
4 Critical Approaches to Sex Determination in Sea Turtles

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4.1 INTRODUCTION

Temperature-dependent sex determination (TSD) was first reported in 1966 in a lizard.¹ Since that time it has been shown to occur in a wide variety of reptiles, including all crocodylians, most turtles, some lizards, and the tuatara.²⁻⁴ The occurrence of TSD in primitive groups of reptiles such as turtles, crocodylians, and tuatara has led some researchers to hypothesize that it may represent an ancestral form of sex determination from which avian and mammalian sex determination systems have evolved.⁵ The adaptive advantage of TSD is not clear, but a number of proposed hypotheses might explain why many reptiles, including sea turtles, have retained TSD.⁶⁻⁸

In sea turtles, TSD was first documented in the loggerhead, *Caretta caretta*.⁹ Since that time it has been shown to occur in all extant species: the green turtle, *Chelonia mydas*;¹⁰⁻¹² the olive ridley, *Lepidochelys olivacea*;¹³⁻¹⁵ the leatherback, *Dermochelys coriacea*;^{12,16,17} the hawksbill, *Eretmochelys imbricata*;¹⁸⁻²¹ Kemp's ridley, *Lepidochelys kempi*;²²⁻²⁴ the black turtle, *Chelonia agassizi*;²⁵⁻²⁷ and the flatback, *Natator depressa*.²⁸ The occurrence of TSD in sea turtles generates a wide variety of questions regarding the physiological, ecological, and conservational implications of this form of sex determination. The purpose of this review is to summarize what is known about TSD in sea turtles and to use that information to address basic questions regarding the biology and conservation of marine turtles.

4.2 CHARACTERISTICS OF TSD IN SEA TURTLES

Although a wide variety of reptiles possess TSD, the effect of a particular temperature may vary depending on the species.^{2,29} Several patterns of sex determination have been described regarding the effects of temperature on sex determination in reptiles.^{2,29} All sea turtles examined to date appear to have a male-female (MF) pattern in which cooler incubation temperatures produce males and warmer incubation temperatures produce females (Figure 4.1). Several terms have been created to describe TSD in reptiles.³⁰ The transitional range of temperatures (TRT) is the range of temperatures in which sex ratios shift from 100% male to 100% female (Figure 4.1). In the case of the MF pattern in sea turtles, temperatures above the TRT will produce all females and temperatures below the TRT will produce all males. Within the TRT, there is temperature referred to as the pivotal temperature, which is the constant incubation temperature that will produce a 1:1 sex ratio (Figure 4.1). The pivotal temperature can vary between and even within a species.³¹ It has also been reported that the TRT may vary among sea turtle populations.³² Therefore, if one is interested in studying sex determination or estimating hatchling sex ratios in a given sea turtle population, it is optimal to determine these parameters for that particular population, rather than extending data from one population to another.

4.2.1 ACCURACY OF TEMPERATURE ESTIMATES

Before delving into the specific temperature estimates from previous studies of TSD in sea turtles, it is necessary to address the potential problems associated with accuracy. The goal of many TSD studies is to estimate parameters that describe TSD

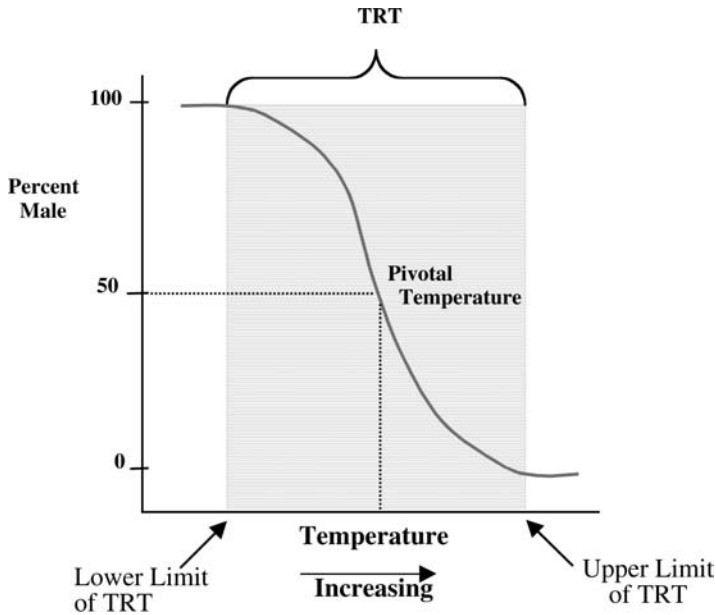


FIGURE 4.1 General pattern of TSD in sea turtles. Relatively cool temperatures produce males and relatively warm temperatures produce females. Pivotal temperatures produce a 1:1 sex ratio. The TRT is the range over which sex ratios shift from all male to all female.

in a species or population (e.g., pivotal temperature, transitional range of temperatures, etc.). Subsequent studies then use those parameters for comparative purposes or even for predicting sex ratios. Therefore, the accuracy of the temperature recordings is of paramount importance. For example, a change of only a few tenths of a degree Celsius can have a significant effect on sex ratio if that change occurs near the pivotal temperature. The accuracy of temperature estimates reported in TSD studies is limited by factors such as the accuracy of the recording equipment, the stability of the incubators, and the experimental protocol. For example, the resolution of typical temperature data loggers is approximately 0.2–0.4°C, and a typical incubator may vary by several tenths of a degree around the selected temperature. Thus, when reviewing data on TSD, one should be cautious when comparing temperature estimates from studies that might use different experimental protocols and temperature recording equipment.

4.2.2 PIVOTAL TEMPERATURES OF SEA TURTLES

A variety of studies have estimated pivotal temperatures in sea turtles (Table 4.1). In general, the reported pivotal temperatures occur over a relatively narrow temperature range from approximately 27.7 to 31°C, depending on the particular species and study, with the majority clustering in the 29.0 to 30.0°C range.³¹ The reason for this narrow range is unknown, but it is plausible that it could relate to factors such as ecological or physiological constraints that may be selecting for this range of pivotal temperatures.

TABLE 4.1
Examples of Pivotal Temperatures Reported for Sea Turtles

Species and Location	Estimated Pivotal Temperature (°C)	Reference
Loggerhead (<i>Caretta caretta</i>)		
U.S.	Approximately 30	44
U.S.	29.0	34
Australia	27.7, 28.7	33
Australia	Approximately 29.0	36
S. Africa	29.7	38
Brazil	29.2	37
Leatherback (<i>Dermochelys coriacea</i>)		
Suriname and French Guiana	29.5	16
		39
Costa Rica	29.4	17
Hawksbill (<i>Eretmochelys imbricata</i>)		
Antigua	29.2	20
Brazil	29.6	101
Green (<i>Chelonia mydas</i>)		
Suriname	28.8	12
Costa Rica	Approximately 28.5–30.3	46
		47
Olive Ridley (<i>Lepidochelys olivacea</i>)		
Costa Rica	Approximately 30	13
Costa Rica	Approximately 31	15
Kemp's Ridley (<i>Lepidochelys kempii</i>)		
Mexico	30.2	23

Despite this relatively narrow range, the variation associated with reported pivotal temperatures often makes it difficult to assign a specific pivotal temperature to the tenth of a degree Celsius for a given population. Table 4.1 shows that the reported pivotal temperatures vary between species and populations. In addition, interclutch variation has been reported for sea turtles and other reptiles with TSD.^{29,33–35} The variation in reported pivotal temperatures is exemplified by the loggerhead turtle, which has received more attention than any other species. Mrosovsky (1988) estimated pivotal temperatures for clutches of loggerhead eggs from three different locations along the coast of the U.S. That study found that all predicted pivotal temperatures were near 29.0°C, but estimates for individual clutches ranged from approximately 28.5 to 29.2°C.^{34,36} Furthermore, a significant

interclutch variation was detected between two clutches from the same nesting beach during that study.³⁴ A study of loggerhead turtles in Australia estimated pivotal temperatures of 27.7 and 28.7°C for two different nesting beaches, and a significant interclutch variation was detected from clutches from one of those beaches.³³ A later study suggested a pivotal temperature of approximately 29.0°C for one of those same nesting beaches. Other estimates for loggerhead pivotal temperatures include 29.2°C for a Brazilian nesting beach³⁷ and 29.7°C for a South African nesting beach.³⁸ Thus, pivotal temperatures within this species have been reported to vary by up to 1.0°C or more.

