rancho Nuevo, Mexico. A positive correlation was observed ($r^2 = 0.148$). (From Rostal et al., 1997).

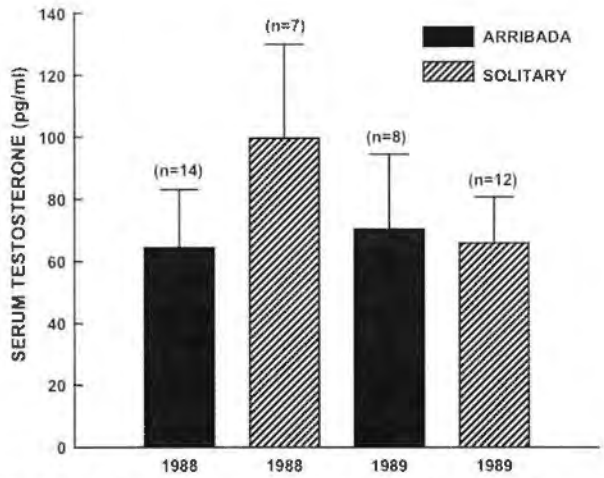

Figure 7. Mean serum testosterone levels for *arribada* versus solitary nesting *Lepidochelys kempii* sampled during May 1988 and 1989. No significant difference was observed. Values are means ± SE. (From Rostal et al., 1997).

their third or final nest (mean = 272.0 ± 42.3 pg/ml; $n = 4$; Fig. 5). 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. 5).

Wild Population Study

Female testosterone levels were plotted versus curved carapace length (CCL) and clutch size for samples collected in May 1988 and May 1989 (Fig. 6). No correlation was observed between testosterone and CCL or clutch size. It was observed, however, that a broad range of testosterone levels (from 5.0 to 218.7 pg/ml) was present for a species that was reported to nest only 1.3 times per season (Márquez et al., 1982). Also, testosterone levels from females classified as solitary versus *arribada* nesters were not significantly different (Fig. 7). A weak positive correlation was observed between CCL and clutch size ($r^2 = 0.148$; Fig. 8). Similar observations have been made in olive ridleys, *Lepidochelys olivacea* (Pritchard, 1969) and *C. mydas* (Bustard, 1973).

A 7.7 km stretch of beach at Rancho Nuevo from Barro Coma to Cachimba was monitored during the 1990 nesting season. Nest site fidelity during the nesting season enhanced recapture results during the study even though we were only able to monitor one-third of the normal range patrolled (ca. 30 km); for more detail see Rostal et al. (1997). Through the use of ultrasonography, detection of oviductal eggs in turtles that false-crawled (female emerged but did not complete nesting; Fig. 9A), determination of ovarian status of the female following nesting (mature versus depleted), and detection of atretic follicles was possible.

Ultrasound scans revealed that 100% of the females scanned in April possessed preovulatory ovaries following their first nesting and were expected to return to nest at least once more (Fig. 9B). Eighty-five percent of the turtles scanned in May 1990 possessed preovulatory ovaries while 15% of the turtles possessed depleted ovaries and atretic

