Research and Management Techniques for the Conservation of Sea Turtles

Prepared by IUCN/SSC Marine Turtle Specialist Group

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Preface

In 1995 the IUCN/SSC Marine Turtle Specialist Group (MTSG) published *A Global Strategy for the Conservation of Marine Turtles* to provide a blueprint for efforts to conserve and recover declining and depleted sea turtle populations around the world. As unique components of complex ecosystems, sea turtles serve important roles in coastal and marine habitats by contributing to the health and maintenance of coral reefs, seagrass meadows, estuaries, and sandy beaches. The *Strategy* supports integrated and focused programs to prevent the extinction of these species and promotes the restoration and survival of healthy sea turtle populations that fulfill their ecological roles.

Sea turtles and humans have been linked for as long as people have settled the coasts and plied the oceans. Coastal communities have depended upon sea turtles and their eggs for protein and other products for countless generations and, in many areas, continue to do so today. However, increased commercialization of sea turtle products over the course of the 20th century has decimated many populations. Because sea turtles have complex life cycles during which individuals move among many habitats and travel across ocean basins, conservation requires a cooperative, international approach to management planning that recognizes inter-connections among habitats, sea turtle populations, and human populations, while applying the best available scientific knowledge.

To date our success in achieving both of these tasks has been minimal. Sea turtle species are recognized as "Critically Endangered," "Endangered" or "Vulnerable" by the World Conservation Union (IUCN). Most populations are depleted as a result of unsustainable harvest for meat, shell, oil, skins, and eggs. Tens of thousands of turtles die every year after being accidentally captured in active or abandoned fishing gear. Oil spills, chemical waste, persistent plastic and other debris, high density coastal development, and an increase in ocean-based tourism have damaged or eliminated important nesting beaches and feeding areas.

To ensure the survival of sea turtles, it is important that standard and appropriate guidelines and criteria be employed by field workers in all range states. Standardized conservation and management techniques encourage the collection of comparable data and enable the sharing of results among nations and regions. This manual seeks to address the need for standard guidelines and criteria, while at the same time acknowledging a growing constituency of field workers and policy-makers seeking guidance with regard to when and why to invoke one management option over another, how to effectively implement the chosen option, and how to evaluate success.

The IUCN Marine Turtle Specialist Group believes that proper management cannot occur in the absence of supporting and high quality research, and that scientific research should focus, whenever possible, on critical conservation issues. We intend for this manual to serve a global audience involved in the protection and management of sea turtle resources. Recognizing that the most successful sea turtle protection and management programs combine traditional census techniques with computerized databases, genetic analyses and satellite-based telemetry techniques that practitioners a generation ago could only dream about, we dedicate this manual to the resource managers of the 21st century who will be facing increasingly complex resource management challenges, and for whom we hope this manual will provide both training and counsel.

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Diagnosing the Sex of Sea Turtles in Foraging Habitats

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Determining the sex of sea turtles on foraging grounds is of interest to biologists and conservationists for a variety of reasons. The fact that sex determination in sea turtles is strongly influenced by the temperature at which the eggs are incubated *(i.e.,* temperature-dependent sex determination or TSD) (see Merchant, this volume) raises numerous questions which are of ecological, evolutionary and/or conservational significance. For example: What are the natural sex ratios in sea turtle populations? Do sex ratios vary between and among populations? What effect does sex ratio have on the reproductive success of a population? Are certain sex ratios optimal for the survival of a population? These types of questions are of particular interest to conservationists, since information of this sort is essential for understanding the reproductive dynamics of a population and thus for generating optimal management strategies for endangered populations. Natural sex ratios produced by TSD can vary widely (reviewed by Wibbels *et al.,* 1991, 1993; Mrosovsky, 1994). Thus, the above questions may not be easily answered, and a comprehensive database of sex ratio information may be required to produce reliable answers.