Although some of this variation may be associated with experimental error, differences in protocols, or divergences in statistical analyses of the data, these findings suggest that pivotal temperatures vary among and even within a population. A reported pivotal temperature simply represents an estimate of the mean pivotal temperature for a sample from a population. Considering the large number of sea turtle populations in the world, there have been only a limited number of pivotal temperature studies. Because of the endangered or threatened status of many of these populations, pivotal temperature studies have usually used a limited number of hatchlings and clutches. To better understand the variations in pivotal temperatures within and between populations, more comprehensive studies using large numbers of clutches would be optimal.

If one is interested in studying sex determination or estimating hatchling sex ratios in a given sea turtle population, it is advantageous to determine the pivotal temperature for that particular population, rather than estimating it based on data from other populations. Because interclutch variation can occur, the accuracy of the estimated pivotal temperature will be dependent on the number of clutches examined. Furthermore, because the estimated pivotal temperature will represent a mean, it should include statistics to describe the variance associated with the mean (see Section 4.2.4).

4.2.3 TRT OF SEA TURTLES

In addition to pivotal temperature, the TRT (Figure 4.1) is a useful parameter for characterizing sex determination in sea turtles. As defined earlier, the TRT represents the range of temperatures in which the sex ratio shifts from 100% male to 100% female.³⁰ For sea turtles (which have an MF pattern of sex determination), temperatures below the lower limit of the TRT will produce only males and temperatures above the upper limit of the TRT will produce only females. A TRT is more difficult to accurately estimate than a pivotal temperature because of the greater number of incubation temperatures necessary to clearly pinpoint the lower and upper bounds of the TRT curve. Most studies have not ruled out the possibility that the TRT could be larger or smaller than reported.

A general characterization of the TRT can be gleaned from past studies by examining maximum incubation temperatures that produce all males and minimum incubation temperatures that produce all females in a particular study (Table 4.2). Many past studies did not precisely determine the TRT because of the number of incubation temperatures and the specific temperatures utilized in the study. However, some studies included a reasonable number of appropriate incubation temperatures, and the results

TABLE 4.2
Examples of Incubation Temperatures Producing All Male or All Female Hatchlings in Previous Studies of Sea Turtle Sex Determination

Species and Location	Temperatures (°C) Producing		Reference
	100% Male	100% Female	
Loggerhead (<i>Caretta caretta</i>)			
U.S.	<27.5	30.4–30.5	34
Australia	26.0	30.0–32.0	33
Australia	<26.0	31.0	36
Brazil	28.0	30.6	37
Leatherback (<i>Dermochelys coriacea</i>)			
Suriname	28.75	29.75	39
Costa Rica	29.0	30.0	17
Hawksbill (<i>Eretmochelys imbricata</i>)			
Antigua	28.5–29.0	30.3	20
Brazil	28.4	30.4	101
Green (<i>Chelonia mydas</i>)			
Suriname	<27.75	>29.25–30.75	12
Costa Rica	<28.0	>30.5	46
Olive Ridley (<i>Lepidochelys olivacea</i>)			
Costa Rica	<28.0	32.0	13
Costa Rica	27.0	32.0	15
Kemp's Ridley (<i>Lepidochelys kempî</i>)			
Mexico	<29.0	31.0	23

Note: All studies are based on constant temperature studies with the exceptions of those for green turtles in Costa Rica, which were based on field studies of natural nests.

provide a general characterization of the TRT in some species. The findings from these studies indicate that the TRT can vary between species and populations.

As with pivotal temperatures, the values of the lower and upper limits of the TRT, as well as the width of the TRT, can vary between species and populations. For example, minimum temperatures producing all females ranged from approximately 29.75 to 32.0°C (Table 4.2), and maximum temperatures producing all males ranged from approximately 26.0 to 28.75°C (Table 4.2), depending on the species and population examined. Studies of loggerhead populations have suggested TRT widths as great as 2.0 to 3.0°C,^{33,34} whereas a study of a leatherback population suggested a TRT width as narrow as approximately 1.0°C.³⁹ It was suggested that the TRT recorded in the latter study was significantly different from that reported for another leatherback population.³²

Collectively, these data indicate that the upper and lower limits of the TRT can vary between sea turtle species and populations. Although data from previous studies are far from comprehensive, they suggest general ranges for the lower limits (approximately 26.0 to 28.75°C) and upper limits (approximately 29.75 to 32.0°C) of TRTs, and they provide a general range of widths for TRTs (approximately 1.0 to 3.0°C). This sort of information has major conservational and ecological implications. For example, this suggests that a temperature change of 3.0°C or less could potentially shift sex ratios from all male to all female (or vice versa). It also provides information on thermal ranges where small changes should not make a difference for sex ratios (e.g., extremely warm temperatures that are well above pivotal). In addition, these data indicate that physiologically, the sex determination cascade switches from male to female (or vice versa) over a relatively narrow temperature window.

4.2.4 METHODS FOR ESTIMATING PIVOTAL TEMPERATURE AND TRT IN A SEA TURTLE POPULATION

Before characterizing TSD in a sea turtle population, one should first address the reasons for doing so. Knowledge of pivotal temperatures and TRTs has a variety of uses ranging from highly applied to theoretical. First, it has direct applications to the conservation of endangered populations. If you know the TRT and pivotal temperature in a population, you can then predict hatchling sex ratios by simply monitoring incubation temperatures in nests. Furthermore, this knowledge provides the basis for potentially manipulating hatchling sex ratios in an effort to enhance reproductive output in a population.^{40,41} Pivotal temperatures and TRTs are also of ecological, physiological, and evolutionary interest because they allow researchers to begin addressing questions regarding variation in TSD and how it might affect the reproductive ecology in a population.

Conservational considerations must also be taken into account before beginning a study of pivotal temperatures and TRTs. In most cases the study will involve an endangered or threatened sea turtle population. Currently, it is impossible to verify the sex of a sea turtle hatchling using external characteristics. The definitive method of determining sex is by histological analysis of the gonads, which requires that the hatchling be killed. Although this sounds counterintuitive to enhancing the recovery of an endangered population, it is quite possible that the knowledge gained will greatly enhance the recovery of the population.

When the effects of temperature on sex in sea turtles are studied, the resolution and accuracy of temperature recordings is of major importance. For example, data from previous studies suggest that pivotal temperatures can vary by tenths of degrees between populations and even between clutches (reviewed by Ref. 31). Therefore, accurate temperature measurements are a prerequisite to obtaining meaningful results. A variety of temperature recording devices are available commercially. For automated recording of temperatures, one of the most commonly used devices is the temperature data logger. These units are battery operated, have an internal and/or external temperature probe, and can be programmed to take temperature recordings at a wide variety of intervals. Many of these data loggers are small enough to be placed among the eggs in a nest or incubator.⁴² The data loggers are connected to a

computer to program the unit and to download data. When purchasing data loggers, one should consider factors such as resolution and accuracy of temperature recordings, amount of memory, size of data logger, battery life, and cost. In general, the resolution of many typical data loggers ranges from approximately 0.2 to 0.4°C. All data loggers and temperature probes should be calibrated before and after use. Calibration requires comparison to a thermometer that is traceable to National Institute of Standards and Technology (NIST) or its equivalent in other countries. Temperature recordings from each data logger and/or temperature probe should be taken simultaneously with the NIST-traceable thermometer in an incubator with a stable temperature in the typical range of sea turtle nest temperatures (e.g., approximately 26 to 35°C). If the values are not consistent, a correction factor should be determined for each data logger and temperature probe.

