SHORTER CONTRIBUTIONS

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Predicted Sex Ratio of Juvenile Kemp's Ridley Sea Turtles Captured near Steinhatchee, Florida

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The Kemp's Ridley (*Lepidochelys kempii*) is one of the most endangered sea turtles in the world, and it possesses temperature-dependent sex determination (TSD). Sex ratios produced under TSD can vary widely and can affect the reproductive ecology of a population. Therefore, sex ratios produced from TSD are of ecological and conservation interest. The current study validated and utilized a testosterone radioimmunoassay (RIA) to examine the sex ratio of juvenile Kemp's Ridleys inhabiting the waters near Steinhatchee, Florida. Testosterone levels were measured in blood samples collected from juvenile Kemp's Ridleys captured over a three-year period. Results of this study indicate that a significant female bias (approximately 3.7:1) occurs in the aggregation of juvenile Kemp's Ridleys inhabiting the waters near Steinhatchee.

SEXUAL determination in many reptiles, including all species of sea turtles, is dependent upon the incubation temperature of the eggs (Janzen and Paukstis, 1991; Mrosovsky, 1994; Wibbels et al., 2000b). Under a temperature-dependent sex determination (TSD) regime, there is the potential of producing biased sex ratios, which do not conform to a 1:1 ratio (Mrosovsky 1994; Wibbels, 2003). Biased sex ratios have the potential of affecting the reproductive ecology in a population. Therefore, sex ratios resulting from TSD are of ecological and evolutionary interest. Biased sex ratios can also affect the recovery of an endangered population, and are therefore of conservation interest.

The Kemp's Ridley, *Lepidochelys kempii*, is one of the most endangered species of sea turtle in the world (Dodd and Byles, 1991). They are commonly found in the coastal and bay waters of the Gulf of Mexico and along the Atlantic coast of the United States (Hildebrand, 1982; Rudloe et al., 1991; Schmid, 2000). It is beneficial to the conservation of this endangered species to determine population demographics including sex ratios, because knowledge of naturally occurring sex ratios provides insight regarding reproductive ecology. Such information is beneficial to the development of an effective management strategy for the recovery of this species.

The purpose of the current study was to examine the sex ratio of juvenile Kemp's Ridley Sea Turtles inhabiting the waters near Steinhatchee, Florida. Determining the sex of juvenile sea turtles is logistically difficult because they do not possess the external characteristics commonly used to identify sex in adult sea turtles (e.g., long tail length in males). Several alternative methods to determine sex have been evaluated including the use of a testosterone radioimmunoassay (RIA), which appears to be an effective and accurate method for determining the sex of juvenile sea turtles (Wibbels et al., 2000b), including the Kemp's Ridley (Coyne, 2000; Gregory and Schmid, 2001). In the current study, a testosterone RIA sexing technique was utilized to determine the sex of juvenile Kemp's Ridleys that were captured over a three-year period near Steinhatchee, Florida.

MATERIALS AND METHODS

The turtles utilized in this study were captured during a population survey study of a juvenile foraging area off the Gulf coast of Florida conducted from 1998–2000 by the U.S. Geological Survey (USGS; Barichivich, pers. comm.). Typically, turtles would be sighted below the surface; then, a small mesh net would be deployed in the area surrounding the last sighting of the turtle. Encircled turtles were retrieved from the net by a snorkeler, generally within five minutes of hitting the net. Some turtles were captured by hand when appropriate (e.g., the water was

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too shallow to run the boat). Immediately after capture, a blood sample was taken from each turtle (see details below) and each turtle was tagged, measured, and then released within 15 minutes of capture. Turtles were designated as juveniles based on their straight carapace length after considering the smallest adult turtles found on the primary Kemp's Ridley nesting beach (59.1–63.0 cm, Chavez et al., 1968). No adults were captured in the survey. Although the survey was conducted year-round, the majority of samples were obtained from turtles that were captured during the summer and fall months (Barichivich, pers. comm.)

