Editorial Comment. – This section has been established as a forum for the exchange of ideas, opinions, position statements, policy recommendations, and other reviews regarding turtle-related matters. Commentaries and points of view represent the personal opinions of the authors, and are peer-reviewed only to the extent necessary to help authors avoid clear errors or obvious misrepresentations or to improve the clarity of their submission, while allowing them the freedom to express opinions or conclusions that may be at significant variance with those of other authorities. We hope that controversial opinions expressed in this section will be counterbalanced by responsible replies from other specialists, and we encourage a productive dialogue in print between the interested parties. Shorter position statements, policy recommendations, book reviews, obituaries, and other reports are reviewed only by the editorial staff. The editors reserve the right to reject any submissions that do not meet clear standards of scientific professionalism.

Chelonian Conservation and Biology, 2000, 3(4):756–761

Sexing Juvenile Sea Turtles: Is There an Accurate and Practical Method?

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Sea turtles have temperature-dependent sex determination (TSD) in which the incubation temperature of the egg is the primary factor controlling the sex of the hatchling (Yntema and Mrosovsky, 1980; Morreale et al., 1982; McCoy et al., 1983; Limpus et al., 1983; Mrosovsky et al., 1984; Limpus et al., 1985; Rimblot et al., 1985; Dalrymple et al., 1985; Spotila et al., 1987; Shaver et al., 1988; Merchant-Larios et al., 1989). The sex ratios produced under different thermal regimens are of interest to biologists for a variety of reasons. For example, naturally occurring sex ratios are of ecological and evolutionary interest since they appear to vary widely and do not always conform to a 1:1 sex ratio predicted by evolutionary theory (Mrosovsky, 1994). Further, hatchling sex ratios produced on nesting beaches are of conservation interest since they may affect the future reproductive success of a population. Thus, there are distinct reasons for studying sex ratios, but such studies have been limited in the past due to the lack of simple methods for sexing immature sea turtles.

Although the sex of adult male sea turtles is clearly indicated by the long and muscular tail, the majority of tail enlargement occurs as males approach sexual maturity (Limpus, 1985). Therefore, tail length does not appear to be an accurate means of sexing immature sea turtles (Limpus, 1985; Wibbels, 1988), with the possible exception of some males as they near sexual maturity (Limpus, 1985). Further, no other external characteristics have been identified which clearly indicate the sex of immature turtles. Therefore, a variety of previous studies have investigated alternative methods which could potentially provide nonlethal techniques for sexing juvenile and hatchling sea turtles. Although a few of these studies have addressed the sexing of hatchling sea turtles (Gross et al., 1995; Wibbels and LeBoeuf, 2000), the majority have focused on juvenile turtles. Methods which have been evaluated as potential sexing techniques for juvenile turtles include karyotype, H-Y antigen, Bkm DNA fingerprinting, laparoscopy, and serum testosterone. The purpose of this article is to provide a general overview of these methods and to highlight the advantages and disadvantages of each.

Karyotype

Karyotypes have been examined in a variety of sea turtles including *Chelonia mydas* (Bickham et al., 1980; Bachère, 1981), *Eretmochelys imbricata* (Bickham, 1981; Kamezaki, 1990), *Lepidochelys olivacea* (Bhunya and Mohanty-Hejmadi, 1986), *Caretta caretta* (Kamezaki, 1989), and *Dermochelys coriacea* (Medrano et al., 1987). Heteromorphic sex chromosomes were not detected in any of these studies. Considering that sea turtles have TSD, the absence of heteromorphic sex chromosomes might be anticipated, although it has been suggested that TSD may simply override an underlying sexual genotype (Zaborski et al., 1982, 1988). Regardless, karyotyping studies have not revealed any heteromorphic sex chromosomes which might be used as an indicator of sex in sea turtles.

H-Y Antigen

Initial studies in mammals suggested that H-Y antigen was a sex-specific antigen which induces testicular differentiation (Wachtel et al., 1975; Ohno et al., 1979). However, subsequent studies have indicated that H-Y antigen is not involved, since testis formation has been shown to occur in the absence of this antigen (McLaren, et al., 1984; Goldberg et al., 1991). Regardless, studies have indicated that this antigen may be sex-specific in many vertebrates (Wachtel and Tiersch, 1994), and thus it could be used as a marker to identify sex.