The majority of previous studies of pivotal temperatures and TRT in sea turtles have involved the incubation of eggs at constant temperatures in laboratory incubators. This represents an efficient means of examining the effects of constant temperatures on sex determination because a wide variety of specific temperatures can be examined. In such studies, the thermal characteristics of the incubator should be evaluated prior to the experiments because they can have a profound effect on the results. Factors such as the thermal stability of the incubator and thermal gradients within the incubator should be examined. For example, incubators often develop “thermal inertia” when the heating or cooling system is activated and will often overshoot the preset temperature. Incubators with the smallest amount of thermal inertia are preferred. An incubator that can maintain a preset temperature to plus or minus a few tenths of a degree should be adequate. The cost of the incubator does not necessarily reflect its effectiveness for this sort of study. It is possible to build incubators using polystyrene (Styrofoam) boxes and aquarium heaters for a relatively modest price that may outperform some of the commercial models.⁴³ Furthermore, the home-built incubators may be the only option if the experiments are performed in the field, where it is not practical to have large commercial incubators.¹⁷

Incubation temperatures for these studies should be chosen in an attempt to resolutely determine the lower and upper limits of the TRT and the pivotal temperature. Multiple temperatures (and thus incubators) will be needed to identify each parameter. Data from previous studies of other populations of the same species should provide insight on appropriate temperatures to examine. Ideally, eggs should be placed into incubators as soon as possible after they are laid. However, this is not a necessity, because previous studies indicate that the temperature-sensitive period of sex determination is approximately the middle third of the incubation period⁴⁴ (reviewed by Ref. 45). Given the increased sensitivity of sea turtle eggs to movement after the first day of incubation, however, it is advisable to place the eggs in the incubators as soon as possible after they are laid.

Because variation in pivotal temperatures has been detected between clutches of eggs, it is important to use multiple clutches of eggs for estimating pivotal temperatures. For example, it is far superior to use 20 eggs from each of five clutches than to use 100 eggs from a single clutch. Ideally, large samples from multiple clutches can be used to evaluate average pivotal temperature and interclutch variation. It is also better to use clutches that have all been laid on the same day, thus

synchronizing the development of the eggs. Depending on the type of incubators, the eggs may need to be placed on a moist substrate (e.g., moistened vermiculite) and kept in covered containers to prevent desiccation.³⁴ Some of the homemade incubators maintain a very high humidity (because of a water-filled heat sink in the bottom of the incubator), so moist substrate is not necessary. Eggs from different clutches should be randomly assigned a position in the incubator to prevent the effect of location within the incubator being misinterpreted as clutch effects. The location of eggs should be rotated periodically (e.g., daily) within the incubator to minimize any position effects in the incubators. Temperature data loggers and/or temperature probes should be placed adjacent to eggs within the incubators, and temperatures should be recorded a minimum of several times per hour throughout the incubation. Upon hatching, the sex of each hatchling must be verified (see Section 4.3.7).

Although most studies have used constant temperatures to analyze pivotal temperature and TRT, studies using fluctuating (e.g., natural) temperature regimes are of distinct interest. Such studies are of particular relevance for sea turtle conservation programs that incubate eggs in natural or translocated nests on natural nesting beaches. Some studies have reported sex ratios relative to natural nest incubation temperatures.^{11,46,47} This subject has been addressed experimentally by Georges et al.,³⁶ and their findings indicate that daily temperature fluctuations can have a profound effect on sex determination, but the effect is dependent upon the magnitude of the fluctuation. The depth of sea turtle nests limits the daily temperature fluctuations (e.g., maximum of 0.3–1.4°C in studies of loggerhead turtles).^{48,49} On the basis of the model by Georges et al.,³⁶ fluctuations of this magnitude would alter the effect of temperature by a maximum of only a few tenths of a degree Celsius in comparison with constant temperatures. This suggests that data from constant-temperature studies are generally applicable to studies examining natural sea turtle nests with fluctuating temperatures. However, this concept is based on a single study that examined temperatures fluctuating around a single mean temperature.³⁶ Thus, it would be useful to have future studies addressing this subject in sea turtles.

Once sex ratio data are obtained from pivotal temperature and TRT studies, they must be analyzed. Analysis in previous studies has varied from simple estimates to elaborate statistical analyses. A rigorous and standardized statistical method of describing pivotal temperature and TRT has been reported.⁵⁰ In addition to describing TSD in a particular population, these statistics allow for comparison of TRT and pivotal temperatures among sea turtle populations.^{32,50}

4.3 SEX RATIOS IN SEA TURTLE POPULATIONS

Evolutionary theory suggests that the primary sex ratio (i.e., the sex ratio of the hatchlings) should be 1:1 if parental investment in both sexes (among other factors) is equal.^{51,52} However, TSD has the potential of producing a wide variety of sex ratios, and numerous questions arise regarding sex ratio produced from TSD. For example, do naturally occurring sex ratios conform to a 1:1 sex ratio, as suggested by evolutionary theory?^{51,52} What range of sea turtle sex ratios occurs in nature? Do sex ratios vary over time and within a population (e.g., seasonal, yearly, by size class, etc.)? What effects does sex ratio have on reproductive success in a population?

Are there optimal sex ratios for the recovery of a population, and should sex ratios be manipulated in an effort to enhance the recovery of an endangered sea turtle population? These are just a few of the questions regarding TSD that confront sea turtle biologists. A prerequisite to answering these questions is the examination of natural sex ratios in sea turtle populations and in conservation programs.

4.3.1 HATCHLING SEX RATIOS FROM NESTING BEACHES

It is beyond the intended scope of this chapter to provide a critical review of all previous reports of naturally occurring sea turtle sex ratios, however, a general overview will be presented. Examples of hatchling sex ratios that have been predicted for sea turtle nesting beaches are shown in Table 4.3. The reader should be cautioned that there is great variability in the methodology and scope of these studies. For example, some predictions are for a single nesting season, whereas others are for up to 14 different nesting seasons, and the amount of temperature data recorded and/or the amount of sexing data on hatchlings vary. Furthermore, some of these studies rely on pivotal temperature data from other sea turtle populations for sex ratio projections. Regardless, several general points can be derived from these data. First and foremost, the great majority of the predicted sex ratios do not conform to a 1:1 sex ratio suggested by evolutionary theory. Although a few of the sex ratios approach 50% female, there is an obvious predominance of beaches that produce female-biased sex ratios, and some of these biases are extreme (greater than 90% female). No reports exist of extreme male biases over an entire nesting season. On the basis of these data, we are confronted with the possibility that TSD in sea turtles may not conform to the predictions of evolutionary theory by Fisher;⁵¹ rather, a predominance of female biases may exist. However, other explanations are possible. It is plausible that some criteria are not fulfilled regarding fisherian sex ratios, such as sex ratios not being at equilibrium.^{31,53} Alternatively, because the sex ratios reported represent only a small sampling of all sea turtle populations and nesting beaches, the results could be affected by sampling bias. Thus, one should be cautious in extrapolating from the limited database that is currently available.

4.3.2 NEST LOCATION AND HATCHLING SEX RATIOS

Several studies have shown that nest location can have a profound influence on hatchling sex ratios. Nesting beaches can have several thermal zones (e.g., beach slope near the water, open beach flat, dune bordering beach, dune with vegetation, etc.) that can influence sex ratio.^{46,47,54} Multiple nesting beaches on islands can also provide a variety of thermal environments. Depending on the specific beach chosen by green turtles nesting on a small island (Heron Island) on the Great Barrier Reef, hatchling sex ratios were shown to vary from 29.5 to 63.1% female.^{33,55} A similar situation was predicted for green turtles on Ascension Island in which one nesting beach was 2.6°C warmer than another.⁵⁶ Indeed, sand color has been shown to be directly related to the thermal properties of nesting beaches.^{57A} Thus, the specific location of nesting can significantly affect sex ratio.