Before a turtle was released, blood samples were drawn from the dorsal cervical sinus in the dorsal portion of the neck, as described by Owens and Ruiz (1980). The blood samples were taken in heparinized vacutainers within five to ten min after capture and kept on ice until they were centrifuged to separate blood cells and plasma. The plasma from each sample was pipetted into cryotubes and frozen at −20 C until they were assayed. A testosterone RIA was utilized in this study due to the practical and logistical difficulties associated with laparoscopy (see review by Wibbels et al., 2000b).

To predict the sex of the juvenile turtles, a testosterone RIA, similar to that described by Wibbels et al. (1991), was utilized to measure circulating testosterone levels (Owens et al., 1978; Wibbels et al., 2000b). Approximately 250 μl aliquots of each sample were assayed in duplicate. Steriod hormones were extracted from each aliquot using three ml of anhydrous diethyl ether. The samples were then dried under nitrogen gas and resuspended in 500 μl of Tris-gel buffer. To assess extraction efficiencies, approximately 1000 cpm of tritiated testosterone (DuPont NEN, MA) were added to each aliquot prior to extraction. From each resuspended sample, 200 μl were assayed by adding 100 μl of testosterone antisera (Endocrine Sciences, CA, Lot #01916M) and approximately 10,000 cpm of tritiated testosterone in a 100 μl volume. The samples were allowed to incubate a minimum of 12 h at 4 C. After incubation, three ml of a dextran-coated charcoal suspension were added to each assay tube. The assay tubes were then incubated for 15 min, and centrifuged for 15 min to separate bound and unbound fractions. The bound fraction was poured into polyethylene scintillation vials and three ml of scintillation cocktail (Scintiverse, Fisher Scientific, GA) were added to each vial. The vials were counted on a beta counter. To generate a standard curve, serial dilutions of testosterone standard were included with each assay and ranged from 15.625 to 2000 pg/ml. A minimum of two control samples was run in each assay to generate intra- and interassay coefficients of variation.

To validate the testosterone RIA, blood samples were obtained from a previous study in which the sex of juvenile Kemp's Ridleys from the western Gulf of Mexico had been verified via laparoscopy (Coyne, 2000; laparoscopy procedure described by Owens, 1999). These blood samples were analyzed using the testosterone RIA methodology described to determine ranges of male and female testosterone levels for juvenile Kemp's Ridleys. These ranges were then used to predict the sex of the juvenile Kemp's Ridleys that were captured near Steinhatchee, Florida. Any samples that fell in the intermediate area between the designated male and female ranges were not assigned a sex. In this method of validation, it is assumed that the relationship of serum testosterone level and sex is the same between (and among) aggregations of juvenile Kemp's Ridley Sea Turtles.

Eleven assays were conducted to complete the evaluation of all samples in duplicate (both validation and Steinhatchee samples). Average assay sensitivity was 6.56 ± 2.1 pg, and intra- and interassay coefficients of variation were 11.7% and 12.6%, respectively, for the male control; 9.27% and 6.79%, respectively, for the female control A; and 4.6% and 6.0%, respectively, for the female control B over all conducted assays. (The female control required the use of two individuals, A and B, due to low collection volume of serum per female).

Results

Forty-eight samples were used to validate the testosterone RIA, 23 of which were immature females and 25 of which were immature males (Fig. 1). Straight carapace lengths of those turtles ranged from 23.9 to 61.9 cm (42.4 ± 11.8 cm, mean ± SD). The ranges of male and female testosterone levels did not overlap. The testosterone levels of the males ranged from 273.4 to 10780.0 pg/ml, whereas the females ranged from 46.1 to 206.1 pg/ml (Fig. 1).

