HATCHLING SEX RATIOS AND NEST TEMPERATURE–SEX RATIO RESPONSE OF THREE SOUTH FLORIDA MARINE TURTLE SPECIES (*CARETTA CARETTA* L., *CHELONIA MYDAS* L., AND *DERMOCHELYS CORIACEA* V.)

by

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ABSTRACT

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South Florida's loggerhead (*Caretta caretta)*, green (*Chelonia mydas)* and leatherback (*Dermochelys coriacea)* sea turtles hatchling have environmentally determined sex. The *in situ* nest mean hatchling sex ratios (SR) were highly female-biased: loggerhead *F*=0.89) and green turtle *F*=0.81; leatherback's SR was nearly balanced (0.55*F*). *N*est temperatures and SRs differed between leatherbacks and loggerhead and green turtles. The latter two did not differ. The loggerhead response parameters were estimated within biological limitations by both 50-65% of incubation and mean middle 1/3 temperature. The maximum middle 1/3 temperature was the best-fit predictor for green turtles. No best-fit sex ratio-temperature response could be identified for leatherbacks. Clutches incubating under natural conditions can vary greatly in SR; TRT differences may account for differences among species' sex ratios.

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CHAPTER 1

HATCHLING SEX RATIOS OF THREE SOUTH FLORIDA MARINE TURTLE SPECIES

INTRODUCTION

Of the U.S. coastal states, Florida is one of the most crucial to marine turtle conservation because of its extensive nesting beach habitat. Three of the world's seven marine turtle species routinely nest on Florida beaches: the loggerhead (*Caretta caretta* L.), green turtle (*Chelonia mydas* L.), and leatherback (*Dermochelys coriacea* V.). Loggerheads overwhelmingly are the most common species nesting in Florida. The Peninsular Florida Recovery Unit (Fig. 1–1) is one of only two very large nesting aggregations in the world that are composed of over 10,000 nesting females per year (National Marine Fisheries Service [NMFS] & U.S. Fish and Wildlife Service [USFWS] 2008). Florida's beaches also host large numbers of nesting green and leatherback turtles each year. Florida's increasing green turtle nesting population is one of the largest in the Caribbean and Western Atlantic, and Florida is the only continental state in which leatherbacks are known to regularly nest (Meylan et al. 1995; Stewart & Johnson 2006; Turtle Expert Working Group 2007). The majority of Florida's marine turtle nests are deposited on the state's east coast in Palm Beach (18%), Martin (17%), and Brevard (39.4%), counties (Fig. 1–1; Meylan et al. 1995) with all three species sharing the beaches during the summer nesting season. Florida's beaches provide the necessary terrestrial habitat for turtles to deposit their eggs; those sands also impact the outcome of the eggs' incubation. Appropriate ranges of temperature, humidity, water potential, salinity and availability of respiratory gases, are necessary for successful embryonic development (Miller 1985; Ackerman 1997). Among these characteristics, nest temperature is of particular importance. Marine turtles lack sex-determining chromosomes and instead exhibit temperature-dependent sex determination (TSD; Mrosovsky & Yntema 1980; Standora & Spotila 1985; Wibbels 2003). Embryos of TSD species are neither sex when eggs are laid; each egg has the potential to become either sex depending on incubation temperatures (Charnier 1966; Bull 1980).

Sex determination in TSD species occurs after fertilization in response to environmental temperatures during a specific portion of development termed the thermosensitive period (TSP; Bull 1980, 1983). The TSP for reptiles occurs during the middle third of development (Bull and Vogt 1981; Yntema and Mrosovsky 1982; Mrosovsky and Pieau 1991). Marine turtle nests that experience cooler temperatures during the TSP incubate longer and have male-biased sex ratios; warmer temperatures produce shorter incubation and female-biased sex ratios (Miller and Limpus 1981; Ackerman 1997). This cool male–warm female pattern is known as TSD type Ia. Other reptiles have cool female–warm male (TSD type Ib systems; e.g., tuataras and some lizard and crocodilian species). Cool female–warm male–warmer female (TSD type II; Ewert et al. 1994, 2004; Viets et al. 1994) is found in some turtles, lizards, and crocodilians (Valenzuela 2004).

Pivotal temperature (PT) and the transitional range of temperatures (TRT) are key concepts that help to describe the consequences of incubation conditions and responses of embryos during sex determination. In general, marine turtles exhibit a TRT with a lower limit of 26.0–28.75^{o}C, below which is predicted to produce100% male, and upper limit of 29.75–32.0^{o}C (depending upon population), above which is predicted to produce 100% female. A relatively narrow range in between, spanning 6^{o}C at most, produces a mixed sex ratio (Wibbels 2003). The PT, the statistical midpoint that produces 50% female: 50% male, tends to be around 29.0–30.0^{o}C. Specific TRTs and PTs vary among species and populations (Wibbels 2003). The TRT for U.S. Southeast Atlantic loggerheads is estimated between 26.5^{o}C –32.0^{o}C in naturally incubated nests (Blair 2005), and the PT is estimated at 29.0^{o}C in laboratory experiments (Mrosovsky 1988). TRT and PT estimations for continental U.S. green and leatherback populations are lacking. Floridian green turtles nest in somewhat similar parts of the beach as those nesting in Tortuguero, Costa Rica. The Tortuguero turtles have an estimated PT of approximately 28.5–30.3^{o}C with temperatures below 28^{o}C producing 90–100% males and above 30.5^{o}C producing 90–100% females (Standora and Spotila 1985). However, the sand at Tortuguero is black while Florida sand is tan. Sand color may influence the thermal conditions in the nests and the developmental responses of these distinct populations. Atlantic leatherback nesting populations in Suriname and French Guiana exhibit a PT of 29.5^{o}C and data from Suriname and Costa Rica give a TRT of 28.75–30.0^{o}C (Wibbels 2003). PT and TRT estimates from Florida and comparable nesting populations are compiled in Table 1–1.

The thermal environment that a marine turtle clutch experiences during incubation is affected by both maternal and environmental factors (Birchard 2004). Rainfall or moisture (Houghton et al. 2007; Leblanc and Wibbels 2009), sand albedo (a measure of reflected light; Hays et al. 2001), geographical beach location, within-beach nest placement (e.g., duneward vs. seaward), time of deposition (Standora & Spotila 1985; Godfrey & Mrosovsky 1999), nest depth and diel solar radiance cycles (reviewed in Birchard 2004) are known sources of variation in the incubation environment.

PT, TRT, and TSP are evolved characteristics of TSD systems that may be due to selection and chance. The evolution of these characteristics likely differs with species, as well as among populations within species. The outcomes may be manifest as maternal effects (Table 1–2) and physiological responses of embryos to incubation temperature (Rhen and Lang 1995). For example, selective pressures acting on clutches laid at the more temperate reaches of a species' range may be different from those acting on clutches incubating at warmer subtropical or tropical latitudes, as evidenced by geographical variation in PTs of some freshwater species, *Chrysemys picta* and *Graptemys pseudogeographica* (Bull 1983). Consequently, hatchling sex ratios for South Florida's loggerhead, green and leatherback turtles may differ as a result of varied selective pressures on PTs and TRTs among species or populations. Sex ratios from different nesting populations, or even at different beaches within the same population, are of limited value to local conservation efforts because nest environment may vary with location. Regional differences can result in very different sex ratios in addition to any potential maternal influences (St. Juliana et al. 2004; reviewed in Birchard 2004).

Deeper nests experience reduced temperature variation due to thermal buffering (insulating effects) compared to shallow nests (reviewed in Birchard 2004). Redfearn (2000) found loggerhead nest depths were significantly shallower when compared to green and leatherback nests on the same beach (Table 1–2). Thus, differences in maternal, spatial, and temporal effects among species may result in marked variation in incubation temperatures, even among nests laid on shared beaches where they have the potential to experience nearly identical environmental conditions.

Estimating sex ratios from PT is challenging in practice (Yntema & Mrosovsky 1982; Georges et al. 1994; Wibbels 2003). Marine turtles' TRTs are relatively narrow and incubation temperatures within natural nests are not constant. Thermal cycles may undergo sufficiently large fluctuation that passes from below to above the PT (and often cycle repeatedly). Developing embryos may experience both male- and female-producing temperatures during the TSP. The use of PT, which lies within the TRT, as a benchmark for sex ratio estimation outside of precisely controlled laboratory conditions is unlikely to lead to realistic results.

Sex ratio estimates are important demographic measures necessary for effective understanding of population trends and conservation management of imperiled species. Sex ratio data allow for identification of shifts in population vigor, aide understanding of mating systems, and establishes the baseline against which the impacts of climate change and other environmental alterations may be compared (Turtle Expert Working Group [TEWG] 2009). In marine turtles, hatchling sex ratios, the primary sex ratio in TSD species, form the baseline from which subsequent sex ratios (by age or stage class) are derived (TEWG 2009). Estimates for Florida's loggerhead hatchlings indicate a female bias (Mrosovsky and Provancha 1989; Mrosovsky and Provancha 1992; Hanson et al. 1998). Blair (2005) reported female-biased loggerhead hatchling ratios of 68% and 75% female, respectively, for samples scaled to production during the 2002 and 2003 nesting seasons from the Southern subpopulation of the Northwest Atlantic. Hatchling sex ratios for Florida's green and leatherback nesting populations are lacking. Thus, there is need for more accurate and up-to-date sex ratio estimates for South Florida's loggerhead, leatherback and green sea turtle hatchlings.

The objectives of this study were to (i) quantify hatchling sex ratios for South Florida's loggerhead, green and leatherback turtles using sex ratios based upon neonates of verified sex from multiple nesting seasons (years) and beaches, and (ii) compare hatchling sex ratios to determine if they differ among species, beaches, and/or years.

METHODS

Study sites and nest selection. — Seven south Florida beaches were sampled for this study: Melbourne Beach, Hutchinson Island, Juno-Jupiter, Boca Raton and Miami Beach on the southeast coast and Sanibel Island and Sarasota on the southwest coast (Fig. 1–1). All sites are located on barrier islands and were chosen based on their relatively high marine turtle nesting densities and available historic nesting data. Gently sloped beaches of open sand backed by vegetated fore-dunes and/or developed properties characterize the sites.

South Florida conditions are hot and wet during May-September (Winsberg 2012) when the majority of incubating embryos are in their TSP. Mean air temperatures are 27.2-28.3^{o}C during the hottest month (usually July); maximum temperatures reach greater than 31.1^{o}C by May on Florida's west coast and June on the east coast. The rainy season typically starts in April and lasts through November or later. In peninsular Florida, the combined nesting season (loggerhead, green and leatherback turtles) runs

March to September (NMFS & USFWS 2008; Florida Fish and Wildlife Conservation Commission [FWC] & Fish and Wildlife Research Institute [FWRI] 2010).

Study nests were selected based on local expectation that each was in a position of minimal risk of loss to tidal inundation, malicious mischief, and predation across the incubation period. The nesting season was partitioned into three subseasons (early, middle, and late) centered on the 1st, 2nd, and 3rd quartiles of historic nest and emergence distributions. Up to four nests/species/beach/subseason were selected for study. Live hatchlings from the first major emergence were collected for rearing until sex identification was completed (Wyneken et al. 2007).

A subset of nests sampled were equipped with temperature data loggers placed in the middle of each clutch that recorded the thermal environment throughout incubation for a related nest temperature study (Rogers 2013).

Sampling and animal rearing. — Study nests were quasi-randomly selected (nests at high risk of washing out were not included in the study). Hatchlings were randomly sampled for approximately 10% of live, normally developed hatchlings. Nest sex ratios were based upon 5 hatchlings/leatherback nest or10 hatchlings/loggerhead or green turtle nest, collected from each nest's first major hatchling emergence, except where fewer emerged. Hatchlings with obvious deformities such as cleft palate, severely deformed shells or missing limbs and those having poor candidacy for captive rearing, such as hatchlings that emerge with skin lesions, were excluded from consideration. All hatchlings were transported under climate controlled conditions to the Florida Atlantic

University Marine Laboratory at the Gumbo Limbo Environmental Complex, Boca Raton, Florida, USA for rearing.

Hatchlings were reared until yolk was absorbed and sex could be identified. Husbandry is detailed by Stokes et al. (2006) and Wyneken et al. (2007). Loggerhead and green hatchlings were housed in individual containers in a flow-through natural seawater system at 26±2^{o}C. Leatherback hatchlings were tethered and maintained singly or in tanks that prevent individuals from interacting with each other or the tank walls (Jones et al. 2000). Leatherback tanks were supplied with water via a closed system at 23±2^{o}C that was partially changed daily and fully changed weekly. All turtles were fed daily using species-specific in-house manufactured diets modified from Stokes et al.(2006) and Jones et al.(2000).

Sex identification. — Neonate sex was determined by visual inspection of a suite of gonadal and accessory duct characteristics via laparoscopic examination once they reached the required 120g minimum size (Wyneken et al. 2007). Gonad size, shape, and attachment to the coelomic wall and paramesonephric duct size, mobility and lumen presence or absence together have been identified as reliable sex-determining characteristics in live loggerhead neonates (Wyneken et al. 2007). Sex of any hatchlings that died during laboratory rearing was determined upon visual inspection of the gonads and ducts during necropsy followed by histological verification (Ceriani & Wyneken 2008; Ceriani & Wyneken, unpublished). After the surgical incisions healed and normal feeding and activity returned, neonates were released into the Gulf Stream Current.

Statistical analyses and terminology. — Sex ratios are reported as proportion female (SE), n = number of nests sampled, given parenthetically. For convenience, we report these sex ratios as hatchling sex ratios, even though the turtles are several weeks old when sex is actually recorded. Sex ratios were calculated for all nests sampled so that sex ratios for each beach and year are the grand mean (SE). Analyses were completed using R software (R Development Core Team 2012). Group comparisons were analyzed using the Kruskall-Wallis test with a 0.05 significance threshold. Where significant differences were found, Tukey-like nonparametric multiple comparisons with probit transformation were used to identify the sources of differences (nparcomp R package, Konietschke 2011).

RESULTS

Loggerhead turtles. — Loggerhead samples were obtained for the years 2002–2004 and 2007–2011 (Table 1–3). Boca Raton was consistently sampled each year that loggerheads were included in the study, with the exception of 2004 (Table 1–4) when many sample nests were lost to storms.

Florida’s loggerhead hatchling sex ratios, across all beaches and years, are highly female-biased. The mean (SE) proportion female of all loggerhead samples is 0.89 (0.01, n=185; Fig. 1–2). Yearly variation is significant and ranges from 0.80 to 1.0, with the minimum in 2002 and the maximum in 2007 (Fig. 1–3 and Table 1–3). The sex ratio for the year 2002 was significantly different from 2004, 2007, and 2010. The year 2003 was significantly different from 2007, 2010, and 2011 (Table 1–5).

Loggerhead sex ratios also differ by beach (Table 1–5 and Fig. 1–4); however, the Tukey-like comparisons analysis was not powerful enough to identify which beach(es) explained the difference. Sarasota and Sanibel Island beaches produced the lower proportions female (SE) giving 0.82(0.05, n=20) and 0.82(0.04, n=28), respectively. While Boca Raton produced the highest proportion female (SE) 0.95 (0.01, n=57).

Green turtles. — Green turtle hatchlings were sampled during the 2004–2011 seasons (Table 1–3). Boca Raton was consistently sampled each year. Melbourne Beach was sampled in 2006 and Juno Beach in 2007 (Table 1–4).

The mean (SE) sample sex ratio for Floridian green turtle hatchlings across all beaches and years is 0.81 (0.05, n=53; Fig.1–2). Most years the sex ratio was female-biased; years vary in the extent of the female bias (range 0.0–1.0, Table 1–3 and Fig. 1–3). The 2006 and 2009 samples were 100% female while no females were present in the 2004 samples. The 2004 sample sex ratios differed significantly from all other years; those from 2008 differed from 2006, 2007, and 2009 (Table 1–5).

Green turtle hatchling mean (SE) sex ratios, analyzed by beach, range from 0.79 (0.05, n=48) in Boca Raton to 1.0 in Melbourne Beach and Juno Beach (Table 1–4 and Fig. 1–4). However, there are no significant differences among beaches.

Leatherback turtles. — Leatherback hatchlings were sampled in the 2006–2010 nesting seasons (Table 1–3). Juno Beach was sampled in 2006-2008. Boca Raton was sampled in 2006, 2007, 2009, and 2010 (Table 1–4).

The mean (SE) sample sex ratio for leatherback hatchlings across beaches and years is 0.55 (0.10, n=15; Fig. 1–2), but years vary significantly in proportion female (range 0.0–1.0, Fig. 1–3). No females were found in 2007 (n=3); no males were found in 2006 (n=1) or 2008 (n=2). All other years' samples included both sexes. Consequently, 2007 differed from all years sampled. The 2006 sex ratio differed from 2010 (Table 1–5).

Beaches did not differ significantly from one another in sex ratios (Table 1–4 and Fig. 1–4). The Juno Beach samples produced a one-to-one sex ratio (mean (SE) = 0.50 (0.21), n=6). The overall mean proportion female in Boca Raton is 0.58 (0.09, n=9), roughly similar to Juno Beach, but varying more across years, spanning 0.33 (0.0, n=2) in 2010 to 0.70 (0.30, n=2) in 2009.

Overall and concurrent incubation comparative results. — Comparisons among species' mean proportion female across all years and beaches together differed significantly (Fig. 1–2). Tukey-like comparisons did not find differences between leatherback and green turtle or loggerhead and green turtle overall mean sample sex ratios. However, the leatherback hatchling mean sex ratios differed from that of the loggerheads. The peninsular Florida mean loggerhead sex ratio is highly female-biased while the mean leatherback sex ratio is nearly balanced (1:1).

Loggerhead, green and leatherback hatchlings were concurrently sampled in Boca Raton during the 2009 and 2010 nesting seasons (Table 1–4). Analysis of nest sex ratios in this smaller subset of concurrently sampled nests also identified a significant difference among species. Trends in these two years were consistent with those found when all species were compared using all years such that leatherback hatchling ratios differed from loggerhead ratios, and green turtle sex ratios did not differ from those of leatherbacks or loggerheads. The loggerhead and green turtle sex ratios (SE) for 2009 and 2010 were highly female-biased at 0.98 (0.01) and 0.87 (0.07), respectively. The leatherback sex ratio for these years was 0.52 (0.16).

Temporal overlap (Fig. 1–5) of TSPs occurred between 2 loggerhead and 2 green turtle nests in 2009, and 1 loggerhead and 2 leatherback nests and 4 loggerhead and 6 green nests in 2010. I found no differences in sex ratio between species by Kruskal-Wallace test in any of these 3 paired comparisons.

DISCUSSION

Systematic documentation of hatchling sex ratios over time helps to establish baselines, facilitate comparisons and identify reproductive trends that are specific to Florida's marine turtles. Such data can give clarity and insight to marine turtle conservation efforts and our understanding of probable impacts of varying climates on TSD species. Here I provide relatively long-term compilation of loggerhead hatchling sex ratios from multiple beaches in South Florida, and the first report for Florida's green and leatherback hatchling sex ratios. I show a female bias in most cases, yet considerable variation among years and species.

The sex ratio samples in this study are all verified and hence add a level of certainly not available in most other studies. Nests included in this study were selected across species' subseasons (early, middle and late) and beach zones (low–middle, middle and high; low nests were not selected because of their increased risk of regular tidal inundation) whenever possible. These selection practices were specifically employed to ensure the thermal effects of temporal and spatial variation were reflected in the samples.