TABLE 4.3
Examples of Hatchling Sex Ratios Predicted for Sea Turtle Nesting Beaches

Location	Method for Predicting Sex	Predicted Sex Ratio	Reference
Loggerhead (<i>Caretta caretta</i>)			
South Carolina and Georgia	GH	56.3% female	Mrosovsky et al., 1984
Florida	GH	>93% female	Mrosovsky and Provancha, 1988
Florida	GH, BT	87.0–99.9% female	Mrosovsky and Provancha, 1992
Brazil	ID	82.5% female	Marcovaldi et al., 1997
Florida	NT	>90% female	Hanson et al., 1998
Cyprus and Turkey	GH	Female-biased	Kaska et al., 1998
Cyprus	NT	Female-biased	Godley et al., 2001
Green (<i>Chelonia mydas</i>)			
Sarawak	ID	74% female	Standora and Spotila, 1985, based on data from Hendrickson, 1958
Suriname	GH, ID	53.9% female	Mrosovsky et al., 1984
Costa Rica	GH	67% female	Spotila et al., 1987
Suriname	GH, BT	68.4% female	Godfrey et al., 1996
Cyprus	GH	Female-biased	Kaska et al., 1998
Cyprus	NT	Highly female-biased	Broderick et al., 2000
Leatherback (<i>Dermochelys coriacea</i>)			
Suriname	GH, ID	44% female	Mrosovsky et al., 1984
French Guiana	GH	Nearly 1:1	Rimblot-Baly et al., 1987
Suriname	GH, BT	53.6% female	Godfrey et al., 1996
Costa Rica	BT, NT	93.5–100% female	Binckley et al., 1998
Hawksbill (<i>Eretmochelys imbricata</i>)			
Antigua	BT, ID	Not likely to be highly female-biased	Mrosovsky et al., 1992
U.S. Virgin Islands	GH	Female-biased	Wibbels et al., 1999
Brazil	ID	>90% female	Godfrey et al., 1999

Notes: The predicted sex ratio is based on the authors' prediction in percent female (if reported) or is the general description given by the authors (e.g., female bias, etc.). The methodology can vary greatly between these studies, so readers should refer to each individual study for information on number of seasons estimated, seasonal variation, interyear variation, etc. GH = gonadal histology; BT = beach temperature; ID = incubation duration; NT = nest temperature.

4.3.3 SEASONAL VARIATION IN HATCHLING SEX RATIOS

A number of previous studies also indicate that hatching sex ratios can vary over the nesting season. An initial study by Mrosovsky et al.⁷³ indicated that sex ratios of hatchling loggerheads in South Carolina and Georgia could vary from less than 10% female during the cooler portions of the nesting season to 80% female during the warmer months. Standora and Spotila⁴⁶ reviewed incubation duration data from Hendrickson^{57B} and predicted seasonal changes in sex ratios of hatchling green turtles at Sarawak, with monsoon season producing predominantly males, whereas nests laid from April through November produced predominantly females. Other examples of seasonal changes in hatchling sex ratios from nesting beaches include loggerheads from Florida,⁵⁴ Brazil,³⁷ and Cyprus,⁵⁸ hawksbills from Brazil, leatherbacks from Suriname,^{12,53} leatherbacks from French Guiana,³⁹ and green turtles from Ascension Island⁵⁹ and Suriname.¹² Of particular interest, several of these past studies have predicted that the temperature decreases associated with periods of rain can have a profound effect on sex ratios, resulting in the production of male biases.^{12,53}

Although seasonal variation in sex ratios may occur on many nesting beaches, it may not occur on all beaches, depending on weather conditions and length of nesting season. For example, beach temperatures were relatively constant at Tortuguero, Costa Rica, during the 1980 nesting season for green turtles, suggesting no seasonal variation in sex ratios.⁴⁷ Furthermore, in some situations, incubation temperatures may be high enough that minor temperature fluctuations have little or no effect, and 100% females are produced, as suggested for the 1993–1994 nesting season of leatherbacks at Playa Grande, Costa Rica.¹⁷

4.3.4 YEARLY VARIATION IN HATCHLING SEX RATIOS

As one might predict, hatching sex ratios from a given nesting beach can also vary from year to year depending on factors such as weather and timing of nesting. Although yearly variations have been reported, these variations are often relatively small for a given nesting beach. For example, sex ratios of hatchling loggerheads from a Florida nesting beach were predicted to be 92.6–96.7% female during 1985, 94.7–99.9% female during 1986, and 87–89% female during 1987.⁵⁴ Godfrey et al.⁵³ predicted a 10% variation between two nesting seasons (1982 vs. 1993) for hatchling sex ratios of both leatherback and green turtles. A study of green turtle nesting beaches on Ascension Island predicted only small interannual differences in beach temperatures, on the basis of 14 years of data.⁵⁶

Variations in annual rainfall have been suggested to affect yearly variations in sex ratios projected during 14 different nesting seasons of leatherback and green sea turtles in Suriname, with annual differences as great as 20–90% female for green turtles.⁵³ It also is possible that physical changes in a nesting beach can affect year-to-year variation. Because of displacement of beach sand by ocean currents, green and leatherback nesting beaches in Suriname and French Guiana have been moving to the west at approximately 2 km per year.⁶⁰ This change in beach location could obviously contribute to yearly variations reported for hatchling sex ratios.⁵³ Human alteration of beach characteristics could also affect sex ratios produced from a nesting

beach. Beach nourishment projects have the potential of changing the thermal characteristics of the beach and thus incubation temperatures.⁴² Furthermore, housing developments on nesting beaches have the potential of limiting the nesting area (and thus the range of thermal environments) and also shading nests.^{42,61}

4.3.5 SEX RATIOS IN IMMATURE AND ADULT PORTIONS OF A POPULATION

Sex ratios in other size classes in sea turtle populations (e.g., immature and adult) are also of interest. These groups represent a condensation of many years of hatchling sex ratios, although it must be kept in mind that these sex ratios could also reflect potential sex-specific mortality during development or as adults. For example, energy expenditures associated with egg production and nesting, together with movements onto nesting beaches, could increase the mortality of adult females relative to males. It is also plausible that sex-specific hatchling and post-hatchling mortality could occur because of seasonal variation in hatchling sex ratios. For example, hatchlings produced early in the nesting season might be predominantly one sex and may experience different food availability and predation from hatchlings produced later in the nesting season (which could be predominantly the other sex). Knowledge of sex ratios in the various size–age classes within a population is necessary to begin understanding the potential dynamics of sex ratios within a population. Ultimately, such knowledge would provide insight on the long-term effects of sex ratios on the reproductive ecology of a population.

Our knowledge of adult sex ratios in sea turtle populations is limited because of a number of factors. First, it is logistically difficult to sample adult sea turtles in the ocean. Another problem relates to sex-specific migration patterns of adult males vs. females. For example, Henwood⁷⁵ found an approximate 1:1 sex ratio of adult loggerheads captured in waters near Cape Canaveral, FL; however, he recorded seasonal differences in the abundance of adult males vs. adult females. A study of green turtles of the southern Great Barrier Reef reported a 1:1 adult sex ratio, but suggested that the ratio may have been due to sex-biased migratory patterns in which adult males stayed closer to the breeding grounds (and sampling areas) than did the adult females.^{62,63}

Human impacts may also affect adult sex ratios. In a study of adult green turtles in Oman, Ross⁶⁸ reported an overall sex ratio that was not significantly different from 1:1, but found an excess of females or males in several locations. In the case of excess of males, it was noted that fishermen in that area preferentially hunted for females.⁶⁸ Several studies have reported female-biased sex ratios of green turtles based on commercial catches,^{64–67} but there is the potential of sampling bias because of fishing practices.^{68A} Thus, a number of potential problems can affect the results of adult sex ratio studies. Accurate evaluation of adult sex ratios requires knowledge of migratory behavior (e.g., timing and specific migratory routes) as well as other factors that might impact the sex ratio of turtles captured in a particular sampling location. Regardless of the associated difficulties, studies of adult sex ratios are necessary to fully understand the potential variation and dynamics of sex ratios within a sea turtle population.

