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Sex Ratios Of Juvenile Green Turtles (chelonia Mydas) In Three Developmental Habitats Along The Coast Of Florida

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SEX RATIOS OF JUVENILE GREEN TURTLES (*CHELONIA MYDAS*) IN THREE DEVELOPMENTAL HABITATS ALONG THE EAST COAST OF FLORIDA

by

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ABSTRACT

The concept of temperature dependent sex determination (TSD) has been somewhat of an evolutionary enigma for many decades and has had increased attention with the growing predictions of a changing climate, particularly in species that are already threatened or endangered. TSD taxa of concern include marine turtles, which go through various life stages covering a range of regions. This, in turn, creates difficulties in addressing basic demographic questions. Secondary sex ratios (from life stages post-hatchling) were investigated by capturing juvenile green turtles (*Chelonia mydas*), 22.6-60.9 cm in straight carapace length (SCL), from three developmental areas along the east coast of Florida (a region known to have important juvenile aggregations) by analyzing circulating testosterone levels. All three aggregations exhibited significant female biases with an overall ratio of 3.2:1 (female: male). The probability of a turtle being female increased as the size of the individual decreased. Ratios obtained in this study were slightly less female-biased, but not significantly different, than those observed in the late 1990s. However, they were significantly more biased than those found in a late 1980s pilot study. The shift to significantly female-biased ratios may be beneficial to a recovering population, an evolutionary adaptation, and is common among juvenile aggregations. A more skewed female bias in smaller size classes may be indicative of recent, warmer periods during incubation on the nesting beaches. This female bias could become more exaggerated if temperatures meet future climate warming predictions.

I dedicate this thesis to certain members of the UCF MTRG. I have depended on the support of Simona Ceriani, Allison Hays, Chris Long, and Andrew Sterner, who were with me every step of the way. Over my three years, we had some epic happenings, such as catching a sawfish in the IRL or counting over 5,000 green turtle nests on the Carr Refuge. I want to also dedicate this to my family, who have always believed in me, and to my biggest supporter, Patrick.

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1. INTRODUCTION

Responses to changing climate have been documented in various organisms, including range shifts (Hill et al., 2002; Wilson et al., 2007; Moritz et al., 2008) and changes in nesting phenology (Crick et al., 1997; Brown et al., 1999; Dunn & Winkler, 1999; Weishampel et al., 2004; 2010). In the Colorado Rocky Mountains, a warmer climate over the past several decades has resulted in earlier arrival dates for migratory birds by two weeks and an earlier emergence of hibernating species by 38 days (Inouye et al., 2000). As concern over anthropogenic climate change has risen over the past two decades, organisms that exhibit temperature-dependent sex determination (TSD) have become a research priority (Janzen, 1994; Schwanz & Janzen, 2008; Kallimanis, 2010; Bickford et al., 2010) due to the ambient temperature's influence on hatchling sex during embryonic development (Mrosovsky & Yntema, 1980; Janzen & Paukstis, 1991).

Many species with TSD have been found to have highly biased primary (hatchling) sex ratios (Bull & Charnov, 1989). Marine turtles have been shown in many cases to deviate from the 1:1 ratio predicted by sex allocation theory (Fisher, 1930) usually towards a female bias (e.g. Mrosovsky & Provancha, 1992; Broderick et al., 2000; Godley et al., 2001; Zbinden et al., 2007). Although not completely understood, there are many theories as to the adaptive significance of TSD (reviewed by Shine, 1999) and possible explanations as to the female bias (Freedberg & Wade, 2001). Higher incubation temperatures produce more females in marine turtles (reviewed by Ewert & Nelson, 1991). As a result, concern for complete population feminization has led to exploration of conservation strategies such as shading nests (Patino-Martinez et al., 2012). Persistently-biased sex ratios could affect ecological and evolutionary processes in a population (e.g. reproductive output, genetic diversity). Thus, knowledge of natural sex ratios is necessary not only to fill a knowledge gap pertaining to threatened and endangered species, but also is necessary if future mitigation strategies are required.

