# Nesting Physiology of Kemp's Ridley Sea Turtles, *Lepidochelys kempi*, at Rancho Nuevo, Tamaulipas, Mexico, with Observations on Population Estimates

DAVID C. ROSTAL<sup>1</sup>, JANICE S. GRUMBLES<sup>1</sup>, RICHARD A. BYLES<sup>2</sup>, RENÉ MÁRQUEZ-M.<sup>3</sup>, AND DAVID WM. OWENS<sup>4</sup>

<sup>1</sup>Department of Biology, Georgia Southern University, Statesboro, Georgia 30460 USA [Fax: 912-681-0845; E-mail: Rostal@gsvms2.cc.gasou.edu]; <sup>2</sup>3115 Campus Boulevard, NE, Albuquerque, New Mexico, 87106 USA; <sup>3</sup>Instituto Nacional de la Pesca, CRIP-Manzanillo, A.P. 591, Manzanillo, Colima 28200 Mexico; <sup>4</sup>Department of Biology, Texas A&M University, College Station, Texas 77843 USA

ABSTRACT. – The nesting physiology of Kemp's ridley turtles, *Lepidochelys kempi*, was studied at Rancho Nuevo, Tamaulipas, Mexico, from 1988 to 1990. Female serum testosterone and estradiol levels were observed to decline over the course of the nesting season in a manner similar to that observed in green turtles, *Chelonia mydas*, and loggerhead turtles, *Caretta caretta*. Female serum progesterone levels did not fluctuate over the course of the nesting season. Ultrasonography was also used to monitor ovarian condition during the 1990 nesting season and reproductive condition was correlated with plasma testosterone levels. Vitellogenesis is completed prior to the beginning of nesting by females at the beach. Follicle size was not correlated with female size. Serum testosterone is a useful predictor of nesting periodicity in *L. kempi*. Based on our physiological data, we estimate *L. kempi* nests approximately three times per season. This supports earlier reports that the nesting population at Rancho Nuevo has been significantly overestimated in past years and that *L. kempi* may be more critically endangered than previously thought.

# KEY WORDS. - Reptilia; Testudines; Cheloniidae; *Lepidochelys kempi*; sea turtle; reproduction; ultrasonography; endocrinology; population estimates; testosterone; nesting; fecundity; Mexico

The high fecundity of sea turtles is unequaled by any other amniotic vertebrate. The green turtle, Chelonia mydas, is capable of producing 150+ eggs per clutch and may lay in excess of six clutches in a nesting season (Moll, 1979). Other species such as loggerhead, Caretta caretta, and leatherback turtles, Dermochelys coriacea, are also capable of high productivity (Moll, 1979; Tucker, 1989, Tucker and Frazer, 1991, 1994). The genus Lepidochelys is unique among the sea turtles, displaying the mass nesting emergences known as arribada and having the longest internesting interval (approximately 28 days; Pritchard and Márquez, 1973). This unique nesting behavior coupled with a long internesting interval, remote location, and widely dispersed nesting have made studying the nesting physiology of the Kemp's ridley sea turtle, Lepidochelys kempi, difficult over the years. In addition, its rapid decline since the 1940s has also skewed our understanding of L. kempi. Lepidochelys kempi is currently considered the most critically endangered sea turtle. The nesting beach at Rancho Nuevo, Tamaulipas, Mexico, has been protected by the Mexican government since 1966 (Pritchard and Márquez, 1973). In 1978, a bi-national team of Mexican and U.S. biologists increased efforts to protect the nesting beach. During the nesting season, the majority of nests located on the beach were collected and transplanted to a fenced corral to protect them from depredation by animals including humans.

Estimates of the size of the adult female population are based solely on nesting data from Rancho Nuevo, Tamaulipas, Mexico (Márquez et al., 1996). From 1978 until 1990, the adult female nesting population was estimated by dividing the total number of nests collected by the average of 1.3 nests per season reported by Márquez et al. (1982). Pritchard (1990) reanalyzed the 1989 nesting data and proposed that L. kempi nested an average of 2.3 times during the 1989 season based on the statistical probability of observing first-, second-, and third-time nesters. More recently, the 1987 population estimate of 374 females based on 748 nests cited in Márquez et al. (1996) appeared to be based on a fecundity index of 2.0 nests per female per season. The basis for this new fecundity index was not cited. Interestingly, individual females nesting up to four times in a season have been observed at Rancho Nuevo over the years (Márquez, 1994). Results from the Cayman Turtle Farm also support that L. kempi produces multiple nests per season (Wood and Wood, 1988; Rostal, 1991; Rostal et al., in press). If a higher fecundity rate is correct, then the previous methodology used to estimate the population would be inappropriate and the population grossly overestimated. Better information is critical to the estimation of the adult population and to the conservation of this species.