These nests were also the most likely to survive and contribute hatchlings to each year's cohort. Thus, the sampling methods utilized in this study provide a comprehensive (exceptions noted in discussion below) and verified sample sex ratio by species and across several beaches in Florida. This study is restricted to comparing systematically sampled sex ratio and is not extrapolated to production by the various beaches.

Florida's loggerhead hatchling sex ratios are highly female-biased. This result is consistent with previous Florida studies. Mrosovsky and Provancha (1989, 1992) estimated 87.0-99.9% female hatchlings at Cape Canaveral in the 1986-1988 nesting seasons. Hanson et al. (1998) predicted 100% female hatchlings from 37 of 40 Hutchinson Island nests. And Blair's (2005) study of the Western Atlantic southern subpopulation loggerheads reports 68–75% females for the 2002 and 2003 seasons. The Hanson et al. study estimates sex ratios from nest sand temperatures and sampled only June and July of one nesting season on one beach. Mrosovsky and Provancha's data cover three seasons, were histologically verified and are generally representative of spatial and temporal variation. However, like Hanson et al., the data are from a single beach and the 1988 mid-season sex ratios (July–August) are estimates based on sand temperatures. The loggerhead data used in this study were collected from seven nesting beaches during eight seasons. My study established that year-to-year variation in Florida loggerhead hatchling sex ratios is significant; however, the variation among samples is relatively small and the data maintained a consistent female bias.

Floridian green Turtle nests contrast with the loggerhead range in mean proportion female by year; they spanned 0.0–1.0 proportion female. Green Turtle nests tend to produce a female bias, but show significant yearly variation that is greater than that found in loggerheads (Table 1–3 and Fig. 1–3). In the single, all-male green turtle nest, TSP temperatures ranged 29.0–30.5°C. Another nearly all-male nest (0.1 proportion female) ranged 27.4–32.0°C. Green turtle all-female TSP nest temperatures ranged 29.9–35.3°C. The TSP temperatures in the loggerhead nest with the greatest number of males (0.2 proportion female) ranged 26.7–30.7°C. Loggerhead all-female TSP nest temperatures ranged 26.0–35.6°C. In green and loggerhead samples, individual nests that produced 100% male or 100% female hatchlings overlap in TSP temperatures and width of TSP temperature ranges. No clear temperature range delineates between all-male and all-female producing nests.

Leatherback hatchling ratios diverged from those of the two hard-shelled species, having a nearly balanced sex ratio (mean (SE)=0.55 (0.10)). Yet, significant yearly variation occurs and the proportion female spans 0.0–1.0. Leatherback nests that produced all male hatchlings were not among those sampled for temperature data. The highest male-producing nest with available temperature data had a 0.33 proportion female; its TSP temperatures ranged 30.3–32.2°C. TSP temperatures in all-female nests ranged 26.5–30.2°C. Similar to the loggerhead and green samples, no clear pattern could be determined regarding the ranges of temperatures producing exclusively male or female nests.

Metabolic heating and thermal inertia. — In TSD species, metabolic heating has the potential to influence sex ratio outcomes. Embryonic metabolic activity increases as development progresses, subsequently increasing the incubation temperature within the nest (Carr & Hirth 1961; Mrosovsky & Yntema 1980; Morreale et al. 1982; Godfrey et al. 1997). If the TSP corresponds with the onset of metabolic heating, the sex ratio of the nest may be affected.

Mrosovsky and Yntema (1980) define the onset of metabolic heating as the temperature of the egg mass increasing more than 1°C higher than the surrounding sand at similar depth. It is unclear if metabolic heating during the TSP is sufficient to alter sex ratios. Several studies on loggerheads (Mrosovsky & Yntema 1980; Maxwell 1988; Milton et al. 1997; Zbinden et al. 2006) report metabolic heating primarily occurs after the TSP and is unlikely to affect sex ratios. In contrast, Broderick et al. (2001) found a marked increase of 0.07–2.61°C in green turtle nests from 30-40% through incubation, coinciding with the beginning of the TSP. Godfrey et al. (1997) directly investigated the onset of metabolic heating in Suriname leatherbacks by staging embryos developing adjacent to temperature recorders. They found a 0.82 (0.09)°C increase in temperature during the TSP and no significant difference between the middle and sides of the sample nests. This increase is enough to feminize embryos if sand temperatures are within, or just below, the TRT. However, their sample size is limited.

Whether metabolic heating during the TSP can alter sex ratios may be dependent on overall clutch mass. The amount of metabolic heating within a nest depends on its thermal inertia, i.e. its clutch and egg size. Nests that have a large overall mass experience greater temperature increases due to collective metabolic activity than those with smaller mass (Mrosovsky & Yntema 1980; Zbinden et al. 2006). Heat in large clutches accumulates slowly but also dissipates slowly. Large clutches might build enough heat by onset of the TSP that nest temperatures sufficiently rise above sand temperatures to affect sex ratios, while metabolic heating in smaller clutches under the same conditions remain at or near ambient sand temperatures.

Loggerhead and green turtle egg and clutch sizes are generally comparable to each other (Table 1–2). Leatherback eggs are relatively large compared to loggerhead and green turtle eggs. Although, leatherbacks lay fewer eggs (Table 1–2), and they also lay varying numbers of small, yolkless eggs called spacers. Based on mean egg and clutch sizes (Table 1–2), loggerhead clutch mass is small (2030–5330g), leatherback clutch mass is generally large (4644–6416g, not including added mass from spacers), and green turtle clutch mass can range from relatively small to very large (3546–7229g). Despite the overlap, differences in clutch mass trends among species may be responsible for the contrasting results on metabolic heating during the TSP in the literature.

Temporal and spatial differences. — Differences in sex ratio among species nesting within the same or on similar beaches, as seen here in Florida's loggerhead, green and leatherback turtles, may be attributed to temporal and spatial differences in maternal behavior (nest site selection, phenology, nest depth, etc.) driving evolution of the TRT.

Significant temporal variation in sex ratio response was found across years in all species. In loggerhead samples 2002 was different from 2004, 2007, and 2010. And 2003 was significantly different from 2007, 2010, and 2011. In green turtles the 2004 sample sex ratios differed from all other years, and 2008 differed from 2006, 2007, and 2009. The 2007 leatherback sample differed from all years sampled and 2006 differed from 2010. These differences among years are likely related to variations in yearly seasonal weather patterns (Southeast Regional Climate Center 2007). The 2002 nesting season was warmer than average with average precipitation, but June experienced higher than average precipitation. Air temperatures in 2004 were average; however, it was a prolific year for tropical weather. Four named storms (Charley, Frances, Ivan, and Jeanne) crossed over peninsular Florida during the end of the season, resulting in higher than average precipitation and few late subseason nest samples due to losses from the storms. The 2006 and 2007 seasons were characterized by average temperatures and below average precipitation. Differences seen in the leatherback samples during these years may be due to within-season sample bias. Four of the six 2006 sample nests were laid during the leatherbacks' mid subseason while the 2007 samples consisted of 2 early and one mid subseason nest. Lastly, 2010 overall seasonal air temperatures were average with low precipitation, but the summer months (June through August) were hotter than normal (NOAA National Weather Service Weather Forecast Office 2010). A similar trend was seen in 2011 weather, with slightly above average temperatures, below average precipitation and hot summer months.

Temporal differences (Fig. 1–5) within the season can also affect nest incubation temperatures and the resultant sex ratio. Leatherbacks start off the South Florida nesting season as early as March and continue laying through July (mean first to last lay dates 22 April–17 June; FWC & FWRI 2010; B. Brost, personal communication 6 November, 2012). Loggerheads overlap with leatherbacks, beginning in May and continue through September (mean first to last lay dates 18 May–11 August; NMFS & USFWS 2008; B. Brost, personal communication, 6 November, 2012). Green turtle nesting begins in July, overlaps with mid–late loggerhead and late leatherback nests, and finishes in late September (mean first and last lay dates 21 June–23 August; FWC & FWRI 2010; B.

Brost, personal communication, 6 November, 2012). Temperature trends over the entire season progressively warm and then start to cool slightly. As a result, mixed sex clutches are more likely to occur in the earlier and later portions of the season, when temperatures are milder. Leatherback and green incubation more frequently extends into these milder periods than loggerhead incubation. My data reflect this in the greater proportion of males found within leatherback and green samples. These species start and end the Florida nesting season, respectively. During the middle portion of the overall season where TSPs among species can overlap (Fig. 1–5), my data showed no differences in hatchling sex ratios. The middle months are the hottest portion of the season when sand temperatures are typically above all three species' reported PTs (Table 1–1).

Spatial differences in nest placement across beach zones and depth within the sand add another layer of complexity that affects incubation temperature profiles. Loggerhead within beach nest placement on southeast Florida beaches appears random (personal observation). However, loggerhead nests on Southwest Florida beaches are located at or near the dune vegetation (Hays et al. 1995; Garmestani et al. 2000). Placement of green and leatherback nests on the Florida east coast beaches was generally consistent with the literature. Green turtles scatter their nests, but on shared beaches they tend to place them higher than leatherback nests (Bjorndal & Bolten 1992). Leatherback nests are scattered across beach zones but are typically laid in open sand (Kamel & Mrosovsky 2004).

Loggerheads have relatively shallow nests (49–69cm; L. Fisher, personal communication November 20, 2012; Redfearn 2000; Tiwari & Bjorndal 2000) compared to green and leatherback turtles. Green turtle nests are moderately deep, ranging from

69-96cm (L. Fisher, personal communication 20 November, 2012; Redfearn 2000), and leatherbacks nests are the deepest of the three species. Leatherback nest depths range from 70–105cm (L. Fisher, personal communication 20 November, 2012; Redfearn 2000; Billes & Fretey 2001). Deeper nests experience less temperature variation compared to more shallow nests because thermal buffering increases with depth (Hanson et al. 1998; reviewed in Birchard 2004). Fisher and Redfearn's data sets are from the same nesting assemblages as nests used in my study. My data show that the deeper nests of green and leatherback turtles, which also tend to be higher on the beach, produced a higher proportion of male hatchlings.

Male production within the loggerhead samples was slightly greater at Melbourne Beach, the northern-most study site, and at the west coast beaches. The shallower nest depth and generally less specific placement of loggerhead nests likely allow greater influence of climatic conditions on incubation temperature at these locations. Melbourne Beach area's average air temperatures and precipitation are slightly lower than at more southerly east coast study sites. Similarly, the west coast sites experience somewhat lower than average air temperatures compared to other sites and average precipitation (1981–2011 averages; NOAA National Weather Service Weather Forecast Office 2006).

Differences in beach morphology may also have influenced higher male production in Melbourne Beach and west coast beaches. Melbourne Beach is narrow, with a tall berm compared to the wider, more gently sloped beaches such as Juno Beach and Boca Raton. Because of Melbourne Beach's narrow width, turtles that climb the tall berm necessarily nest near or in the dune vegetation where shading is more likely to occur. Nests laid below the berm are necessarily near the tide line. Below the berm nests that do not wash out likely also experience somewhat lower temperatures due to higher moisture content in the sand. On the west coast, Sanibel Island and Sarasota's natural beaches are also relatively narrow. Like Melbourne Beach, nests laid on these beaches are more likely to be exposed to lower, male-producing temperatures than wider east coast beaches.

The spatial and temporal differences in nesting among species (Table 1–2) are likely to be the result of multiple selection pressures. Marked variation in incubation temperatures clearly occurs, even among nests laid on shared beaches where they have the potential to experience otherwise nearly identical environmental conditions. The species-specific TRTs likely evolved separately and so direct differences in hatchling sex ratios among Florida's loggerhead, green and leatherback turtles. The loggerhead TRT is the widest of the three species with up to a 10.3^{o}C range (Blair 2005; Rogers 2013), followed by the green TRT with a range up to 7.7^{o}C wide (Standora & Spotila 1985; Rogers 2013) and the leatherback with the narrowest TRT range of only a 1.25^{o}C span (Wibbels 2003). A wide TRT can both allow for a broad sex ratio resiliency at a wide range of latitudes and produce males and females even in shallow nests that can experience greater temperature fluctuations. In shallow loggerhead nests, the highly female-biased sex ratios are not surprising; their eggs are insulated by less overlying sand and most subjected to the effects of the warming sun in comparison to the deeper nests of the other species that are more insulted. The few males I found in the loggerhead sample indicate that males are possible at high temperatures even though females are more common. Assuming the sigmoid sex ratio response curve (Bull 1980; Mrosovsky & Yntema 1980) reflects the responses of embryos in natural nests, then a wide TRT, along with some individual variation in response, may produce such results. Fully male nest samples are extremely rare because the lower boundary of the TRT is lower than typical Florida sand temperatures during incubation.

A narrow TRT in nests with large temperature fluctuation is likely to be selected against due to the potential for skewing ratios during periods of more extreme climatic conditions that could last for many years. Under conditions in which the incubation temperature varies little, narrower TRT are essential if both sexes are to occur. Narrow TRT give greater potential for minimal fluctuations to result in all males or all females, and some nests that produce both. The sex ratios reported here provide supporting evidence for differing selective pressures on the TRT of each species.

The overall female bias in green turtle hatchlings is great but individual nest sex ratios vary over the possible range. This variation is likely due to their narrower TRT, which can increase chances of an all one-sex response within any given nest. The female bias is likely maintained because the majority of green turtle nesting occurs during the hottest months of the year, and their nests are shallow enough that unshaded nests experience mostly all female-producing temperatures.

South Florida leatherbacks do not show a hatchling sample bias toward either sex. Their even narrower TRT can predispose clutches to develop as 100% of one sex with slight deviations of nest temperatures. However, at leatherback nest depth, the minimal temperature fluctuation allows two sex clutches to occur, particularly during seasons with historically average weather patterns (e.g., Winsberg 2012).

Climate effects. — The 2010 and 2011 nesting seasons experienced atypical weather patterns. The summer of 2010 was Southeast Florida's hottest on record with average temperatures 1.1–1.4^{o}C above normal and the second hottest on Florida's west coast at similar latitudes (NOAA National Weather Service Weather Forecast Office 2010). In 2011, South Florida's Spring and Summer were both warmer than normal (NOAA National Weather Service Weather Forecast Office 2012). Both years resulted in 100% female loggerheads from all east coast (Boca Raton) nests that were sampled. On the west coast, Sanibel Island samples produced the only male loggerheads each year. Boca Raton's green turtle sex ratios were female-biased in these warmer years. The somewhat lower female bias found in 2011 may be attributed to nest location. Several were in areas known to be shaded for the majority of the day. In spite of the hot season, the 2010 mean leatherback ratio was male biased. This result likely is due to the earlier nesting period and the depth of the nests. Leatherbacks were not sampled during the 2011 nesting season.

Based on historical records, the 2010 and 2011 nesting seasons were warmer than normal but such occurrences may become common place or more extreme if climate change increases. The effects of predicted rapid climate change (Intergovernmental Panel on Climate Change 2007) could greatly impact the primary sex ratios of TSD species. The lack of male loggerheads in the record hot seasons identifies that loggerheads, with their shallower nests, may be at higher risk of decline due to fewer males hatching than their deeper-nesting counterparts. However, the loggerhead's wider TRT may imply greater adaptability; in other turtle species with TSD Ia, wider TRTs are positively correlated with the proportion of mixed sex nests (Hulin et al. 2009). South

Florida's already highly female-biased hatchling sex ratios would need a right-shifted TRT to continue to produce males under rapidly warming conditions. Hawkes et al. (2007) modeled predictions for climate change effects on loggerhead hatchling sex ratios in Cape Canaveral, located along Florida's central East coast. They estimate total feminization would occur with a 2^{o}C warming of air temperatures. Average temperatures for 2010's record hot summer that produced all 100% female loggerhead samples were only 1.1–1.4^{o}C above normal (NOAA National Weather Service Weather Forecast Office 2010).

Nesting phenology harbors potential for adaptation to warming climate. Under a warmer climate regime, a shift toward earlier onset of the nesting season may allow for continued production of male hatchlings in South Florida. It could also provide protection from potentially lethal sand temperatures later in the season. Evidence of a shift toward earlier nesting has already been documented in Florida's loggerheads. Weishampel et al. (2004) found the median date of loggerhead nesting between Sebastian Inlet and the southern border of Patrick Air Force Base moved ahead 10 days between 1989 and 2003 in response to warming sea surface temperatures during the month of May. Pike et al. (2006) also found loggerhead nesting at Canaveral National Seashore moved ahead 9 days during the same time period. Weishampel et al. (2010) found that both loggerhead and green turtle nesting shifted approximately 4.5 days earlier per 1^{o}C rise in May sea surface temperature. However, shifting nesting phenology still may not be able to counteract the effects of climate change. The freshwater turtle *Chrysemys picta* shows plasticity in nesting date in response to winter temperatures, but the plasticity is not repeatable within individuals, suggesting that the timing of nesting onset is not a trait on which selection can act (Schwanz & Janzen 2008) within short time frames. Visser (2008) argues that phenotypic plasticity in temperature response will not be sufficient; reaction norms will no longer be adaptive due to climate change disrupting correlations between temporally spaced environmental variables. For example, a large shift to earlier nesting of Florida sea turtles could mean nests begin incubating during the end of the dry winter months. Because nest moisture level is a known influence on incubation temperature (Houghton et al. 2007; LeBlanc & Wibbels 2009), drier sands could allow incubation temperatures to rise faster than normal for these early season nests. Such a scenario could shift Florida leatherback hatchling sex ratios away from their effective equilibrium.

Summary. — The need for long-term hatchling sex ratio baselines assessing the effects of climate change is widely recognized (e.g., Hawkes et al. 2009; Witt et al. 2010). The initial baselines provided here support the value of establishing systematic sex ratio sampling to identify a normal range of sex ratios that are sampled, with even this limited sampling scheme, across the season. However, as robust as this comparative sampling is, there are limitations. This study compares sample sex ratios across years and does not scale the sample sex ratios by hatchling production. Extrapolation to population level impacts of sex ratio requires those hatchling production measures. Secondly, leatherback hatchling sex ratio sample sizes are low. Their nests have relatively small clutch sizes (Tucker & Frazer 1991; Stewart & Johnson 2006) and low emergence success (Spotila et al. 1996; Bell 2004; Perrault et al. 2011), resulting in a lower number of sampled hatchlings.

In summary, I report relatively long-term trends in marine turtle hatchling sex ratios sampled across the nesting season and for multiple beaches in South Florida. Additionally, this study is the first to report green and leatherback hatchling sex ratios in Florida. Loggerhead and green turtle hatchling sample sex ratios are highly female-biased while leatherback hatchling sex ratios are effectively 1:1. The sample sex ratios are varied among years, beaches, and within individual beaches at the nest level. The northern-most beaches produce slightly more males in the loggerhead samples. Sex ratio variation is more pronounced in green and leatherback turtles and we propose it is likely due to the narrower TRTs when compared to the TRT of loggerheads. Leatherback turtles show potential for the widest variation in sex ratios under current environmental conditions, but more samples are needed. Our data provide a baseline against which South Florida's future primary sex ratios may be compared using the same sampling regimes and they suggest the loggerhead may be at higher risk to increasing temperatures than green and leatherback populations due to their shallow nests and wide TRT.