Wellins (1987) used an H-Y antigen cytotoxicity assay to examine blood cells of two species of sea turtles, *C. mydas* (3 adult males and 3 adult females), and *C. caretta* (2 adult

males and 2 adult females). The assay indicated that blood cells of males in each species had higher levels of H-Y antigen than those in females. These results suggested that H-Y antigen could prove to be an accurate marker for indicating the sex in sea turtles. However, several points should be mentioned. First, as with all of the techniques reviewed in this article, the H-Y technique is labor intensive and includes a series of biochemical procedures followed by the visual identification of approximately 200 cells per sample using a hemacytometer. Further, the results of this initial study are based on a sample size of only ten adult animals. Therefore, prior to using such a technique for sexing individuals, rigorous validation is required involving large sample sizes of juvenile turtles.

There are also potential problems associated with this technique. The results of the study by Wellins (1987) indicated that only recently sampled blood could be used in the assay; blood that had been drawn more than 24 hours prior to analysis demonstrated high variability in the assay and was therefore not used in the data analysis. It was also reported that in some of the cytotoxicity tests, erratic titers or reversal of expected results were obtained (Wellins, 1987). Lastly, a study of H-Y antigen in a freshwater turtle (*Emys orbicularis*) with temperature-dependent sex determination indicated that both H-Y positive and H-Y negative females could be obtained at certain incubation temperatures (Zaborski et al., 1988). Thus, while the initial study of H-Y antigen in sea turtles suggested that this antigen could potentially be used as an indicator of sex, rigorous validation of the assay is warranted prior to widespread usage.

Bkm DNA Fingerprinting

The use of DNA fingerprinting to identify sex has been examined in sea turtles (Demas and Wachtel, 1989; Demas et al., 1990) using a "banded krait minor" (Bkm) DNA probe. The structure of the probe was based on DNA which was originally isolated from the W chromosome of a snake, the banded krait, *Bungarus fasciatus* (Singh et al., 1981). Bkm-related sequences have been found in a variety of vertebrates and are preferentially concentrated on their sex chromosomes (Jones and Singh, 1981a, 1981b). Further, the Bkm probe has been used to reveal sex-specific bands in DNA fingerprints of some mammals, birds, and reptiles (Wachtel and Tiersch, 1994).

The study by Demas et al. (1990) examined DNA fingerprints in two species of sea turtles, *C. mydas* (5 males and 5 females) and *Lepidochelys kempi* (15 males and 15 females). For this analysis, DNA was extracted from blood cells, digested with restriction enzymes, and then used in Southern hybridizations with the Bkm probe. The study included the evaluation of 20 different restriction enzymes in the procedure. The results indicated the presence of male-specific fragments in the fingerprints of both species when certain restriction enzymes were utilized. Therefore, this technique could potentially be used as a sexing technique for sea turtles. Again, it should be noted that prior to widespread usage, rigorous validation is needed, including the examination of large sample sizes of juvenile turtles. DNA fingerprinting is a routine molecular procedure, but it is labor intensive. Each blood sample requires DNA extraction followed by restriction enzyme digestion. Multiple samples are then subjected to electrophoresis, membrane transfer, and hybridization. Following hybridization, the DNA fingerprints are visualized by autoradiography or nonradioactive development procedures. As such, the logistics and cost of this procedure could hinder the analysis of large sample sizes.

Laparoscopy

Laparoscopy represents an accurate method for sexing juvenile sea turtles (Wood et al., 1983; Limpus and Reed, 1985; Limpus, 1985; Owens, 1999). This is a surgical procedure in which a laparoscope is inserted through the body wall (in the inguinal area near a hind flipper) and into the body cavity. The morphology of the immature gonads, as seen through the laparoscope, clearly indicates the sex of a juvenile (Limpus and Reed, 1985). A detailed description of the laparoscopic procedure is provided by Wood et al. (1983) and a technical overview is provided by Owens (1999). While laparoscopy represents an accurate means of sexing juvenile sea turtles, it is logistically difficult. This procedure requires specialized equipment and should not be attempted without proper veterinary training. Further, since it is an invasive surgical procedure, there is greater risk to the turtle in comparison to techniques which require only a blood or tissue sample. Laparoscopy is also difficult to perform in the field, and it requires a relatively large amount of time and effort for each turtle. Nevertheless, laparoscopy has been, and is currently used successfully by a number of researchers. In fact, in at least one research program it has been used to sex thousands of sea turtles (CJL). Further, the use of laparoscopy is currently a prerequisite for positively sexing juvenile turtles in order to evaluate other sexing techniques. Thus, laparoscopy represents an accurate method for sexing juvenile sea turtles, but logistical difficulties associated with this technique limit its widespread use.