The complex life history of marine turtles creates difficulties in obtaining sex ratios of breeding adults. Problems include biases due to sex-specific migration routes (Henwood, 1987) and logistical challenges of working with adults at foraging grounds. Hence, most studies have focused on hatchling sex ratios; however, ratios can change seasonally and annually (Mrosovsky & Provancha, 1992; Godfrey et al., 1996) and survival in general, as well as potential survival differences between sexes, is poorly understood. Juvenile aggregations in developmental habitats provide an additional sampling opportunity, because they represent a conglomeration of hatchlings from an accumulation of years (Wibbels et al., 1991). These aggregations typically represent individuals from multiple rookeries (Lahanas et al., 1998; Bass, 1998; Bass et al., 2006; Naro-Maciel et al., 2007). Although this creates difficulties for exploring one specific genetic stock, it provides information on the species as a whole. Since marine turtles are not sexually dimorphic as juveniles, various methods have been developed to determine sex. Methods such as laparoscopy are direct (Wood et al., 1983), yet are logistically difficult. Successful characterization of sex through testosterone radioimmunoassay (RIA) has created added opportunity to quantify juvenile sex ratios (Owens et al., 1978). Although not as direct as laparoscopy, numerous studies have found success in using RIA with juvenile marine turtle species (e.g. Geis et al., 2003; 2005; Witzell et al., 2004; Blanvillain et al., 2007).

The Atlantic green turtle (*Chelonia mydas*) is listed as endangered under the U.S. Endangered Species Act (1973) and by the IUCN (2012), yet several green turtle populations around the world have been increasing over the past 30 years (Chaloupka et al., 2007). Adult green turtles nesting on Florida's east coast beaches have been tracked to foraging grounds in the region of the Florida Keys (Schroeder et al., 2008). Juvenile green turtles have been found in developmental habitats along the US east coast and have become a research priority since some return to Florida nesting grounds as adults (NMFS & USFWS, 2007). These juveniles have been genetically linked mostly to rookeries in Florida, Mexico and Costa Rica (Bass & Witzell, 2000; Bagley, 2003). For the current study, sex ratios of juvenile green turtles were examined in three developmental habitats along the east coast of Florida, therefore contributing to demographic information on the Atlantic green turtle. Our objectives were to 1) characterize the sex ratios of all three developmental habitats and 2) compare sex ratios obtained from two of the sites in the current study to ratios obtained in the late 1980s and 1990s from the same sites. Long-term comparisons of juvenile sex ratios are rare. One study by Limpus et al. (2005) in Australia reported juvenile green turtle sex ratios for a 5-year period and found a female bias, yet an additional look reported 6 years later did not find continued feminization of these juvenile aggregations (Limpus & Chaloupka, 2011). We linked snapshots from multiple studies and elucidated how sex ratios have fluctuated temporally over two decades on Florida's east coast.

2. METHODS

2.1 Study Areas and Data Collection

Three ecologically different, known juvenile green turtle developmental habitats were sampled (Fig. 1) from 2011 to 2012 from Florida's east coast; each site used different capture methods. The Trident Basin is an artificial basin located near the mouth of the Port Canaveral Shipping Channel, created for nuclear submarine maintenance. Water depth ranged from 0.5-13 m and juvenile green turtles mainly foraged on algae growing on the rip-rap lining the basin (Redfoot, 1997). Turtles were caught in large and small-mesh tangle nets and were also actively caught with large-hoop dip nets. Netting trips were conducted over two-day periods, twice per year, once in the spring/summer and once in the fall/winter.

South Bay is found within the Indian River Lagoon (IRL) system, which extends over 260 km from Ponce Inlet to Jupiter Inlet. South Bay is approximately 3 km south of Sebastian Inlet and approximately 60 km south of the Trident Basin. Water depth ranges from two to four meters, and juvenile green turtles have been found to forage mainly on beds of drift algae within the area (Holloway-Adkins, 2001). Turtles were captured with a large mesh tangle net (see Ehrhart et al., 2007), and trips were conducted twice per month, year-round.