Understanding the nesting physiology of *L. kempi* is crucial to determining the true fecundity of this species. Conventional tagging studies involving several species of sea turtles have previously proven problematic in estimating fecundity (Green, 1979; Balazs, 1982; Mrosovsky, 1983; Alvarado and Figueroa, 1992). Serum testosterone, however, has proven a reliable index of the reproductive status of captive nesting *L. kempi* (Rostal, 1991; Rostal et al., in press), as well as in wild nesting *C. caretta* (Wibbels et al., 1990). Serum testosterone can provide information as to



Figure 1. Lepidochelys kempi nesting at Rancho Nuevo, Tamaulipas, Mexico, in May 1990.

whether a particular female has nested previously that season (either unobserved or nesting at another beach) or is a new arrival to the nesting beach. Rostal et al. (1990) validated the application of ultrasonography for studying the reproductive biology of female *L. kempi*. Ultrasonography is a noninvasive technique by which the ovarian status of a female can be monitored directly. The combination of ultrasonography and serum testosterone levels has provided an accurate evaluation of the reproductive status of female *L. kempi*.

Between 1988 and 1990, wild nesting *L. kempi* at Rancho Nuevo, Mexico (Fig. 1) were studied in collaboration with the Mexican Instituto Nacional de Pesca and the U.S. Fish and Wildlife Service. The objective was to collect baseline data on the nesting physiology of *L. kempi* and to evaluate the estimates of nesting fecundity. Low-risk and noninvasive techniques (blood samples and ultrasonography) were used to study nesting females at the beach. This study presents physiological data that indicate that the fecundity of *L. kempi* is greater than previous estimates, more similar to other sea turtle species, and that the adult female population has been overestimated in the past.

## METHODS

Study Area. - The nesting beach at Rancho Nuevo, Tamaulipas, Mexico, has been patrolled annually from April to August since 1966 by Mexican biologists and since 1978 by a bi-national Mexican - US team of biologists. Approximately 30 km of beach was patrolled from Barra del Tordo to Barra Aparejo (Burchfield et al., 1989; Fig. 2). In 1990, an additional camp was established from which to patrol an additional 30 km to the north of the sanctuary (Burchfield et al., 1990). The beach is composed of light colored, calcareous sand with areas of sedimentary rocks and crushed shell. The width of the beach averages 43 m to the primary dune structure. The dune structure is predominantly low (1 to 2 m above sea level) and covered with vegetation. Seasonally open, brackish estuaries are extensive behind the dune structure. Beach patrols were conducted three times daily (at approximately 0600, 1100, and 1500 hrs) using four-wheel all-terrain motorcycles. The central camp was located at the approximate mid-point of the sanctuary at Barra Coma. One person was assigned to patrol north and another to patrol south from this central camp. A beach patrol required approximately 1.5 to 2 hours to complete. If an *arribada* occurred, all members in the camp would go to the beach to maximize coverage and try to record all nesters.

When a nesting female was encountered, she was tagged using a monel flipper tag and her curved carapace length (CCL) was measured. Some females were also implanted with passive integrated transponder (PIT) tags during the 1990 season. Data were collected regarding orientation during nesting, location on the dune structure, and specific location along the beach. Following completion of nesting, the eggs were collected, counted, and transferred to a protected corral for incubation. In 1990, approximately 40% of actual nesting events were not observed; the majority of such nests were identified by fresh turtle tracks on the beach. These nests were also transferred to the protected corral.

Blood Sampling and Hormonal Analysis. — Nesting females were sampled when encountered during a nesting emergence. After completion of nesting, blood samples (15 ml) were obtained from the cervical sinus using a 3.8 cm, 21gauge needle and a sterile blood collection vacuum tube (Owens and Ruiz, 1980). Samples were obtained from both



Figure 2. Map of the beach at Rancho Nuevo, Tamaulipas, Mexico. showing nest site fidelity displayed by female *L. kempi* K0124. Asterisks (\*) represent dates female's blood was sampled and ultrasonographic examinations were conducted; BC = base camp. NT = nest, FC = false crawl.