Plasma samples from 42 individual juvenile Kemp's Ridleys captured near Steinhatchee were examined in the current study (recaptures were excluded from analysis). The turtles ranged in size from 22.2 to 48.2 cm (33.5 ± 7.2 cm, mean ± SD). Although the range of straight carapace lengths of the Steinhatchee turtles overlaps the range of the turtles used as validation, the means were found to be significantly different (t-test, $t = -4.39$, $P < 0.05$). Of

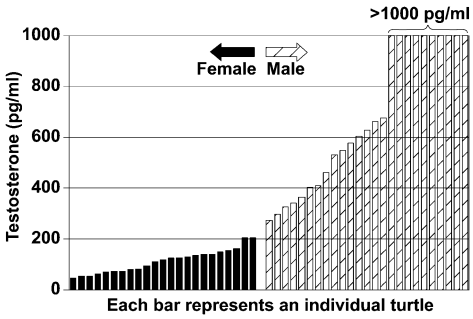

Fig. 1. Serum testosterone levels of juvenile Kemp's Ridley Sea Turtles whose sex was verified by laparoscopic examination (Coyne and Owens, unpubl. data). Each bar, in order of increasing testosterone concentration, represents an individual turtle.

Fig. 2. Serum testosterone levels and predicted sex of juvenile Kemp's Ridley Sea Turtles that were captured foraging near Steinhatchee, Fl. Each bar, in order of increasing testosterone concentration, represents an individual juvenile.

the Steinhatchee samples, 33 were determined to be female, and nine were male. The testosterone range for the Steinhatchee female turtles was 38.5 to 204.8 pg/ml, whereas the range for the male turtles was 408.4 to 1903.0 pg/ml (Fig. 2). No samples fell into the intermediate area between the male and female ranges. Testosterone concentration did not significantly correlate to the straight carapace length of the turtles ($r^2 = 0.03$ females and males, $P > 0.05$). The resulting sex ratio of juvenile turtles captured near Steinhatchee is 3.7:1.0 (female:male). This ratio was significantly different from a 1:1 sex ratio (replicated goodness of fit test, $G = 14.6$, $P < 0.005$).

To evaluate possible variation of sex ratio relative to size (and potentially age), turtles were arbitrarily divided into three size classes based on ten cm intervals of carapace length, with the smallest size class starting at 20.0 cm (Fig. 3). One turtle was excluded from this evaluation because the carapace length was not known. The sex ratios in the two groups of the smallest turtles (6.0:1.0 and 5.6:1.0, female:male) were significantly different from a 1:1 ratio ($N = 14$, $G = 7.9$, $P < 0.005$ and $N = 20$, $G = 10.8$, $P < 0.005$ respectively), but the sex ratio in the largest group of turtles (1.0:1.3, female:male) was unbiased ($N = 7$, $G = 0.14$, $P > 0.05$).

Discussion

The sex of many turtles is determined by the temperature experienced during embryonic development (reviewed by Bull, 1980; Janzen and Paukstis, 1991). As a consequence of TSD, sex ratios have the potential to vary between populations (Mrosovsky, 1994; Wibbels, 2003). Determination of juvenile sex ratios is crucial to population demographics, and is beginning to receive greater attention from researchers. This study used a testosterone RIA to evaluate the sex ratio in an assemblage of juvenile Kemp's Ridleys inhabiting the waters near Steinhatchee, Florida.

Previous studies have demonstrated the effective use of RIAs as a method for determining the sex of several juvenile sea turtle species, including Kemp's Ridleys (Owens et al., 1978; Wibbels et al., 2000b; Geis et al., 2003). The results of this study verify that a testosterone RIA can be used to determine the sex of juvenile Kemp's Ridley Sea Turtles. Circulating testosterone levels were easily detected in this assay when evaluating 250 μl of serum. The testosterone ranges designated for male and female juveniles during validation did not overlap, and all of the unknown turtles examined had testosterone levels that fell within either the male or

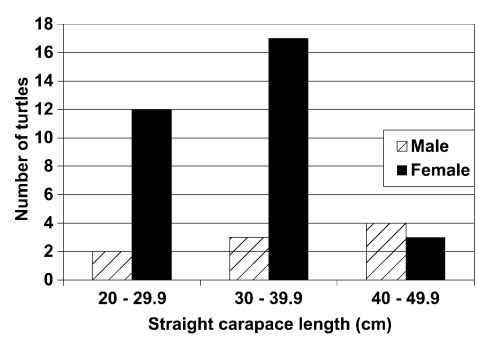

Fig. 3. Sex ratios of Kemp's Ridley Sea Turtles grouped by size using ten cm intervals. The ratios for 20–29.9 cm group (6.0:1.0, female:male) and 30–39.9 cm group (5.6:1.0, female:male) of turtles are significantly female biased ($P < 0.005$), while the sex ratio of the 40–49.9 cm group (1.0:1.3, female:male) is unbiased ($P > 0.05$). One turtle was excluded from analysis because the size was not available to the author.