CHAPTER 1 TABLES

TSP temperature range °C (SE)	Parameter Estimate °C	Location	Study type	Source
		Caretta caretta		
25.6–35.6	TRT_L Und. TRT_U 33.3[a] PT 28.9[a] TRT_L Und TRT_U 30.4–31.1 [b] PT 27.6–29.1 [b]	South Florida	Field	Rogers 2013
N.R.	TRT 26.5–32.0 [b]	North Carolina–Florida	Field	Blair 2005
27.5(0.5)–30.0(1.0)	TRT <27.5–30.5 [c] PT 29.2 [c]	North Carolina–Florida	Lab	Mrosovsky 1988
26.0–34.0(1.0)	PT 30.0 [c]	Georgia	Lab	Yntema & Mrosovsky 1982
		Chelonia mydas		
27.4–35.3	TRT_L 29.7–30.8 [d] TRT_U 33.7–35.4 [d] PT 32.5–32.6 [d] TRT_L 29.1-30.1[e] TRT_U 32.3–32.8[e] PT 30.8–31.8 [e]	South Florida	Field	Rogers 2013
25.4–36.5	TRT <28–≥30.5 [b]	Atlantic Costa Rica	Hatchery & Field	Morreale et al. 1982 Morreale 1983
28.8–34.1	PT < 29.2 [b]	Northern Cyprus	Field	Broderick et al. 2000
		Dermochelys coriacea		
26.6–32.4	TRT Und. PT 30.9–31.4 [b]	South Florida	Field	Rogers 2013
28.0–35.1(0.2)	TRT 29.0–30.0 [c] PT 29.4 [c]	Pacific Costa Rica	Lab	Binckley et al. 1998
27.0(0.2)–32.0(1.8)	TRT 28.75–29.75 [c]	Suriname & French Guiana	Lab	Rimblot et al. 1985
29.250.5–29.75(0.2)	PT 29.5 [c]	French Guiana	Lab	Rimblot-Baly et al. 1987

Table 1–1. PT and TRT values for loggerhead (*C. caretta*), green (*C. mydas*) and leatherback (*D. coriacea*) turtles in Florida and comparable nesting populations. TRT_L=lower bound and TRT_U=upper TRT bound. N.R.=not reported.

a. Estimated from 50-65% TSP maximum temperature models. b. Estimated from middle third TSP mean temperature models. c. Calculated from constant incubation temperatures. d. Estimated from middle third and 50-65% TSP maximum temperature models. e. Estimated from middle third and 50-65% TSP mean temperature models.

Characteristic	*Caretta caretta*	*Chelonia mydas*	*Dermochelys coriacea*
Nesting season with mean first & last lay dates	April-September [1] May 18-August 11 [3]	June-late September [2] June 21-August 23 [3]	March-July [2, cited in 4] April 22-June 17 [3]
Nest placement	Within or near supra-littoral vegetation [5,6]	Scattered among zones; higher than leatherbacks on shared beaches [7]	Scattered in open sand [8]
Mean nest depth (cm)	44.7-53.7 [9] 53-69 [10] 44-60 [11]	69-96 [10] 57-83 [11]	75-105 [10] 75-91 [11] 70 [12]
Mean annual estimated clutch frequency	4-7 [2] 3.49 [13] 2.81-4.18 [14]	3-5 [2] 2.93 [13]	6.17 [12] 5.8-7.5 [14]
Mean # eggs/clutch	112.4 [13] 114.4-127.54 [14] 100-126 [16]	115 [2] 88-136.4 [17]	54.74-91.26 [4] 64.5-73.6 [15]
Mean Incubation duration and range in days	60 [2] 53-68 [18]	54-88 [18] 43-60 [19] 62 [20]	55-75 [18]
Egg diameter (mm)	37.61-42.53 [9] 37.5-42.2 [13]	40.0-54.6 [13] 39.1-48.4 [17]	51-53.8 [13]
Egg mass (g)	20.3-41.8 [13]	40.3-53.0 [13]	72-84 g [13] 73.9-87.9 [21]

Table 1–2. Comparison of nesting and incubation characteristics of loggerhead, green and leatherback turtles.

1. NMFS & USFWS 2008. 2. FWC & FWRI 2010. 3. B. Brost, personal communication, 6 November 2012. 4. Stewart & Johnson 2006. 5. Garmestani et al. 2000. 6. Hays et al. 1995. 7. Bjorndal & Bolten 1992. 8. Kamel & Mrosovsky 2004. 9. Tiwari & Bjorndal 2000. 10. Redfearn 2000. 11. L. Fisher, personal communication 20 November, 2012. 12. Billes & Fretey 2001. 13. Van Buskirk & Crowder 1994. 14. Frazer & Richardson 1985. 15. Tucker & Frazer 1991. 16. Dodd 1988. 17. Bjorndal & Carr 1989. 18. Coles 2003. 19. Broderick et al. 2000. 20. Fowler 1979. 21. Wallace et al. 2007.

Year	*Caretta caretta*	*Chelonia mydas*	*Dermochelys coriacea*
	Mean proportion female (SE) # of nests, # of hatchlings		
Overall	0.89 (0.01) 185, 1511	0.81 (0.05) 53, 271	0.55 (0.10) 15, 48
2002	0.80 (0.02) 76, 746	---	---
2003	0.87 (0.03) 27, 263)	---	---
2004	0.93 (0.05) 23, 209)	0 2, 12	---
2005	---	0.75 (0.10) 6, 58	---
2006	---	1 2, 19	0.69 (0.10) 6, 25
2007	1.0 15, 1)	0.95 (0.05) 19, 19)	0 3, 3
2008	0.96 (0.04) 3, 28	0.64 (0.17) 3, 26	1 2, 8
2009	0.94 (0.03) 8, 80	1 2, 18	0.70 (0.30) 2, 6
2010	0.99 (0.01) 18, 166	0.84 (0.08) 11, 100	0.33 (0.0) 2, 6
2011	0.97 (0.02) 15, 227	0.64 (0.15) 8, 61	---

Table 1–3. Sex ratios for South Florida hatchling samples by year.

Year	Melbourne		Hutchinson Is.	Juno–Jupiter			Boca Raton			Miami	Sanibel Is.	Sarasota
	Cc	*Cm*	*Cc*	*Cc*	*Cm*	*Dc*	*Cc*	*Cm*	*Dc*	*Cc*	*Cc*	*Cc*
All	0.86(0.03) n=21	1 n=2	0.88(0.05) n=17	0.93(0.04) n=28	1 n=3	0.50(0.21) n=6	0.95(0.01) n=57	0.79(0.05) n=48	0.58(0.09) n=9	0.80(0.06) n=14	0.82(0.04) n=28	0.82(0.05) (20)
2002	0.86(0.04) n=11	---	0.81(0.07) n=11	0.91(0.04) n=11	---	---	0.87(0.04) n=13	---	---	0.77(0.06) n=12	0.55(0.11) n=6	0.75(0.07) n=12
2003	0.85(0.06) n=9	----	---	---	---	---	0.95(0.02) n=9	---	---	---	0.81(0.08) n=9	---
2004	---	---	1 n=6	0.90(0.10) n=9	---	---	---	0 n=2	---	---	---	0.89(0.07) n=8
2005	---	---	---	---	---	---	---	0.75(0.13) n=6	---	---	---	---
2006	---	1 n=2	---	----	---	1 n=1	---	---	0.63(0.11) n=5	---	---	---
2007	---	---	---	1 n=8	1 n=3	0 n=3	1 n=7	0.94(0.06) n=16	---	---	---	---
2008	0.89 n=1	---	---	---	---	1 n=2	1 n=2	0.64(0.17) n=3	---	---	---	---
2009	---	---	---	---	---	---	0.93(0.03) n=7	1 n=2	0.70(0.30) n=2	---	1 n=1	---
2010	---	---	---	---	---	---	1 n=13	0.84(0.08) n=11	0.33 n=2	---	0.96(0.04) n=5	---
2011	---	---	---	---	---	---	1 n=6	0.64(0.15) n=8		1 n=2	0.94(0.04) n=7	---

Table 1–4. Sex ratios for each species by beach and year. Data are mean proportion female (SE). n=nests sampled. *Cc*=*C. caretta*, *Cm*=*C. mydas*, and *Dc*=*D. coriacea*. The total hatchlings examined were 1511 *Cc* from 185 nests, 271 *Cm* from 53 nests, and 48 *Dc* from 15 nests.

Comparison	Kruskal-Wallace $H_{(df)}$ with Tukey-like contrasts (t)	*p* value
	Caretta caretta	
All years	$H_{(7)} = 52.31$	< 0.001
2002 vs. 2004	$t = 3.97$	< 0.001
2002 vs.2007	$t = 9.02$	< 0.001
2002 vs. 2010	$t = 5.98$	< 0.001
2003 vs. 2007	$t = 4.47$	< 0.001
2003 vs. 2010	$t = 3.54$	0.008
2003 vs. 2011	$t = 2.54$	< 0.001
All Beaches	$H_{(6)} = 22.62$	< 0.001
	Chelonia mydas	
All years	$H_{(7)} = 22.30$	0.002
2004 vs. 2005, 2006, 2008, 2009 and 2010	$t = 23.95$	< 0.001
2004 vs. 2007	$t = 4.49$	< 0.001
2004 vs. 2011	$t = 3.02$	0.046
2008 vs. 2006	$t = -23.95$	< 0.001
2008 vs. 2007	$t = -3.30$	0.018
2008 vs. 2009	$t = 23.95$	< 0.001
	Dermochelys coriacea	
All years	$H_{(4)} = 11.06$	0.026
2006 vs. 2010	$t = 12.74$	< 0.001
2007 vs. 2006	$t = -12.74$	< 0.001
2007 vs. 2008, 2009 and 2010	$t = 12.74$	< 0.001
	Overall and Concurrent Comparative Results	
All species	$H_{(2)} = 10.02$	0.007
Dc vs. *Cc*	$t = -2.40$	0.040
2009 and 2010 all species	$H_{(2)} = 8.48$	0.014
2009 and 2010 *Dc* vs. *Cc*	$t = -2.33$	0.047
All species by year	$H_{(5)} = 16.42$	0.005
2009 *Cc* vs. 2009 *Cm* and 2010 *Cc*	$t = 2.80$	0.046
2009 *Cc* vs. 2010 *Dc*	$t = -959.80$	< 0.001
2009 vs. 2010 *Cm*	$t = -3.46$	0.005
2009 *Cm* vs. 2010 *Dc*	$t = -959.80$	< 0.001
2009 vs. 2010 *Dc*	$t = -959.80$	< 0.001
2010 *Cc* vs. *Cm*	$t = -3.46$	0.005
2010 *Cc* vs. *Dc*	$t = -959.80$	< 0.001
2010 *Cm* vs. *Dc*	$t = -4.50$	< 0.001

Table 1–5. Comparison statistics for loggerhead (*Cc*=*C. caretta*), green (*Cm*=*C. mydas*) and leatherback (*Dc*=*D. coriacea*) turtles. $H_{(df)}$ = Kruskall-Wallis test statistic; t=nonparametric Tukey-like test statistic.

CHAPTER 1 FIGURES

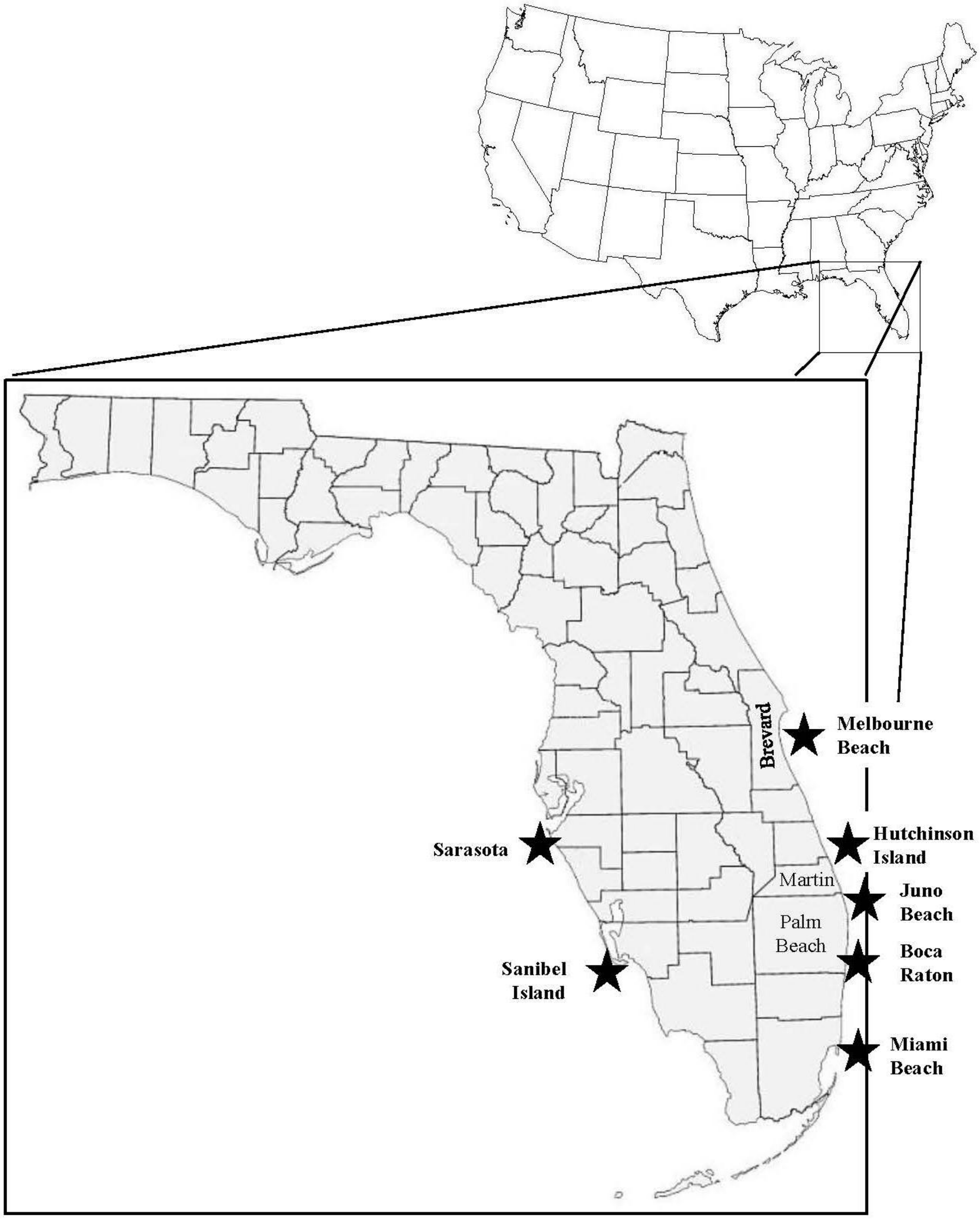

Figure 1–1. South Florida study beaches. Counties hosting the highest percentages of sea turtle nesting are indicated: Brevard, Palm Beach and Martin.

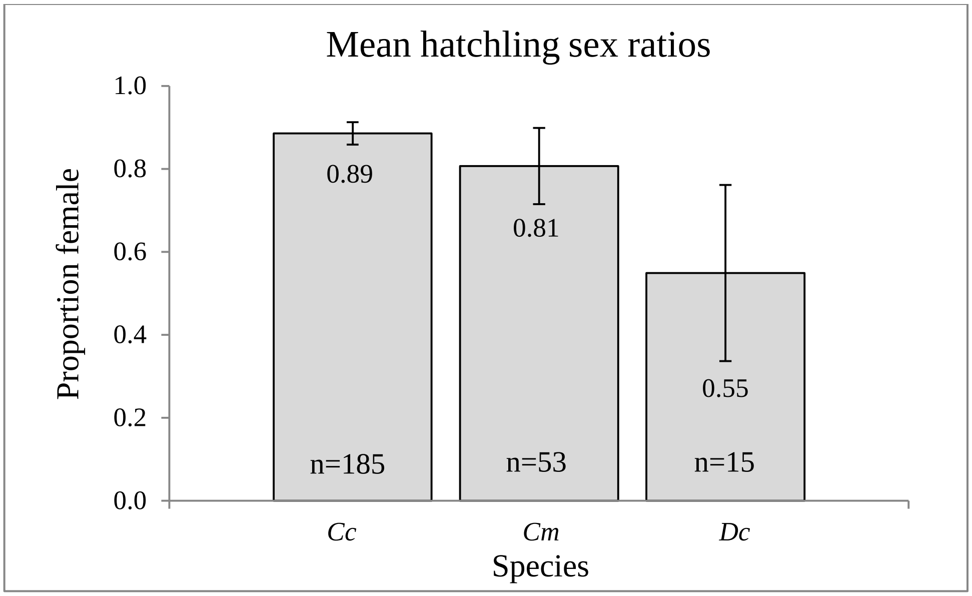

Figure 1-2. Species comparison of mean hatchling sex ratios with 95% confidence intervals. *Cc*=*C. caretta*, *Cm*=*C. mydas* and *Dc*=*D. coriacea*. *Cc* differs from *Dc*; *Cm* does not differ from either *Cc* or *Dc*. n=number of nests sampled.

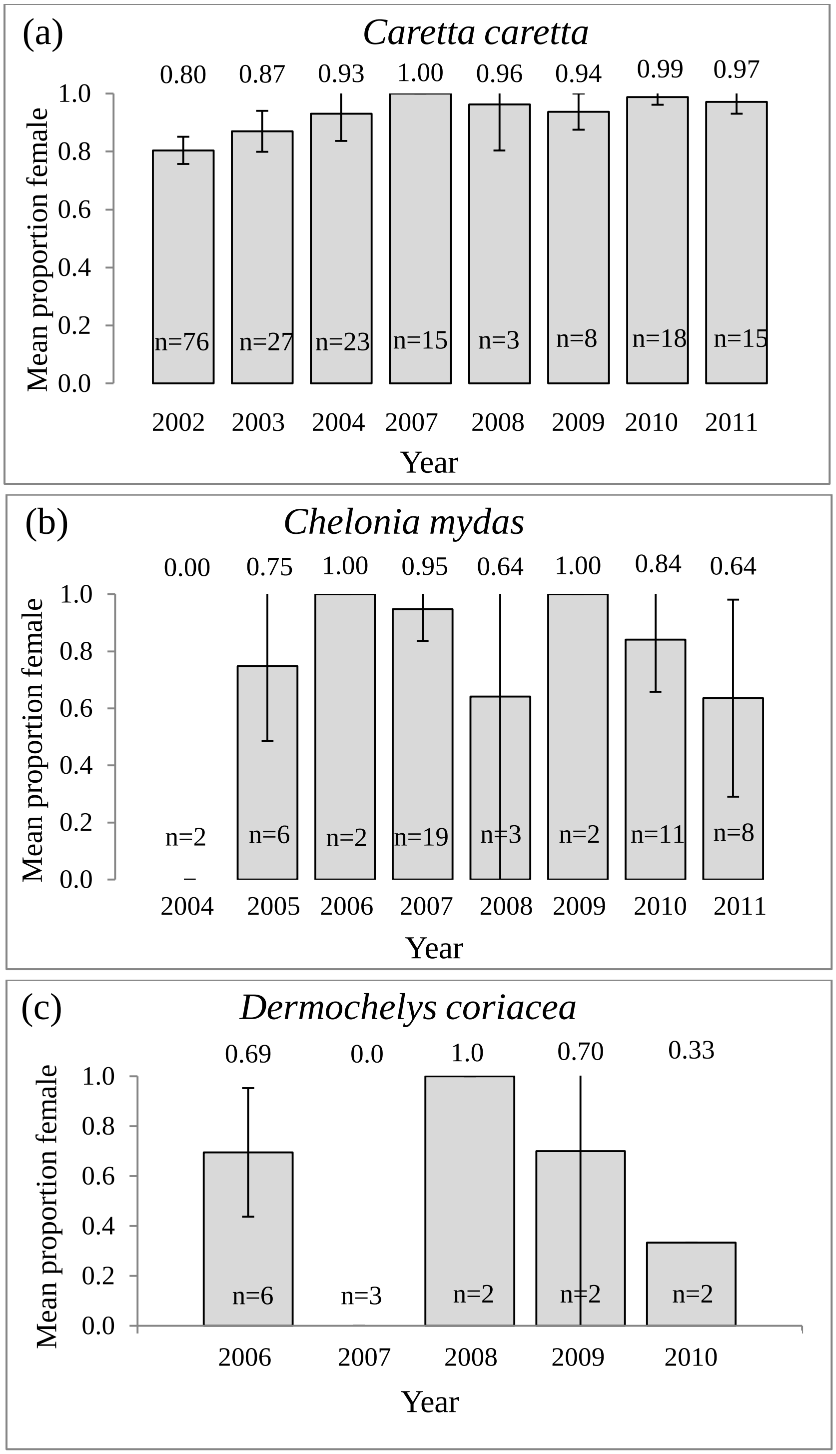

Figure 1-3. Loggerhead (a), green (b), and leatherback (c) mean proportion female (above each bar) by year with 95% confidence intervals (CI). n=number of nests sampled. CIs that extend beyond the axes are years with greatly varied sample sex ratios. The 2010 leatherback samples shared the same sex ratio.