*arribada* nesters and solitary nesters. *Arribada* nesters were classified as groups (scattered or clustered) of 20 or more turtles nesting on a single day. Solitary nesters were all other nesting turtles. Twenty blood samples were obtained in 1988 between 14 and 28 May (7 solitary and 14 *arribada*). Twenty-six blood samples were obtained in 1989 between 22 May and 16 July (18 solitary and 8 *arribada*). The total number of samples obtained per year was regulated by Mexican permits. Serum was separated from the red blood cells after centrifugation at 3000 rpm and subsequently stored in liquid nitrogen until analyzed.

During the 1990 nesting season, 54 blood samples were obtained from 47 turtles following completion of nesting (21 in April, 18 in May, and 15 in June). Animals with known nesting histories were sampled preferentially when possible during May and June. During this nesting season, we concentrated our search for nesting turtles to a 7.7 km stretch of beach at Rancho Nuevo from Barra Coma to Barra Cachimba (Fig. 2). Nesting site fidelity of the turtles enhanced our recapture results during this portion of the study even though we were only able to monitor approximately one-third of the range that was patrolled by the camp crew. Blood samples were obtained and handled as described above.

Serum testosterone and progesterone were measured using an H3 radioimmunoassay (RIA) technique as described by Wibbels et al. (1990). For testosterone and progesterone, 250 or 500 µl of serum was extracted using anhydrous ether. Samples were run in duplicate. Extraction efficiencies averaged 93.2%. Sensitivity of the assays were 2.3 and 21 pg/tube at 80% B/B0. Intra-assay coefficients of variation were 4.8% and 2.4%, and interassay coefficients of variation were 17.5% and 19.6%, respectively.

Serum estradiol was measured using an Iodine kit provided by Diagnostic Products Co., 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 was 5.1% and interassay coefficient of variation was 13.6%.

Total calcium was monitored as an indicator of vitellogenesis. Increased levels of serum total calcium were correlated with vitellogenesis in *L. kempi* (Heck et al., 1990; Heck et al., 1997; Rostal et al., in press). Serum total calcium was measured by flame atomic absorption spectrophotometry as described in Rostal et al. (in press).

*Ultrasonography.* — Once nesting and blood sampling were completed, the female was carried over the primary dune and her reproductive tract examined using ultrasonography. The equipment used for ultrasonography was a portable Aloka 500V B-mode real-time ultrasound scanner with a 5.0 mhz convex linear transducer (Corometrics Inc., CT) powered by a Honda EX1000 portable generator (American Honda Motor Co., CA) via a Stabiline Uninterruptible Power Supply with battery backups (Superior Electric, CT). The equipment was transported in the back of a four-wheel-drive vehicle to the location of the nesting turtle. A path which ran behind the primary dune structure facilitated this



**Figure 3. A.** Ultrasound image of a preovulatory ovary with multiple large vitellogenic follicles (mean diameter = 2.5 cm, n = 6 measured) from *L. kempi* K0124 recaptured following her first successful nesting emergence of the season on 28 April 1990. **B.** Ultrasound image of a depleted postovulatory ovary with small previtellogenic and large attretic follicles (mean diameter = 2.4; n = 4 measured) from *L. kempi* K0124 recaptured following her third recorded successful nesting emergence on 12 June 1990.

movement. The female was placed in dorsal recumbency on a 33 cm diameter automobile tire and the front flippers were restrained by hand. Sand and debris were rinsed off the plastron and inguinal regions with water. Water-soluble coupling gel provided good contact in the inguinal region cranial to the hindflipper and enhanced imaging. A permanent record of all observations was made using a Sony UP-850 video graphic printer (Classic Medical Supply, FL). The ultrasound procedure required approximately 20 min per animal. Each ovary and oviduct was scanned independently.

Oviductal eggs, vitellogenic follicles, and atretic follicles were identified using ultrasonography as described by Rostal et al. (1990). Ovaries were classified as preovulatory vs. postovulatory based on the overall image of the ovary (Fig. 3). In a preovulatory ovary, multiple large vitellogenic follicles (> 1.5 cm in diameter) were readily imaged and follicles were randomly chosen for measurement (four to six per female as an estimate of follicle size; Fig. 3A). 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 (Fig. 3B). A total of 56 ultrasound scans were conducted on a total of 50 turtles (20 in April, 21 in May, and 15 in June). Blood samples were not obtained from three turtles in May. Turtles with known nesting histories for the 1990 nesting season (i.e., first, second, or third nest) were analyzed separately.