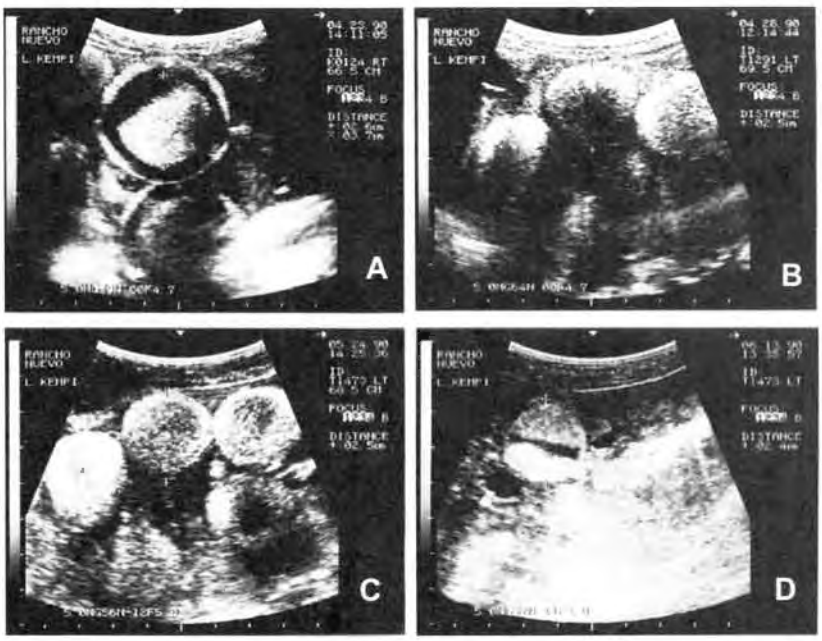

Figure 9. A. Ultrasound image of oviductal egg from wild female *Lepidochelys kempii* recaptured following its first unsuccessful nesting emergence of the season on April 23, 1990. Ultrasonography revealed well calcified eggs with distinct anechoic albumin layer and echogenic yolk in both oviducts. **B.** Ultrasound image of multiple large vitellogenic follicles from a wild female *L. kempii* recaptured following its first successful nesting emergence of the season on 28 April 1990. Ultrasonography revealed large vitellogenic follicles (dia. = 2.5 cm; $n = 6$ measured) in both ovaries, no oviductal eggs were observed. **C.** Ultrasound image of multiple large vitellogenic follicles from a wild female *L. kempii* recaptured following its second successful nesting emergence of the season on 13 May 1990. Ultrasonography revealed large vitellogenic follicles (dia. = 2.5 cm; $n = 6$ measured) in both ovaries, no oviductal eggs were observed. **D.** Ultrasound image of a postovulatory ovary with a large atretic follicle from wild female *L. kempii* recaptured following its third recorded successful nesting emergence on 12 June 1990. Ultrasound revealed postovulatory ovaries containing only small previtellogenic and several large atretic follicles (dia. = 2.4; $n = 4$).

follicles (Fig. 9C). Finally, 20% of the turtles scanned in June possessed preovulatory ovaries while 80% of the turtles possessed depleted ovaries and atretic follicles (Fig. 9D).

Mean follicular diameter did not vary over the course of the nesting season (April = 2.5 ± 0.03 cm, $n = 20$; May = 2.5 ± 0.02 cm, $n = 17$; June = 2.5 ± 0.05, $n = 3$; $H = 0.424$, df = 2, $p = 0.809$). Mean follicular diameter per female ranged from 2.1 to 2.6 cm with an overall mean of 2.5 ± 0.02 cm ($n = 40$; Fig. 10). There was no correlation between mean follicular size and female CCL observed ($r^2 = 0.012$, $F = 0.451$, df = 1, 38, $p = 0.506$; Fig. 11).

Similar to the observations in the CTF nesting females, serum testosterone was observed to decline over the nesting season (Fig. 12) and correlated with *arribada* events in 1990. Estimates of follicle numbers are also plotted based on known clutch sizes and nest number. Female testosterone levels were significantly elevated (mean = 139.8 + 19.0 pg/ml, $n = 20$; $H = 34.38$, df = 2, $p < 0.001$) during the first third of the nesting season (first nests) when females displayed large preovulatory ovaries, declined during the middle-third of the nesting season (mean = 48.4 ± 7.2 pg/ml, $n = 14$) when most females displayed preovulatory ovaries and were mini-

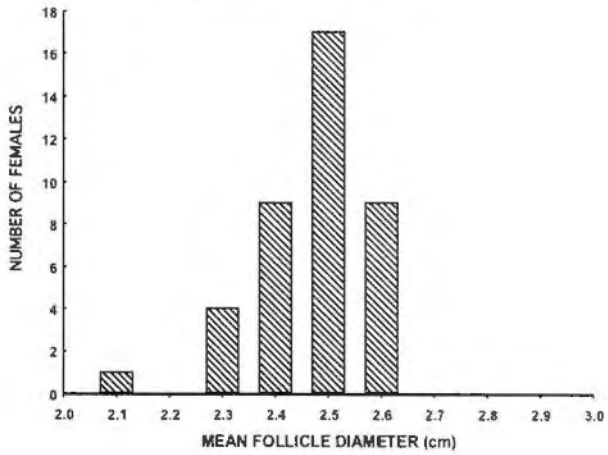

Figure 10. Histogram of mean follicle size classes observed for 40 wild female *Lepidochelys kempii* at Rancho Nuevo, Mexico, during the 1990 nesting season. Four to six vitellogenic follicles were measured per female. (From Rostal et al., 1997).