Studies of sex ratios in the immature portion of a population (i.e., juvenile to subadult turtles) may provide more accurate estimates of sex ratios, because these studies may not be hampered by sampling bias problems associated with adult breeding migrations (although it is possible that sex-specific behavior may be expressed by juvenile and subadult turtles). A number of studies have examined sex ratios in the immature portion of sea turtle populations, and the results provide insight on the potential variability of sex ratios within a sea turtle population. For loggerheads along the Atlantic coast of the U.S., one study used blood testosterone levels to sex 218 immature turtles in the Atlantic waters off Florida and found a 2:1 (female–male) sex ratio.⁶⁹ A similar study used blood testosterone levels to sex loggerheads from four different locations along the Atlantic coast of the U.S., and also found an approximate 2:1 (female–male) sex ratio.⁷⁰ Another study used necropsy to sex 139 immature loggerhead turtles that had stranded on the Georgia coast, and also found an approximate 2:1 (female–male) sex ratio.⁷¹ These studies suggest that an approximate 2:1 (female–male) sex ratio may exist in the immature portion of the loggerhead population along the Atlantic coast of the U.S. An approximate 2:1 (female–male) sex ratio has also been reported for immature loggerheads in the Gulf of Mexico.^{68B} Although distinctly female-biased, this 2:1 sex ratio is far less than the approximate 9:1 (female–male) hatchling sex ratios suggested for Florida nesting beaches.^{54,72}

A number of suggested hypotheses could account for this discrepancy. First, the two Florida beaches examined for hatchling sex ratios may not be representative of all nesting beaches for the loggerheads in the U.S. It is possible that other loggerhead nesting beaches produce greater numbers of males. For example, a study of hatchling loggerhead sex ratios on South Carolina and Georgia beaches (which are part of the northern nesting subpopulation) suggested a sex ratio of approximately 1:1 (female–male).⁷³ However, the two beaches examined in Florida (Cape Canaveral and Hutchinson Island) represent major nesting beaches for the south Florida nesting subpopulation, which produces approximately 90% of all hatchling loggerheads from U.S. beaches.⁷⁴ If other beaches in the south Florida nesting subpopulation also produce hatchling sex ratios of approximately 9:1 (female–male), then even strong male biases on northern or Gulf of Mexico nesting beaches could not contribute enough hatchlings to account for an overall 2:1 (female–male) sex ratio that was suggested for the immature portion of the population. Therefore, other factors must account for the difference. One possibility is that some nesting beaches of the south Florida subpopulation may produce more males. It is also possible that some previous years had relatively cool periods that produced more males. Another hypothesis would be that males may be produced during the early portion of the nesting season⁷³ and have greater survival rates because of factors such as food availability or longer growth period prior to first winter. An additional possibility is that turtles captured off the U.S. Atlantic coast come from other nesting beaches (e.g., Mexico) that could produce more male-biased ratios. No attempts were made to assign beaches of origin to the samples of turtles in those studies. Adult sex ratios in this population have not been adequately addressed because of the logistical difficulty of sampling adult loggerheads in coastal waters, and also sampling biases associated with sex-specific migration patterns of adult sea turtles.⁷⁵ Regardless, the discrepancy between the

sex ratios of hatchling vs. immature loggerheads in U.S. waters is intriguing and worthy of future studies.

A similar scenario may occur with the Kemp's ridley sea turtle. Data suggest a strong female-bias (possibly 80–90% female or greater) of hatchling sex ratios produced over 4 recent years,⁷⁶ and this could be indicative of previous years because almost all nests have been translocated to the same protected egg "corral" for more than 20 years. Blood testosterone levels were used to predict the sex of 39 immature Kemp's ridley turtles captured along the Gulf coast of Florida and predicted a 1.7:1.0 (female–male) sex ratio.⁷⁷ A similar study examined blood testosterone in 42 Kemp's ridley turtles captured along the Gulf coast of Florida and reported a sex ratio of approximately 3.7:1 (female–male).⁷⁸ Laparoscopy was used to sex 231 Kemp's ridley turtles captured off the coast of Texas and Louisiana, and found a sex ratio of 1.3:1 (female–male).⁷⁹ Necropsy was used to sex 89 stranded Kemp's ridley turtles from the Texas coast, and found a sex ratio of 3:1 (female–male).⁸⁰ A similar study examined 89 stranded immature Kemp's ridleys from the Texas coast and found an approximate 2.0:1.0 (female–male) sex ratio.^{68B} Thus, the sex ratios reported for the juvenile portion of this population appear female-biased, but the bias is distinctly less than that predicted for recent hatchling sex ratios. As with the loggerhead sex ratios described above, the discrepancy between the sex ratios of hatchling vs. immature ridley turtles is worthy of future studies.

Although female-biased sex ratios have been reported in many populations, a near 1:1 sex ratio has been predicted for the immature portion of the Hawaiian green turtle population on the basis of two different studies. One study used blood testosterone levels to sex 63 juvenile turtles captured in waters off the coasts of Molokai and Hawaii, and predicted a sex ratio of approximately 1:1.⁸¹ A second study used necropsy to examine 421 immature and adult turtles, and the sex ratio of juveniles did not significantly differ from a 1:1 sex ratio.⁸² The Hawaiian green turtle represents an interesting population for sex ratio studies because it is an isolated group of green turtles,⁸³ and data suggest relatively cool incubation temperatures in comparison to those of other green turtle populations.⁸⁴ It is possible that this population represents an excellent example of how the thermal environment of a nesting beach may select for a specific pivotal temperature.

Blood testosterone levels were also used to predict the sex of 111 immature green turtles from Bermuda.⁸⁵ The results indicated a 1.4:1.0 (female–male) sex ratio, which did not significantly differ from a 1:1 ratio. Genetic data from that group of turtles suggest that the majority originated from Caribbean nesting beaches, particularly Tortuguero, Costa Rica.⁸⁶ A previous study at Tortuguero predicted an approximate 2:1 (female–male) hatchling sex ratio,⁴⁷ but a more recent study suggested a male-biased (2:3, female–male) hatchling sex ratio from Tortuguero.⁸⁷ Compared to the loggerhead and Kemp's ridley data reviewed above, these represent a relatively small difference between predicted hatchling and immature sex ratios; each study involved only a single nesting season and may not be indicative of all nesting seasons at Tortuguero. Nevertheless, this is another example of possible variation of sex ratios within a population.