Figure 1-4. Sex ratios by beach across all years each beach was sampled. *Cc*=*C. caretta*, *Cm*=*C. mydas*, and *Dc*=*D. coriacea*. Mean proportions of each sex are given within the shaded female and white male sections.

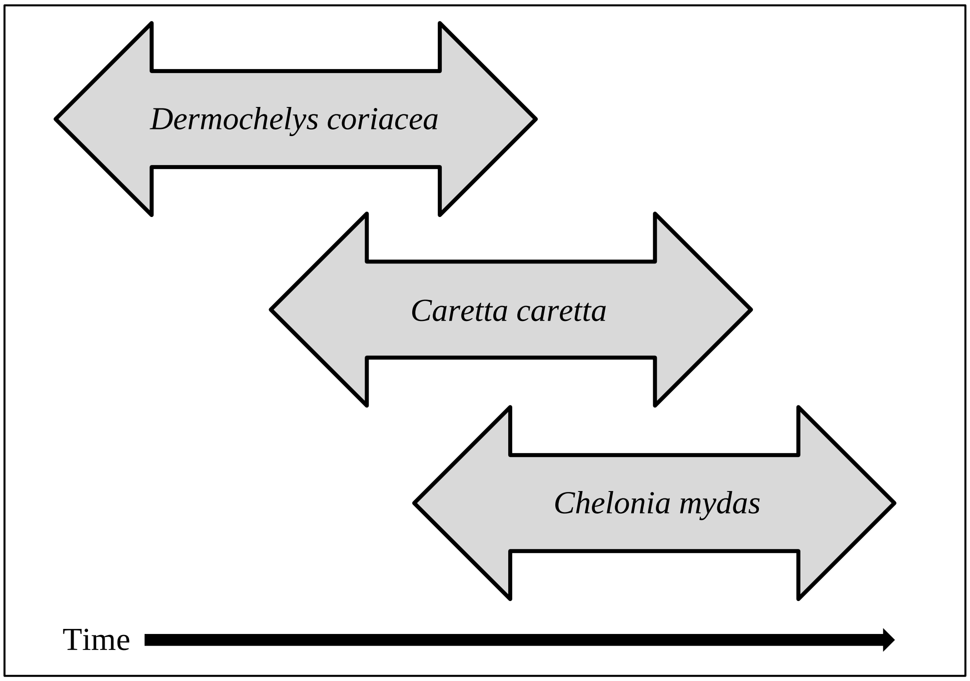

Figure 1-5. Diagram of temporal relationships and overlap among species incubation periods (nesting season through the latest hatch-outs) in Florida.

CHAPTER 2

NEST TEMPERATURE–SEX RATIO RESPONSE OF THREE SOUTH FLORIDA MARINE TURTLE SPECIES

INTRODUCTION

Nest temperatures direct marine turtle sex during embryonic development, as temperature-dependent sex determination (TSD; Bull 1980, 1983). Generally, sex determination occurs during the middle third of development (the thermosensitive period (TSP); Mrosovsky & Yntema 1980; Yntema & Mrosovsky 1980; Standora & Spotila 1985). The timing of the loggerhead (*Caretta caretta*) TSP has been narrowed to 50-65% of incubation (Blair 2005). Three patterns of temperature response have been documented in reptiles with TSD. Marine turtles exhibit cool male–warm female patterns (MF) or type Ia (Mrosovsky 1980; Miller & Limpus 1981; Ackerman 1997; Ewert et al. 2004). Cool female–warm male (FM; type Ib) is seen in *Sphendon* spp.,some lizards and some crocodilian species (Viets et al. 1994; Valenzuela 2004). Cool female–warm male–warmer female patterns (FMF; TSD II) are found in some turtles, lizards, and crocodilians (Ewert & Nelson 1991; Viets et al. 1993; Lang & Andrews 1994; Valenzuela 2004).

The nest temperature–sex ratio relationship at constant incubation is characterized by a transitional range of temperatures (TRT), within which sex ratio changes from 100% M to 100% F, and a pivotal temperature (PT), at which a 1:1 sex ratio occurs (Wibbels

2003). Many studies describe this relationship under laboratory conditions (e.g., Yntema & Mrosovsky 1980, 1982; Morreale et al. 1982; Mrosovsky 1988; Binckley et al. 1998). The value of such studies is that they allow comparisons of sex response to temperature alone and show that PT and TRT vary among species and populations (Wibbels 2003). However, many factors influence nest incubation temperatures in the field (Standora & Spotila 1985; Hays et al. 2001; Birchard 2004; Houghton et al. 2007; LeBlanc & Wibbels 2009) so laboratory estimates of PT and TRT rarely reflect *in situ* responses.

All marine turtle species frequenting U.S. waters are listed as endangered or threatened under the Endangered Species Act of 1973, as amended (ESA; U.S. Department of the Interior, U.S. Fish and Wildlife Service [USFWS] 2003), and the IUCN Red List. Population demographics and demographic models are used to inform conservation management decisions; sex ratios are important components. Quantifying sex ratio trends can coarsely identify a population's current and future reproductive potential. In species with TSD, hatchling sex ratios represent primary sex ratios; they form the baseline from which subsequent age- or stage-class sex ratios arise (Turtle Expert Working Group [TEWG] 2009). Hatchling sex ratios may vary depending on the embryo-thermal-sex response and any natural or anthropogenically-induced modifiers of those responses. For example, beach management methods such as sand nourishment, dune removal, vegetation removal and building construction may alter sand temperatures of sea turtle nesting grounds, thus altering sex ratios (Mrosovsky et al. 1995; Schmid et al. 2008) and potentially impacting species survival, simply by changing color and shading patterns. Similarly, rising air temperatures due to climate change (Intergovernmental Panel on Climate Change 2007) may also alter sand temperatures in nesting grounds (Hays et al. 2003; Hawkes et al. 2007, 2009; Fuentes et al. 2010; Witt et al. 2010).

South Florida beaches are productive nesting grounds for three species of sea turtles, the loggerhead (*Caretta caretta*; Ehrhart et al. 2007), green (*Chelonia mydas*; National Marine Fisheries Service [NMFS] & U.S. Fish and Wildlife Service [USFWS] 2007a) and leatherback (*Dermochelys coriacea*; NMFS & USFWS 2007b). Yet, studies documenting Florida nest temperature–sex ratio relationships, in the lab or field, are few for loggerhead turtles (Mrosovsky 1988; Milton et al. 1997; Hanson et al. 1998; Blair 2005) and none address Floridian green and leatherback turtles.

The objectives of this study were to (i) document and characterize nest temperature profiles during incubation for South Florida's loggerhead, green and leatherback turtles and to (ii) model each species' temperature–sex ratio response based upon nest sample sex ratios across time and space.

METHODS

Study sites and nest selection. — Seven peninsular Florida nesting beaches were sampled this study. Loggerheads were collected at Melbourne Beach, Hutchinson Island, Juno–Jupiter, Boca Raton and Miami Beach on the southeast coast and Sanibel Island and Sarasota on the southwest coast (Fig. 1–1). Green turtle and Leatherback data were sampled in Boca Raton. All sites are located on barrier islands and were chosen based on their relatively high marine turtle nesting densities and available historic nesting data. Gently sloped beaches of open sand backed by vegetated fore-dunes and/or developed properties (homes, condominiums, public parks, and a power plant) characterize the sites.

Up to four nests/species/beach/subseason were selected for study based on local expectation that each was likely to complete development and produce hatchlings. Nests were selected across each species' complete nesting seasons and to represent all subseasons (early, middle and late; centered on the 1st, 2nd–3rd and 4th quartiles of historic nest and emergence distributions) and beach zones (low–middle, middle and high). Nests in the low zone were not selected because of their increased risk of regular tidal inundation); similarly nests in areas with malicious mischief and high predation were avoided. Nest selection practices were employed to ensure the thermal effects of spatial and temporal variation were reflected in the samples. Thus, the consistent sampling methods used in this study provide a multi-year hatchling sex ratio for each species by site.

Nest temperatures. — Marine turtle nest temperatures were recorded throughout incubation in selected nests during the 2002-2012 nesting seasons. Temperature data loggers (Onset Computer Corporaton HOBO® models H8 (2011a) and U22 (2011b), TidbiT (2011c), or VEMCO® Minilog 12-bit (2011)) were placed in up to 12 study nests per species/beach/season. Data loggers were programmed to record nest temperature no less than every hour and positioned in the middle of the clutch either (i) during egg deposition as indicated by deposition of approximately 50 cheloniid or 35 dermochelyid eggs or (ii) the morning following nesting, when nests were marked by survey crew. If the latter occurred, a column of approximately 20-40 eggs were removed, without rotation, the data logger was placed, the displaced eggs were then replaced, and the clutch recovered with its sand and resealed by packing the sand firmly. The data loggers were recovered during nest inventory excavations.

The range of temperatures nests experience is well within the best resolution ranges of the data loggers (Table 2–1). While the data loggers differ in resolution and accuracy, data were treated as accurate to the resolution of the least sensitive model (HOBO® H8 series).

Sampling and animal rearing. — Hatchlings were randomly sampled for approximately 10% of live, normal hatchlings. Each nest's sex ratio was based upon 5 leatherback hatchlings or10 loggerhead or green turtle hatchlings collected from each nest's first major hatchling emergence. In several cases, fewer turtles emerged so all were included and in a few other instances more were sampled (up to 34) for inclusion in other studies. Hatchlings with obvious deformities such as cleft palate, severely deformed shells or missing limbs and those emerging with extensive lesions were excluded from consideration. All hatchlings were transported under climate-controlled conditions for rearing at the Florida Atlantic University Marine Laboratory at the Gumbo Limbo Environmental Complex, Boca Raton, Florida.

Hatchlings were raised until yolk was absorbed and sex could be identified laparoscopically. Husbandry is detailed by Stokes et al. (2006) and Wyneken et al. (2007). Loggerhead and green hatchlings were housed in individual containers in a flow-through natural seawater system at 26±2^{o}C. Leatherback hatchlings were tethered and maintained singly or in tanks that prevent individuals from interacting with each other or the tank walls (Jones et al. 2000). Leatherback tanks were supplied with water via a closed system at 23±2^{o}C that was partially changed daily and fully changed weekly. All turtles were fed daily using species-specific in-house manufactured diets modified from Stokes et al.(2006) and Jones et al.(2000).

Sex identification. — All turtle sexes were verified by laparoscopy and in a small number of cases biopsy or histology collected at necropsy. Neonate sex was determined by visual inspection of a suite of gonadal and accessory duct characteristics via laparoscopic examination once they reached the required 120g minimum size (Wyneken et al. 2007). Gonad size, shape, and attachment to the coelomic wall and paramesonephric duct size, mobility and lumen presence or absence together have been identified as reliable sex-determining characteristics in live loggerhead neonates (Wyneken et al. 2007). Sex of any hatchlings that died during laboratory rearing was determined upon visual inspection of the gonads and ducts during necropsy followed by histological verification (Ceriani & Wyneken 2008; Ceriani & Wyneken, unpublished). After the surgical incisions healed and normal feeding and activity returned, neonates were released into the Gulf Stream Current. Some dead hatchlings also were included to provide morphologically verified samples if they died in quarantine or occasionally predators killed hatchlings before they reached the water.

Statistical analyses and parameter estimations. — The experimental unit for this study was each nest's sex ratio, reported as proportion female. For convenience, posthatchling sex ratios are termed hatchling sex ratios because the turtles were collected as hatchlings. Sex ratios were calculated for all nests sampled for each species, beach and year; these data were then summarized as grand means ± standard errors (SE).

Descriptive statistics (mean, maximum, minimum, and mode) were calculated for temperatures of each nest during two estimates of the TSP: (i) the middle third and (ii) 50-65% of incubation. Incubation in days (deposition date to date of first hatchlings' emergence) was multiplied by (i) 0.33 and 0.66 and (ii) 0.50 and 0.65, respectively, to determine the start and end dates of the two TSP periods.

Maximum likelihood logistic regression (Dalgaard 2008; Zar 2010; UCLA: Statistical Consulting Group 2013) with probit transformation, weighted by hatchling sample size of each nest, was used to analyze the response relationships between (i) each of the TSP descriptors and sex ratio and (ii) incubation days and sex ratio. Analyses were completed using R software (R Development Core Team 2012) (termed "model selection in R" or "R model") and the TSD program (hereafter termed TSD program) developed by Girondot (1999) and Godfrey et al. (2003), available at http://http://max2.ese.u-psud.fr/epc/conservation/Girondot/Publications/TSD.html.

The logistic regression model (Equation 1) used in the R model was described as follows.

$$F = \Phi(\beta_0 + \beta_1 t_1) \tag{1}$$

F is the sex ratio as proportion female and t is incubation temperature. Φ is the cumulative distribution function of the standard normal distribution. The model parameter estimates for intercept, β_0, and the TSP descriptor (mean, maximum, minimum or mode), β_1, influence the model's shape. Equation 2 is a reorganization of Equation 1 to solve for t.

$$t = \frac{\Phi^{-1}(F) - \beta_0}{\beta_1} \tag{2}$$

Resultant model TSP descriptor and intercept parameters were input into Equation 2 to determine PT and TRT temperatures. F=0.5 was used to estimate PT. The upper bound for the TRT was calculated using F=0.95, above which no males were found in the samples and the lower bound was calculated using F=0.05, below which no females were found in the loggerhead and green samples. All leatherback samples were small and included females. To remain consistent among species, the lower bound for the leatherback was also set to F=0.05. In analyses of incubation days, day number, d, was used in place of t and β_1 is the estimate of the number of incubation days' influence on the model shape. Sex ratios used to estimate the inflection point and transition period of the incubation days models were the same as in the temperature-based models.

As described by Godfrey et al. (2003), the TSD program includes A-logistic, Richards, Hill, modified Hill (Hill*), Weibull and modified Weibull (Weibull*) equations that can be used to describe the sigmoidal relationship of sex ratio to temperature data. The program estimates PT (termed P in the program), the curve representing the relationship between sex ratio and temperature during transition from feminizing to masculinizing or masculinizing to feminizing temperature *(S)*, and symmetry of the curve *(K)*. If K=0 the shape of the curve is symmetrical on either side of the PT and uses the unaltered form of the equations (logistic). If K is greater or less than 0 the shapes on either side of the PT are asymmetrical (A-logistic). Upper and lower TRT bounds were estimated from the program's graph tab using F=0.95 and F=0.05, respectively.

Model selection was based on minimum Akaike Information Criteria (ΔAIC; Akaike 1974) values. The model with the smallest ΔAIC and models with $\Delta AIC<3$ values of the minimum model were retained as good candidates. Models with ΔAIC values 3–7 above the minimum ΔAIC value have considerably less support; models with $\Delta AIC >7$ were rejected. Fit of selected maximum likelihood models from R analyses was determined by the chi-squared (χ^2) goodness-of-fit test using the residual deviance and degrees of freedom (df) from the model (reported χ^2 (residual deviance, df)). Where $p\geq0.05$, the model cannot be rejected. The logistic equation used in R analyses assumes symmetry around the curve's inflection point, the PT. Fit of selected models from the TSD software was determined by measuring the alpha risk (Type I error) using parameters from the model in 1000 iterations. A p value of ≥0.05 indicates the model cannot be rejected. An estimate of the power to detect if the shape of the curve is symmetrical ($K=0$) or not ($K\neq0$) was also conducted to test whether sample size influenced model selection in 100 iterations. If the power to detect $K\neq0$ is ≤0.05 the sample size is too small to differentiate between symmetric and asymmetric shape.

Incubation temperature comparisons among species, years and beaches were analyzed using the Kruskall-Wallis test with a 0.05 significance threshold in R. Where significant differences were found, Tukey-like nonparametric multiple comparisons with probit transformation were used to identify the sources of differences (nparcomp R package, Konietschke 2011).

RESULTS

Model fitting. — Overall and individual beach sex ratios and ranges of the middle third TSP temperatures are reported in Table 2–2. The 50-65% TSP range of temperatures is encompassed within the middle third TSP temperatures. Table 2–3 and 2–4 summarize the ΔAIC-selected models with their estimates of PT and TRT based on the R models. Table 2–5 summarizes the models and their PT and TRT estimated by the TSD software.

Nest temperature variation. — Loggerhead temperatures and sex ratios span multiple nesting seasons across multiple nesting beaches in South Florida. Green turtle and leatherback incubation temperatures were recorded during multiple nesting seasons at Boca Raton. Yearly nest temperature comparisons were conducted on the temperature descriptors of the middle third and 50-65% TSPs.

Empirically measured incubation temperatures of loggerhead middle third TSP ranged from 25.6–35.6^{o}C. Mean temperatures ranged 27.5–34.1^{o}C, and modes ranged 26.3–34.5^{o}C. Incubation temperatures during the 50-65% TSP ranged from 26.0–35.6^{o}C. Mean temperatures of the 50-65% TSP ranged from 29.5–32.6^{o}C and modes ranged 26.3–34.8^{o}C. Temperatures that produced mixed sex ratio samples ranged 25.6–34.9^{o}C during the middle third TSP and 26.0–34.4^{o}C in the 50–65% TSP. The minimum loggerhead TSP temperature was recorded in nests sampled from Melbourne Beach (2002 and 2003) and Sarasota (2002) and the maximum was recorded in Boca Raton (2011). Yearly variation among mean temperatures (Table 2–6) was significant when using data from all beaches and when Boca Raton was examined alone. The single 2012 nest sample was excluded from yearly comparisons. For both the middle third and 50-65% TSPs, the year 2002 was different from all other years sampled and 2010 differed from 2011. Additionally, 2003 50-65% TSP temperatures differed from 2010. Differences in maximum, minimum and modal TSP temperatures (Tables 2–7 to 2–9) and days of incubation (Table 2–10) gave similar results; the 2002 and 2010 seasons were consistently set apart from the other seasons.

Green turtle middle third TSP incubation temperatures ranged from 27.4–35.3^{o}C and means ranged 30.0–33.8^{o}C. Middle third modes ranged 27.8–34.3^{o}C. Temperatures in the 50-65% TSP ranged 28.6–35.3^{o}C; the means ranged 30.1–34.5^{o}C and modes ranged 30.1–35.1^{o}C. The temperature ranges that produced mixed nest sex ratio samples ranged 27.4–34.9^{o}C during the middle third TSP and 28.6–34.9^{o}C in the 50–65% TSP. The green turtle lowest and the highest TSP temperatures were recorded during the 2010 nesting season. Green turtle yearly temperature variation was not significant during either of the TSP periods.

