

## Sex Ratio of an Immature Hawksbill Seaturtle Aggregation at Mona Island, Puerto Rico

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**ABSTRACT.**—With nest incubation temperature determining sex in marine turtle hatchlings, sex ratios among populations and intermediate aggregations are likely to exhibit more geographic and temporal variability than for vertebrates with genotypic sex determination. In this study, we examined the sex ratio of an immature Hawksbill Seaturtle (*Eretmochelys imbricata*) aggregation at Mona Island, Puerto Rico, using serum testosterone level criteria that were validated through laparoscopy in a subset of turtles. Measured serum testosterone for female turtles ranged between 0.32 and 17 pg/ml and for males between 18.2 and 262 pg/ml. Of 120 turtles sampled, 53 individuals were classified as females and 66 as males, with one individual undetermined. The resulting F:M ratio of 0.80:1 is not significantly different from 1:1. This result contrasts with the highly female-biased sex ratios reported from surrounding Caribbean immature hawksbill aggregations, suggesting that the Mona near shore habitat recruits male turtles from a source uncommon to other aggregations.

**RESUMEN.** Las proporciones sexuales en tortugas marinas tienen gran importancia para la creación de planes de manejo efectivos, ya que en estas especies el sexo es determinado por la temperatura ambiental y no por diferenciación de cromosomas. Esta característica ha creado poblaciones de tortugas marinas con las proporciones sexuales sesgadas a un género en particular y no la proporción sexual de 1:1 propuesta por la teoría de alocaión sexual. Varios trabajos han demostrado que estudios de proporciones sexuales en agregaciones de juveniles y sub-adultos de tortugas marinas son los más apropiados para obtener la razón sexual real de dicha agregación. Para determinar la razón sexual de una agregación de la altamente amenazada tortuga marina carey (*Eretmochelys imbricata*) en la Reserva Natural de Isla de Mona, Puerto Rico se utilizaron los niveles de testosterona como técnica para distinguir el sexo de cada individuo. De 120 individuos, el 56% fueron machos y el 44% fueron hembras. Estos resultados no reflejaron una diferencia estadísticamente significativa de la razón sexual 1:1. Sin embargo, investigaciones en otras agregaciones de la tortuga carey en el Caribe reportan sesgos sexuales hacia un género en particular. Estudios de proporciones sexuales tanto de neonatos en playas de anidación como de juveniles en áreas de alimentación de la tortuga carey en el Caribe son necesarios para ayudar a explicar la diferencia en las proporciones sexuales de las agregaciones de tortugas carey en el Caribe.

Marine turtles are subject to environmental sex determination (Bull, 1980), through sensitivity to temperature during embryonic development (Mrosovsky and Yntema, 1980). As a result, sex ratios among turtle hatchlings can differ significantly from 1:1 (Yntema and Mrosovsky, 1982; Limpus et al., 1983; Mrosovsky et al., 1984). Incubation temperatures of two other Caribbean Hawksbill Seaturtle (*Eretmochelys imbricata*) rookeries produce substantial variation in the sex ratios hatchlings (Mrosovsky et al., 1992; Wibbels et al., 1999). The way in which hatchlings of different rookeries influence the sex ratios in aggregations of immature turtles, and eventually affect sex ratios of breeding populations, remains largely unknown, because this process is governed by poorly understood factors such as migration patterns, differential survival, growth, and maturation and temporal variation in rookery hatchling sex ratios (Wibbels, 1999).

One problem in measuring sex ratio of any mature turtle population is the potential for strong sampling bias caused by behavioral differences in reproductively active turtles. Adult male hawksbills at Mona Island, for example, tend to be highly inquisitive and,

therefore, more easily encountered than the shy females on the nearshore breeding grounds (pers. obs.). Any sex ratio estimates based purely on sightings or capture frequencies would be strongly biased by this behavioral difference. Previous studies lacking controls to eliminate the potential biases caused by sexual differences in turtle behavior include those on the sex ratios among harvested adult Green (*Chelonia mydas*; Ross, 1984) and Hawksbill Seaturtles (Carrillo et al., 1999). By studying immature turtles, at least some of the behaviorally related sampling biases may be avoided, and the premise of equal catchability can be tested realistically.