Serum Testosterone Levels

A variety of studies have examined circulating testosterone levels (i.e., serum or plasma testosterone levels) in juvenile sea turtles (Owens et al., 1978; Morris, 1982; Wibbels et al., 1987; Wibbels, 1988; Bolten et al., 1992; Coyne et al., 1994; Gregory, 1996; Casale et al., 1998), including several studies which have verified sex through laparoscopy (Wibbels et al., 1987; Wibbels, 1988). The most comprehensive study, which included laparoscopic verification of sex, was conducted on juvenile sea turtles inhabiting Heron Atoll on the Great Barrier Reef (Wibbels, 1988). The data from that study are summarized in Table 1. In all three species examined, male levels were significantly higher than those of females. With *C. caretta* (n = 61) and *E. imbricata* (n = 25), the ranges of male and female testoster-

Table 1. Serum testosterone levels (pg/ml) of three species of juvenile sea turtles captured on Heron Atoll, Great Barrier Reef, Australia. Data summarized from Wibbels (1988). Status of juveniles as pubescent or nonpubescent was determined from laparoscopy as described by Limpus and Reed (1985). Testosterone levels in some female samples were non-detectable (ND; less than approximately 2.3 pg/ml). Means ± standard errors were calculated using the lowest amount detectable in the assay for values that were non-detectable.

Species	Sex	Status	*n*	Mean	Range
C. caretta	Male	pubescent	17	95.4 ± 7.5	52.5 – 188.0
C. caretta	Male	nonpubescent	30	87.1 ± 7.4	30.0 – 180.3
C. caretta	Female	pubescent	8	11.1 ± 1.7	ND – 18.0
C. caretta	Female	nonpubescent	7	11.1 ± 1.2	ND – 16.8
E. imbricata	Male	nonpubescent	5	114.8 ± 27.9	68.5 – 206.4
E. imbricata	Female	pubescent	2	5.0 ± 0.2	ND – 5.2
E. imbricata	Female	pubescent	18	5.9 ± 0.4	ND – 10.9
C. mydas	Male	pubescent	11	166.1 ± 50.2	16.9 – 565.8
C. mydas	Male	nonpubescent	60	56.3 ± 5.1	7.8 – 230.3
C. mydas	Female	pubescent	9	6.8 ± 1.6	ND – 17.4
C. mydas	Female	nonpubescent	117	5.3 ± 0.4	ND – 13.3

one levels did not overlap. In the case of *C. mydas* ($n = 197$), 3 of the 72 males had levels which were lower than the highest female level.

In a similar study which examined juvenile *C. caretta* from Florida waters (Wibbels et al., 1987), testosterone levels were comparable to *C. caretta* from Heron Atoll and the ranges of male and female levels did not overlap ($n = 22$, sex verified by laparoscopy). Additionally, an unpublished study examined testosterone levels in approximately 80 yearling "head-started" *C. mydas* from Florida (Meylan, Wibbels, and Owens, unpubl. data). The sex of these turtles was verified by laparoscopy and the study was conducted in a blind fashion in which the assayist was not informed as to the sex of the turtles. The results showed distinct male and female ranges of testosterone levels, which overlapped to some extent; approximately 15% of these yearlings had testosterone levels in the overlap zone.

The results of the studies reviewed above suggest that circulating testosterone levels can be used to predict the sex in the majority of juvenile sea turtles. The exception would be turtles which have levels which fall in the intermediate or overlapping zone between male and female levels. However, this appears to be a small percentage of turtles and their testosterone levels clearly indicate that they cannot be sexed by this technique. That is, testosterone level can indicate that a turtle is either a male, a female, or that it cannot be sexed. Subsequent studies have used this technique to predict the sex of relatively large numbers of juvenile turtles (Wibbels et al., 1991, 1993; Bolten et al., 1992; Coyne et al., 1994; Casale et al., 1998).

The use of testosterone level for sexing juvenile sea turtles offers several advantages. The testosterone radioimmunoassay (RIA) is conducted in the laboratory, so the field component is limited to the capture and blood sampling of turtles. A single testosterone RIA can include a relatively large number of samples, thus providing a practical means of sexing large numbers of sea turtles. Additionally, steroid hormones such as testosterone are very stable, so serum samples from turtles can be stored for prolonged periods of time (i.e., at least several years) at -20°C or below with little or no degradation.