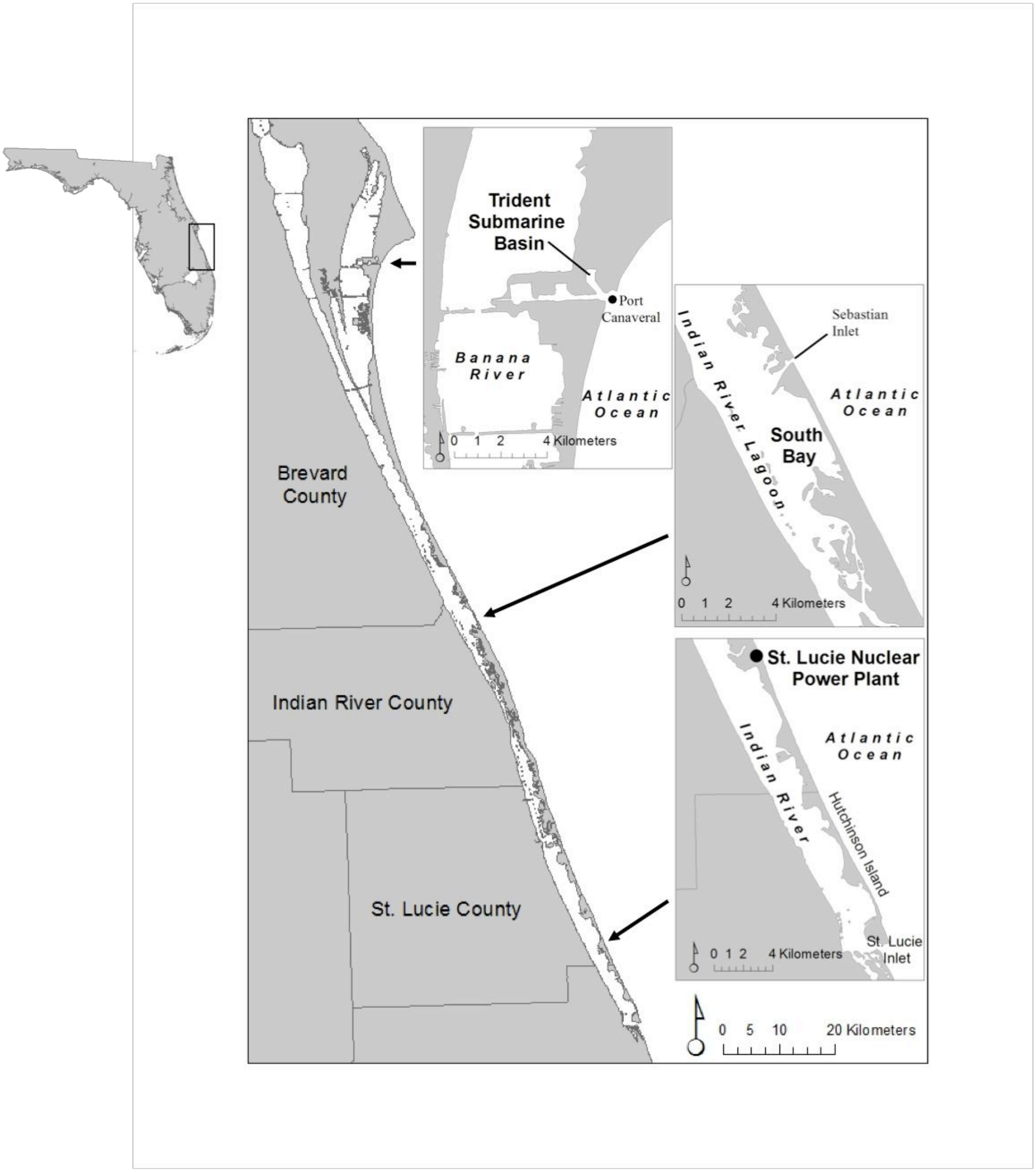


Fig. 1. Three study sites along the Florida east coast: the Trident Submarine Basin, the Indian River Lagoon (IRL) and the St. Lucie Power Plant.

The St. Lucie Nuclear Power Plant is located on the ocean-side of the barrier island, approximately 60 km south of South Bay. Three intake pipes (two 3.7 m and one 4.9 m in diameter) extend nearly 365 m offshore (Bresette et al., 1998). Just southwest of the pipes is a system of sabellariid worm rock reefs. It was created by polychaete worms, *Phragmatopoma lapidosa,* and extends from Cape Canaveral to Biscayne Key (Kirtley & Tanner, 1968). Juvenile green turtles have been found to selectively forage on macroalgae provided by this system (Holloway-Adkins, 2001; Gilbert, 2005). Turtles that actively swim into the pipes are transported into the canal, where they remain until caught via dip net, tangle net, or hand capture (Bresette et al., 1998; Quantum Resources Inc., 2011).