Statistical Analysis.—We identified significant changes in serum testosterone, progesterone, estradiol, total calcium, and clutch size using a Kruskal-Wallis One Way Analysis of Variance on Ranks (H) followed by a Dunn's Pairwise Multiple Comparison test.

Serum testosterone levels were compared between *arribada* and solitary nesters using a Mann-Whitney Rank Sum Test (T). Linear Regression Analysis (F) was used to test the relationship of mean follicle diameter vs. female carapace length and clutch size vs. female carapace length. Values reported are means  $\pm$  standard error, with significance set at  $p \le 0.05$ .

#### RESULTS

*Ovarian Cycle.* — Nesting females began arriving at Rancho Nuevo in early April and continued nesting into July. Ultrasonographic examinations revealed that 100% of the 20 females scanned in April possessed preovulatory ovaries following their first nesting and therefore were expected to return to nest at least once more (Fig. 4; Table 1). In May, 86% of the 21 turtles scanned possessed preovulatory ovaries while 14% had postovulatory ovaries with atretic follicles (Fig. 4; Table 1). Finally, in June, 20% of the 15 turtles scanned possessed preovulatory ovaries while 80% had postovulatory ovaries with atretic follicles (Fig. 4, Table 1).

Large previtellogenic follicles were observed in most females scanned in April and May. For turtles determined to be preovulatory, mean follicular diameter did not vary during the nesting season (April =  $2.5\pm0.03$  cm, n = 20; May =  $2.5\pm0.02$  cm, n = 17; June =  $2.5\pm0.05$  cm, 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 diameter of  $2.5\pm0.02$  cm (n=40; Fig. 5). Intermediate size previtellogenic follicles were not observed in any female scanned. Atretic follicles were more frequently observed in females during May and June. Many of the females scanned in May and June had already laid two clutches of eggs. No correlation was observed between mean follicle size and CCL ( $r^2 = 0.012$ , F = 0.451, df = 1, 38, p = 0.506; Fig. 6).

Serum Testosterone, Progesterone, and Estradiol. — In 1988 and 1989, nesting females were sampled primarily during May. Serum testosterone levels obtained from these females were compared with CCL; no correlation was observed ( $r^2 = 0.0008$ , F = 0.0305, df = 1, 37, p = 0.862). We did observe, however, a broad range of testosterone levels (from 5 to 219 pg/ml) in both years. There was no significant difference between testosterone levels for solitary vs. *arribada* nesters (T = 439.5, n = 19, 22, p = 0.296; Fig. 7), with levels for solitary nesters ranging from 5 to 204 pg/ml



**Figure 4.** Ultrasonography results of ovarian scans from *L. kempi* at Rancho Nuevo, Mexico, during the 1990 nesting season. A total of 56 scans were obtained on 50 turtles (April = 20 scans, May = 21 scans, June = 15 scans). Ovaries were classified as pre- or postovulatory based on the presence or absence of vitellogenic preovulatory follicles.

 $(\bar{x} = 66.6 \pm 14.4 \text{ pg/ml}, n = 22)$  and testosterone levels for *arribada* nesters ranging from 14 to 219 pg/ml ( $\bar{x} = 78.3 \pm 14.5 \text{ pg/ml}, n = 19$ ).

Circulating testosterone and estradiol levels declined over the course of the 1990 nesting season while serum progesterone levels remained relatively low (Fig. 8). Serum testosterone levels declined significantly (H = 32.4, df = 2, p < 0.001) over the course of the nesting cycle from elevated levels at the beginning when ovaries contained multiple preovulatory follicles (139.8  $\pm$  19.0 pg/ml, n = 20) to intermediate levels midway through  $(48.4 \pm 7.2 \text{ pg/ml}, n =$ 14) to low levels in females displaying postovulatory ovaries at the end  $(12.1 \pm 0.6 \text{ pg/ml}, n = 10; \text{Fig. 9})$ . Plasma estradiol levels declined in a similar fashion over the course of the nesting cycle (H = 28.06, df = 2, p < 0.001). Estradiol levels were highest in females with early, preovulatory ovaries (8.3  $\pm$  1.1 pg/ml, n = 20), intermediate midway through the nesting cycle  $(3.0 \pm 0.9 \text{ pg/ml}, n = 14)$ , and lowest in females displaying postovulatory ovaries at the end of the nesting

Table 1. Ultrasonography predictions of nesting reoccurrence and recapture results at Rancho Nuevo, Tamaulipas, Mexico, during the 1990 nesting season. Note the similarity of results between the predicted number of renesters (No. 4) and the accuracy prediction (No. 7).