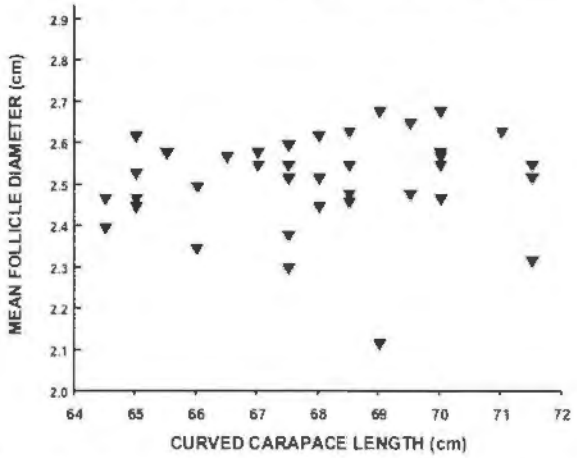

Figure 11. Mean follicle diameter versus curved carapace length of wild *Lepidochelys kempii* at Rancho Nuevo, Mexico, during the 1990 nesting season. Four to six vitellogenic follicles were measured per female. (From Rostal et al., 1997).

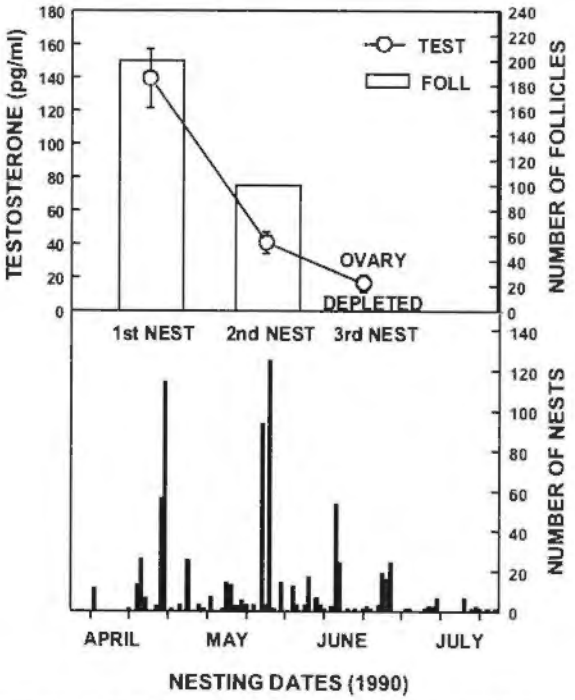

Figure 12. Plot of serum testosterone and number of ovarian follicles compared with number of nests for wild *Lepidochelys kempii* sampled at Rancho Nuevo, Mexico, during the 1990 nesting season. Number of follicles estimates based on ultrasound results and mean clutch size. (From Rostal et al., 1997).

mal in turtles that had completed nesting for the season (mean = 12.1 ± 0.6 pg/ml, n = 10; Fig. 12) when females displayed postovulatory ovaries. Serum estradiol levels also declined in a similar fashion over the nesting season (H = 28.06, df = 2, $p < 0.001$) but were much lower than testosterone levels. Estradiol levels were highest in females with large preovulatory ovaries (mean = 8.3 ± 1.1 pg/ml, n = 20), intermediate midway through the nesting cycle (mean = 3.0 ± 0.9 pg/ml, n = 14) and lowest in females displaying postovulatory ovaries at the end of the nesting cycle (mean = 0.4 ± 0.2 pg/ml, n = 10; Fig. 12). Progesterone levels were highly variable (ranging from < 95 to 906 pg/ml) and not

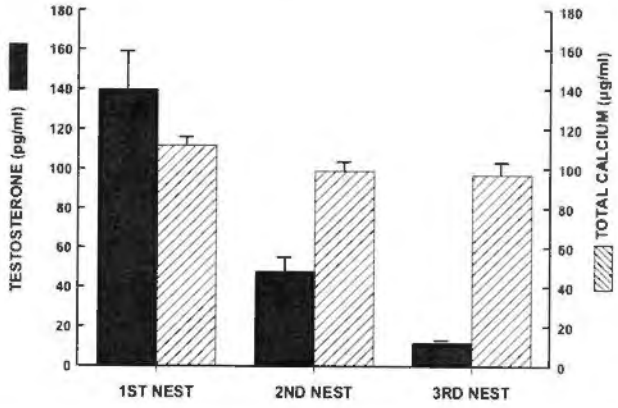

Figure 13. Mean total calcium and serum testosterone levels per nesting occurrence based on nesting history and ultrasonography results for wild *Lepidochelys kempii* at Rancho Nuevo, Mexico, during the 1990 nesting season. (From Rostal et al., 1997).

correlated with reproductive state (H = 3.445, df = 2, p = 0.179; Fig. 12). Nesting results were independently confirmed from 1990 beach census records.