An important consideration of feeding ground studies of immature marine turtles is that the sex ratios encountered do not necessarily represent any one breeding population. Aggregations of immature hawksbills may be composed of individuals originating from various nesting colonies, such as in the aggregation studied at Mona Island, where animals from at least six breeding sites in the Caribbean are represented (Bass, 1999). Sex ratios of immature turtle aggregations thus can be considered the amalgamated result of the prevailing hatchling production of contributing colonies.

Reported sex ratios vary greatly between marine turtle aggregations, presumably because of differences in the thermal nest incubation conditions from where

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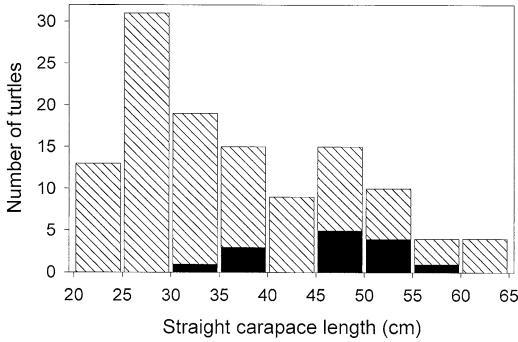


FIG. 1. Size distribution of the 120 immature Hawksbill Seaturtles studied. Black bars indicate turtles examined through laparoscopy and by blood serum testosterone assay. Hatched bars represent turtles examined by laparoscopy and through blood serum testosterone level assays. Hatched bars indicate turtles subjected only to testosterone measurement. Turtles sampled on more than one occasion are included only once, using the mean of straight carapace length measurements.

the turtles originated. Limpus et al. (1983), Wibbels et al. (1991), and León and Diez (1999) encountered bias toward either males or females on turtle feeding grounds, whereas Wibbels et al. (1993) reported an unbiased sex ratio of immature Green Seaturtles in the Hawaiian Archipelago. Limpus and Reed (1985) suggested that the mechanism of temperature-dependent sex determination could produce nonbiased sex ratios in marine turtle populations. In the current study, we validated the serum testosterone level criteria used for assigning sex and then tested the working hypothesis of an unequal sex ratio for the aggregation of immature hawksbills inhabiting the feeding grounds of Mona Island, Puerto Rico. Finally, we tested whether there were sexual differences in catchability for the group of turtles studied.

#### MATERIALS AND METHODS

**Study Area.**—The Mona Island Natural Reserve incorporates two islands, Mona and Monito. These islands are located midway in the Mona Passage between the Dominican Republic and Puerto Rico. The near-shore zones of both islands are known feeding grounds for juvenile and adult Hawksbill Seaturtles and are visited by adults nesting on Mona. Turtles from three sections of these feeding grounds are included in this study, encompassing two major habitat types: the cliff walls dominating the coast of Monito and the northern half of Mona Island; and the coral reef patches along the south-west coast of Mona.

**Data Collection.**—Every year from 1992 to 2000, we surveyed the study area for periods of from one to four months, generally during summer. Turtle surveys included capture of sighted Hawksbill Seaturtles by hand, supplemented by free diving or with the aid of SCUBA. All captured turtles were brought aboard a small boat for tagging and measurement, with a subset sampled for blood in the years 1993 to 1995. In addition, during a one-week period in 1993, turtles

were taken ashore for laparoscopic examination. All turtles were subsequently returned to the location of capture. Turtles with straight carapace lengths (SCL) between 20.0 and 65.0 cm were classified as immatures (van Dam and Diez, 1998).