The concept of examining sex ratios in sea turtle populations seems straightforward. However, to successfully complete such studies, one must make several decisions regarding experimental design and then overcome a number of logistical difficulties presented by sea turtle biology and life history. First, one must decide which portion of the population to examine *(e.g.,* embryonic, hatchling, immature, adult). Differential survival relative to sex could occur in sea turtles, and thus sex ratios could vary between various age classes within a population. For example, hatchlings emerging early in a nesting season could experience different water conditions and food availability in comparison to hatchlings emerging late in a nesting season, and hatchling sex ratios can change significantly during a nesting season (Mrosovsky *et al.,* 1984). Therefore, optimal sex ratio studies would include the various age classes within a population. This chapter reviews nonlethal methods for identifying the sex of sea turtles from foraging grounds *(i.e.,* immature and adult turtles) and analysis of sex ratio data.

Identifying the Sex of Adult Sea Turtles

One of the fundamental necessities in sea turtle sex ratio studies is a valid means of identifying the sex of individual turtles. This is normally not a problem with adult sea turtles since males develop secondary sexual characteristics *(e.g.,* tail length, carapace morphology, morphology of the nails on the front flippers) during puberty. The most obvious secondary sexual characteristic is the large and muscular prehensile tail which extends well beyond the carapace in an adult male (Figure 1). While actual tail lengths will vary with species and possibly populations, the tail of female sea turtles is short and, at most, will project only slightly beyond the edge of the marginal scutes. However, one should be cautious when using tail length to indicate the sex of sea turtles that are near the minimum adult size for a given population; some large immature or pubescent males may not have yet developed long tails and could therefore be mistaken as small adult females (Limpus, 1985; Limpus and Reed, 1985).

Sexing Techniques for Immature Turtles

In contrast to adults, the sexing of immature and hatchling turtles represents a significant logistical hurdle. Tail length is not an accurate sexing technique for immature sea turtles (Limpus, 1985; Wibbels, 1988). However, tail length may be indicative of sex in some males as they near sexual maturity (Limpus, 1985).

A variety of nonlethal methods has been proposed and/or developed for determining the sex of immature sea turtles. The most definitive method is the direct observation of the gonads by laparoscopic examination. In addition, several techniques have been evaluated as physiological or molecular markers of sex. These include karyotyping (Bickham *et al.,* 1980), H-Y antigen assay of blood cells (Wellins, 1987), Bkm DNA fingerprinting (Demas *et al.,* 1990), and assay of blood testosterone levels (Owens *et al.,* 1978; Wibbels *et al.,* 1987; Wibbels, 1988). Karyotyping has yet to reveal sex specific differences, whereas H-Y antigen assay of blood cells and Bkm DNA fingerprinting have been proposed as potential sexing techniques but have not been well validated. Further, the logistics and costs of these three methods would hinder their widespread use for examining large numbers of turtles; therefore, they will not be discussed in detail in this chapter. Laparoscopy and testosterone RIA are also characterized by logistical difficulties and expensive equipment, but they have proven to be practical in successfully sexing large numbers of immature turtles. For a sexing technique to be useful it should be accurate, logistically practical, and cost effective.

Laparoscopy

Laparoscopic examination has been shown to be an effective method of sexing immature sea turtles (Wood *et al.,* 1983; Limpus and Reed, 1985; Limpus, 1985) since the gonads can be viewed directly through the laparoscope (Figure 2). A detailed description of immature and mature gonads is in Limpus and Reed (1985) and Wibbels (1988), and several photographs of immature gonads are shown by Rainey (1981). A detailed description of the laparoscopic procedures is provided by Wood *et al.* (1983). Owens (this volume) provides a technical overview. The main disadvantage is that the procedure is invasive and potentially hazardous to the turtle. Moreover, it is logistically difficult and should not be attempted without proper veterinary training. Despite the caveats, laparoscopy has been used successfully by a number of researchers and has been used to sex thousands of sea turtles (C. Limpus, Qld. Dept. Environment, pers. comm.). Further, the use of laparoscopy is currently a necessity for evaluating the effectiveness of other nonlethal sexing techniques for immature sea turtles.