Three milliliters of blood was drawn from juvenile turtles within 30 minutes of capture from the left or right dorsal cervical sinus (Owens & Ruiz, 1980) into a heparinized vacutainer with a 22-gauge 1” needle. Blood was placed on ice for a maximum of eight hours, then centrifuged. Plasma was pipetted into cryogenic vials and stored at -80°C until processed through radioimmunoassay (RIA). In cases where -80°C was not available, plasma was stored at -20°C (maximum 4 weeks), then transferred to the -80°C. Samples were not collected when water temperatures dropped below 20°C due to positive negative effects on testosterone levels (Braun-McNeill et al., 2007). Straight carapace length (SCL), measured from the nuchal to the longest pygal, and body mass were recorded for each individual. Each turtle received a unique passive integrated transponder (PIT) tag for further identification if re-caught, then was released. To exclude adults, the maximum size (SCL) considered for this project was 76.7 cm, found to be the minimum size at sexual maturity for green turtles in Bermuda, based on 178 laparoscopies (Meylan et al., 2011).

2.2 Testosterone Radioimmunoassay (RIA)

Procedures similar to Geis et al. (2003; 2005) were used to measure circulating testosterone levels for predicting the sex of immature turtles (Owens et al., 1978). For each sample, 400 µl aliquots were used when possible. One hundred µl of extraction trace and 3 ml of diethyl ether were added to extract the steroid hormones. Liquid nitrogen was used to freeze the aqueous phase, and the ether (now containing the hormones) was decanted into a new tube. The diethyl ether was dried with a nitrogen manifold while in a 37 °C water bath. Samples were resuspended in 500 µl of Tris-gel buffer (pH=7). Two hundred µl were pipetted into new tubes (duplicates made of each sample), and combined with both 100 µl of antibody (Fitzgerald Industries International, Acton, MA) and 100 µl of tritiated trace (Perkin Elmer) at approximately 10,000 cpm. These were left to incubate overnight at 4°C. Twenty µl of the resuspended samples and 100µl of extraction trace were combined and counted in a beta counter to measure extraction efficiencies. One ml of charcoal suspension comprised of 50 mg Dextran, 500 mg charcoal, and 100 ml Tris-gel buffer, was added to all samples and duplicates after incubating overnight. Samples incubated in the 4°C refrigerator for 15 minutes, then were centrifuged for 15 minutes. The bound portion, located in the top, liquid layer was decanted into polyethylene scintillation vials with 3 ml of scintillation blend (Scintiverse, Fisher Scientific, GA). The vials were then processed in a beta counter. A standard curve was generated for each assay, and two adult female control samples (200 µl used instead of 400 µl) were included in each assay to compute coefficients of variation (CV).

Plasma samples from Kemp's ridley marine turtles that had previously been verified through laparoscopy were used to calibrate the RIA. Additionally, testosterone levels from juvenile green turtle samples, previously verified through laparoscopy, were used to determine female and male ranges (Wibbels, 1988; Wibbels et al., 2000; Wibbels unpub.).

2.3 Historic Conversion

Sex ratio data exist from the late 1990s from the Trident Basin and the IRL (Bagley, 2003) and from the late 1980s in the IRL (Leupschen, 1987). Water temperatures were not recorded during these previous sampling periods, therefore it is possible that a female bias may be due to reduced testosterone levels during times of lower water temperatures, possibly causing males to be mistaken as females (Braun-McNeill et al., 2007). To account for this, I determined the relationship between surface water temperatures recorded from previous netting trips in the IRL and the Trident Basin with air temperatures from the NOAA National Climatic Data Center station USC00085612 (2012) in Brevard County, for both sites. From this relationship, the air temperature at 20°C water temperature was determined, designated as the threshold air temperature. Next, air temperature was obtained from NOAA for the days that historic samples were collected, samples were discarded if they fell below the threshold air temperature, and a modified sex ratio was created. These sex ratios were then compared to the sex ratios obtained in the current study using Fisher’s Exact test.