	April	May
Observations used for calculations:		
1. Number of turtles scanned =	20	21
2. Number of turtles predicted to renest =	20	18
3. Number of turtles observed renesting =	13	7
Calculations:		
4. Percentage of turtles predicted to renest =	100%	86%
5. Percentage of predicted turtles observed renesting =	65%	39%
<ol> <li>Percentage of total nesting events observed =         <ul> <li>(# nesting events observed ÷ total # nests laid)</li> </ul> </li> </ol>	64%	48%
<ul> <li>7. Estimated accuracy of predictions =</li> <li>(% of predicted turtles observed renesting ÷</li> <li>% nesting events observed)</li> </ul>	1.02	0.81



Figure 5. Histogram of mean follicle size classes observed for 40 female *L. kempi* at Rancho Nuevo, Mexico, during the 1990 nesting season. Four to six vitellogenic follicles were measured per female.



**Figure 6.** Mean follicle diameter vs. curved carapace length of *L. kempi* at Rancho Nuevo, Mexico, during the 1990 nesting season. Four to six vitellogenic follicles were measured per female.



Figure 7. Mean serum testosterone levels for *arribada* versus solitary nesters sampled during May 1988 and 1989. No significant difference was observed.

cycle ( $0.4 \pm 0.2$  pg/ml, n = 10; Fig. 9). Plasma progesterone levels were highly variable between individuals (ranging from < 95 to 906 pg/ml) and were not correlated with reproductive condition (H = 3.445, df = 2, p = 0.179; Fig. 9).

Serum Total Calcium. — Serum calcium levels remained relatively constant over the course of the nesting season, however, a slight decline was observed from females laying their first nest ( $x = 111.4 \pm 4.1 \ \mu g/ml$ , n = 20) in April to females laying their final nest ( $x = 90.6 \pm 6.9 \ \mu g/ml$ , n =10) in June (H = 10.874, df = 2, p < 0.004). Serum testosterone levels declined more sharply in comparison with serum calcium over the course of a female's nesting cycle (Fig. 10).

Clutch Size. — Clutch size in our data set was not significantly different between years. Females we sampled in May 1988, 1989, and 1990 were compared. Mean clutch size was 106.8 ± 3.9 eggs (n = 21) in 1988, 112.4 ± 3.5 eggs (n = 20) in 1989, and 111.4 ± 2.6 eggs (n = 20) in 1990. Clutch size remained relatively constant throughout the 1990 nesting season (H = 5.321, df = 2, p < 0.070). When compared with nesting chronology for females with known histories, mean clutch size ranged from 99.3 ± 4.4 eggs (n = 20) for the first nest, 105.0 ± 5.9 eggs (n = 17) for the second nests, and 88.2 ± 6.7 eggs (n = 10) for the third nests during 1990. A weak positive correlation was observed between CCL and



Figure 8. Plot of measured serum testosterone, progesterone, and estradiol levels over time for *L. kempi* at Rancho Nuevo, Mexico, during the 1990 nesting season.



Figure 9. Mean serum testosterone, progesterone, and estradiol levels per nesting occurrence based on nesting history and ultrasonography results for *L. kempi* at Rancho Nuevo, Mexico, during the 1990 nesting season.

clutch size ( $r^2 = 0.148$ ; Fig. 11). A similar observation has been made on the olive ridley, *Lepidochelys olivacea* (Pritchard, 1969) and *C. mydas* (Bustard, 1973).

*Fecundity Estimates.* — Ultrasonography was used to determine reproductive status as well as predict future nesting capabilities. The nesting records for the 1990 season were analyzed for recaptures (Table 1). The estimated accuracy of predictions based on ultrasonography was analyzed using a formula which takes into account the ability to observe all turtles nesting on the beach during any given period:

Estimated accuracy of predictions = % of predicted turtles observed renesting ÷ % of actual nesting events observed

The mean internesting interval for the 1990 nesting season was  $25 \pm 0.4$  days (n = 139). Based on this value, the nesting season was divided into 25-day segments and the total "% of actual nesting events observed" during each period was determined as the number of actual nesting events observed (turtles observed) divided by the total number of nests laid. Analysis of renestings was conducted for turtles sampled