Testosterone Radioimmunoassay (RIA)

Serum testosterone level can be used as an accurate indicator of sex of immature sea turtles (Owens *et al.,* 1978; Morris, 1982; Wibbels *et al.,* 1987; Wibbels, 1988). For example, in a study of sea turtles on Heron Atoll, serum testosterone was examined in immature green (n=197), loggerhead (n=61) and hawksbill (n=25) turtles in which the sex was verified through laparoscopy (Wibbels, 1988). In all three species, males exhibited significantly higher testosterone levels than females. In all hawksbill and loggerhead turtles, as well as 98% of green turtles, testosterone levels were an accurate indicator of sex *(i.e.,* the ranges of male and female testosterone levels did not overlap). A study of immature loggerheads captured in the Cape Canaveral channel (Florida, U.S.) provided similar results with no overlap of the ranges of male and female testosterone levels (Wibbels *et al.,* 1987).

More recently, testosterone levels have been used in studies to sex relatively large numbers of immature

Figure 1. Adult male green turtle showing muscular tail which extends well beyond the margin of the carapace.
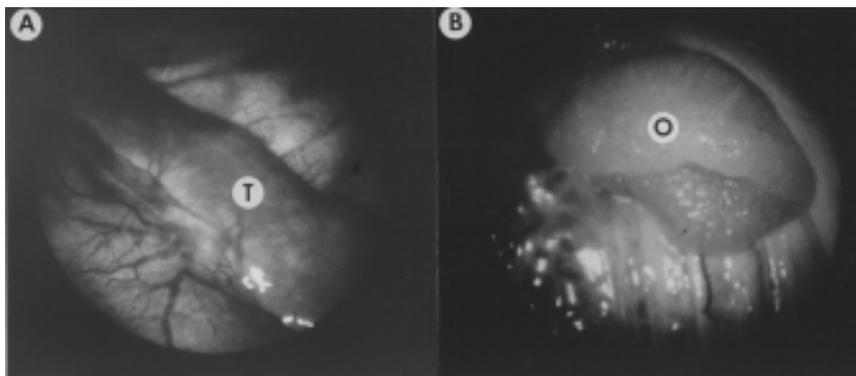
Figure 2. Appearance of immature testis and ovary through a laparoscope. 2A) Immature testis (T) is shown running diagonally through photograph. 2B) Immature ovary (O) is shown.

green and loggerhead sea turtles captured in the wild (Wibbels *et al.,* 1991, 1993; Bolten *et al.,* 1992). The minimum size limits of sea turtles that can be sexed by testosterone RIA has not been well documented. However, an unpublished study (A. Meylan, Florida Dept. Environ. Protection, pers. comm.) suggests that it could be used to sex green turtles with straight carapace lengths as short as approximately 25 cm.

There are several advantages in using a testosterone RIA to sex sea turtles. The RIA is conducted in the laboratory, so the field component is limited to the capture and blood sampling of turtles. Testosterone is a rather stable hormone, so serum samples from turtles can be stored for prolonged periods of time (at least several years) at -20 C or below with little or no degradation. A single testosterone RIA can easily include 50 to 100 samples or more, thus providing a practical and cost effective means of sexing relatively large numbers of sea turtles.

There are also limitations to using an RIA to sex sea turtles. First, as with any sexing technique for sea turtles, an RIA should be well-validated. For example, results from RIAs may vary within and between laboratories. Additionally, testosterone levels can vary slightly between sea turtle species, and possibly between populations (Wibbels, 1988). Therefore, whenever possible, a particular RIA should be validated using serum samples from turtles of known sex *(e.g.,* laparoscopically verified sex) from the species and/or population which is to be analyzed. In those analyses, various size classes of turtles should be examined to validate the size range of turtles that can be accurately sexed. Second, the results described above for green turtles (Wibbels, 1988) show that in some populations the testosterone levels of a small percentage of males and females can overlap. This again shows the necessity for accurate valida-

tion of the RIA. Female-only and male-only ranges must be determined. Only turtles falling within those ranges can be accurately sexed as male or female. Finally, once validated, the RIA should include interassay controls to verify assay reliability over time.