2.4 Statistics

Where data did not pass tests of normality (using the Shapiro Wilk test), correlations were determined using Spearman’s rank test and differences between variables were determined using a Kruskal Wallis 1-way ANOVA, similar to Hawkes et al. (2013). Each site was tested against a 1:1 (female:male) with a replicated G test goodness-of-fit, as well as tested for heterogeneity between the sites. Logistic regression was run to determine the relationships between size and sex as determined by the testosterone assay.

3. RESULTS

3.1 Characterizing aggregations

A total of 243 juvenile green turtles were sampled among the three sites. Average SCL was smallest at the Trident Basin (29.92 cm) while the IRL (41.8 cm) and St. Lucie Power Plant (42.11 cm) were more similar. Welch's ANOVA was used to compare means since variances were unequal between the three sites, and there was a significant difference between The Trident Basin and the two other sites (*F* ratio = 141.36, $P < 0.0001$). Carapace length data were partitioned into 10 cm increments, and the resulting distribution was visibly different among the three sites (Fig. 2).

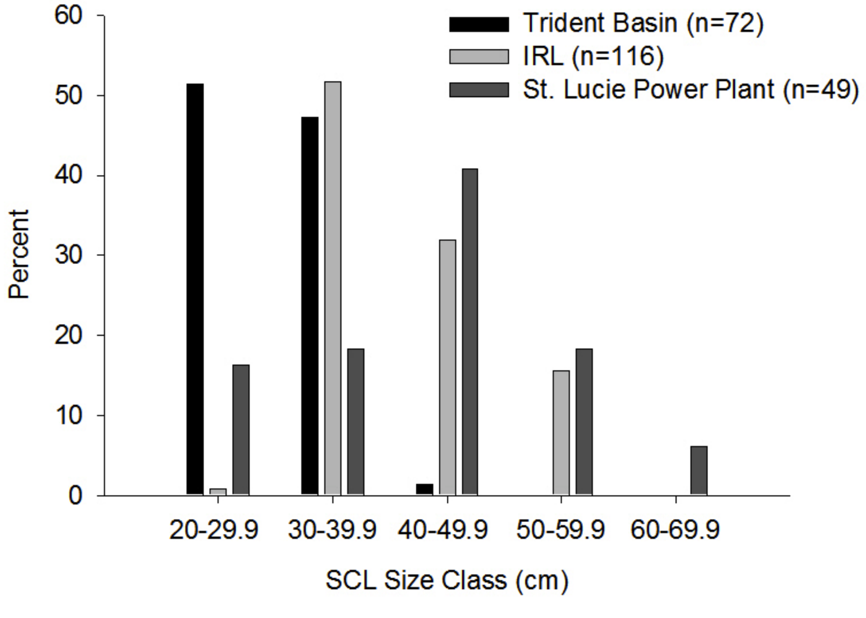


Fig. 2. Percent of each size class within the three sites: The Trident Basin (n=72), IRL (n=116), St. Lucie Power Plant (n=49). Six turtles were excluded from the IRL site due to unknown SCL.

Nine assays were run, 25-35 individual samples and two controls in each assay in duplicate, resulting in an intra- and inter-assay coefficient of variation of 4.87 and 17.75, respectively. The average minimum sensitivity was 6.61 ± 4.51 pg. Eight samples fell between the highest verified (18 pg/ml) female and the lowest verified male (13 pg/ml). These eight samples were classified as unknown (Table 1). No significant relationships were found between season and testosterone for the IRL (Kruskal Wallis 1-way ANOVA: $X^2 = 5.64$, df = 3, $P = 0.13$) or St. Lucie Power Plant (Kruskal Wallis 1-way ANOVA: $X^2 = 2.95$, df = 3, $P = 0.49$). The Trident Basin was only sampled during the summer because water temperatures fell below 20°C during the fall/winter sampling periods. Data were pooled by site, and sex ratio ranges were created for each site by counting all the unknowns as females, then as males. All sites and scenarios were significantly female-biased (differing from a 1:1 sex ratio) and pooled data resulted in a female-biased 3.2:1 sex ratio (Table 1). Heterogeneity was not significant, indicating the three sites were not significantly different from one another. The logistic regression (Fig. 3) suggests that smaller turtles had a higher probability of being female, while the probability of being female decreased as size increased. Out of the samples collected, a 60 cm SCL turtle had a 55% chance of being a female while a 30 cm SCL turtle had an 80% chance of being female.