TABLE 1. SEX RATIOS REPORTED FOR IMMATURE KEMP'S RIDLEY SEA TURTLES. Sexing methods included necropsy of dead turtles that had stranded, or the evaluation of blood testosterone levels of turtles that had been captured alive during netting projects. In an attempt to exclude adult turtles from this table, data from turtles with straight carapace lengths greater than 60 cm are excluded, with the exception of the data from Shaver, 1991. In the study by Shaver, 1991, the data is reported as single sex ratio for a sample composed primarily of immature turtles (approximately 90% of the turtles examined were immature). All sex ratios are reported as female:male and were tested for significance against a 1:1 sex ratio (Chi Square).

Location	Sampling period	Sexing method	n	Sex ratio	Significance	Reference
Cape Cod, MA	1977–1987	Necropsy	48	1.4:1.0	$P > 0.05$	Danton and Prescot, 1988
South TX Coast	1983–1989	Necropsy	81	1.0:1.0	$P > 0.05$	Shaver, 1991
Long Island Sound, NY	1985–1987	Necropsy	30	3.2:1.0	$P < 0.05$	Morreale et al., 1992
Upper TX Coast	1986–1992	Necropsy	08	2.9:1.0	$P < 0.05$	Stabenau et al., 1996
Cedar Keys, FL	1992	Testosterone	36	1.8:1.0	$P < 0.05$	Gregory and Schmid, 2001
Upper TX and LA Coast	1992–1997	Testosterone	229	1.4:1.0	$P < 0.05$	Coyne, 2000
Upper TX and LA Coast	1994	Necropsy	88	1.0:1.2	$P > 0.05$	Cannon, 1998

female range. Assay sensitivity and precision was typical for a testosterone RIA. As such, the results of this study indicate that testosterone level provides a practical and effective means of estimating the sex ratios in the immature portion of the Kemp's Ridley population.

The results indicate a 3.7:1.0 (female:male) sex ratio of juvenile Kemp's Ridleys captured near Steinhatchee. This female-biased sex ratio differed significantly from a 1:1 sex ratio. Furthermore, an ongoing study of juvenile Kemp's Ridleys captured near Ten Thousand Islands, Florida, also suggests a female bias (National Marine Fisheries Service, Wayne Witzell, pers. comm.). Previous studies have investigated sex ratios of juvenile Kemp's Ridleys from a variety of locations in the Gulf of Mexico and Atlantic Ocean (Table 1). These studies reported a variety of sex ratios ranging from near 1:1 to strong female biases. The reasons for the disparity in reported sex ratios are unknown, but female-biased sex ratios predominate in these studies.

Variations in sex ratios of juvenile sea turtles could relate to many factors. It is possible that sex ratios could change over time in a population. This could account for the difference between the sex ratio in current study (3.7:1.0, female:male) and that estimated in a study conducted six to eight years earlier (1.8:1.0, female: male) in the same general area (Gregory and Schmid, 2001). This suggests that young turtles recruited into the juvenile aggregation near Steinhatchee caused an increase in the female bias during the mid to late 1990s. As such, it is possible that sex ratio fluctuation over time could account for at least some of the variation in sex ratios reported for Kemp's Ridleys. There are also alternative factors that could account for the diversity of sex ratios reported. First, there may be a sampling bias associated with the sampling technique. Netting studies often exclude the smallest size range of turtles due to the fact that wide mesh nets are used to decrease bycatch. Optimally, sex ratio comparisons should be made between the same size classes of turtles. Second, it is possible that sex ratio may vary between sampling locations. Seasonal or yearly variations of winds and currents in the Gulf of Mexico could affect the recruitment of young Kemp's Ridleys into specific foraging areas (e.g., west coast of Florida versus Texas coast). It is also possible that certain foraging areas may be more attractive to certain size classes of juveniles due to characteristics such as depth and current.