**Visual Sex Determination.**—In 1993, 14 immature Hawksbill Seaturtles were examined internally by laparoscopy to determine sex by visual assessment of the gonads. Because of the size of the available laparoscopy equipment, only hawksbills greater than 34 cm SCL could be examined safely. Turtles were immobilized, and an endoscope was introduced into the peritoneal cavity for visual inspection of the gonads (Wood et al., 1983). The criteria used for assigning sex to an individual followed those established by Limpus and Reed (1985) for *C. mydas*. After laparoscopy, a highly visible piece of tape was glued to the carapace of the turtles to permit behavioral observations and prevent recapture.

**Blood Sampling and Processing.**—From 1993 to 1995, blood was collected from 120 immature Hawksbill Seaturtles, typically 30 min after capture. Up to 10 ml blood was drawn from each animal through one of the dorsal cervical sinuses using a Vacutainer tube (without additives) and a 3.8-cm 22-ga needle. Whole blood was stored on ice until completion of each daily survey. Following centrifugation for five minutes, two 2-ml samples of blood serum per animal were extracted by pipette, labeled, and stored in a  $-10^{\circ}\text{C}$  freezer. The testosterone level from one of each duplicate serum sample was measured in the laboratory of D. W. Owens using a sensitive radio-immunoassay (Owens et al., 1978; Wibbels et al., 1987).

#### RESULTS

The size distribution (Fig. 1) of the 120 turtles sampled for testosterone was similar to that of 276 Hawksbill Seaturtles captured during 1993–1996 (van Dam and Diez, 1998) and can be considered representative of the aggregation of immature turtles in the study area. As only larger hawksbills were selected for laparoscopy, an underrepresentation of animals < 35 cm SCL exists in this subset of turtles (Fig. 1).

**Validation of Testosterone Criteria for Determining Sex.**—Of the 14 hawksbills examined by laparoscopy, six individuals were classified as females and eight as males, after detecting ovarian follicles and testes, respectively. Serum testosterone concentrations of the six females ranged between 3.35 and 16.1 pg/ml, whereas testosterone of the eight males ranged between 68.4 and 262 pg/ml (Fig. 2A). Six of the turtles examined by laparoscopy were resampled for testosterone in 1994 or 1995 or both, allowing an extension of the validated hormone level range between to 3.1 and 16.1 pg/ml for females and 56.7–262 pg/ml for males (Fig. 2A', A'').

During 1993–1995, an additional 32 turtles were captured and sampled on two or more occasions, yielding a range of testosterone levels for each individual. Where these individual ranges overlapped with those of the laparoscoped animals, they allowed for a further stepwise widening of the validated testosterone ranges for each of the sexes. For example, the serum testosterone level of turtle 94-067 was 14.4 pg/ml in 1994, establishing it as a female; in 1995 this individual had a level of 17.0 pg/ml, extending the

