

THE PREDICTED SEX RATIO OF HEAD-STARTED KEMP'S RIDLEYS
DETERMINED BY TESTOSTERONE RADIOIMMUNOASSAY

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The discovery of temperature-dependent sex determination in sea turtles has caused concern that current conservation practices might inadvertently alter the population sex ratios. The Kemp's ridley, Lepidochelys kemp, has been the focus of a concerted effort between Mexico and the United States to establish a new nesting beach on Padre Island, Texas (Klima and McVey, 1982). Under certain conditions, the use of styrofoam boxes as incubation chambers may favor testicular development, as has been demonstrated in the green sea turtle, Chelonia mydas (Mrosovsky, 1982). A predicted sex ratio for the head-started Kemp's ridleys was determined by the measurement of circulating testosterone in 32 animals. This technique was used with complete accuracy to determine sex in immature green turtles (Owens et al., 1978).

The experimental animals were part of the 1978 Kemp's ridley head starting program in which the animals were ^{head starting} at the National Marine Fisheries Lab in Galveston. Twenty-two ridleys were selected in March 1979 and sent to Seaquarium in Miami, FL. Another ten turtles were moved to SeaArama (Galveston, TX) in August. Blood samples, collected by the dorsal cervical sinus sampling technique (Owens and Ruiz, 1980), were taken every 3-4 months over a 20-month period from the SeaArama turtles beginning at two and one-half years of age. Each Seaquarium ridley was sampled at least once and as many as three times during the same period. Testosterone levels were determined for each sample by a specific radioimmunoassay.

In both the Seaquarium and SeaArama groups there was a significant dichotomy in testosterone (T) levels between the predicted males and females at all sampling times (Table 1). Using these consistent differences, the predicted sex ratio for the 1978 year class is 21M:11F. The predicted sex of two of the Seaquarium individuals was also verified by autopsy (both males).

Generally each sample set from a group of ridleys gave the same predicted sex ratio. However, because T levels fluctuate seasonally, the threshold level of T separating males from females also varies. For example, 200 pg/ml was used as the arbitrary limit in the spring 1981 samples. The separation was not well defined at the September 1981 sampling period. At that time, two of the suspected females

TABLE 1

A COMPARISON OF THE CIRCULATING TESTOSTERONE LEVELS IN THE
SUSPECTED MALE AND FEMALE KEMP'S RIDLEYS

Sampling Date	Testosterone (pg/ml) (Mean $\pm$ SE)	N	Level of Significance	Temp. °C
SeaArama:				
22 Jan 81	M: 72.50 $\pm$ 19.45 F: 14.25 $\pm$ 7.09 (cutoff 25 pg/ml)	4 4	$\alpha = .025$	20.6
26 May 81	M: 315.25 $\pm$ 31.50 F: 13.33 $\pm$ 0.88 (cutoff 200 pg/ml)	4 6	$\alpha = .0005$	25.6
11 Sept 81	M: 761.43 $\pm$ 112.46 F: 78.67 $\pm$ 1.28 (cutoff 146 pg/ml*)	4 6	$\alpha = .0005$	26.7
4 Dec 81	M: 146.88 $\pm$ 21.43 F: 35.90 $\pm$ 10.58 (cutoff 95 pg/ml)	4 6	$\alpha = .0005$	21.7
13 Feb 82	M: 236.00 $\pm$ 72.94 F: 18.33 $\pm$ 0.99 (cutoff 35 pg/ml)	4 6	$\alpha = .005$	22.8
18 May 82	M: 922.75 $\pm$ 129.01 F: 32.33 $\pm$ 4.84 (cutoff 200 pg/ml)	4 6	$\alpha = .0005$	27.8
14 Sept 82	M: 652.75 $\pm$ 144.74 F: 26.33 $\pm$ 5.20 (cutoff 200 pg/ml)	4 6	$\alpha = .0005$	28.3
Seaquarium:				
4 June 81	M: 1281.29 $\pm$ 167.36 F: 7.60 $\pm$ 0.98 (cutoff 200 pg/ml)	17 5	$\alpha = .0005$	28.0
27 May 82	M: 1095.00 $\pm$ 68.27 F: 14.00 (cutoff 200 pg/ml)	5 1	$\alpha = .005$	27.0

* see text for details

had relatively high levels of serum testosterone (138 and 146 pg/ml). Concurrently, one suspected male, with consistently lower T levels than the other males, also had serum T levels of 146 pg/ml. Although discrimination of the two sexes would have been difficult to do based on that data set alone, subsequent sampling confirmed the original sex prediction.

Measurement of tail lengths, a secondary sex characteristic for marine turtles, also revealed a significant difference between the predicted males and females. At three and one-half years of age the mean tail lengths ($\pm$ SE) for the SeaArama ridleys were 10.33 ± 0.49 cm and 7.18 ± 0.39 cm for males and females respectively. The Seaquarium turtles demonstrated a similar dichotomy in tail lengths at four years of age (M: 19.04 ± 1.59 ; F: 9.94 ; $\alpha = .05$).

Comparison of serum testosterone between the two subsamples reveals that the Miami Seaquarium males have levels four times those of the Galveston SeaArama animals sampled at the same time of year. At least two factors may have contributed to these differences. First, temperature is highly correlated with T levels in the predicted SeaArama males ($r^2 = 0.928$). Therefore the 2.4°C difference in water temperature could account for the disparity in serum testosterone. This observation suggests a cause/effect relation-

ship important with regards to reproductive cycles and circannual rhythms. Second, the Miami animals were fed more, resulting in much larger individuals of the same age.

The two subgroups also had different predicted sex ratios (Miami 17M:5F; Galveston 4M:6F). This discrepancy was shown to be statistically significant by the binomial test ($p < .005$; Bhattacharyya and Johnson, 1977) and by the log likelihood ratio test ($p < .005$; Sokal and Rohlf, 1969). The turtles ⁵went to Miami were the largest in the head-start program at that time and may have been several weeks older than those that went to the Galveston SeaArama. If this scenario is correct, the older, earlier hatched animals may have experienced cooler spring temperatures resulting in the production of more males.

During the 1978 season, incubation time was about five days longer in the artificial nests than on the beach. Marquez (1978) speculated that the cause was a 2-3°C temperature difference between the two types of incubation environments. If so, the total estimated sex ratio of 21M:11F (combined SeaArama and Seaquarium populations) supports the hypothesis that lower temperatures bias male differentiation. However, Mrosovsky and Yntema (1980) formulated a general working rule that a 1°C drop in temperature extends incubation time by five days. They concluded that the average incubation temperature in the artificial nests of the head-starting program for the Kemp's ridley was 1°C cooler

than the sand at the nesting beach. They suggested that the 2-3°C difference seen by Marquez was an extreme rather than the average temperature difference.

In conclusion, several lines of evidence, including repeatability over time, autopsy verification and developing tail lengths all indicate that measurement of circulating T levels may be a reliable method of predicting sex in immature animals. The predicted sex ratio of 21M:11F for the head-started population suggests that incubation in styrofoam boxes may be influencing sex differentiation in favor of males, assuming a "normal" 1:1 ratio. Finally, careful comparisons of tail lengths may prove to be useful as a means of predicting the sex of immature animals in field studies.

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