While the studies reviewed above suggest that circulating testosterone represents a useful predictor of sex in most juveniles, there are problems associated with this technique which could drastically affect its accuracy (Wibbels, 1988; Gregory, 1996). It is important to note that the majority of the studies mentioned above used the same testosterone RIA which had been validated with hundreds of blood samples from turtles which had been sexed by laparoscopy. A problem arises when attempting to use an RIA which has not been validated with blood samples from turtles of known sex. Ideally, all testosterone RIAs should generate comparable values for a given sample. Unfortunately, in reality, the values generated by a given RIA are normally very precise, but the accuracy can vary between laboratories (Constantini et al., 1975; Bolelli et al., 1982; Boots et al., 1998). That is, a typical testosterone RIA will consistently generate a value for a given blood sample, but that value may be somewhat higher or lower than a value generated for the same sample in an RIA in another laboratory. This creates a problem, since the ranges of male and female testosterone levels in juvenile sea turtles are not widely separated and they appear to overlap in at least some species. Therefore, it is difficult to accurately predict the sex of a juvenile sea turtle by comparing testosterone levels generated in one laboratory to those published by another laboratory. A variation of approximately 10 to 50 picograms per ml or less could mean the difference between identifying a turtle as a male versus a female (Table 1). For example, the data from Casale et al. (1998) shows a distinct bimodal distribution of testosterone levels suggesting male and female ranges. However, due to the lack of laparoscopic data for validating their RIA, the authors were cautious in interpreting the testosterone data. Thus, it is important that a testosterone RIA be "validated" before it can be used to accurately predict the sex of individual juvenile sea turtles. Ideally, an RIA should be validated with samples from juvenile sea turtles of known sex (e.g., sex verified via laparoscopy). Alternatively, it might be sufficient to directly compare values generated by an unvalidated RIA to one in another laboratory which has been validated (i.e., aliquots of the same samples could be run in both assays and the resulting values would be compared). In that way, the values generated in one RIA could be cross-referenced to an RIA which had been validated with samples of known sex.

Validating a testosterone RIA with samples from the population of interest can avoid several other potential problems. One potential problem is the possibility of inter- and intraspecific variations in testosterone levels (Wibbels, 1988; Gregory, 1996). Testosterone levels have been reported for juveniles in only four species of sea turtles (*C. caretta*, *C. mydas*, *E. imbricata*, and *L. kempi*) and minor interspecific variations in the ranges of male and female testosterone levels have been recorded (see Table 1). It is also plausible that testosterone levels could vary between populations of sea turtles. Although this subject has not been adequately addressed in previous studies, large variations might not be expected since even interspecific variations appear minor (Table 1). Additionally, it has been suggested that testosterone levels could vary seasonally in juveniles (Gregory, 1996). Again, this subject has not been adequately addressed, but one study revealed no significant seasonal changes in testosterone levels in juvenile *C. caretta* or *C. mydas* from Florida waters (Luepschen, 1987). Regardless, validation of an RIA with samples from juveniles of known sex, from the population of interest, can circumvent potential problems relating to possible inter- or intraspecific variation in testosterone levels.

Finally, blood samples should (ideally) be taken immediately after capture to avoid possible effects of stress on testosterone levels. Corticosterone levels increase significantly over a several hour period following capture (Wibbels et al., 1987; Gregory 1994), but it is not clear if significant changes in testosterone levels occur after capture (Wibbels et al., 1987; Luepschen, 1987; Gregory, 1996). Regardless, obtaining blood samples immediately after capture circumvents potential problems associated with stress. Blood samples can be quickly drawn from the bilateral cervical sinus located in the dorsal portion of the neck (described by Owens, 1976; Owens and Ruiz, 1980; Bentley and Dunbar-Cooper; 1980; Wibbels, 1999).

Development of New Sexing Technology

It is plausible that new biochemical markers could be identified which might provide a clear indication of sex in juvenile sea turtles. It would be optimal to identify a marker (e.g., hormone, DNA, etc.) which only occurs in one sex or is produced at distinctly higher levels in one sex, thus avoiding any overlapping values such as reported for testosterone in juvenile *C. mydas* (Wibbels, 1988). Testosterone is the only sex steroid which has received extensive investigations in juvenile sea turtles. Other sex steroids or their metabolites could potentially prove to be indicators of sex (Gregory, 1996). Further, other reproductive hormones such as luteinizing hormone (LH) or follicle stimulating hormone (FSH) might show sex-specific levels in the blood. A sex-specific hormone, mullerian inhibiting hormone (which causes the oviducts to degenerate in male vertebrates), has recently been isolated in turtles, but its circulating levels have not been examined in juvenile sea turtles (Wibbels et al., 1998; Wibbels and LeBoeuf, 2000).