Figure 10. Mean total calcium and serum testosterone levels per nesting occurrence based on nesting history and ultrasonography results for *L. kempi* at Rancho Nuevo, Mexico, during the 1990 nesting season.

during April and May only since 80% of females scanned in June were postovulatory and not expected to renest. Renestings during the subsequent 25-day nesting periods were analyzed (Table 1). Analysis of June results was not possible because after the June sampling period heavy rains greatly impeded beach patrols, particularly to the north. Ultrasonography was found to be a relatively accurate tool for monitoring reproductive status and predicting future nesting based on renesting results (April: predicted = 100% renesting, estimated accuracy = 1.02; May: predicted = 86% renesting, estimated accuracy = 0.81; Table 1).

A fecundity index (FI) was calculated using ultrasonography results from turtles with known nesting histories from the 1990 season (i.e., a confirmed second- or third-time nester; Table 2). The results of 46 ultrasound scans were used in the analysis (20 first-time nesters, 16 second-time nesters, and 10 third-time nesters). A fecundity index of 3.075 nests per female was calculated on the basis that 100% of first-time nesters were expected to lay a second nest, 87.5% of second-time nesters were expected to lay a third nest, and only 20% of third-time nesters were expected to lay a fourth nest (Table 2).

During the 1990 nesting season, four *arribadas* were observed (Fig. 12). However, the third and fourth *arribadas* during June were composed of separate subgroups of females observed in the second large *arribada* of the season in May. The mean nesting interval between *arribadas* was 24.9  $\pm 0.4$  days (n = 100). Of the 311 individual females identified in the beach census, 113 were recaptured nesting twice and

Table 2. Fecundity index determination for wild Kemp's ridleys at Rancho Nuevo, Tamaulipas, Mexico, based on ultrasonography imaging results.

Nesting Event	Number of Turtles Examined	Number Predicted to Renest	Percent Predicted to Renest	Fecundity Index
0		100		1.000
1	20	20	100.0%	1.000
2	16	14	87.5%	0.875
3	10	2	20.0%	0.200



Figure 11. Clutch size vs. curved carapace length (CCL) for *L. kempi* at Rancho Nuevo, Mexico, during the 1988, 1989, and 1990 nesting seasons. A weak positive correlation was observed (clutch = -31.6 + (2.06 x CCL);  $r^2 = 0.148$ ).

19 were recaptured nesting three times. During the 1990 season, fewer than 61% of the total nesting events were actually observed by the beach patrols.

### DISCUSSION

Nesting Physiology. — The nesting physiology of the Kemp's ridley sea turtle (L. kempi) is similar to other sea turtles studied (C. mydas: Licht et al., 1979; Wibbels et al., 1992; C. caretta: Wibbels et al., 1990, 1992; L. olivacea: Licht et al., 1982; and D. coriacea: Rostal et al., 1996). Lepidochelys kempi lays multiple clutches (approximately 3 per nesting season) and is capable of higher fecundity than previously reported. As well, vitellogenesis is completed prior to the first nesting by the female.

The reproductive condition of the nesting females changed over the course of the nesting season. The pattern observed was similar to what would be expected for a multiclutch species. Ultrasonography revealed a marked decline in pre-ovulatory females as the nesting season progressed. Females began arriving at the nesting beach in April 1990 with large, robust ovaries containing multiple preovulatory follicles. As the season progressed, the percentage of females with large ovaries declined. While many still maintained large preovulatory ovaries, the follicles were not as densely grouped and viscera mostly hidden by the ovaries became more readily visible. By the end of the nesting season, the majority of females observed were postovulatory.

Serum calcium levels remained relatively constant over the nesting season. These results along with observations from reproductively active captive *L. kempi* also support the conclusion that vitellogenesis is essentially complete prior to the first nesting (Rostal, 1991; Rostal et al., in press). Increases in total calcium levels have been correlated with vitellogenesis and ovarian follicular growth in a variety of turtle species (see reviews in Rostal et al, 1996; Rostal et al., in press). In addition, ultrasonography revealed that ovarian follicle size remained relatively constant over the course of the nesting season.