Chaloupka and Limpus⁶³ conducted a comprehensive examination of sex ratios within the immature and adult portions of green and loggerhead turtles inhabiting the

waters of the southern region of the Great Barrier Reef from 1985 through 1992. Sex ratios were determined for 954 green turtles and 271 loggerheads using laparoscopy. In greens, the sex ratio of immatures fluctuated over the 8-year study, but was consistently female biased. This was contrary to the unbiased sex ratio recorded for immature greens during an earlier study.⁸⁸ The yearly fluctuations in these immature sex ratios could be due to sampling bias, but could also reflect factors such as year-to-year variability in hatchling sex ratios. The consistent female bias of immature green turtles recorded over the 8-year study probably reflects an overall female bias of hatchlings produced from the southern Great Barrier Reef.⁸⁹ The adult sex ratio of greens from that 8-year study fluctuated significantly and was consistently male-biased, but it was speculated that the male bias could be due to sex-specific migratory patterns of adults, which could cause a sampling bias.⁶³ In contrast to the green turtle data, the sex ratios of both immature and adult loggerheads from that region remained relatively constant over the 8-year study, and both were significantly male biased.⁶³ Similarly, in a study on foraging grounds along the Queensland mainland, immature greens had a female-biased sex ratio and immature loggerheads had a male-biased sex ratio.^{90,91A}

In summary, male-biased, female-biased, and unbiased sex ratios have been reported in the immature portions of sea turtle populations. Data from a long-term study of green sea turtles suggest that immature sex ratios within a population can fluctuate over time.^{63,88} The sex ratio in the immature portion of the population most certainly reflects hatchling sex ratios from previous years, but the exact relationship is not clear because variations between immature and hatchling sex ratios are suggested in some populations. Potential factors such as sex-specific survival rates and sex-specific behavioral differences could enter into the equation. A thorough understanding of sex ratio dynamics within a population may require long-term evaluation of hatchling sex ratios coupled with long-term evaluation within various size classes of immature turtles within a population.

4.3.6 PROBLEMS ENCOUNTERED WHEN ESTIMATING SEX RATIOS IN A SEA TURTLE POPULATION

A variety of hurdles must be surmounted to accurately predict a sex ratio in a sea turtle population. First, a basic need exists to accurately verify or predict the sex of individual turtles. Fortunately, this is not a problem with adult sea turtles because males develop a large muscular tail during puberty (see photo by Wibbels^{91B}). However, one should be cautious when assigning sex to minimum-sized adults on the basis of tail length because it is possible to mistake large immature males for small mature females.⁹² The primary problem associated with accurately predicting adult sex ratios is potential sampling bias due to sex-specific migratory behavior (specific examples were discussed previously). One might be able to decrease or avoid sampling bias by sampling on a foraging ground during a nonmigratory time of year (e.g., several months after nesting season). Regardless, when estimating adult sex ratios, one should attempt to interpret the results relative to what is known about the migratory behavior in a given population.

Evaluation of sex ratios in the immature portion of the population avoids the problem of sex-specific breeding migrations, but a difficulty arises in identifying the

sex of live turtles. A variety of methods have been evaluated for sexing immature sea turtles (reviewed by Ref. 84). Laparoscopy represents a definitive method for verifying sex, but it is difficult in the field and requires surgical training.^{88,93} The most widely used method uses testosterone levels in the blood (determined by radioimmunoassay) to predict the sex of individual turtles (reviewed by Ref. 84). The advantage of this method is that the assay is performed in the laboratory, so the field component is limited to obtaining and storing blood samples. Radioimmunoassays can vary among laboratories, so the accuracy of this method is dependent on the validation of a particular radioimmunoassay using samples from turtles of known sex (e.g., sex identified by laparoscopy). In that way, male and female ranges of testosterone levels can be determined and then used to predict the sex of other turtles. It is optimal to validate the assay with samples from turtles from the population being studied. Thus, for accurate predictions, it is imperative to validate the radioimmunoassay that will be used in a particular study.

4.3.7 PREDICTING HATCHLING SEX RATIOS

4.3.7.1 Direct Methods for Predicting Hatchling Sex Ratios

Several difficulties are associated with estimating hatchling sex ratios. Again, a major problem is identifying the sex of individual turtles because they do not possess external characteristics that can be used to distinguish sex. Basic techniques used to sex hatchlings have been reviewed in detail.⁹⁴ The traditional method has required the dissection of hatchlings and evaluation of the gonads. The most resolute way of doing this is through histological study, in which the gonads show sex-specific characteristics.^{94,95} Female hatchlings have a well-developed cortex (i.e., a thickened cortical layer) and poorly organized medullary portion of the gonad, whereas males have little or no cortex and show organization in the medulla (Figure 4.2). Some researchers have suggested that gross morphology of the gonads can be used to determine the sex of hatchlings.^{13,15,96} Others have suggested that histology is preferred over gross morphology to prevent errors.^{41,97}

Although these methods can provide valuable data, they require either that hatchlings be killed or that hatchlings found dead in nests be used. The availability of an accurate and nonlethal sexing technique for hatchling sea turtles would greatly enhance our ability to investigate sex ratios in sea turtle populations. One method has been proposed for sexing hatchling loggerheads by examining both testosterone and estrogen levels in the blood or chorioallantoic fluid via radioimmunoassay.⁹⁸ That study found significantly higher estrogen–testosterone ratios in female hatchlings, with only a minor overlapping of male and female ranges. However, that method did not prove to be effective for sexing pre- and post-hatchling olive ridley turtles.⁹⁴ Thus, the accurate use of that technique requires further validation.

Ideally, a nonlethal hatchling sexing technique should be accurate and practical for sexing large numbers of hatchlings. Because sea turtles have TSD, there may not be underlying genetic differences in the DNA that can be used to identify phenotypic sex. Therefore, physiological differences may be the best avenue. Furthermore, if physiological differences can be detected in the blood (e.g., sex-specific

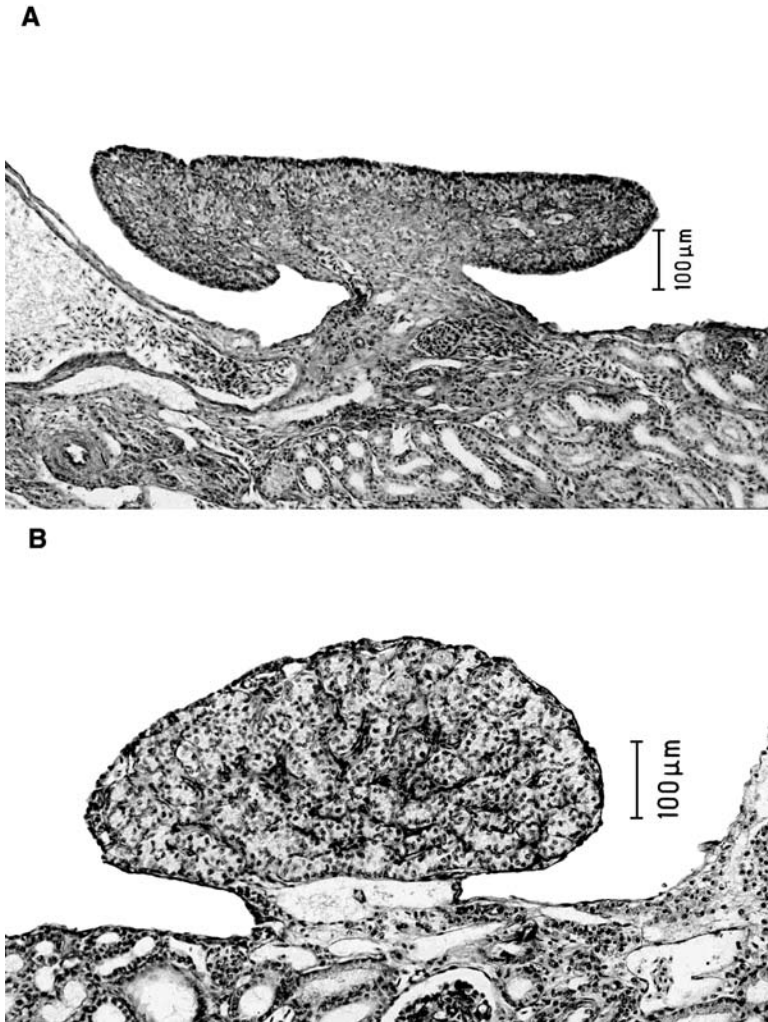


FIGURE 4.2 Hatchling ovary (A) vs. testis (B) in the olive ridley sea turtle. The ovary has a well-developed cortex that stains heavily. The medullary region of the ovary consists of degenerating sex cords. In contrast, in the testis the cortex has degenerated and the medulla has groups of cells (sex cords) that will continue to develop into seminiferous tubules.

hormones), it could prove logistically feasible to sex large numbers of hatchlings, because blood sampling from hatchlings is a relatively simple procedure.⁹⁹ However, for now, histological evaluation of the gonads appears to be the most accurate method for sexing individual hatchlings.