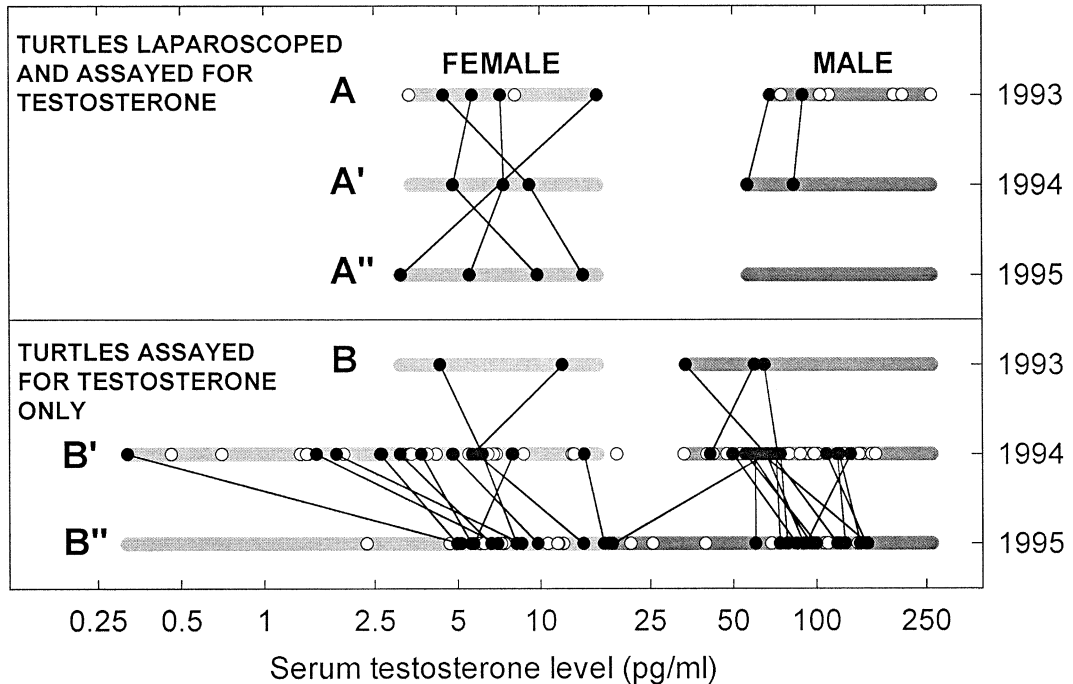


FIG. 2. Construction of testosterone level criteria for assigning sex to immature Hawksbill Seaturtles captured at Mona Island, Puerto Rico. Turtles with sex determined laparoscopic examination (A) yielded an initial range (shaded) of associated testosterone levels; extension of range through subsequently measured testosterone levels of these laparoscoped individuals (connecting lines: A, A' and A''). Assignment of sex to turtles sampled for testosterone only (B) using range from A'', and the expansion of the female and male range limits from turtles sampled on multiple occasions (connecting lines: B, B', B''). Hormone levels of turtles sampled only once are plotted as open circles.

previous upper testosterone limit for females to this value. Such extension by individual duplicates resulted in a serum testosterone range of between 0.32 and 17 pg/ml for females and between 18.2 and 262 pg/ml for males (Fig. 3B, B', B''). These extensions yielded no inconsistencies or overlap in the serum testosterone ranges assigned to females and males.

**Sex Ratio.**—With the established serum testosterone ranges, only one hawksbill (with the intermediate level of 17.7 pg/ml) of the 120 turtles sampled remained of undeterminable sex. Exclusion of this individual from the dataset resulted in a total count of 53 females and 66 males. This F:M proportion of 0.80:1 was not significantly different from 1:1 ( $\chi^2 = 1.42$ ,  $P = 0.233$ ).

As the lower limit of the male range of testosterone levels was large (from the 56.7 pg/ml validated by laparoscopy down to 18.2 pg/ml by comparison of individual duplicates), we tested the sensitivity of the overall resulting sex ratio through stepwise reversals of this extension. Because the lower limit for males was raised from 18.2 to 56.7 pg/ml, progressively more turtles were assigned to the undetermined sex category, yielding new calculated sex ratios (Fig. 3). With more conservative settings for the lower limit, the F:M sex ratio steadily approached a value of 1:1, (not significantly different from 1:1). Similarly, exclusion from the dataset of hawksbills smaller than 34.0 cm SCL (size of the smallest laparoscoped turtle), resulted in a sex ratio

F:M of 0.72:1 among the remaining 52 individuals. This ratio was not significantly different from 1:1 ( $\chi^2 = 1.473$ ,  $P = 0.225$ ).

**Testing Equal Catchability.**—An assumption implicit in studying aggregation characteristics is an even probability of sampling the different subgroups of interest. To test for a possible difference in catchability between the sexes, we compared the number of times turtles of each sex were caught (counting any number of captures within a single year as one). From 1993 to 2000, the 53 female and 66 male turtles examined in the present study were captured on average 3.04 and 3.05 times, respectively. The most parsimonious explanation for the closeness of these values is that there was no sex-related catchability bias for this group of immature hawksbill turtles.