Advances in molecular biology during recent years have provided new avenues which could prove useful for developing sexing techniques for juvenile sea turtles. For example, polymerase chain reaction (PCR) has been used to identify the sex of animals by amplifying sex-specific DNA (Aasen and Medrano, 1990; Appa Rao et al., 1993; Griffiths and Tiwari, 1993). The PCR-based sexing techniques described for other animals appear quite powerful and practical. The problem associated with the development of such techniques is the identification of sex-specific DNA, and it is presently not clear whether sex-specific DNA is present in turtles with TSD (Wibbels et al., 1994). It has been suggested that TSD may simply override underlying genetic sex differences (Zaborski et al., 1982, 1988), and the studies reviewed above regarding H-Y antigen and Bkm DNA in sea turtles suggest that sex-specific differences in DNA may occur. Therefore, the investigation and development of PCR-based sexing technology for sea turtles could prove worthwhile. However, as with any of the techniques reviewed above, to be useful, such a technique must prove to be accurate and practical.

Which Technique is Best?

Considering the methods reviewed above, is there an accurate and practical means of sexing juvenile sea turtles? If accuracy is the only concern, laparoscopy would be the optimal method. Unfortunately, it is currently practical for only a handful of well-trained researchers who are willing to use this labor-intensive surgical procedure. Examining testosterone levels provides a more practical means of predicting sex for large numbers of juvenile turtles, but the accuracy of this technique is dependent upon how well the RIA has been validated with samples from turtles of known sex. Since it requires validation through laparoscopy, the testosterone sexing method is not a technique which can be quickly implemented into a new laboratory. For this reason, the majority of testosterone sexing data has been generated by a single laboratory (DWO). Other methods such as H-Y antigen and Bkm DNA fingerprinting have only received initial evaluations and would require extensive validation prior to use. Further, it is not clear if these techniques would be practical for sexing large numbers of juvenile turtles.

Thus, there is currently no "ideal" method for easily sexing juvenile sea turtles. Of the methods available, laparoscopy and serum testosterone are the best, and both are capable of producing accurate sex data. It is plausible that future studies will be able to identify a sex-specific marker (e.g., hormone, DNA, etc.) which will clearly identify sex. Identification of such a marker would facilitate the widespread adoption of the technique and would assure accuracy.

Acknowledgments. — The completion of this manuscript was made possible through funding by the National Oceanic and Atmospheric Administration, U.S. Department of Commerce, Mississippi-Alabama Sea Grant Consortium under Grant #NA56RG0129 and the Texas A&M Sea Grant College Program under Grant #NA86RG0058.

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Received: 26 October 1998
Reviewed: 8 February 2000
Revised and Accepted: 24 February 2000

Chelonian Conservation and Biology, 2000, 3(4):761–767

A Response to Nicholas Mrosovsky's *Sustainable Use of Hawksbill Turtles: Contemporary Issues in Conservation*

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Nicholas Mrosovsky of the University of Toronto has been a leading figure in the field of the philosophy, practice, and goals of marine turtle management for a generation. He is also a valued colleague and friend. This friendship has withstood — perhaps even been enhanced by — our sometimes energetic disagreements on the specifics of how turtles should be managed and saved. I believe that such valued and lively — even disputatious — relationships within the overall context of friendship constitute the essence of truly civilized behavior. In this essay, I propose to indulge in a further elaboration of this eminently sophisticated and respectful relationship by responding to the issues raised in his latest book (Mrosovsky, 2000).

There has long been a striking difference between the *modi operandi* of the IUCN/SSC Crocodilian Specialist Group and the Marine Turtle Specialist Group. The former, with close ties to the hide industry, is notorious for espousing "rational exploitation," whereas members of the latter generally advocate "protection." The reason for the opposite approaches may lie in differences in the biological specifics and population dynamics of crocodiles versus turtles; or perhaps in the different responses that they evoke from human beings; or perhaps even in the different kinds of people who direct or belong to the two groups. The turtle