4.3.7.2 Indirect Methods for Predicting Hatchling Sex Ratios

The logistical difficulty of sexing individual hatchlings combined with the conservation dilemma associated with the killing of hatchlings for sex ratio studies has

resulted in a variety of studies adopting indirect methods for predicting hatchling sex ratios. Beach temperatures, nest incubation temperatures, and incubation durations have been used in a variety of studies to predict hatchling sex ratios.^{12,37,48,49,53,54,56,58,59,72,100–102} Previous studies indicate that temperature during the middle third of incubation determines sex in sea turtles.^{44,103} Therefore, if the pivotal temperature and TRT are known for the population being studied, nest incubation temperature or even beach temperature (e.g., at nest depth) can be used to predict hatchling sex ratios. For example, an average incubation temperature during the middle third of incubation that is above the upper limit of the TRT would be predicted to produce all females, whereas an average incubation temperature below the lower limit of the TRT would be predicted to yield 100% males. Average incubation temperatures between the pivotal temperature and the upper limit of the TRT would be predicted to produce a female-biased sex ratio, and average temperatures between the pivotal temperature and the lower limit of the TRT would be predicted to produce a male-biased sex ratio.

The availability of small data loggers for recording temperatures provides a practical means of directly recording incubation temperatures within sea turtle nests.^{48,72} If direct measurement of incubation temperature is not feasible, then incubation duration provides an alternative method for predicting sex ratios for a given nest.^{37,101,102} The total duration of incubation will reflect temperatures during the middle third of incubation, and assuming that temperatures are relatively constant throughout the entire incubation period, incubation duration should be a reliable predictor of sex ratio. In general, longer incubation durations are indicative of male-biased sex ratios, and shorter incubation durations are indicative of female-biased sex ratios.

It is significant that the accuracy of these indirect methods is dependent on validation data that are based on direct sexing techniques. For example, when incubation temperatures are used, the accuracy of the sex ratio predictions is dependent on the validity of the pivotal temperature and TRT data for that population.

4.3.7.3 Predicting an Overall Sex Ratio for a Nesting Beach

One of the primary reasons for developing the sexing techniques discussed above is to provide a means of examining the overall hatchling sex ratios produced from nesting beaches. Once a method has been chosen for estimating sex ratios from nests, you then need to develop an effective strategy for evaluating the sex ratio produced from the nesting beach of interest. This subject has been reviewed previously in detail.²¹ Major points that should be considered include the spatial and temporal diversity of nests during a nesting season. In most situations, it is not possible to monitor temperature in all nests, so subsets of nests that accurately represent the diversity and abundance of nest locations on the nesting beach can be used (e.g., beach flat, dune, and vegetation zone). In addition, nests should be examined throughout the nesting season to evaluate any temporal variation in sex ratio during a nesting season. As indicated previously, seasonal variation in hatchling sex ratios has been detected in a number of studies. Weather parameters such as air temperature, rainfall, and cloud cover should be recorded throughout the nesting season for possible correlation with sex ratios. Studies have also shown annual

variations, which suggest that multiyear data are critical to understanding sex ratio dynamics of sea turtle populations. Multiyear studies of sex ratios from a nesting beach should consistently address spatial and temporal diversity of nests during each of the nesting seasons to make year-to-year comparisons meaningful.

4.3.8 MANIPULATION OF HATCHLING SEX RATIOS

Because sea turtles possess TSD, it is possible to artificially alter hatchling sex ratios. However, there has been considerable debate as to whether sex ratio manipulation is an appropriate conservation strategy considering our limited knowledge of TSD and the effects of sex ratios on reproductive ecology in sea turtles.^{40,41,104,105} Historically, sex ratio manipulations began as an unintentional byproduct of conservation practices for protecting eggs. In some conservation programs, eggs were transferred to polystyrene boxes and incubated in egg hatcheries to prevent predation and poaching, and to enhance hatching success.¹⁰⁶ This practice can skew sex ratios, often by cooling the eggs and thus increasing the production of males.^{24,106} Some conservation programs use egg hatcheries in which eggs are moved to protected locations and buried. This practice has the potential of altering incubation temperatures.¹⁰⁷ As an example, the majority of Kemp's ridley eggs have been moved to egg hatcheries for several decades.^{108,109} Evaluation of hatchling sex ratios from those hatcheries in recent years has indicated a strong female bias in this recovering species.⁷⁶ It is not clear whether this bias reflects the natural sex ratio that would be produced if nests were left *in situ*, but the egg corrals most certainly do not contain the diversity of incubation temperatures that are present on the natural nesting beach. Regardless, it is clear that sex ratios can be manipulated, so the question is whether sex ratios should be intentionally manipulated to enhance the recovery of a population.

On the surface it seems logical that strong male biases would not be advantageous for the recovery of a population, and that the production of female biases could enhance recovery by increasing egg production.⁴⁰ However, several factors should be considered prior to such manipulations. First, it is not clear what proportion of males is needed for successful fertilization of most or all females in a population.^{41,104} Even if a small minority of males could fertilize a large majority of females, the artificial skewing of sex ratios could alter ecological factors such as intra- and intersexual competition, sperm competition, and multiple paternity, which might affect the reproductive ecology and evolution of a species.¹⁰⁴ Thus, intentionally producing a female-biased sex ratio may have both advantages and disadvantages.

At the very least, nesting beach conservation programs should attempt to monitor hatchling sex ratios, especially if nests are translocated to hatcheries or to safer locations on the beach (e.g., above high tide line). If egg hatcheries are used, the resulting sex ratio should be considered when choosing the location of the hatchery.¹⁰⁷ It may be useful to have two hatcheries with different thermal environments that would allow the production of males or females, depending on the hatchery in which eggs are placed.^{40,41} For egg hatcheries, a decision must be made about a desired sex ratio. One option would be to monitor incubation temperatures in natural nests on the nesting beach and attempt to duplicate the natural sex ratio. If the natural sex ratio is not known or cannot be predicted, an alternative is to choose a sex ratio based on its potential for enhancing the recovery of the population (discussed above) or based on sex ratios from other populations. As

reviewed previously, a variety of sex ratios have been reported in sea turtle populations, with many having female biases. It is not clear, however, if those data represent a random sampling of all sea turtle nesting beaches. It is plausible that highly biased sex ratios are more often reported because there is an inherent interest to find and publish extreme sex ratios. As such, this issue is still speculative.

In summary, the manipulation of hatchling sex ratios can most certainly have an effect on the reproductive ecology and recovery of sea turtle populations. Unfortunately, we do not understand all the ecological and evolutionary ramifications of artificially skewing a sex ratio. Although it seems logical that female biases would be preferred over male biases in a conservation program for a recovering population, one should be cautious when manipulating sex ratios until more data are available on this subject.

4.4 PHYSIOLOGY OF TSD

The majority of studies of sex determination in sea turtles have focused on pivotal temperatures, TRTs, and sex ratios. Although some studies have addressed the physiology underlying sex determination in sea turtles, the majority of information regarding the physiology of TSD in turtles has been documented in freshwater turtles, many of which have a similar pattern of sex determination (i.e., MF pattern) and similar pivotal temperatures and TRTs.^{110,111} As such, this section of the chapter will focus on sea turtles, but also include information from freshwater turtles when applicable.