Leatherback middle third TSP incubation temperatures ranged 26.6–32.4^{o}C and means ranged 28.3–31.5^{o}C. Modes for the middle third TSP ranged 28.31–31.31^{o}C. Temperatures during the 50-65% TSP ranged 28.1–32.4^{o}C. Means of the 50-65% TSP ranged 28.8–31.5^{o}C and modes ranged 28.7–32.1^{o}C. Temperatures that produced mixed sex ratio samples ranged 27.9–32.4^{o}C during the middle third TSP and 28.7–32.4^{o}C in the 50–65% TSP. Both the lowest and highest TSP temperatures were recorded in 2012. Similar to the green turtles, leatherback yearly temperature variation did not differ significantly during either TSP period.

Species and beach comparisons. — Comparisons among species (Table 2–11) were conducted on nest temperatures of the middle third and 50-65% TSPs and incubation length (days). Boca Raton was the only beach sampled for nest temperature and sex ratio data from all three species. Mean, minimum, maximum, and modal nest temperatures and the days of incubation were significantly different among species. The loggerhead and green turtle TSP descriptors and days of incubation were not different ($p>0.05$ in all comparisons). Leatherback TSP descriptors and days of incubation differed significantly from those of both loggerheads and green turtles.

Loggerhead data were sampled from seven South Florida beaches. TSP nest temperature descriptors differed significantly among beaches during both TSP periods (Tables 2–6 to 2–10). Paired comparisons regularly identified Boca Raton as different from the other beaches, such that the collective paired comparisons set it apart from every beach. Only one of the significant paired comparisons did not involve Boca Raton: the maximum temperatures of the middle third TSP for Hutchinson Island and Sanibel Island were also different.

Loggerhead turtles. — Loggerhead sex ratios and nest temperatures were documented for Melbourne Beach 2002 and 2003, Hutchinson Island 2002, Juno Beach 2002, Boca Raton 2002, 2003, 2010–2012, Miami Beach 2002 and 2011, Sanibel Island 2002, 2010 and 2011 and Sarasota 2002 nesting seasons. There were 992 hatchlings examined from 93 nests. The proportion female ranged 0.0–1.0 with mean (SE)=0.87 (0.02). The single, all male nest was laid in the early subseason of 2012 in Boca Raton. It was located in the mid-beach near the spring tide line and its egg chamber was of typical of other loggerhead nests on the same beach. Fifty of the 93 nests were 100% female and 88 had female-biased sex ratios (proportion *F*>0.5).

The loggerhead R model curves fit the upper portion of pattern Ia, cool males–warm females. No nests experienced temperatures that fit the lower half of the curve. Minimum ΔAIC values indicate the 50-65% TSP maximum temperature (Fig. 2–1) as the best-fit descriptor of loggerhead sex ratio response to nest temperature. The 95% confidence intervals around the parameter estimates (intercept: -13.56, -9.05; maximum temperature parameter: 0.32, 0.46) were relatively narrow, but its fit was poor (χ^2 (152.76, 91df), $p<0.001$). The model estimates the loggerhead PT=28.9^{o}C and the upper TRT bound =33.3^{o}C; no lower bound could be calculated.

Previous works (Mrosovsky & Provancha 1989, 1992; Hanson et al. 1998) used the mean temperature of the middle third of development to relate field-based sand and nest temperatures to sex ratio outcomes. My curve resulting from mean middle-third TSP model (Table 2–3, Fig. 2–2; 95% confidence intervals: intercept (-15.87, -10.37) and mean (0.38, 0.57); χ^2 (152.86, 91df), $p<0.001$) estimates the upper TRT bound at 31.1^{o}C and PT at 27.6^{o}C. In neither case was the TRT lower bound model estimate supported due to the lack of male-dominated nests in the data.

Because few nests in the sample produced males, an additional model was fitted using the middle third mean that weighted nests *F*<0.5 equal to the weights of nests *F*>0.5 (Fig. 2–3). This model was an exploratory fit in attempt to better anchor the male-biased curve at the lower temperatures. The male-weighted model (intercept (SE)=-26.99 (1.26), z=-21.45 and mean (SE)=0.92 (0.04), z=21.25) was also highly significant ($p<0.001$); however, its ΔAIC (826.6) was substantially higher than the other models.

The male-weighted model PT was 29.4^{o}C, and the lower and upper bounds of the TRT were 28.3^{o}C and 30.4^{o}C, respectively. The 95% confidence intervals were (-29.49, -24.62) around the intercept and (0.84, 1.00) around the mean. The model fit was poor (χ^2 (719.24, 91df), p<0.001).

The TSD program selected type Ia models using the middle third TSP mean. The Hill equation's ΔAIC was lowest, although the Weibull equation's was only slightly higher (Table 2–5). The Hill equation estimates a PT of 29.0^{o}C and the upper bound of the TRT at 30.4^{o}C; the TRT lower bound could not be determined due to too few cool-temperature nests in the dataset. Its power to detect $K \neq 0$ was 0 and the alpha risk was <0.001. The middle third TSP mean Richards (ΔAIC=122.9963), Hill* (ΔAIC=123.2424) and Weibull* (ΔAIC=123.836) model equations were also good candidates with similarly low ΔAIC values (within 3 units of the minimum ΔAIC model). Some TSD type II ΔAIC values were low; however this response model was rejected based multiple laboratory studies that identify marine turtles as having type Ia response curves (Mrosovsky 1980; Miller & Limpus 1981; Ackerman 1997; Ewert et al. 2004) and the lack of supporting data at male-producing temperatures. The days of incubation model and Type Ib models gave high ΔAIC values and so were rejected.

Green turtles. — Green turtle nest temperatures were recorded in Boca Raton during the 2009-2012 nesting seasons. A total of 172 hatchlings from 20 nests were examined. The proportion female ranged 0.0–1.0 with mean (SE)=0.76 (0.32). The only nest that gave an all-male sample was laid in the late subseason of 2011. It was located at the edge of the foredune vegetation and had a deep egg chamber with a shallow, oval-shaped egg mass. The single 2012 nest was mostly male; its sample gave a 0.1 proportion female. It was also located near the foredune edge and was in a dune restoration (replanted) area that was watered daily in early mornings. Ten of the 20 nest samples were 100% female and 14 of the 20 were female-dominated (F>0.5). Sample nests were predominately female in the 2009-2011 seasons.

R models' ΔAIC values indicate the maximum nest temperature of the middle third TSP (Fig. 2–4) as the best-fit descriptor for the green turtle nest temperature sex ratio response. The model estimates the PT for green turtle maximum middle third TSP temperatures at 32.5°C and the TRT range is estimated at 30.9–34.1°C. The model's 95% confidence limits around the parameter estimates were (43.21, 25.36) for the intercept and (0.78, 1.33) for the maximum temperature. The fit was poor (χ^2 (35.999, 18df), p=0.007). The TSP model based upon the mean temperature of the middle third of incubation is also shown. Its TRT and PT estimates are 29.1–32.6°C and 30.8°C, respectively.

The green turtle model selected by the TSD program reflected type Ia based upon maximum temperature of the 50-65% TSP model and using the Weibull equation. This model estimates the green turtle PT at 32.6°C with a TRT range of 30.8–33.7°C. The power test resulted with K=0 and the model fit was good (alpha risk= 0.954). However, the middle third and 50-65% TSP maximum and mean Weibull and Hill models' ΔAICs were within 1 unit of each other. The models with the four lowest ΔAIC values are given in Table 2–5. Several other type Ia models gave ΔAICs within 3 units from the maximum temperature 50-65% TSP model and must also be considered as possible candidates. The incubation days–sex response model and TSD type Ib and II models were rejected; all had high ΔAIC values.

The selected model curves were all characteristic of pattern Ia, cool males–warm females, but I was unable to confirm the Florida green turtle TSD pattern. The sample size is relatively small and samples may not be fully representative of some years; e.g., only 2 nests were sampled in the 2009 season.

Leatherback turtles. — Leatherback nest temperatures were recorded in Boca Raton during the 2006, 2010 and 2012 nesting seasons. Twenty five hatchlings from 6 nests were examined; the proportion female ranged 0.0–1.0 with a mean (SE)=0.69 (0.51). Three nest samples were female-dominated, two *F*=1 (one from 2006 and one from 2012) and one *F*=0.88 female (2012). Two nest samples in 2006 had 1:1 ratio (*F*=0.5) and the single 2010 nest was male-dominated (*F*=0.33).

Leatherback sample sizes are small and do not fully represent the entire nest incubation season. The nest temperature–sex ratio response relationship could not be determined using either model selection process.

Model selection in R was not able to distinguish among neither minimum, maximum or mean descriptors of the middle third or 50-65% TSPs nor incubation days as the best indicator for sex ratio response. The models' ΔAIC values differed from one another by ~3 or less. ΔAIC values of modal temperature models of both the middle third and 50-65% TSP were ~5 units higher than the minimum ΔAIC model. The modal temperature based models remain candidates but have considerably less support than the other models. The minimum temperature model of the 50-65% TSP had the lowest

ΔAIC. The temperature model curves most closely resembled TSD type Ib, warm males–cool females (e.g., Fig. 2–5). Mean temperature model estimates give a PT range of 30.9–31.4°C. Upper and lower bounds for the TRT could not be determined. The model relationship between days of incubation and sex ratio (Table 2–4; Fig. 2–6) suggests day 62 as the developmental length that produces a 1:1 sex ratio. It estimates incubation times shorter than 54 days produces 100% females and longer than 70 days produces 100% males. In practice, Florida leatherback nests incubate for at least 52 days.

Analyses of the leatherback data using the TSD modeling program were also inconclusive. The type Ib 50-65% minimum Weibull model gave the lowest ΔAIC. However, all type Ib Hill and Weibull models, type Ia Hill and Weibull maximum middle third TSP models, and type Ia minimum, maximum and mean 50-65% TSP models had $\Delta \mathrm{AIC}<1$. Many more of the models were similarly close, with ΔAICs ≤ 3 units from the Ib 50-65% minimum Weibull model. Four of the models with ΔAICs within 1 unit of each other, including the type Ib 50-65% minimum Weibull model, are outlined as examples in Table 2–5. They give PT estimates (30.6–31.4°C) but TRT bounds could not be determined. TSD type II models gave the highest ΔAIC values and so the pattern was rejected.

DISCUSSION

Here I present estimates of field-based nest temperature–sex ratio responses of Florida’s loggerhead, green and leatherback turtles using data sets spanning 10 loggerhead nesting seasons and several green and leatherback nesting seasons.

Nest temperature–sex response variation. — The overall range of middle third and 50-65% TSP nest temperatures for South Florida's loggerhead, green and leatherback turtles was 25.6–35.6^{o}C. Loggerhead nests spanned the entire 10^{o}C range, which represents the lowest and highest TSP incubation temperatures that yielded live hatchlings. In contrast, green turtle TSP temperatures spanned 7.9^{o}C (27.4–35.3^{o}C) and leatherback TSP temperatures spanned 5.9^{o}C (26.6–32.4^{o}C).

South Florida's hatchling sex ratios differ among loggerhead, green and leatherback turtles (Rogers 2013) nesting on the same beach. Differences among species in TSP temperature descriptors coincided with the sex ratio results; loggerhead and green turtle nest temperatures do not differ from each other, but leatherback TSP descriptors differ from both. Seasonal differences among nest temperatures were also identified. Yearly nest temperatures varied significantly in loggerhead samples, but such differences were not found in green or leatherback samples. Thermal differences among species likely are a function of several sources of variation including climactic influences and spatial and temporal differences in nesting behavior and nest depth (Carthy et al. 2003; Rogers 2013), as well as individual clutch responses to temperature (Dodd et al. 2006).

Individual clutch responses varied greatly from one another in loggerhead and green turtles. Loggerhead nests whose mean TSP temperatures were within the estimated PT range (27.6–29.1^{o}C) produced 0–1F. Similarly, green turtle nests with TSP means within their estimated mean temperature PT range (30.8–31.8^{o}C) gave 0.25–1F. Additionally, wide variation in temperatures that produce a single sex ratio response occurred throughout the range of TSP temperatures (e.g. Figs. 2–1 through 2–4), and the effect increased as sex ratios moved toward F=1.

High variation outside of model expectations may be due to the effects of metabolic heating and thermal inertia. Temperature data recorders were placed in the center of the clutch, where metabolic heating has the greatest effect (Godfrey et al. 1997). Clutches with large total egg mass may generate and hold enough heat that it affects incubation temperatures during the TSP (Rogers 2013). Large clutches that incubated at temperatures near the PT may have produced unexpected results compared to clutches with smaller mass. Eggs at the edges of a clutch with large mass would maintain cooler temperatures than eggs in the center, while smaller-massed clutches dissipate heat more easily, resulting in a more uniform temperature throughout. However, metabolic heating may only explain some of the temperature variations that produce mixed sex ratios. The wide temperature range that produced 100% female nests remains unexplained.

In both loggerhead and green turtles, the mean TSP temperature ranges that produced 100% female nests (28.5–33.9^{o}C in loggerhead nests and 31.2–33.8^{o}C in green turtle nests) start within the estimated PTs of the mean temperature based models, where mixed sex would be expected. Nesting beach sand temperatures tend to be relatively close to the pivotal temperatures (Kraemer 1979), thus the overall beach production generally provides a mixed sex ratio. However the extreme variation that is possible in individual clutches incubating near the PT suggests the nest temperature–sex ratio response is not a straight forward response. Bull et al. (Bull et al. 1990) found that variation in all-female producing temperatures shows a potency effect in their ability to feminize embryos. Consequently, estimating sex ratios from nest or sand temperatures based on PT values alone is ill advised. Where verified sex ratios are not attainable, utilizing the TRT bounds along with the PT to estimate sex response may be more appropriate. However, this method likely still produces only rough estimates.

Model selection and fit. — Modeling sex response to nest incubation temperatures under natural conditions is challenging. The thermal environment that a marine turtle clutch experiences during incubation is affected by both maternal and environmental factors (Birchard 2004). Rainfall or moisture (Houghton et al. 2007; Leblanc and Wibbels 2009), sand albedo (a measure of reflected light; Hays et al. 2001), geographical beach location, within-beach nest placement (e.g., duneward vs. seaward), time of deposition (Standora & Spotila 1985; Godfrey & Mrosovsky 1999), nest depth and diel solar radiance cycles (Birchard 2004) are known sources of temperature variation in the incubation environment.

Temperature variation from some sources, such as geographical location and sand albedo generally are not highly dynamic. However, diel solar radiance has the potential to dynamically affect sex ratio response where nests are not deep in the soil. Georges et al. (1994) proposed a model for predicting hatchling turtle sex ratios under fluctuating diel temperature regimes. To demonstrate the model's use they chose to incubate loggerhead turtle eggs in the laboratory, but they noted the model is most beneficial in shallow nesting freshwater species where nests experience wider diel fluctuations. Marine turtle nests are sufficiently deep that they do not generally experience large temperature shifts from diel solar cycles (reviewed in Birchard 2004). The diel fluctuation within my sample nests was small, typically <1°C. Although greater changes in daily temperatures were occasionally recorded, such instances were relatively rare.

The temperature ranges in my sample nests reflected longer-term effects such as seasonal warming and metabolic heating as well as episodic cooling due to multi-day storm events.

Mean TSP nest temperatures were considered an appropriate measure to accommodate within-nest temperature variation. However, some studies report mean temperature is not the best predictor of sex response (Pieau 1982; Bull 1985; Georges et al. 1994); thus, I also investigated the minimum, maximum and modal TSP temperatures as potential predictors. The Loggerhead models selected from R analyses identified the maximum temperature of the 50-65% TSP as the best fit while the TSD program selected the mean temperature of the middle third TSP. Green turtle models also differed so that models in R selected the maximum temperature of the middle third TSP while the TSD software selected the 50-65% TSP's maximum temperature. Although mean temperature was not excluded as a predictor for the loggerhead response, both loggerhead and green turtle analyses identified maximum temperature as a possible best-fit descriptor. The maximum temperature may better reflect of the effects of incubation temperatures on overall developmental responses during the TSP because development occurs at a faster rate, within bounds, at warmer temperatures (Ewert 1985).

Both of the ΔAIC-selected loggerhead models resulted in a significant but weak fit to the observed data with many points falling outside the 95% CI. While fit of the green turtle models was conflicted; the R model fit poorly but TSD program model fit well. The difference in which thermal metric best describes the species' sex response to incubation temperatures likely reflects both small differences in the equations but also the variability of responses in nature.

Leatherback model selection was inconclusive by either model fitting approach. Analyses in R rejected only modal temperatures of both the middle third and 50-65% TSPs as predictors. However, minimum, maximum and mean temperatures and incubation days all remain statistically valid. TSD software analyses were also unable to distinguish among predictor variables, and no leatherback model fit tests resulted in model rejection, hence I could make no discrimination. It is likely that the somewhat more stable leatherback-depth temperature regimes coupled with the small sample size of sex ratios makes distinguishing among response curves difficult.

Previous incubation temperature–sex ratio relationships indicate marine turtles have TSD type Ia, cool males–warm females, at least in the laboratory (Yntema & Mrosovsky 1980; Mrosovsky et al. 1984; Rimblot et al. 1985; Rimblot-Baly et al. 1987). The upper portion of my loggerhead and green turtle models selections are consistent with the literature; both the R and TSD program model curves show the type Ia response pattern. However both datasets lack sufficient numbers of male-biased nests to be confident in the lower portion of the model curves. The green turtle mean sex ratio (F=0.76) found in this study is similar to those found in Ascension Island (F=0.75; Godley et al. 2002), Tortuguero, Costa Rica (F=0.67; Standora & Spotila 1985; Spotila et al. 1987) and Suriname (F=0.68% 14 yr average; interannual range: F=0.20–0.90; Godfrey et al. 1996). Likewise, my loggerhead mean hatchling sex ratio (F=0.87) is comparable to similarly highly female-biased estimates from Florida and other western Atlantic nesting sites (Mrosovsky & Provancha 1989, 1992; Hanson et al. 1998; Blair 2005; LeBlanc et al. 2012). Similarities in sex ratios among multiple nesting assemblages provides evidence that the lack of male-biased loggerhead and green turtle nests is not restricted to South Florida and indicates logistic regression may not be the most suitable approach to understanding marine turtle nest temperature–sex ratio response curves.

The leatherback response curve was undetermined. I found many leatherback models were possible and the group of best fit models (lowest ΔAICs) represented both types Ia or Ib. Laboratory studies (Rimblot et al. 1985; Rimblot-Baly et al. 1987; Chan & Liew 1995; Binckley et al. 1998) found that leatherbacks tested under controlled conditions exhibit the type Ia response pattern, typical of other sea turtles. The highly variable responses I found suggest that leatherback sex ratio–temperature responses are either subject to many modifying factors or are more responsive to small changes in the nest environment. Such plasticity suggests that that leatherback hatchling sex ratios may be more adaptable to environmental shifts (Rogers 2013) than Florida's green and loggerhead nesting stocks. The leatherback sex ratios were based upon fewer animals and fewer nests than the other Floridian species. Consequently, additional seasons of data may improve model selection.

The variation in model selection results highlights the complexity of modeling TSD responses under natural conditions. The loggerhead dataset used in this study is large and broadly scaled both temporally and spatially, yet selection between programs did not agree and all model fits were poor. The Floridian loggerhead data are highly female-biased and even the coolest temperatures measured are near or slightly below published PTs and those lower temperatures frequently resulted in highly female-biased samples. The lack of clearly male-producing temperatures limits the models' ability to predict sex ratio outcomes below the PT and thus increases the possible model outcomes.