Serum calcium was measured as an indicator of vitellogenesis during the nesting season. While serum testostrone declined over the course of the nesting seaon, serum calcium remained relatively constant and only showed a slight decline from April (111.4 ± 4.1 µg/ml, n = 20) to June (90.6 ± 6.9 µg/ml, n = 10; Fig. 13). These levels were similar to post-nesting levels (86.4 ± 6.5 µg/ml, n = 10) in CTF female *L. kempii* while pre-mating vitellogenic levels were significantly higher (201.8 ± 12.7 µg/ml, n = 10).

Clutch size was not significantly different for first, second, or third time nesters. During the 1990 nesting season, the overall mean clutch size remained relatively constant (100.3 ± 2.9 eggs, n = 44 nests).

DISCUSSION

The Kemp's ridley sea turtle displays a seasonal reproductive cycle with a distinct spring mating period (March) followed by a three month nesting period (mid-April to mid-July). Seasonal patterns were observed in serum testosterone, estradiol, progesterone, thyroxine, total calcium, and vitellogenosis (Fig. 14). Serum testosterone appears to function in regulating seasonal reproduction in both male and female sea turtles. 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).

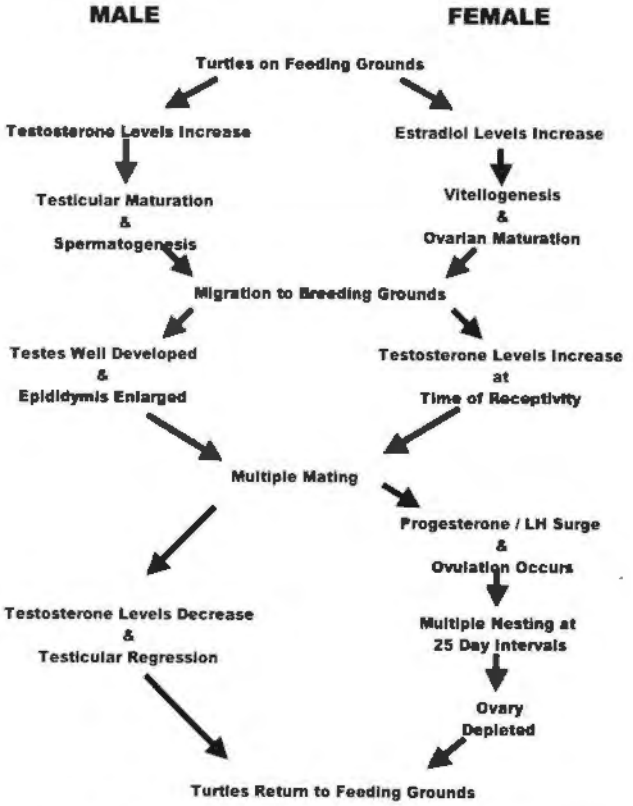

Figure 14. Outline of reproductive events during the seasonal reproductive cycle for the Kemp's ridley sea turtle.