During the nesting season, circulating testosterone, estradiol, and progesterone levels had similar patterns to those of other sea turtle species. Serum testosterone declined in a stepwise fashion as each clutch of eggs was produced. Similar patterns of decline have been observed in other multiclutch sea turtle species, C. mydas (Licht et al, 1979), C. caretta (Wibbels et al., 1990), and D. coriacea (Rostal et al., 1996), but the stepwise pattern has been less discernible due to the higher number of clutches laid by these species. Rostal et al. (in press) also observed a similar pattern in testosterone in nesting captive L. kempi at the Cayman Turtle Farm. Estradiol levels also declined slightly during the nesting season but were relatively low throughout. This pattern was similar to observations on C. mydas and C. caretta during the nesting season (Licht et al., 1979; Wibbels et al., 1990). Nesting season serum estradiol levels from wild females were markedly reduced relative to levels observed in captive females during the vitellogenic period four to six months prior to mating (Rostal et al., in press). Elevated levels of progesterone have been correlated with luteinizing hormone (LH) and ovulation in L. olivacea (Licht et al., 1982), C. mydas (Wibbels et al., 1992), and C. caretta (Wibbels et al., 1992) approximately 24 to 48 hours postnesting in females capable of nesting again during the season. Progesterone levels in females we sampled at the time of nesting remained relatively constant over the course of the nesting season.

Based on serum testosterone levels, *arribada* nesters were not discernible from solitary nesters. This observation was not unexpected since many solitary nesters were later observed during *arribadas* and vice versa. The physiological and behavioral mechanisms that regulate *arribada* be-



Figure 12. Histogram of *L. kempi* nesting at Rancho Nuevo, Mexico, during the 1990 season. Three *arribada* periods were observed (late April, late May, and mid-to-late June). Females were repeatedly observed during all three periods.

havior still remain a mystery. Eckrich and Owens (1995) demonstrated that the evolution of *arribada* nesting appears to support the role of predator satiation in *L. olivacea*. However, the nesting physiology of solitary and *arribada* nesters appears similar, with the exception of the duration of the internesting interval. Solitary nesters have a 14-day interval while *arribada* nesters have approximately a 28-day interval. The physiological mechanism regulating this increased egg retention in *arribada* nesters has not been elucidated.

The step-wise decline observed in serum testosterone suggests that following mating, approximately one-third of the preovulatory vitellogenic follicles are ovulated in preparation for nesting. Following nesting, ovulation is reported to occur within 48 hours in response to LH and progesterone (Licht et al., 1979; Wibbels et al., 1992). With each subsequent clutch produced, serum testosterone was observed to decline. These observations support the hypothesis that the granulosa cells around the preovulatory follicles are the source of steroid production and that following ovulation this source is lost (Owens, 1997).

While clutch size has been correlated with female size in most sea turtle species (Hirth, 1980; Van Buskirk and Crowder, 1994), including L. kempi, the relationship between follicle size and female size has largely gone unstudied. Early embryonic development can occur within the oviduct of sea turtles (Miller, 1985). Embryos from L. olivacea are reported to be at late gastrula stage at the time of nesting (Crastz, 1982). Concurrent with this oviductal embryonic development, the yolk changes size and shape (unpublished data). Thus, studying follicle size was only possible from necropsy or by ultrasonography. Using ultrasonography, we were able to confirm that follicle size is independent of female size in L. kempi. Similar observations have been made for D. coriacea (Rostal et al., 1996). In other reptiles, larger hatchlings do not directly equate to increased fitness (Congdon, 1989). Thus far, in the two sea turtle species studied to date (L. kempi and D. coriacea), larger females do not produce larger follicles.

Population Estimates. - It was previously thought, based on a fecundity index of 1.3 to 1.5 nests per female per season, that each arribada of the season was largely a separate population or sub-population arriving at the nesting beach independently (Márquez et al., 1982; Márquez, 1994). Pritchard (1990) reanalyzed the 1989 nesting season data for L. kempi at Rancho Nuevo taking into account the probability of observing a given turtle on all of its nesting emergences and argued that the population was being overestimated. His analysis suggested that L. kempi laid at least 2.3 nests per season and that tag loss would push this figure higher. We found that the majority of the nesting population apparently arrives at Rancho Nuevo in mid- to late April and remains in the vicinity through June until nesting is completed. 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  $24.9 \pm 0.4$  days).

The pattern of nesting during the 1990 season consisted of three *arribadas* and solitary nestings (Fig. 12). Multiple recaptures of nesting females throughout the nesting season further support the calculation of 3+ nests per female from the ultrasonography and testosterone data. These observations have significant implications with regard to monitoring and managing this critically endangered species. Tucker (1989) and Steyermark et al. (1996) have also elucidated the effects of underestimating fecundity on overestimating population size in *D. coriacea*. It also is important to note that there has been a positive trend in increased numbers of nests since 1990 suggesting an increase in the adult female population (Márquez et al., 1996).