The green turtle dataset contains a small but higher percentage of male-dominated nest samples than the loggerhead dataset, allowing for a better curve fit at lower temperatures. However, the dataset is still relatively small so the curve is fit to fewer data points. Further, in loggerheads the relatively large variation in temperatures that produced the same sex ratio makes model fitting more challenging than for the green turtles' often tighter responses (Fig. 2–1 compared with Fig. 2–4).

Many of the nests in the early years (≤2006) of this study were outfitted with data loggers (Onset H8 series) that are accurate to ±1.5°C in the temperature ranges that are typical of incubating sea turtles nests. In nests incubating within or near the limits of the TRT, the potential for a precision error may account for some of the variation found in this study.

Laboratory vs. field approaches.— Laboratory incubation studies have proved valuable in defining TSD response patterns (Ia, Ib and II; Ewert & Nelson 1991; Ewert et al. 1994) and standardizing terminology (PT and TRT; Yntema & Mrosovsky 1982; Mrosovsky 1988; Mrosovsky & Pieau 1991). However, PT and TRT temperature estimates obtained from laboratory studies clearly have limited use in predicting sex ratios from naturally incubated nests. Nest temperatures are influenced by many different variables (e.g. Standora & Spotila 1985; Godfrey & Mrosovsky 1999; Hays et al. 2001; Birchard 2004; Houghton et al. 2007; Leblanc & Wibbels 2009) interacting to create the thermal environment. Such interactions make it nearly impossible to mimic natural conditions in the lab. For example, in a split-clutch design using TSD freshwater red-eared sliders, Paitz et al. (2010) found the half clutches incubated in the lab produced significantly more females than their field-incubated counterparts. Even the level of variation introduced by experiments using fluctuating temperatures is stable and predictable, unlike nature.

Loggerhead turtles TRT and PT estimates. — The loggerhead data suggest the TRT is wider than previous reports. Blair (2005) estimated the U.S. Southeast Atlantic loggerhead TRT between 26.5^{o}C and 32^{o}C (a 5.5^{o}C span). Mrosovsky's (1988) TRT estimate from the same population, but smaller sample, is <27.5–30.5^{o}C (less than a 3^{o}C span). My mean TSP temperature models estimate the upper TRT bound =30.4–31.1^{o}C. My range is generally consistent with Mrosovsky's upper bound estimate but is 0.9–1.6^{o}C lower than Blair's estimate (Fig. 2–7). However, the full range of TSP temperatures experienced by mixed-sex nests was 25.6–34.9^{o}C, covering 9.3^{o}C. The mean temperatures have a 6.6^{o}C span (27.5–34.1^{o}C). Blair's TRT estimate spans 5.5^{o}C and Mrosovsky's spans >3^{o}C. Blair's TRT, sampled from naturally incubated nests, was calculated using mean nest temperatures of the middle third of development. My data too were field-based and the raw data she used were included in my study. Although my overall sample and geographic scope size is smaller than Blair's, my data span more nesting seasons and thus provide a more robust representation of sex ratio response over time.

To my knowledge no other field-based studies have investigated whether minimum, maximum and mode TSP temperatures are candidates for describing nest temperature–sex ratio response relationships. Analyses of loggerhead and sex ratio data in R identified the maximum temperature descriptor of the 50-65% TSP as the best fit model. The model estimate for the upper TRT bound was 33.3^{o}C, but the lower bound was unsupported due to lack of male data. Using the coolest, maximum temperature nest producing at least some males as a lower bound and the upper bound from the model, the maximum temperature TRT spans 5.4^{o}C, which is consistent with Blair's (2005) TRT. Empirical measures of the mixed sex nest maximum 50-65% TSP temperatures spanned 7.7^{o}C span and ranged 27.9–35.6^{o}C, also suggesting that previous TRT estimates likely underestimate the South Florida loggerhead TRT width. The maximum 50-65% TSP model estimates a PT of 28.9^{o}C.

The loggerhead PT estimates using the mean temperature middle third TSP models range 27.6–29.1^{o}C. The range is lower than previously reported PTs in the western Atlantic population. Yntema and Mrosovsky (1982) reported the loggerhead PT at 30.0^{o}C in Georgia nests and Mrosovsky (1988) documented 29.2^{o}C for Florida loggerheads. Both were from laboratory studies in which eggs were incubated at a series of set temperatures. The Georgia study was designed to identify the TSP and provided the first loggerhead TRT and PT estimates from eggs incubated at constant temperatures over 2^{o}C intervals spanning 26–34^{o}C. The Florida eggs were individually incubated over a narrower range to better resolve the PT (temperatures were set at 27.5–30.5^{o}C, 0.2–0.5^{o}C apart). The temperature ranges in my study involved whole clutches that experienced a limited range of temperatures so while comparisons identify that my ranges exceeded those of both studies, the conditions are substantially different. Blair's (2005) field-based study of Atlantic coast loggerheads (11 beaches) did not directly estimate the PT but reported 29–30^{o}C as the temperature range that had maximum effect on changes in sex ratio; sex ratios at 29^{o}C ranged from 0.2–1.0 in 2002 and 0.0–1.0 at 30^{o}C in 2003.

Although the estimated PT range in my study is lower, nests with TSP means within the PT range gave similarly wide sex ratios (*F*=0–1), but the majority of these nests were female-biased. The female bias and wide variation in sex response around the PT in natural nests highlights the importance of field-based data. My study's estimates reflect the temperature–sex ratio response of South Florida loggerhead hatchlings, and include the influences of multiple natural variables that likely affect incubation temperatures collectively.

The male-weighted loggerhead curve's TRT (28.3^{o}C lower bound and 30.4^{o}C upper bound) is narrower than Blair's findings, but wider than Mrosovky's. The male-weighted PT of 29.4^{o}C is consistent with previous reports. Although the male-weighted model is consistent with lab-based studies and Blair's (2005) findings, its value has only demonstrative purposes. Across all years that loggerheads were sampled, just two nest samples were documented with $F<0.5$: one from Sarasota 2002 at 0.2*F* (n=10 hatchlings) and one fully male nest (0.0*F*, n=10 hatchlings) from Boca Raton 2012 (Fig. 2–1).

The lack of male-biased loggerhead sample nests is not likely an artifact of sampling bias. Sampling biases generally reflect nest washouts from storms, usually in the early and late part of the season or losses due to predators. Both types of sample loss do have the potential to be male-biased: nests that are laid nearer to the high tide line are likely cooler due to higher moisture content in the sand (Foley et al. 2000) and predator attacks are typically more frequent near the dune vegetation (personal observation) where nests are often shaded (Mrosovsky et al. 1995; Schmid et al. 2008). Arguably, the losses of these nests from the sample are not a bias because they are less likely to contribute to the population. The loggerhead dataset is large enough, covering 7 nesting beaches over

5 seasons with nearly 1000 hatchlings of verified sex, that such losses are likely representative of the overall loss from the sampled beaches.

The 10% sample is not an adequate sample to characterize the nest as a whole, based upon binomial probabilities alone (Zar 2010). The sex ratios are instead consistently sampled sex ratios of eggs representing those beaches. The bias represented in those samples is likely to vary across the season as the expectation of eggs in the hottest parts of the season becoming male hatchlings is not represented by a typical binomial distribution.

Green turtles TRT and PT estimates. — This study provides the first TRT or PT estimates for Florida green turtles. The green turtle nests sampled in this study were all *in situ* nests that together produced mixed sex ratios spanning 7.5°C over 27.4–34.9°C during the middle third TSP, or spanning 6.7°C over 28.6–35.3°C from the 50-65% TSP. In the Caribbean, green turtles from Tortuguero, Costa Rican have a TRT range of 28–30.5°C, with a 2.5°C span (Morreale et al. 1982; Morreale 1983). The 1982 study was hatchery-based with 5 nests each in controlled zones of the hatchery designated as: cold (100% shaded), cool (50% shade) and warm (full sun) that produced both sexes, with progressively more females in the warm section. The 1983 study was field-based.

Model TRT estimates for the South Florida population were relatively consistent. The three models spanned 3.2°C or 3.3°C (R model maximum middle third TSP 30.9–34.1°C; TSD program maximum TSP model 30.5–33.7°C and mean TSP model 29.5–32.8°C). Field measures indicate that Florida green turtles appear to have a wider TRT than is reported for other populations. The criteria defining the TRT in this study were slightly different from those used in other studies and set such that no mixed sex samples were below or above the lower and upper bounds, respectively. Morreale (1983) and Morreale et al. (1982) used TRT cutoffs of 90-100% males on the cool end and 94-100% females on the warm end and so may have underestimated the Costa Rican green turtle TRT.

Modeled green turtle PT estimates from mean TSPs ranged 31.2–31.8^{o}C. Broderick et al. (2000) estimated the Mediterranean green turtle PT<29.2^{o}C using dead-in-nest samples taken from naturally incubated nests. They were not able to pinpoint a discrete PT value due to the lack of males in their data. Even so, their estimated PT is lower than the PT range I found among South Florida's green turtle models. The Broderick et al. (2000) estimate may be reasonable; their samples were from Cyprus which is at a more northerly latitude (35^{o}N) than any of the South Florida's nesting beaches (25–29^{o}N). The range of mean TSP temperatures in Cyprus nests (29.8–32.5^{o}C) is within the range found in South Florida (27.4–35.3^{o}C). It is possible that the green turtle nest temperature–sex ratio response is slightly left-shifted at more temperate sites.

Leatherback turtles TRT and PT estimates. — The leatherback TRT could not be determined from the small sample size. Mixed sex nests occurred over the narrowest TSP range 26.6–32.4^{o}C, covering 5.8^{o}C. Mean TSP temperatures spanned 3.2^{o}C (range 28.3–31.5^{o}C). Comparable leatherback TRT estimates are more narrow than measured here, spanning just 1^{o}C, in French Guiana and Suriname (28.75–29.75^{o}C; Rimblot et al. 1985) and Pacific Costa Rica (29.0–30.0^{o}C; Binckley et al. 1998). Both of those studies were laboratory based. My field-based data suggest the Florida leatherback TRT may be wider than those of the Eastern Pacific and Southern Caribbean populations. However, leatherback sample size likely does not yet represent a robust spatial and temporal sample. Leatherback nests usually have fewer, larger eggs than cheloniid clutches (Tucker & Frazer 1991; Stewart & Johnson 2006) and low emergence success (Spotila et al. 1996; Bell 2004; Perrault et al. 2011), resulting in a lower number of sampled hatchlings. Additionally, rearing of captive leatherback hatchlings is challenging and expensive such that the number of animals available for laparoscopic verification is very limited.

Leatherback PT estimates from the mean temperature example models (middle third and 50-65% TSPs, Tables 2–3 and 2–4) range 30.9–31.4^{o}C. This estimate is warmer than the lab-based estimates from Pacific Costa Rica (29.4^{o}C; Binckley et al. 1998) and Atlantic French Guiana (29.5^{o}C; Rimblot-Baly et al. 1987). The warmer PT range in naturally incubated South Florida nests may imply leatherbacks have undergone local adaptation. The Florida data also includes potential effects of metabolic heat from eggs clustered together in a clutch.

South Florida's beaches are relatively newly colonized nesting grounds for the leatherback turtle (Stewart & Johnson 2006). They host the northernmost known nesting assemblage of leatherback turtles in the world (Mast et al. 2006) at 25–29^{o}N (sample nests from Boca Raton were located at approximately 26^{o}N). The only other leatherback nesting assemblage that is comparable in latitude is that of the southern extreme in iSimangaliso Wetland Park, South Africa where nesting occurs between 28–29^{o}S. To my knowledge, no reports of the nest temperature–sex ratio response in South African leatherbacks are available. Even if PTs among nesting sites are not likely to differ

(Mrosovsky 1988), it is possible that TRT widths vary. Florida’s subtropical location may select for a wider range in TSP incubation temperatures than at other, more equatorial, nesting sites. South Florida’s leatherback nests undergo a climatic transition (Winsberg 2012) during development. They are typically deposited in the dry late winter/early spring months but continue to incubate into, and hatch during, the hot, wet tropical weather of the summer season. Shifts in temperature associated with climatic transitions such as happens in South Florida could drive selection to widen the TRT, ensuring mixed sex production. Naturally incubated nest temperature–sex ratio response studies that are representative of the full distribution of leatherback nesting beaches are needed.

Limitations of the Models. — Creation of mathematical models to approximate a natural response is possible, but mathematics do not necessarily ensure that the model predictions are biologically sound. A normal, individual embryo’s sex is a binomial male or female response. However, the developmental physiological mechanism that determines sex in TSD species is not a simple on/off switch. Neuwald and Valenzuela (2011) found that sex in red-eared sliders (a freshwater TSD turtle species) can be reversed from the expected outcome at 100% female- and male-producing mean temperatures if variation around the mean is high. Temperature initiates the sex directing response but the cascades of developmental signals and processes that define gonadal differentiation are complicated and still not largely understood. Known influences in gonadal differentiation include aromatase levels (Crews et al. 1991; Desvages et al. 1993; Pieau et al. 1999; Pieau & Dorizzi 2004; Wibbels et al. 2005; Ramsey & Crews 2009) and gene expression (e.g., AMH, DAX, SF, SOX, and WT1; Pieau et al. 1999; Fleming et al. 1999; Shoemaker & Crews 2009). Additionally, there may be a combined effect of temperature and genetic influences that direct the developmental cascade. For example, in Mrosovsky's 1988 constant temperature study on loggerhead PTs, Clutch G consistently produced more males than the other clutches, and the response held even at typically all-female producing temperatures.

Summary. — Sex ratio estimates are important measures that are increasingly being incorporated in demographic models of imperiled marine turtles. Understanding of sex ratio trends helps to estimate the current and future reproductive potential of a population. My study provides relatively long-term and verified data that estimate the nest temperature–sex response relationship from naturally incubated nests of South Florida's loggerhead, green and leatherback nesting assemblages. It further develops understanding of responses and their descriptive parameters in Florida's loggerhead and green turtle nesting population. Simultaneously, my analyses challenge our understanding of field-derived (real-world) sex ratios. When direct sex identification methods are not feasible, the fitted model curves should become useful tools to estimate hatchling sex ratios of loggerhead and green turtles from nest or sand temperatures. However, this study identifies a great deal of variation in responses at given temperatures and inconsistencies in metrics that might be used. At this time, the Florida leatherback model is in need of further data.

This study identifies that sex ratio responses measured in laboratory-based studies are not representative of field-based sex ratio–temperature responses. This discovery highlights that we understand just a portion of marine turtle environmental sex determination under natural incubation conditions and extrapolation from the lab is quite limited.

CHAPTER 2 TABLES

Model	Temperature range (^{o}C)	Accuracy (^{o}C)	Resolution (^{o}C)
HOBO® H08-001-02	-20–70	1.5 over 20–40^{o}	0.4 at 21^{o}
HOBO® H08-002-02	-20–70	1.5 over 20–40^{o}	0.4 at 21^{o}
TidbiT UTBI-001	-20–70	0.2 over 20–40^{o}	0.02 at 25^{o}
HOBO® U22-001	-40–70	0.2 over 20–40^{o}	0.02 at 25^{o}
VEMCO® Minilog	-30–>80	0.2 over -30–40^{o}	0.2 over -30–40^{o}

Table 2–1. Specifications of temperature data loggers used in nests.

Year	Boca Raton			Hutchinson Is.	Juno–Jupiter	Melbourne	Miami	Sanibel Is.	Sarasota
Species	*Cc*	*Cm*	*Dc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*
Overall	0.94(0.03) 26.7-35.6°C n=31	0.76(0.32) 27.4-35.3°C n=19	0.69(0.12) 26.5-32.4°C n=6	---	---	0.87(0.05) 25.6-34.0°C n=10	0.82(0.04) 26.1-34.1°C n=13	0.88(0.04) 26.0-35.0°C n=15	---
2002	0.91(0.04) 26.7-35.6°C n=9	---	---	0.85(0.05) 26.7-33.2°C n=8	0.96(0.92) 26.7-34.0°C n=5	0.90(0.05) 25.6-34.0°C n=5	0.79(0.04) 26.1-33.3°C n=11	0.60(0.06) 26.0-32.8°C n=3	0.75(0.07) 26.5-34.9°C n=12
2003	1 31.5-34.9°C n=3	---	---	---	---	0.84(0.10) 25.6-33.2°C n=5	---	---	---
2006	---	---	0.67(0.17) 26.7-31.9°C n=3	---	---	---	---	---	---
2009	---	1 30.3-34.3°C n=2	---	---	---	---	---	---	---
2010	1 29.1-35.2°C n=13	0.84(0.26) 27.4-35.3°C n=10	0.33 30.3-32.3°C n=1	---	---	---	---	0.96(0.04) 26.6-34.2°C n=5	---
2011	1 30.1-35.6°C n=5	0.58(0.40) 30.5-35.1°C n=7	---	---	---	---	1 28.8-34.1°C n=2	0.94(0.04) 26.7-35.0°C n=7	---
2012	0 26.6-31.4°C n=1	---	0.90(0.10) 26.5-32.4°C n=2	---	---	---	---	---	---

Table 2–2. Mean proportion female (SE) and middle third–50-65% TSP range of temperatures by beach and year. *Cc*=*C. caretta*, *Cm*=*C. mydas*, and *Dc*=*D. coriacea*. n=the number of nest sampled. The total hatchlings examined were 992 *Cc* from 93 nests, 172 *Cm* from 20 nests, and 25 *Dc* from 6 nests.

TSP & Model	AIC	Parameters (SE)	Test statistics	PT °C	TRT °C
Caretta caretta					
Middle 1/3 Mean	256.52	Intercept = 13.04 (1.39) Mean = 0.47 (0.05)	$z = -9.41$, $p < 0.001$ $z = 10.15$, $p < 0.001$	27.6	Lower Und. Upper 31.1
50-65% Maximum	252.07	Intercept = -11.27 (1.14) Maximum = 0.39 (0.04)	$z = -9.86$, $p < 0.001$ $z = 10.81$, $p < 0.001$	28.9	Lower Und. Upper 33.3
Chelonia mydas					
Middle 1/3 Mean	65.78	Intercept = -28.75 (4.10) Mean = 0.93 (0.13)	$z = -7.014$, $p < 0.001$ $z = 7.123$, $p < 0.001$	30.8	Lower 29.1 Upper 32.6
Middle 1/3 Maximum	56.91	Intercept = -33.67 (4.68) Maximum = 1.04 (0.14)	$z = -7.199$, $p < 0.001$ $z = 7.289$, $p < 0.001$	32.5	Lower 30.9 Upper 34.1
Dermochelys coriacea					
Middle 1/3 Mean	16.57	Intercept = 17.59 (8.37) Mean = -0.57 (0.28)	$z = 2.10$, $p = 0.036$ $z = -2.04$, $p = 0.041$	30.9	Und.
Middle 1/3 Minimum	15.07	Intercept = 17.82 (7.67) Minimum = -0.61 (0.27)	$z = 2.33$, $p = 0.020$ $z = -2.26$, $p = 0.024$	29.2	Und.
50-65% Mean	15.90	Intercept = 22.07 (10.18) Mean = -0.71 (0.33)	$z = 2.17$, $p = 0.030$ $z = -2.12$, $p = 0.034$	31.1	Und.
50-65% Minimum	14.25	Intercept = 33.41 (14.0) Minimum = -1.13 (0.48)	$z = 2.39$, $p = 0.017$ $z = -2.35$, $p = 0.019$	29.5	Und.

Table 2–3. Selected model parameters, PT and TRT upper and lower bound estimates from maximum likelihood logistic regression in R. TSPs are the middle third and 50-65% of total incubation rounded to the nearest day. Models are based on the TSP temperature descriptors minimum, maximum, mean, and the number of incubation days (lay date–date of first emergence). Undetermined=Und.