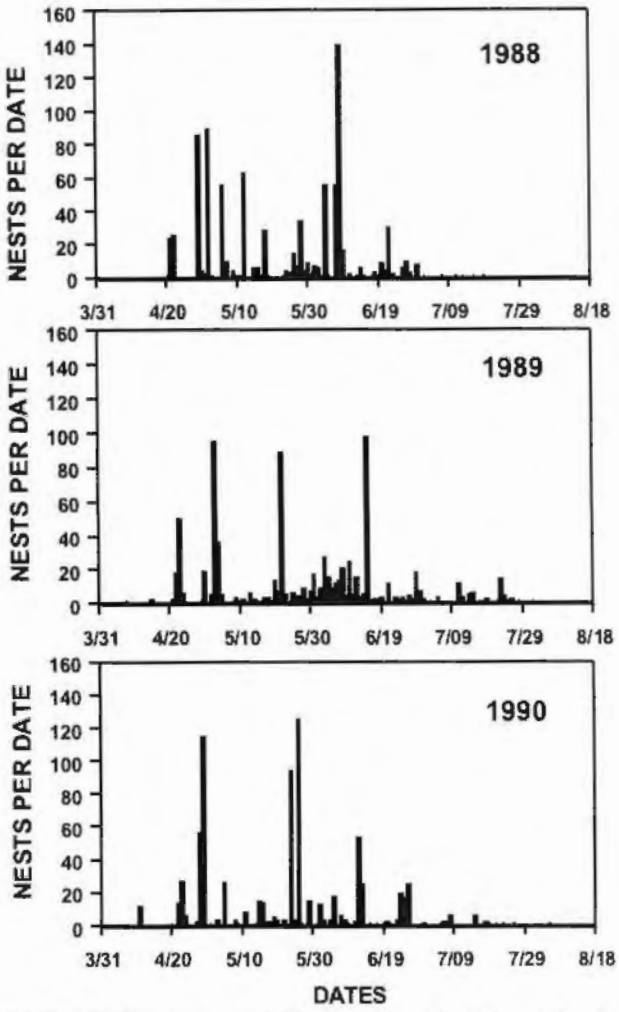

Figure 15. Histogram of *Lepidochelys kempii* nesting at Rancho Nuevo, Mexico, during the 1988, 1989, and 1990 seasons. Three *arribada* periods were observed (late April, late May, and mid-to-late June) during 1989 and 1990. Females were repeatedly observed during all three periods.

Male Reproductive Cycle. — Male *L. kempii* displayed a prenuptial rise in testosterone four to five 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 behavior (courtship or mounts) or increased activity. The long term elevation of testosterone in males appears to primarily have a physiological role, but may also have a behavioral role in priming specific regions of the brain. 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 CTF (Licht et al., 1979). Serum calcium levels remained constant throughout the year as expected. Similar seasonal reproductive patterns have been observed in captive male *C. mydas* (Licht et al., 1985b) and wild *C. caretta* (Wibbels et al., 1990). Spermatogenesis was confirmed to be seasonal during a pilot study with male *L. kempii* 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. Seminiferous tubules had regressed by May 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).

Thyroxine levels in males were observed to increase during March (mating) and coincided with the observed decline in male testosterone. Licht et al.(1985b) did not, however, observe a cycle in captive male *C. mydas* in which male thyroxine levels were measured at CTF. Licht et al.(1985a), however, observed a seasonal peak in thyroxine in summer coincident with the nadir in testosterone of male *Chrysemys picta*. Testosterone / thyroxine interactions have been reported in other male reptiles (*Naja naja*, Bona-Gallo et al., 1980; *Vipera aspis*, Naulleau et al., 1987; *Calotes versicolor*, Kar and Chadola-Saklani, 1985). These observations support the suggestion of a negative interaction between androgens and thyroxine in male reptiles (Bona-Gallo et al., 1980). The lack of a seasonal pattern in captive male *C. mydas* at CTF may be the result of males having been collected from multiple populations with different seasonality obscuring the potential cycle. The captive male *L. kempii* at CTF came from the only population in the world.

Female Reproductive Cycle. — Female *L. kempii* 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 six months prior to the mating period (March), increased levels of serum estradiol, vitellogenin protein, and total calcium were observed in CTF female *L. kempii* and are indicators of vitellogenesis. Vitellogenesis has been demonstrated to be estradiol-17-fl-(E_2)-dependent in a variety of reptiles (Ho, 1987). Follicular maturation and ovarian recrudescence were confirmed using ultrasonography in *L. kempii*. Estradiol is thought to be secreted by the granulosa cells of the pre-vitellogenic follicles in response to gonadotropin secretion by the pituitary. Wibbels et al. (1990) measured elevated levels of E_2 in wild female *C. caretta* one to two months prior to migration. In addition, injections of E_2 in captive immature *C. mydas* and *L. kempii* stimulated vitellogenesis (Owens, 1976; Heck et al., 1997).