#### DISCUSSION

The even sex ratio measured for Mona Island's immature hawksbill aggregation contrasts with the strongly female-biased sex ratios reported for other aggregations off the southwest coast of the Dominican Republic (F:M = 2.71:1,  $N = 143$ , 1996–1998; León and Diez, 1999), and around Buck Island in the U.S. Virgin Islands (F:M = 4:1,  $N = 72$ , 1996–1998; Geis et al., in press). Although the current study pre-dates the others by several years, a strictly temporal explanation for the

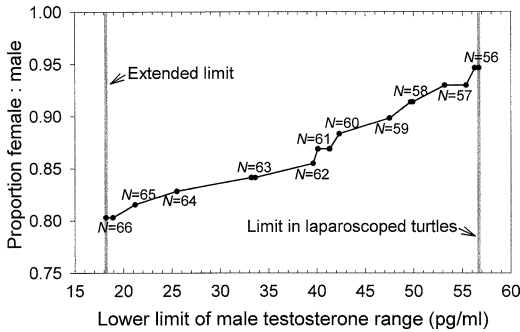


FIG. 3. Sensitivity of the calculated sex ratio to the lower limit of testosterone for designating Hawksbill Seaturtles as males. As the limit is set more conservatively (higher), turtles with hormone levels lower than the limit (but above 17 pg/ml, the firm female upper limit) are progressively assigned to the category "sex unknown," reducing the number (N) of turtles determined as males. For all points in the graph, the resulting F:M sex ratio remains statistically not different from 1:1.

observed differences is implausible, because all studies included a range of turtle sizes corresponding to animals recruited to the feeding grounds over a period of around a decade (assuming recruits initially measured < 30 cm SCL and had somatic growth rates of around 3 cm SCL/year). The effect of any hypothetical influx of highly female-biased turtles recruiting to the region's feeding grounds after 1995 is unlikely to have been detected because of their dilution within the resident pool of animals and the relatively short sampling periods.

The contrasting sex ratios are more probably attributable to dissimilar sourcing, that is, differences in the nesting rookeries contributing individuals to the feeding ground aggregations. With nest incubation temperatures determining hatchling sex in Hawksbill Seaturtles (Mrosovsky et al., 1992), the Mona aggregation appears to have received a high proportion of turtles hatched on cooler, male-generating nesting beaches. We are aware of only two studies indicating the sex ratios of hawksbill hatchlings produced on Caribbean nesting beaches, at Jumby Bay, Antigua (Mrosovsky et al., 1992) and Buck Island, U.S. Virgin Islands (Wibbels et al., 1999). In Antigua, the sand at nest depth was generally cooler than the measured pivotal temperature, suggesting a male bias in the hatchlings produced. In contrast, examination of the sex of hawksbill hatchlings that died from natural causes at Buck Island, suggested a strongly female-biased turtle output there. Bowen et al. (1996) indicated that a high proportion of the Mona Island immature aggregation had mtDNA haplotypes found primarily in hatchlings at Buck Island, with Bass (1999) calculating a contribution of 41.5% of this rookery to the Mona aggregation from an expanded data set. The same studies suggested that only a small contribution (4.14%) was made by Antigua to the Mona foraging aggregation. Any male bias in the Antigua hatchlings would consequently have little effect on the sex ratios of immature hawksbills at Mona Island.

With sex ratios in the surrounding immature foraging grounds so highly female biased and a strong female bias present in a major contributing rookery, the question remains what explains the balanced sex ratio of immatures at Mona Island. The observation that Mona Island beaches, in contrast to other rookeries, are used year-round by nesting hawksbills (CED, pers. obs.), raises the possibility that the additional males are produced locally during the low temperature winter months. Genetic analyses have yielded estimates that the Mona nesting females are represented in the local feeding ground immatures at levels of 12.7% (Bowen et al., 1996) and 41% (Diaz-Fernandez et al., 1999). A contribution as large as the latter figure, combined with a high overall proportion of males in the Mona hatchlings, would reconcile the genetic profile of the Mona foraging ground immature turtles with the observed non-female-biased sex ratio of the aggregation. We are currently conducting a sex ratio study of the hawksbill hatchlings produced on Mona Island, which should clarify whether local sourcing plays a significant role in determining the sex ratio of the Mona feeding ground immatures.

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