Table 1

Sampling summary for the three sites and results from a replicated G test goodness-of-fit analysis.

	No. sampling days	No. sampled turtles	No. females	No. males	No. Unk	Sex Ratio Range (F: 1M)	Predicted Ratio without Unk (F: 1M)
Trident Basin	6	72	57	12	3	5.0 – 2. 8***	4.75 ***
IRL	26	122	89	30	2	3.07 – 2.7 ***	2.97 ***
St. Lucie Power Plant	44	49	33	14	2	2.5 – 2.06 **	2.36 **
Pooled	76	243	180	56	7	3.34 - 2.8 ***	3.2 ***
Heterogeneity						$G = 0.25, 0.34$ $P = 0.25, 0.34$	$G = 0.26$ $P = 0.26$

(*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$

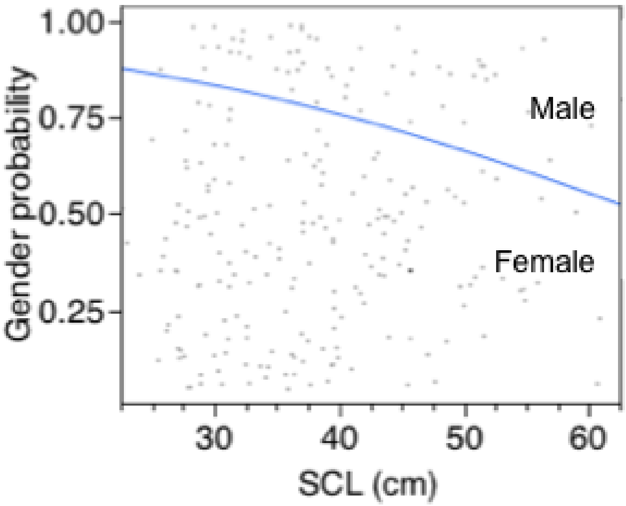


Fig. 3. Logistic plot of the probability of being male (above the blue line) or female (below the blue line) based on SCL (X^2 = 6.87, R^2 = 0.028, DF = 1, P = 0.0088).

3.2 Historic Comparison

There was a significant correlation between air and water temperature in the Trident Basin (72 data points, R^2 = 0.75, P < 0.0001) and in the IRL (413 data points, R^2 = 0.79, P < 0.0001). Prediction intervals were generated for each regression. For both sites, air temperature at the lower 95% prediction interval was determined, for water temperature at 20°C. New ratios from the historic samples were calculated, discarding samples below the air temperature at the lower 95% confidence interval for the Trident Basin (22.5°C air temperature) and the IRL (22.5°C air temperature). These newly calculated ratios were compared to the original ratios from Bagley (2003) where water temperature was not taken into account (Table 2). When comparing ratios obtained (taking temperature into account) to the original ratios, there was no significant difference for the IRL (P = 1.0) or for the Trident Basin (P = 1.0). Historic ratios from Leupschen (1987) and original sex ratios from Bagley (2003) were compared to sex ratios obtained in the current study (Table 3).

Table 2

Ratios obtained from a previous study (Bagley, 2003) of juvenile green turtle sex ratios in the Trident Basin and IRL. Listed are the original, overall ratios and the modified ratios from that same study, taking temperature into account.

	No. turtles sampled	Female	Male	Unk	Sex Ratio Range (F:1M)	Predicted Ratio without Unk (F:1M)
Trident Basin						
Overall	100	80	14	6	6.14 - 4.0	5.71
Above Air Temp 22.5°C	92	75	13	4	6.08 - 4.41	5.77
IRL						
Overall	100	79	15	6	5.67 - 3.76	5.27
Above Air Temp 21.5°C	31	26	5	0	none	5.21

Table 3

Historic and current sex ratio comparison using a Fisher's Exact test.