The synthesis of testosterone increases in association with ovarian follicular maturation and increase in size prior to the mating period. At the time of mating, the ovary is fully developed in *L. kempii* and the entire complement of follicles for the nesting season is present. Serum testosterone and estradiol are also at their maximum levels at the onset of mating. In female *L. kempii* 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 = 82.0 ± 9.0 pg/ml; n = 10) to March (mean = 378 ± 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 (for more detail on the behavioral role of testosterone, see Rostal et al., 1998). As nesting progresses, subsequent clutches are ovulated and serum testosterone and estradiol levels are observed to decline to their nadir in June and July (late nesting). 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, E_2, and progesterone declines. Similar declines in testosterone, E_2, and progesterone over the nesting season have been observed for wild *C. caretta* (Wibbels et al., 1990; Whittier et al., 1997) and *Dermochelys coriacea* (Rostal et al., 1996, 2001). In *L. kempii*, the E_2-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, indicating that vitellogenesis is completed prior to mating in this species. Wibbels et al. (1990) suggested that in 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 ten 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, 2001).

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). A significant increase in progesterone was first observed 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 hrs post-nesting 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.

Increased thyroxine levels observed in female *L. kempii* during December (pre-mating) may be related to 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., 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).

Wild Population Study. — Lepidochelys kempii is capable of much higher fecundity than previously reported for the species. During the 1990 nesting season, *L. kempii* were capable of nesting 3.075 times with an average clutch size of 100+ eggs. Also based on testosterone results, its appears that the nesting physiology of *L. kempii* is similar to other Testudines (Licht et al., 1979; Wibbels et al., 1990).

The primary source of testosterone is the granulosa cells of the vitellogenic follicles. Testosterone results from the 1990 nesting season support the conclusion that ovarian recrudescence is complete at the time of mating. The three stage decline observed in serum testosterone suggests that following mating, one-third of the pre-ovulatory vitellogenic follicles are ovulated in preparation for nesting. Following nesting, ovulation is reported to occur within 48 hours post-nesting in response to luteinizing hormone (LH) and progesterone (Licht et al., 1979). With each subsequent clutch produced, serum testosterone was observed to decline as the source of testosterone was removed by ovulation of the follicles.

It was previously thought, based on a fecundity index of 1.3 nests per female, that each *arribada* of the season was a separate population or sub-population arriving at the nesting beach independently. This, however, does not appear to be the case. The pattern of decline in serum testosterone over the nesting season has implications regarding the nature of the nesting population. Its appears that the majority of the nesting population arrives at Rancho Nuevo in mid- to late April and remains in the vicinity through June until they have completed nesting. This is further supported by the fact that females have been observed to nest up to four times during the season, although these were previously thought to be rare events (Márquez, 1994). During June 1990, 10 of 15 turtles sampled were confirmed third-time nesters and two more of these females were probably third-time nesters since the recorded internesting interval was 45 days for each female (approximately 2 times the mean internesting interval of 25 ± 4.2 days, SD).

The pattern of nesting during the 1988, 1989, and 1990 seasons displayed multiple periods of increased nesting or *arribadas* (Fig. 15). While nesting was less synchronized during the 1988 nesting period, there were three distinct periods of nesting during 1989 and 1990. Multiple recaptures of nesting females throughout the nesting seasons further support the ultrasonography / testosterone results of 3 or more nests per female during the 1990 nesting season.

These observations have significant implications with regard to monitoring and managing this critically endangered species. Since 1978, the nesting female population has often been estimated simply by dividing the total number of nests collected by the fecundity index of 1.3 (Márquez et al., 1982; Márquez, 1990). Pritchard (1990) reanalyzed the 1988 nesting season data for *L. kempii* at Rancho Nuevo taking into account the probability of observing a given turtle on all of its nesting emergences. His analysis suggested that females may be capable of 2.3 nests per season. This fecundity index has, however, come under more recent scrutiny. Tucker (1989) elucidated the effect of underestimating fecundity on overestimating population size in *D. coriacea*. Our results support an estimate of 3.075 nests per female per season (Rostal et al., 1997). Applying this fecundity estimate would result in a 30 to 38% reduction in the female population estimate with fewer than 546 adult females surviving in 1990 in the Gulf of Mexico as compared with the previous estimate of 790 to 875 (Márquez, 1990). This would represent a significant reduction in population estimates for this critically endangered species. It has been nearly 15 years since these studies were conducted at Rancho Nuevo. Further studies are needed to determine how the wild population of *L. kempii* is responding as the population recovers.

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