Model	AIC	Parameters (SE)	Test statistics	Inflection day	Transition period (days)
Days of incubation	15.87	Intercept = -13.34 (6.80) Incubation = 0.22 (0.11)	z = -1.96, *p* = 0.0498 z = 2.04, *p* = 0.0413	62.0	Lower 54.3 Upper 69.6

Table 2–4. *D. coriacea* sex ratio model based upon days of incubation (lay date–date of first emergence). Model parameters and SE, inflection day (where sex ratio shifts from female-biased to male-biased) and transitional period upper and lower bound estimates from maximum likelihood logistic regression in R.

TSP & Model	AIC	Parameters (SE)	Likelihood (-ln)	PT °C (SE)	TRT °C
Caretta caretta					
Middle 1/3 Mean Type Ia, Hill	121.09	*S*= -0.0162 (0.0) *K*= 0.1	L= 57.55	29.0 (0.0)	Lower Und. Upper 30.4
Middle 1/3 Mean Type Ia, Weibull	121.80	*S*= -0.0318 (0.0)	L= 57.90	29.1 (0.0)	Lower Und. Upper 30.4
Chelonia mydas					
Middle 1/3 Max Type Ia, Weibull	23.11	*S*= -0.0243 (0.0)	L= 8.56	32.5 (0.0)	Lower 30.5 Upper 33.7
Middle 1/3 Mean Type Ia, Weibull	23.21	*S*= -0.0214 (0.0)	L= 8.66	31.2 (0.0)	Lower 29.5 Upper 32.3
50-65% Max Type Ia, Weibull	23.03	*S*= -0.0224 (0.0)	L= 8.52	32.6 (0.0)	Lower 30.8 Upper 33.7
50-65% Mean Type Ia, Weibull	23.32	*S*= -0.0208 (0.002)	L= 8.72	31.8 (0.04)	Lower 30.1 Upper 32.8
Dermochely coriacea					
Middle 1/3 Max Type Ia, Hill	13.70	S= -0.0046 (0.004) *K*= 0.1	L= 3.85	30.8 (0.02)	Und.
Middle 1/3 Max Type Ia, Weibull	13.50	*S*= -0.0069 (0.0)	L= 3.73	30.8 (0.0)	Und.
50-65% Min Type Ib, Weibull	13.33	*S*= 0.0530 (0.0)	L= 3.67	30.6 (0.0)	Und.
50-65% Mean Type Ib, Weibull	13.33	*S*= 0.0478 (0.0)	L= 3.67	31.4 (0.0)	Und.

Table 2–5. Selected model parameters, PT and TRT upper and lower bound estimates from the TSD software (Girondot 1999; Godfrey et al. 2003) with 1.5°C uncertainty. TSPs are the middle third (Middle1/3) and 50-65% of total incubation rounded to the nearest day. Models are based on the TSP temperature descriptors minimum (Min), maximum (Max), and mean. Type=TSD pattern, followed by fitted equation name. Undetermined=Und.

Comparison	Kruskal-Wallace ($H_{(df)}$) with Tukey-like contrasts (t)	*p* value
	Middle third TSP	
Years	$H_{(3)} = 36.47$	< 0.001
2002 vs. 2003	t = 2.19	0.013
2002 vs. 2010	t = 6.17	< 0.001
2002 vs. 2011	t = 4.62	< 0.001
2010 vs. 2011	t = 1.50	0.046
Years: BR only	$H_{(3)} = 14.90$	0.002
2002 vs. 2003	t = 18.02	< 0.001
2002 vs. 2010	t = 3.98	< 0.001
2002 vs. 2011	t = 2.60	0.048
Beaches	$H_{(6)} = 29.29$	< 0.001
BR vs. HI	t = -3.54	0.007
BR vs. MB	t = -3.98	0.002
BR vs. ME	t = -4.58	< 0.001
BR vs. SA	t = -3.46	0.009
	50-65% TSP	
Years	$H_{(3)} = 34.49$	< 0.001
2002 vs. 2003	t = 1.72	0.033
2002 vs. 2010	t = 6.48	< 0.001
2002 vs. 2011	t = 3.37	< 0.001
2003 vs. 2010	t = 1.61	0.040
2010 vs. 2011	t = -1.63	0.039
Years: BR only	$H_{(3)} = 15.498$	0.001
2002 vs. 2003	t = 18.02	< 0.001
2002 vs. 2010	t = 4.01	< 0.001
Beaches	$H_{(6)} = 23.75$	0.001
BR vs. HI	t = -3.45	0.009
BR vs. MB	t = -3.64	0.005
BR vs. ME	t = -4.17	0.001

Table 2–6. *C. caretta* mean TSP temperatures significant yearly and beach comparison statistics. $H_{(df)}$=Kruskall-Wallis test statistic; t = nonparametric Tukey-like test statistic. Beach codes are BR=Boca Raton, HI=Hutchinson Island, MB=Miami Beach, ME=Melbourne and SA=Sarasota.

Comparison	Kruskal-Wallace ($H_{(df)}$) with Tukey-like contrasts (t)	*p* value
	Middle third TSP	
Years	$H_{(3)} = 36.96$	< 0.001
2002 vs. 2003	t = 1.55	0.043
2002 vs. 2010	t = 6.07	< 0.001
2002 vs. 2011	t = 5.34	< 0.001
2003 vs. 2010	t = 1.505	0.045
Years: BR only	$H_{(3)} = 15.82$	0.001
2002 vs. 2003	t = 18.02	< 0.001
2002 vs. 2010	t = 3.49	0.003
2002 vs. 2011	t = 3.00	0.015
Beaches	$H_{(6)} = 26.49$	< 0.001
BR vs. HI	t = -4.23	< 0.001
BR vs. MB	t = -3.51	0.008
BR vs. ME	t = -3.74	0.004
HI vs. SAN	t = 3.01	0.043
	50-65% TSP	
Years	$H_{(3)} = 38.415$	< 0.001
2002 vs. 2003	t = 1.855	0.026
2002 vs. 2010	t = 6.63	< 0.001
2002 vs. 2011	t = 3.956	< 0.001
2003 vs. 2010	t = 1.605	0.040
Years: BR only	$H_{(3)} = 16.20$	0.001
2002 vs. 2003	t = 18.02	< 0.001
2002 vs. 2010	t = 4.268	< 0.001
2002 vs. 2011	t = 2.996	0.015
Beaches	$H_{(6)} = 26.82$	< 0.001
BR vs. HI	t = -4.11	0.001
BR vs. MB	t = -3.68	0.004
BR vs. ME	t = -4.17	0.001
HI vs. SAN	t = 2.80	0.076

Table 2–7. *C. caretta* maximum TSP temperatures significant yearly and beach comparison statistics. $H_{(df)}$=Kruskall-Wallis test statistic; t=nonparametric Tukey-like test statistic. Beach codes are BR=Boca Raton, HI=Hutchinson Island, MB=Miami Beach, ME=Melbourne and SAN=Sanibel Island.

Comparison	Kruskal-Wallace ($H_{(df)}$) with Tukey-like contrasts (t)	*p* value
	Middle third TSP	
Years	$H_{(3)} = 23.67$	< 0.001
2002 vs. 2010	t = 4.29	< 0.001
2002 vs. 2011	t = 3.43	0.003
Years: BR only	$H_{(3)} = 15.67$	0.001
2002 vs. 2003	t = 18.02	< 0.001
2002 vs. 2010	t = 3.42	0.004
2002 vs. 2011	t = 3.39	0.004
Beaches	$H_{(6)} = 25.38$	< 0.001
BR vs. HI	t = -3.20	0.021
BR vs. MB	t = -3.81	0.002
BR vs. ME	t = -3.96	0.001
BR vs. SA	t = -3.43	0.010
BR vs. SAN	t = -3.57	0.006
	50-65% TSP	
Years	$H_{(3)} = 29.74$	< 0.001
2002 vs. 2003	t = 1.67	0.035
2002 vs. 2010	t = 5.52	< 0.001
2002 vs. 2011	t = 2.91	0.002
2003 vs. 2011	t = 1.62	0.039
2010 vs. 2011	t = -1.67	0.035
Years: BR only	$H_{(3)} = 12.04$	0.007
2002 vs. 2003	t = 2.655	0.038
2002 vs. 2010	t = 3.23	0.006
Beaches	$H_{(6)} = 27.97$	< 0.001
BR vs. HI	t = -3.38	0.011
BR vs. JU	t = -3.15	0.025
BR vs. MB	t = -3.66	0.004
BR vs. ME	t = -4.35	0.001
BR vs. SA	t = -3.52	0.007
BR vs. SAN	t= -3.38	0.012

Table 2–8. *C. caretta* minimum TSP temperatures significant yearly and beach comparison statistics. $H_{(df)}$=Kruskall-Wallis test statistic; t=nonparametric Tukey-like test statistic. Beach codes are BR=Boca Raton, HI=Hutchinson Island, JU=Juno–Jupiter, MB=Miami Beach, ME=Melbourne, SA=Sarasota and SAN=Sanibel Island.

Comparison	Kruskal-Wallace ($H_{(df)}$) with Tukey-like contrasts (t)	*p* value
	Middle third TSP	
Years	$H_{(3)} = 23.535$	< 0.001
2002 vs. 2003	t = 1.47	0.047
2002 vs. 2010	t = 5.44	< 0.001
2002 vs. 2011	t = 2.32	0.009
2003 vs. 2010	t = 1.657	0.036
Years: BR only	$H_{(3)} = 11.22$	0.011
2002 vs. 2003	t = 18.02	< 0.001
2002 vs. 2010	t = 3.24	0.006
Beaches	$H_{(6)} = 26.56$	< 0.001
BR vs. MB	t = -3.61	0.006
BR vs. ME	t = -4.856	< 0.001
ME vs. SAN	t = 3.26	0.018
	50-65% TSP	
Years	$H_{(3)} = 25.596$	< 0.001
2002 vs. 2010	t = 6.38	< 0.001
2002 vs. 2011	t = 1.466	0.048
2003 vs. 2010	t = 1.666	0.035
2010 vs. 2011	t = -1.765	0.030
Years: BR only	$H_{(3)} = 13.50$	0.004
2002 vs. 2003	t = 3.49	0.003
2002 vs. 2010	t = 3.95	< 0.001
Beaches	$H_{(6)} = 17.40$	< 0.001
BR vs. MB	t = -3.27	0.017
BR vs. ME	t = -3.51	0.008

Table 2–9. *C. caretta* modal TSP temperatures significant yearly and beach comparison statistics. $H_{(df)}$=Kruskall-Wallis test statistic; t=nonparametric Tukey-like test statistic. Beach codes are BR=Boca Raton, MB=Miami Beach, ME=Melbourne and SAN=Sanibel Island.

Comparison	Kruskal-Wallace ($H_{(df)}$) with Tukey-like contrasts (t)	*p* value
Years	$H_{(3)} = 15.26$	0.002
2002 vs. 2010	t = -4.13	< 0.001
Beaches	$H_{(6)} = 15.21$	0.019
BR vs. ME	t = 3.79	0.003

Table 2–10. *C. caretta* yearly and beach comparisons of days of incubation that differed significantly. $H_{(df)}$=Kruskall-Wallis test statistic; t=nonparametric Tukey-like test statistic. Beach codes are BR=Boca Raton and ME=Melbourne.

Comparison	Kruskal-Wallace ($H_{(df)}$) with Tukey-like contrasts (t)	*p* value
Incubation days	$H_{(2)} = 5.59$	< 0.001
Dc vs. *Cm*	t = 24.84	< 0.001
Dc vs. *Cc*	t = 4.982	< 0.001
	Middle third TSP	
Mean	$H_{(2)} = 8.25$	0.016
Dc vs. *Cm*	t = -3.10	0.005
Dc vs. *Cc*	t = -3.50	0.002
Maximum	$H_{(2)} = 11.45$	0.003
Dc vs. *Cm*	t = -3.49	0.001
Dc vs. *Cc*	t = -4.74	< 0.001
Minimum	$H_{(2)} = 7.01$	0.030
Dc vs. *Cm*	t = -3.06	0.007
Dc vs. *Cc*	t = -2.48	0.037
Mode	$H_{(2)} = 6.96$	0.031
Dc vs. *Cm*	t = -2.65	0.023
Dc vs. *Cc*	t = -3.40	0.002
	50-65% TSP	
Mean	$H_{(2)} = 8.36$	0.015
Dc vs. *Cm*	t = 3.66	0.001
Dc vs. *Cc*	t = -3.61	0.001
Maximum	$H_{(2)} = 10.74$	0.005
Dc vs. *Cm*	t = -3.65	< 0.001
Dc vs. *Cc*	t = -4.62	< 0.001
Minimum	$H_{(2)} = 7.54$	0.023
Dc vs. *Cm*	t = -3.75	< 0.001
Dc vs. *Cc*	t = -3.127	0.005

Table 2–11. Significant species comparisons statistics for the number of incubation days and mean, maximum, minimum and mode TSP descriptors. *Cc*=*C. caretta*, *Cm*=*C. mydas* and *Dc*=*D. coriacea*. The all-species comparisons were from Boca Raton alone.

CHAPTER 2 FIGURES

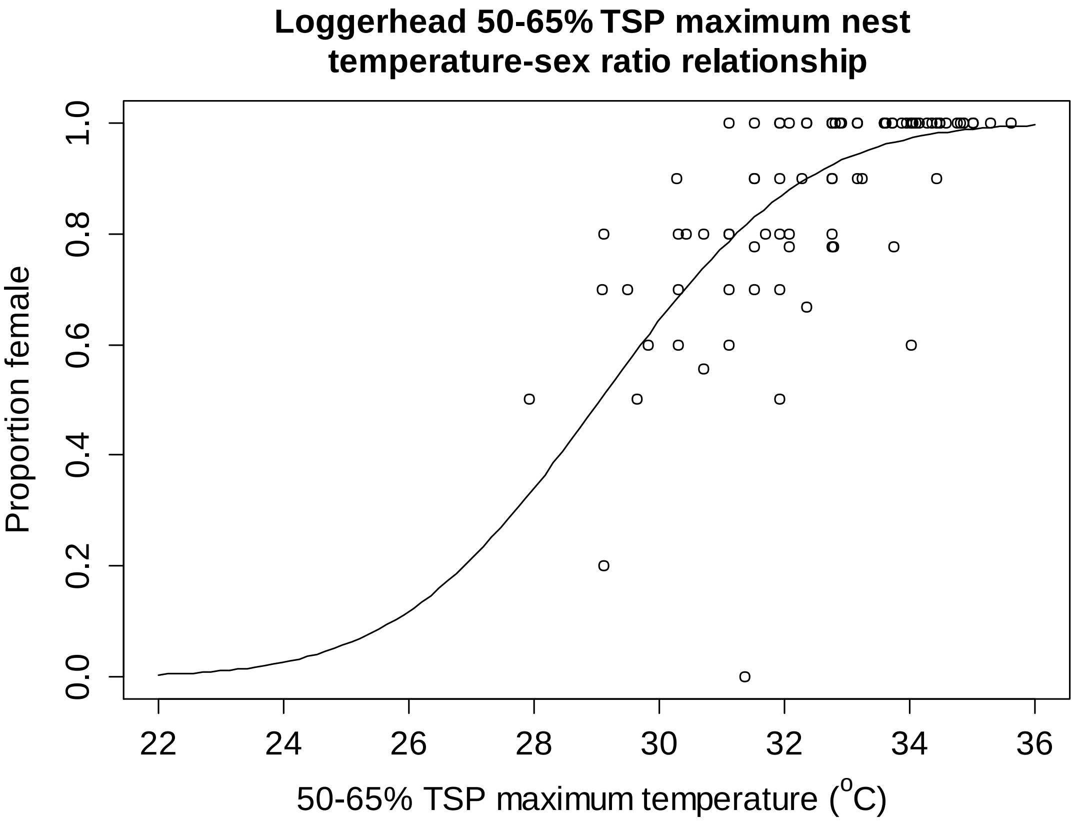

Figure 2–1. Predicted loggerhead (*C. caretta*) 50-65% TSP maximum temperature–sex ratio response curve.

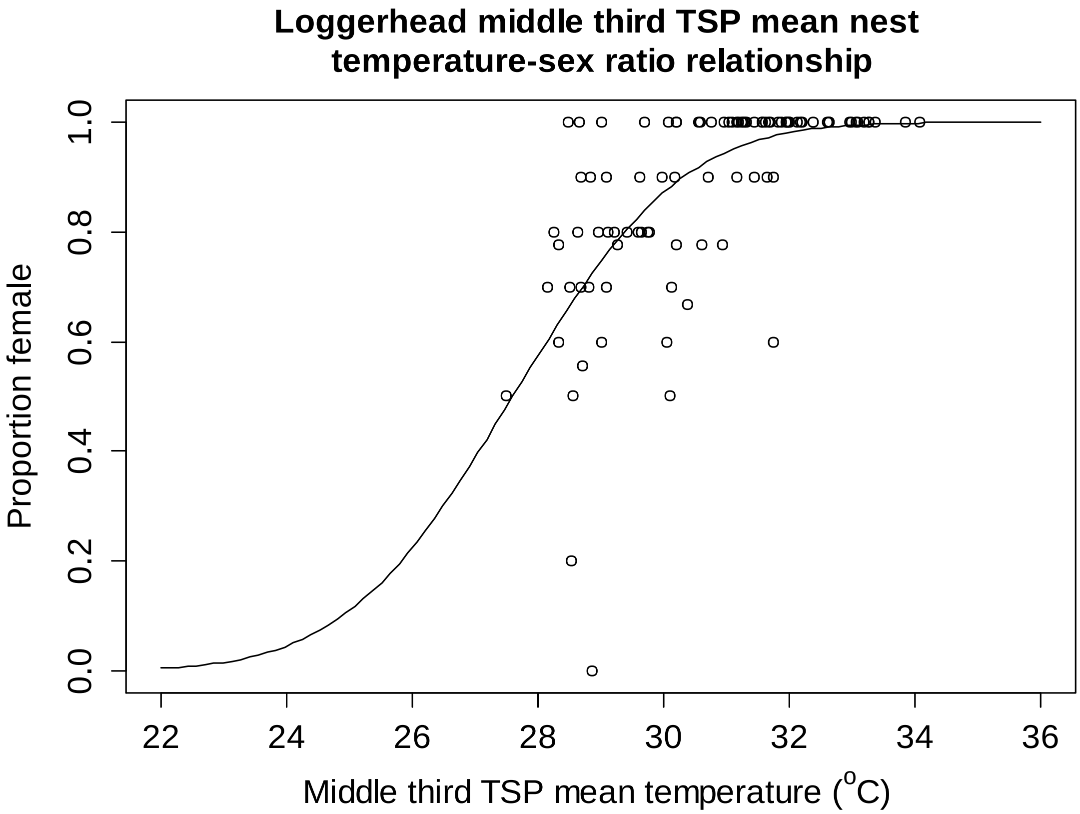

Figure 2–2. Predicted loggerhead (*C. caretta*) middle third TSP mean temperature–sex ratio response curve.