A formula for more accurate estimation of the adult female population size has been validated by the National Research Council Committee on Sea Turtle Conservation (Magnuson et al., 1990):

$$\mathbf{P}_{\mathrm{nf}} = \frac{\mathbf{N}_{\mathrm{t}}}{\mathbf{N}_{\mathrm{f}}} \div \boldsymbol{p}_{\mathrm{nf}}$$

where  $\mathbf{P}_{nt}$  = total population of adult females,  $\mathbf{N}_{t}$  = total number of nests per year,  $\mathbf{N}_{r}$  = average number of nests per reproductively active female (fecundity index), and  $p_{nr}$  = proportion of females that nest in a given year.

Using this formula for the 1978 to 1988 nesting season results, Magnuson et al. (1990) noted that population estimates may be overestimated based on which value for the fecundity index (N<sub>c</sub>) is used (650 females if  $N_f = 1.3$  nests per female [Márquez et al., 1982]; or 350 females if  $N_f = 2.3$  nests per female [Pritchard, 1990]). Our 1990 results based on ultrasonography suggest N, = 3.075 nests per female which results in an even lower value for the female nesting population: 317 nesting females, based on 977 nests laid (vs. 425 females if N, = 2.3 or 751 females if  $N_r = 1.3$ ). In this calculation,  $p_{re}$  was assumed to be 100% or 1.00 (each female nests annually). Magnuson et al. (1990) noted that a firm estimate of  $p_{nf}$  is still not available. Tagging studies have been utilized over the years in an attempt to estimate this value, however, tag loss has not been accurately estimated for this species. A total of 407 monel flipper tags were recorded or placed on nesters in 1988, however, only 87 of these tags (20.64%) were observed during the subsequent 1989 and 1990 seasons, possibly due to tag loss. Alternatively, this may also reflect a longer interannual nesting interval than previously suggested. or increased mortality. PIT tag results will hopefully help refine our estimate of  $p_{nf}$ .

The interannual nesting interval is the best available estimate of  $p_{nf}$ . An accurate estimate for all females in the population is not possible until a more accurate value for  $p_{nf}$  is available. For example, based on 1990 nesting records, a

value of 100% or 1.0 (annual nesters) results in an estimate of 317 adult females, a value of 75% or 0.75 (1.5 year interval) results in an estimate of 423 adult females, and a value of 50% or 0.50 (2 year interval) results in an estimate of 634 adult females. The  $p_{\rm nf}$  value clearly has a significant role in the estimation of sea turtle populations. It is well established that other sea turtles such as *C. mydas* and *C. caretta* nest on a multi-annual basis and it is probable that *L. kempi* requires a longer interannual nesting interval as well.

Natural sex ratios for sea turtle populations have been the subject of significant study. Márquez (1994) noted that no data exist to estimate the adult sex ratio in L. kempi. While Limpus and Reed (1985) demonstrated a 1:1 sex ratio for adult and immature C. mydas (n = 187) in waters of Heron Island, Australia, Limpus (1985) also found a significantly male-biased sex ratio for C. caretta (n = 106) inhabiting the same reef system. Wibbels et al. (1987) demonstrated that the sex ratio of immature C. caretta (n = 272) along the Atlantic coast of the US is female-biased. Owens (1997) reviewed the studies of sex ratio currently completed on C. caretta, C. mydas, and Eretmochelys imbricata and demostrated that both male- and female-biased populations may exist within the same species. Thus, further studies on the sex ratio of L. kempi are necessary before the male population can be legitimately estimated. Although we do not know the sex ratio of L. kempi, it is apparent that sufficient males still survive for adequate mating based on the relatively high hatching success (75% in 1988, 79% in 1989, and 79% in 1990) of relocated wild nests laid at Rancho Nuevo, Mexico (Burchfield et al., 1990).

The results of our study support the conclusions that *L. kempi* is physiologically capable of 3+ nests per nesting season and that potentially fewer adult females may survive than previously estimated. It must be stressed, however, that these data reflect the fecundity during the 1990 nesting season only. More analysis of the existing 30-year nesting data base is required to determine the average fecundity and changes in response to annual fluctuations in ecological variables.

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