Blood Sampling

Blood samples for RIA analysis (or for other sex determination techniques) can be obtained from blood vessels located parallel to the spinal cord on the dorsal portion of the neck of sea turtles (Owens and Ruiz, 1980). The turtle can be placed in a slanted orientation with head down for optimal results, but in many cases blood samples can be readily obtained from turtles that are in a horizontal position. The optimal length and size of the needle required for blood sampling may vary depending of size of the turtle and the species, but a 3.8 cm, 21 gauge needle works well for most immature sea turtles. To obtain a sample, the needle is attached to vacutainer or syringe and then inserted into the neck at a steep angle at the approximate locations shown in Figure 3. Blood should be collected in sterile vacutainers if serum will be used in the assay or in treated, sterile tubes *(e.g.,* heparin or sodium citrate tubes) if plasma will be used in the assay. A minimum of several milliliters of blood should be taken, so that enough serum can be obtained for running samples in duplicate. Sample tubes should be placed on ice until
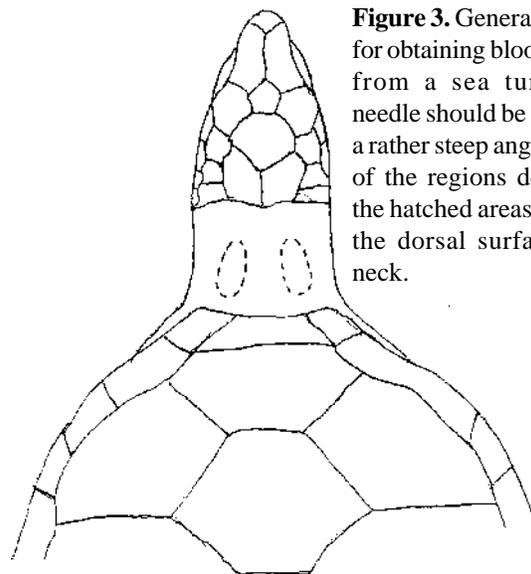

Figure 3. General locations for obtaining blood samples from a sea turtle. The needle should be inserted at a rather steep angle into one of the regions denoted by the hatched areas shown on the dorsal surface of the neck.

they can be centrifuged. The serum or plasma is separated from the blood cells by centrifugation and then transferred to a separate container and frozen.

Analysis of Sex Ratio Data

Once sex ratio data have been collected from a foraging ground, appropriate methods must be chosen for analysis. Specific questions relating to the sex ratio data should be formulated so that meaningful analyses can be conducted. For example, it may be insightful to examine whether a sex ratio differs from a 1:1 ratio, or whether sex ratios from different feeding grounds vary. Further, in addition to examining pooled data from a population, it may be advantageous to subdivide the data based on such factors as size classes of turtles, time of year when sampled, and sampling location.

Once specific questions have been generated, appropriate statistical analyses can be conducted. The sex of a sea turtle represents a qualitative rather than a quantitative variable, and a sex ratio is a derived variable. Therefore, when examining a single sex ratio from a population, many of the familiar statistics and their descriptive parameters *(e.g.,* mean, variation, confidence limits) do not apply (Siegel, 1956; Sokal and Rohlf, 1969; Zolman, 1993). However, there are statistical tests that are appropriate for sex ratio data. To compare observed frequencies of males and females in a population to a predicted value (1:1 for example), the chi-square goodness of fit test is appropriate when working with moderate to large sample sizes (all expected cell frequencies should be 5 or greater). Additionally, a Fisher's exact test can be used for these analyses and should be used instead of a chi-square test when working with small sample sizes (Siegel, 1956; Sokal and Rohlf, 1969; Zolman, 1993). These goodness of fit tests allow researchers to examine whether the observed sex ratio in a given population differs significantly from a 1:1 ratio. These tests can also be used to compare sex ratio data. For example, it may be useful to compare sex ratio data collected at different times of the year from a particular feeding ground, to compare sex ratios from different feeding grounds, or to compare sex ratios of different size classes of sea turtles within a population. Such analyses can also be accomplished with a replicated goodness of fit test (Sokal and Rohlf, 1969). This test generates "G" statistics which will indicate if the sex ratios are homogeneous and if the pooled male and female frequencies significantly differ from predicted values.

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