	Female	Male	Predicted Ratio without Unk (F:1M)	*P*-value
Trident Basin				
Late 1990s (Bagley 2003)	80	14	5.71	0.67
Current study	57	12	4.75	
IRL				
Late 1980s (Leupschen 1987)	18	19	0.9	0.0043
Current study	89	30	2.97	
Late 1990s (Bagley 2003)	79	15	5.27	0.13
Current study	89	30	2.97	

4. DISCUSSION

This study used testosterone RIA to predict sex ratios of juvenile green turtles, finding an overall sex ratio of 3.2:1 (female:male). The juvenile green turtle female bias coincides with reported sex ratios from green turtle nesting beaches in the Atlantic (Spotila et al., 1987; Godfrey et al., 1996; Godley et al., 2002), although there are surprisingly very few studies that have investigated this topic for green turtles. A female bias has been found in previous juvenile marine turtle studies as well for hawksbills (Wibbels et al., 1989; Limpus, 1992; Leon & Diez, 1999; Geis et al., 2003; Blanvillain et al., 2007), loggerheads (Wibbels et al., 1987; 1989; Bass et al., 1998; Braun-McNeill et al., 2007; Delgado et al., 2010; Maffucci et al., 2013), Kemp's ridley turtles (Morreale, 1992; Stabeneau et al.,1996; Coyne, 2000; Gregory & Schmidt, 2001; Witzell et al., 2004; Geis et al., 2005), and green turtles (Wibbels et al., 1989; Bass et al., 1998; Limpus et al., 2005; Foley et al., 2007). For juvenile green turtles in the Atlantic, sex ratios have been reported to be female-biased since the late 1990s (Table 3). Although many studies have found a female bias, there are accounts of unbiased ratios for juvenile green turtles (Limpus & Reed, 1985; Bolten & Bjorndal, 1992; Meylan et al., 1992; Wibbels et al., 1993), loggerheads (Casale et al., 2006), hawksbills (Diez & Van Dam, 2003), and Kemp's ridley turtles (Danton & Prescott, 1988; Cannon, 1998; Shaver, 1991).

Table 4

Summary of sex ratio studies done on juvenile green turtles in the Atlantic with a sample size greater than ten.

Ratio (F:1M)	N	Method	Location	Reference
0.9	37	RIA	IRL	Leupschen, 1987
1.4	120	RIA	Bahamas	Bolten et al., 1992
Not sig.	56	Laparoscopy	Bermuda	Meylan et al., 1992
1.75*	66	Necropsy	Mosquito Lagoon	Schroeder & Owens, 1994
5.57*	46	RIA	North Carolina	Bass et al., 1998
5.27*	100	RIA	IRL	Bagley, 2003
5.71*	100	RIA	Trident Basin	Bagley, 2003
2.62*	100	RIA	Reef location, FL	Bagley, 2003
3.25*	51	Necropsy	Saint Joseph Bay, FL	Foley et al., 2007
3.2*	243	RIA	FL east coast	Current study

(*) Denotes significant female bias

When looking at size, I found a higher female bias in smaller turtles, with that probability getting smaller as size increases (Fig. 3). Those smaller size classes were mostly found at the Trident Basin (Fig. 2). The three sites are ecologically different, comprised of different size classes, and there may be several reasons why there is a higher female bias at the smaller size classes. A possibility is that testosterone may increase with increased size, but no significant relationship was found in green turtles (n=197), whose testosterone levels had been verified through laparoscopy (Wibbels, 1987). Further examination of testosterone levels in smaller individuals would provide further insight. The female bias found may not be related to size, but instead to behavioral differences between females and males.

Juveniles migrating to and from developmental habitats may not encounter similar threats. Differences in sex specific survival have not been found (Chaloupka & Limpus, 2002; 2005), although there has been a report of possible sex specific migrations for loggerheads

(Casale et al., 2002). Juvenile green turtle migrations have been documented as extensive as 2800 km (Meylan et al., 2011), therefore juveniles traveling greater distances or through areas of greater risk may have lower survival (Bjorndal et al., 2003). Smaller size classes have been found to make up a large percentage of stranded samples in Bermuda, suggesting they have a higher mortality rate (Meylan et al., 2011). It is possible that our smaller size classes (with the stronger female bias) have not yet experienced this type of selection. Future sequencing of biopsy samples from turtles in the current study will be done and compared to those seen in the 1990s at the same sites to address some of these concerns. Long-term examination of genetics in certain developmental habitats should be done to see if genetic composition changes and if sex ratios remain similar throughout several years.