If a female bias exists, it may be influenced by the intense conservation efforts that have been directed toward this endangered species over the past several decades at the primary nesting beach located near Rancho Nuevo, Mexico. These efforts include the moving of most nests to protected egg hatcheries over the past several decades (Marquez-M., unpubl. data). The current juvenile sex ratio is a direct result of incubation temperatures in the egg hatcheries during previous years, and studies suggest that those egg hatcheries produce sig-

nificant female biases (Geis et al., 2000; Wibbels et al., 2000a; Geis et al., 2002). However, this does not preclude the possibility that the juvenile sex ratio recorded in this study might be similar to a ''natural'' sex ratio if all nests were incubated *in situ* on the nesting beach. Data from the nesting beach near Rancho Nuevo suggest that nests incubated *in situ* could produce a female-biased sex ratio (Geis et al., 2002).

Regardless of whether the female-biased sex ratio is similar to a ''natural'' sex ratio, it is of conservation significance because it will affect reproduction during future years. A female bias has the potential of increasing egg production and thus enhancing the recovery of an endangered species (Vogt, 1994). However, caution should be used when considering such a hypothesis without support from empirical data, since males could be a limiting factor in reproduction (Mrosovsky and Godfrey, 1995; Lovich, 1996; Wibbels, 2003). Nonetheless, the female bias reported in the current study should be considered when developing management strategy for the recovery of the Kemp's Ridley.

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Literature Cited

Bull, J. J. 1980. Sex determination in reptiles. Quart. Rev. Biol. 55:3–21.

Cannon, A. C. 1998. Gross necropsy results of sea turtles stranded on the upper Texas and western Louisiana coasts, 1 January–31 December 1994. NOAA Technical Report NMFS– 143:81–85.

Chavez, H., M. Contreras-G., and T. P. E. Hernandez-D. 1968. On the coast of Tamaulipas. Internat. Turt. Tort. Soc. J. 2:20–29.

Coyne, M. S. 2000. Population sex ratio of the Kemp's Ridley Sea Turtle (*Lepidochelys kempii*): problems in population modeling. Unpubl. Ph.D. diss., Texas A&M University, College Station, Texas.

Danton, C., and R. Prescott. 1988. Kemp's Ridley research in Cape Cod Bay, Massachusetts–1987 Field Research, p.17–18. *In*: Proceedings of the Eighth Annual Workshop on Sea Turtle Conservation and Biology. B.A. Schroeder (comp.). NOAA Technical Memorandum NMFS–SEFC-214.

Dodd, C. K., Jr., and R. Byles. 1991. The status of Loggerhead, *Caretta caretta*; Kemp's Ridley, *Lepidochelys kempi*; and Green, *Chelonia mydas*, Sea Turtles in U.S. waters: a consideration. Mar. Fish. Rev. 53: 30–31.

Geis, A., T. Wibbels, R. Marquez-M., M. Garduno-D., P. Burchfield, and J. Pena. 2000. Evaluation of hatchling Kemp's Ridley sex ratios using nest incubation temperatures at Rancho Nuevo, Mexico. Amer. Zool. 40:1026–1027.

———, T. Wibbels, B. Phillips, Z. Hillis-Starr, A. Meylan, P. Meylan, C. Diez, and R. VanDam. 2003. Predicted sex ratio of juvenile Hawksbill Seaturtles inhabiting Buck Island Reef National Monument, U.S. Virgin Islands. J. Herpetol. 37:400–404.

———, T. Wibbels, M. Garduno-D., R. Marquez-M., P. Burchfield, and B. Schroeder. 2002. Evaluation of Kemp's Ridley hatchling sex ratios within egg corral and *in situ* nests at the primary nesting beach. Integr. Comp. Biol. 42:1234.

Gregory, L. F., and J. R. Schmid. 2001. Stress responses and sexing of wild Kemp's Ridley Sea Turtles (*Lepidochelys kempii*) in the northeastern Gulf of Mexico. Gen. Comp. Endo. 124:66–74.