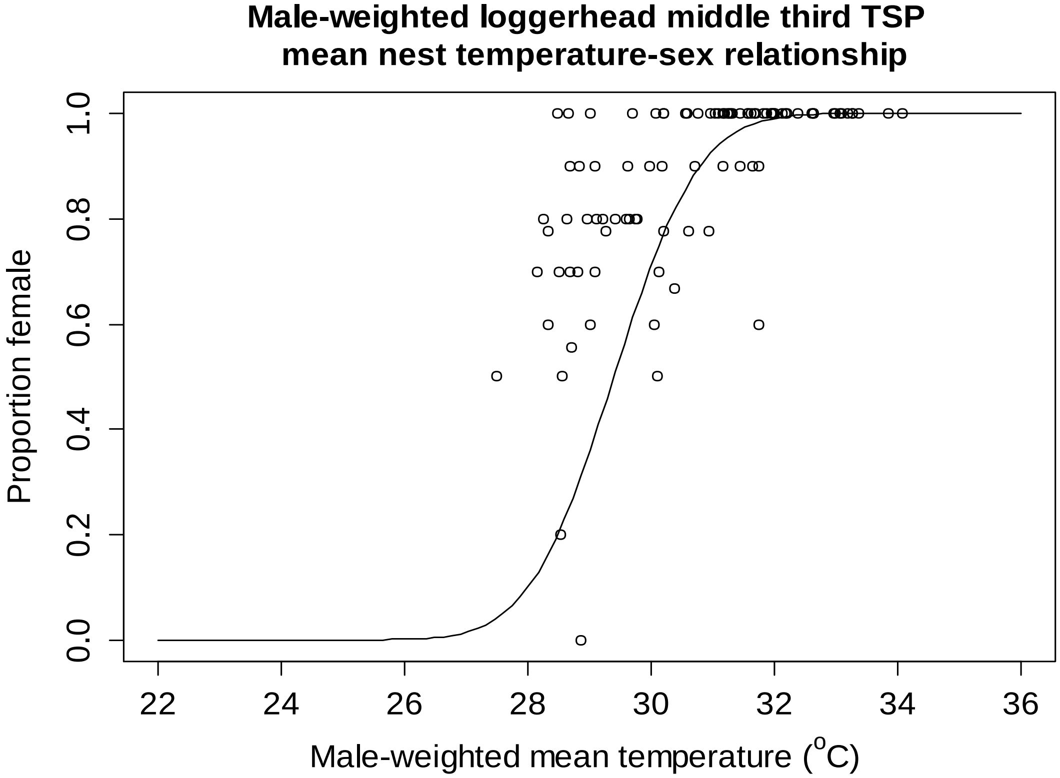

Figure 2–3. Male-weighted loggerhead (*C. caretta*) middle third mean nest temperature–sex ratio response curve.

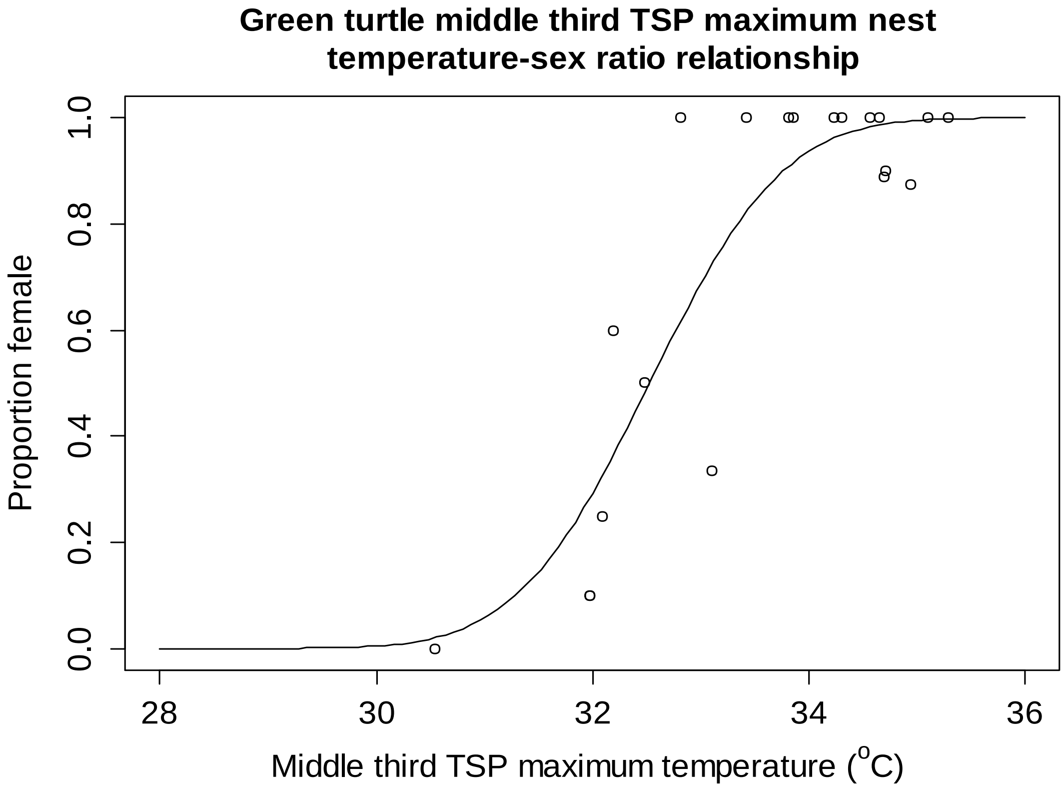

Figure 2–4. Green turtle (*C. mydas*) middle third maximum nest temperature–sex ratio response curve.

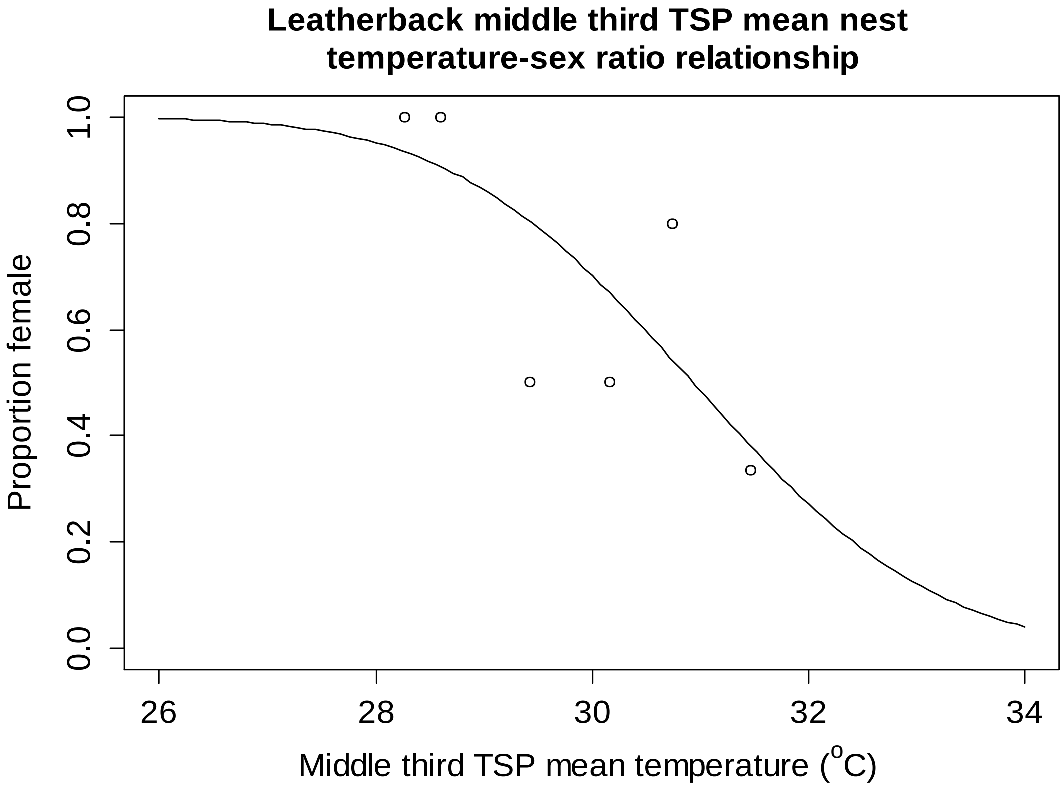

Figure 2–5. Leatherback (*D. coriacea*) middle third mean nest temperature–sex ratio response curve.

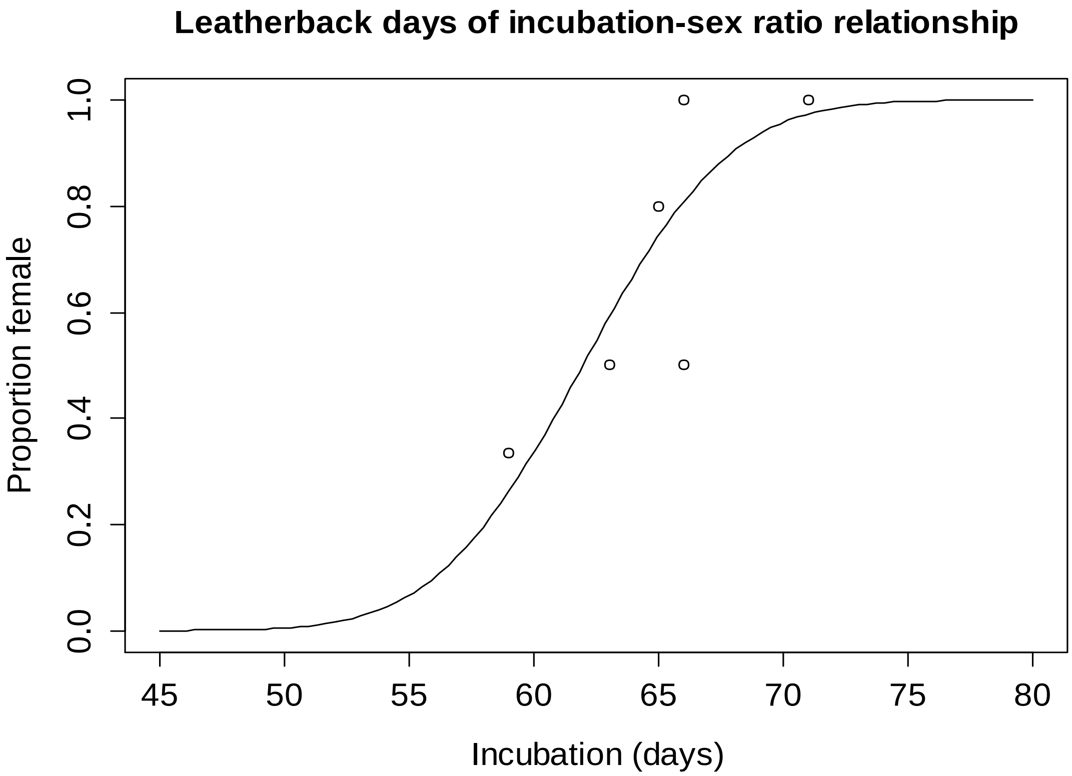

Figure 2–6. Leatherback (*D. coriacea*) incubation days-sex ratio response curve.

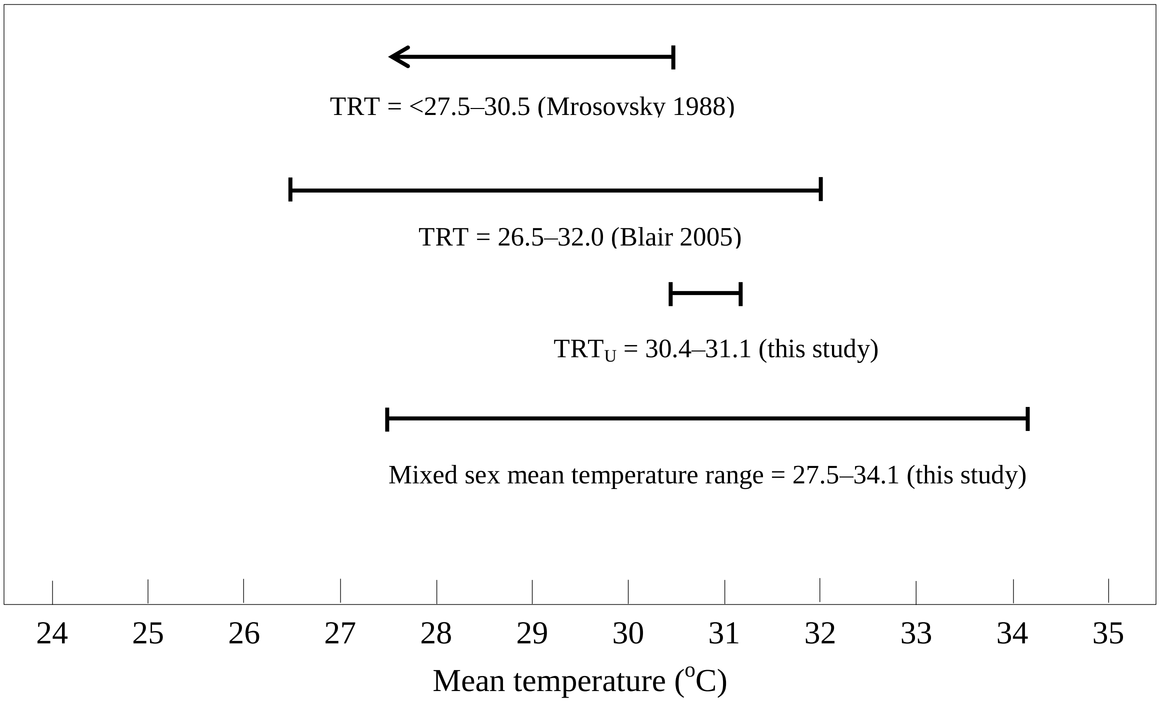

Figure 2–7. Diagrammatic comparison of loggerhead TRT estimates from the literature and this study with the range of mean nest temperatures that produced mixed-sex nests. TRT$_U$=upper bound of the TRT.

APPENDIX

Model	*Caretta caretta*		*Chelonia mydas*		*Dermochelys coriacea*	
Days of incubation	282.71		83.477		15.87	
	TSP temperature descriptors					
TSP	Middle 1/3	50-65%	Middle 1/3	50-65%	Middle 1/3	50-65%
Min	287.60	312.05	80.68	79.84	15.07	14.25
Max	262.33	252.07	56.91	65.18	16.87	17.57
Mean	256.52	265.88	65.78	69.82	16.57	15.90
Mode	326.49	288.86	97.52	100.61	19.51	19.18

A–1. R model AIC values. Estimated TSP periods are the middle third (Middle 1/3) and 50-65% of incubation length (rounded to the nearest full day).

	Caretta caretta	*Chelonia mydas*	*Dermochelys coriacea*
Genotypic sex determination			
	1066.8910	201.2207	22.0742
Days of incubation			
Model equation			
Richards	430.9302	167.1807	30.0742
Hill	406.1695	162.2776	18.0220
Hill*	1061.0280	1904.9500	317.7788
Weibull	396.8482	160.3616	17.6817
Weibull*	398.4862	162.2712	20.2987

A–2. TSD program genotypic sex determination and days of incubation AIC values.

Model	*Caretta caretta*		*Chelonia mydas*		*Dermochelys coriacea*	
TSP	Middle 1/3	50-65%	Middle 1/3	50-65%	Middle 1/3	50-65%
Richards equation						
Max	131.5728	139.7434	25.0305	25.1231	15.7151	17.5976
Min	137.4209	178.2895	26.7905	28.0501	17.5976	15.3472
Mean	122.9963	141.2668	25.8946	25.5980	17.5976	15.7344
Mode	169.8594	163.0163	31.2836	31.2468	20.5383	15.6718
Hill equation						
Max	129.9730	141.0244	23.5896	23.8943	13.7130	13.8187
Min	135.5692	181.1153	25.3147	26.0471	22.1582	13.9546
Mean	120.9156	139.4932	23.9051	24.6245	20.5331	14.1545
Mode	168.2007	161.2618	29.2910	29.5347	18.7454	20.3825
Hill* equation						
Max	130.3054	138.9610	24.9967	25.0555	15.8928	15.8010
Min	137.7824	184.1117	26.8999	28.0352	24.2751	15.3899
Mean	123.2424	141.0979	25.8015	25.5463	22.6289	16.1375
Mode	169.4142	162.9059	31.6051	32.0564	20.6848	22.3665
Weibull equation						
Max	126.9361	134.0719	23.1143	23.0340	13.4987	13.5715
Min	137.0695	182.9602	24.0547	26.660	22.2670	13.4501
Mean	121.7989	139.3211	23.3154	23.3190	20.5731	13.7370
Mode	167.8542	161.6958	32.5665	32.3061	18.5866	20.3251
Weibull* equation						
Max	133.0868	151.5986	26.3210	26.3445	15.8434	17.7081
Min	137.1178	181.2064	28.7050	28.3996	24.0858	15.5183
Mean	123.8360	140.3757	26.4556	27.5737	22.5204	16.3539
Mode	170.6056	163.5983	31.5357	31.3249	21.0348	22.3872

A–3. TSD program type Ia TSP temperature descriptor AIC values. All models were analyzed with 1.5^{o}C uncertainty. Estimated TSP periods are the middle third (Middle 1/3) and 50-65% of incubation length (rounded to the nearest full day).

Model	*Caretta caretta*		*Chelonia mydas*		*Dermochelys coriacea*	
TSP	Middle 1/3	50-65%	Middle 1/3	50-65%	Middle 1/3	50-65%
Richards equation						
Max	395.9860	398.1589	88.61308	114.9527	15.3555	15.3596
Min	395.3395	395.7313	139.9060	114.9527	15.3382	15.3325
Mean	390.9737	397.7310	141.2923	150.8094	15.3594	15.3603
Mode	380.6181	397.6619	114.9527	149.1313	15.3402	15.3480
Hill equation						
Max	394.2357	396.3215	159.9735	159.9323	13.3553	13.3473
Min	392.8568	393.5206	159.9850	159.9548	13.3484	13.3402
Mean	388.5578	395.7361	154.4018	150.7156	13.3435	13.3494
Mode	377.7670	395.5999	159.9643	149.5705	13.3928	13.4372
Hill* equation						
Max	395.3084	398.2167	154.8111	150.9466	15.3695	15.3445
Min	396.0708	396.1281	161.2334	153.3074	15.3468	15.3332
Mean	391.6342	397.7083	156.7189	153.9467	15.3389	15.3567
Mode	382.0694	397.8646	158.6776	153.8849	15.3414	15.3331
Weibull equation						
Max	394.6434	396.5797	153.7370	149.6562	13.3352	13.3342
Min	392.5490	393.4427	159.7926	149.0671	13.3314	13.3305
Mean	387.8372	395.9944	153.9099	148.4478	13.3384	13.3336
Mode	376.3497	395.7545	156.5475	146.4459	13.3328	13.3308
Weibull* equation						
Max	396.3015	398.2627	154.9425	150.9788	15.3590	15.3429
Min	394.9751	395.5273	161.6890	152.8261	15.3468	15.3561
Mean	390.6521	397.6756	156.5015	153.3373	15.4234	15.3526
Mode	380.0467	397.5454	158.6024	152.4900	15.3691	15.4902

A–4. TSD program type Ib TSP temperature descriptor AIC values. All models were analyzed with 1.5°C uncertainty. Estimated TSP periods are the middle third (Middle 1/3) and 50-65% of incubation length (rounded to the nearest full day).

Model	*Caretta caretta*		*Chelonia mydas*		*Dermochelys coriacea*	
TSP	Middle 1/3	50-65%	Middle 1/3	50-65%	Middle 1/3	50-65%
			Richards equation			
Max	121.7060	137.2234	31.3119	31.2256	21.3306	21.3304
Min	119.7910	117.2372	31.5899	31.8485	21.3339	21.3311
Mean	116.2959	121.4343	31.3059	31.2851	21.3344	21.3304
Mode	126.9368	121.5543	32.4668	31.5022	21.3304	21.3304

A–5. TSD program type II TSP temperature descriptor AIC values. All models were analyzed with 1.5^{o}C uncertainty. Estimated TSP periods are the middle third (Middle 1/3) and 50-65% of incubation length (rounded to the nearest full day).

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