4.4.1 THERMOSENSITIVE PERIOD AND GONADAL DIFFERENTIATION

Studies of loggerhead⁴⁴ and olive ridley sea turtles¹⁰³ indicate that temperature affects sex determination during the approximate middle third of incubation. This is consistent with data reported for freshwater turtles.⁴⁵ Temperature appears to have both a cumulative and a quantitative effect on sex determination.¹¹² That is, an egg must be exposed to a male-producing or a female-producing temperature for an extended time period before sex is determined, so a short-duration spike of male-producing or female-producing temperature will not irreversibly determine sex. For turtles with the MF pattern of sex determination, the warmer the temperature, the more potent it is for producing females, and the cooler the temperature, the more potent it is for producing males.¹¹²

Gonadal differentiation has been described in the olive ridley sea turtle, and the first signs of sexual differentiation occur toward the end of the thermosensitive period.¹⁴ Sexual differentiation at female-producing temperatures includes the thickening of cortical epithelial tissue (i.e., a proliferation of the gonad's cortex) and degeneration of medullary cords (i.e., regression of the gonad's medulla). In contrast, at male-producing temperatures, the cortex does not proliferate, and the medullary cords do not regress and eventually will develop into seminiferous tubules. By the time of hatching, an ovary can be histologically distinguished from a testis (Figure 4.2).

4.4.2 ESTROGEN HYPOTHESIS

The physiological cascade underlying TSD is not well understood, but the leading hypothesis is that female-producing incubation temperatures stimulate the production of estrogen by the gonad, and the estrogen then stimulates the gonad to differentiate into an ovary.¹¹⁰ This hypothesis is supported by a number of findings. Numerous studies of turtles have shown that exogenous estrogen injected into or applied topically to eggs can cause the production of females from eggs incubated at male-producing temperatures.⁴⁵ This includes a study of the olive ridley in which females were produced by treating eggs with estrogen.¹⁰³ Furthermore, treatment of turtle eggs with aromatase inhibitors (aromatase is the enzyme that produces estrogen from androgens) results in the masculinization of gonads.^{113–116} In the European pond turtle, *Emys orbicularis*, higher estrogen levels and higher aromatase enzyme activity occur in the developing ovaries in comparison to the testes.^{117,118} A study in the leatherback sea turtle also revealed higher aromatase enzyme activity in developing ovaries.¹¹⁹ In a study of the diamondback terrapin, *Malaclemys terrapin*, higher levels of aromatase mRNA were recorded at female-producing temperatures, in comparison to male-producing temperatures, during the thermosensitive period of TSD.¹²⁰ Collectively, the findings from these studies support the hypothesis that estrogen production may play a pivotal role in the sex determination cascade of reptiles with TSD.

Although there is strong support for the involvement of estrogen in TSD, the results from studies of several reptiles do not support this hypothesis. Studies of the olive ridley sea turtle;¹²¹ the red-eared slider turtle, *Trachemys scripta*;^{122–124} the saltwater crocodile, *Crocodylus porosus*;^{125,126} and the American alligator, *Alligator mississippiensis*^{127,128} did not detect female-specific elevations in estrogen levels, aromatase levels, and/or aromatase enzyme activity in the gonads during the thermosensitive period of TSD. However, some of these studies did detect elevations after the thermosensitive period. Thus, although the estrogen hypothesis has substantial support, other studies suggest that elevated estrogen levels may be only a downstream event that occurs after sex determination in the ovary.

Alternatively, some findings suggest that the brain (rather than the gonads) may be a source of estrogen production during early development.^{120,121} Consistent with the hypothesis of brain involvement in TSD, a study of the olive ridley indicates that the nervous system innervates the gonads prior to their sexual differentiation.¹²⁹

4.4.3 GENETICS OF TSD

In contrast to birds and mammals, heteromorphic sex chromosomes have not been identified in reptiles with TSD, including the sea turtles.⁸⁴ In fact, it has been hypothesized that the XX/XY sex-determining system in mammals and the ZZ/ZW system in birds have evolved from autosomes of ancestral vertebrates with TSD.¹³⁰ A number of genes have been evaluated as potential factors in the sex determination–gonadal differentiation cascade of TSD in reptiles. The testis-determining gene in mammals (i.e., sex-determining region Y [SRY]) has not been detected in reptiles or birds, and is believed to have evolved well after the mammalian ancestors diverged

from stem amniotic vertebrates.¹³⁰ In fact, SRY has not even been identified in primitive mammals; i.e., monotreme mammals.¹³⁰

SOX9 is an SRY-related gene that appears to have a conserved role in testis differentiation.^{131,132} Studies of the olive ridley indicate that SOX9 is expressed in gonads that are differentiating as testes, but its expression is downregulated in gonads developing as ovaries.^{133–135} Although these findings suggest that SOX9 may be involved in testis differentiation, studies of TSD in the alligator indicate that SOX9 expression is a downstream event and is not the testis-determining gene.¹³⁶

DMRT-1 is another gene that exhibits elevated expression in the differentiating testis of mammals¹³⁷ and birds,¹³⁸ and is required for testis differentiation in mammals.¹³⁹ DMRT-1 could potentially be involved in TSD because it has been shown to be expressed in the testis of the red-eared slider,¹⁴⁰ but it has not been studied in sea turtles.

Anti-müllerian hormone (AMH, also called müllerian inhibiting substance [MIS]) is produced by the differentiating testis and causes the müllerian ducts to degenerate in male vertebrates.¹⁴¹ AMH has been identified in reptiles with TSD,^{136,142} and it is expressed during the thermosensitive period in the alligator.¹³⁶ AMH has not been investigated in sea turtles.

Steroidogenic factor-1 (SF-1) appears to be a master regulator of steroidogenic genes, and it is required for the development of the gonads, the adrenal glands, and the ventromedial hypothalamus.^{143–147} SF-1 also regulates the AMH gene.¹⁴⁸ SF-1 has been shown to have a sex-specific pattern of expression in mammals^{149,150} and birds.¹⁵¹ One of the steroidogenic genes regulated by SF-1 is the aromatase gene, so SF-1 could have a role in TSD if estrogen is involved (see Section 4.4.2). SF-1 has been identified in reptiles with TSD,^{111,152} and has been shown to have a sex-specific expression pattern in the alligator and in a freshwater turtle with TSD.^{152,153} SF-1 has not been examined in sea turtles.

The gene producing DAX1 has been implicated in mammalian sex determination because overexpression of DAX1 is associated with male-to-female sex reversal.^{154,155} DAX1 has been identified in a reptile with TSD (the alligator), but no sex-specific pattern of expression was detected.¹⁵² DAX1 has not been investigated in sea turtles.

The gene producing Wilms tumor 1 (WT1) is necessary for the proper development of the kidneys and gonads in mammals.¹⁵⁶ WT1 has also been hypothesized to act synergistically with SF-1 in regulating AMH, whereas DAX1 antagonizes this synergy.¹⁵⁷ WT1 has been identified in the alligator, but no sex-specific pattern of expression has been detected during TSD.¹⁵² WT1 has not been investigated in sea turtles.

In summary, the genetics of TSD is not well understood, but a number of potential factors in the sex determination cascade have been identified. Although the testis-determining gene in mammals (SRY) does not appear to be present in reptiles, many other genes in the sex determination–sex differentiation cascade are conserved in amniotic vertebrates, and some could potentially be involved in TSD. In addition, as the specific functions of these genes are elucidated, the results will provide insight into the putative involvement of estrogen in TSD. Although only a few studies have addressed the genetics of sex determination in sea turtles, information from other reptiles with TSD can act as a template for designing studies that can efficiently evaluate the potential role of specific genes in the sex determination of sea turtles.

From a conservational viewpoint, some freshwater turtles may represent a more practical subject for TSD studies (in comparison to sea turtles) because the eggs of some species are available commercially in large numbers from captive breeding operations.¹¹¹

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