Hildebrand, H. H. 1982. Biology and conservation of sea turtles, p. 447–453. *In*: A Historical Review of the Status of Sea Turtle Populations in the Western Gulf of Mexico. K. A. Bjorndal (ed.). Smithsonian Inst. Press, Washington, D.C.

Janzen, F., and G. L. Paukstis. 1991. Environmental sex determination in reptiles: ecology, evolution and experimental design. Quart. Rev. Biol. 66:149–179.

Lovich, J. E. 1996. Possible demographic and ecologic consequences of sex ratio manipulation in turtles. Chel. Conserv. Biol. 2:114–117.

Morreale, S. J., A. B. Meylan, S. S. Sadove, and E. A. Standora. 1992. Annual occurrence and winter mortality of marine turtles in New York waters. J. Herpetol. 26:301–308.

Mrosovsky, N. 1994. Sex ratios of sea turtles. J. Exp. Zool. 270:16–27.

———, and M. H. Godfrey. 1995. Manipulating sex ratios: turtle speed ahead! Chel. Conserv. Biol. 1: 238–240.

Owens, D. W., J. R. Hendrickson, V. Lance, and I. P. Callard. 1978. A technique for determining sex of immature *Chelonia mydas* using radioimmunoassay. Herpetologica 34:270–273.

———, and G. J. Ruiz. 1980. New methods for obtaining blood and cerebrospinal fluid from marine turtles. *Ibid.* 36:17–20.

———. 1999. Reproductive cycles and endocrinology, p. 119–123. *In*: Research and Management Techniques for the Conservation of Sea Turtles. K. L. Eckert, K. A. Bjorndal, F. A. Abreu-Grobois, and M. Donnelly (eds.). IUCN/SSC Marine Turtle Specialist Group Publication 4.

Rudloe, A., J. Rudloe, and L. Ogren. 1991. Occurrence of immature Kemp's Ridley Turtles, *Lepidochelys kempi*, in coastal waters of northwest Florida. N.E. Gulf Sci. 12:49–54.

SHAVER, D. J. 1991. Feeding ecology of wild and head-started Kemp's Ridley Sea Turtles in South Texas waters. J. Herpetol. 25:327–334.

SCHMID, J. R. 2000. Activity patterns and habitat associations of Kemp's Ridley Turtles, *Lepidochelys kempi*, in the coastal waters of the Steinhatchee, Florida. Unpubl. Ph.D. diss., University of Florida, Gainesville, Florida.

STABEANAU, E. K., K. S. STANLEY, AND A. M. LANDRY, JR. 1996. Sex ratios from stranded sea turtles on the upper Texas coast. J. Herpetol. 30:427–430.

VOGT, R. C. 1994. Temperature controlled sex determination as a tool for turtle conservation. Chel. Conserv. Biol. 1:159–162.

WIBBELS, T. 2003. Critical approaches to sex determination in sea turtles, p. 103–134. *In*: The Biology of Sea Turtles. Volume 2. P.L. Lutz, J. A. Musick, and J. Wyneken (eds.). CRC Press, Boca Raton, Florida.

———, R. E. MARTIN, D. W. OWENS, AND M. J. AMOSS. 1991. Female-biased sex ratio of immature Loggerhead Sea Turtles inhabiting the Atlantic coastal waters of Florida. Can. J. Zool. 69:2973–2977.

———, R. MARQUEZ-M., M. GARDUNO-D., P. BURCHFIELD, AND J. PENA. 2000a. Incubation temperatures in Kemp's Ridley nests during the 1998 nesting season, p. 133–134. *In*: Proceedings of the 19th Annual Symposium on Sea Turtle Conservation and Biology. H. Kalb and T. Wibbels (eds). NOAA Technical Memorandum NMFS-SEFSC-443.

———, D. W. OWENS, AND C. J. LIMPUS. 2000b. Sexing juvenile sea turtles: is there an accurate and practical method? Chel. Conserv. Biol. 3:756–761.

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