Genetically, juvenile aggregations are comprised of a mixture of turtles from various nesting beaches (Lahanas et al., 1998; Bass, 1998; Bass et al., 2006; Naro-Maciel et al., 2007). Genetic studies from the late 1990s (Bass & Witzell, 2000; Bagley, 2003) found the majority of turtles from all three sites had haplotypes I (Florida and Mexico) and III (FL, Costa Rica, MX, Aves Is.); however, percentages were different among the sites. It is possible that the nesting beaches contributing to these juvenile aggregations are producing different ratios, some more female-biased than others. Recruitment may vary between the three different sites, resulting in dissimilar ratios.

Seeing a female bias in hatchlings and in juvenile aggregations may be due to other factors, excluding a warming climate. There are “differential fitness” models proposed for adaptive significance for reptiles with TSD (summarized by Shine, 1999). One theory is that the sex ratio is female-biased due to cultural inheritance (Freedberg & Wade, 2001), where if a nest site produces an excess of females, that site will continually increase due to philopatry. If this is the case, an excess of females will allow a population to increase. This is because green turtles are polyandrous and exhibit multiple paternity (Peare & Parker, 1996; Fitzsimmons, 1998; Ireland et al., 2003; Lee & Hays, 2004). Thus, fewer males may be needed than females to maintain an increasing population. A satellite tracked male green turtle has been found to travel along various rookeries, presumably mating with reproductively active females (Wright et al., 2012b), which would promote gene flow in a population dominated by females. It has been assumed that males also mate more often than females, and recently through microsatellites, it was found that males in Cyprus did not mate annually (Wright et al., 2012a). Regardless, sex ratios of reproductively active adults in the same region were higher for males at 1:1.4 (female:male) during the 2008 nesting season (Wright et al., 2012b). The same was seen with male loggerheads, where the interval for a male to mate was shorter than for a female (Hays et al., 2010).

The female bias seen in the current study was also seen in previous sex ratio results at two of the same locations (Table 3). At the Trident Basin, sex ratios were also significantly female-biased in the late 1990s (Bagley, 2003). Meanwhile, sex ratio results from the late 1980s were balanced (Leupschen, 1987) and results from the late 1990s were significantly female-biased (Bagley, 2003). These results, including from the current study, represent snapshots of sex ratios. We do not know what is happening in between these snapshots. It is possible that there are natural oscillations in sex ratios, and we were seeing the snapshots when they are female-biased. On the other hand, these aggregations may be maintaining a female bias.

Advantages of having a female bias for population recovery was seen when Coyne (2000) created a population model over a 50-yr period for Kemp’s ridleys, with varied ratios. The greatest increase was seen with 3:1 (female:male), with very slow recovery when using 1:3

(female:male), and the Fisherian baseline of 1:1 (female:male) falling between the skewed ratios. When looking at six green turtle rookeries with over 25 years of nesting data available for each site, Chaloupka et al. (2007) found 4-14% increases per year, suggesting these populations are recovering at incredible rates. It is possible that the female biases seen in nests and juvenile populations are aiding this recovery. Additionally, temperature may already be playing a role in the sex ratios seen.

The female biases seen in the current and historic studies could be a product of temperatures that have been warming over the previous six decades. The IPCC (2007) stated that from 1956 to 2005, there was a linear warming trend, which was about twice more than what was seen from 1906 to 2005. This indicates that the past 50 or so years have seen a much higher increase in temperature than in the early 1900s (IPCC, 2007). If this is the case, and temperatures have risen enough to influence hatchling sex in the past 50 years, sex ratios would have already become more female-biased in the 1980s and the late 1990s, when the first two studies on juvenile sex ratios on green turtles were conducted. Von Holle et al. (2010) documented regions in Florida having significantly warmer summer and fall months, and significantly cooler winter months due to the changing climate. Warming summer and fall months, during green turtle nesting season, strongly suggest that this could influence marine turtle sex ratios. Reconstructed green turtle nest temperatures for the past 100 years on Ascension Island have increased between 0.36 and 0.49°C (Hays et al., 2003), and future projections for green turtles in Australia predict a complete feminization of hatchlings by the year 2070 (Fuentes et al., 2010). Evidence of a changing climate has also been observed in the water.

Changes in behavior have already been documented in adults with warming sea surface temperatures. Weishampel et al. (2004) found both loggerheads and green turtles nested earlier with warmer sea surface temperatures in Florida, but just north of that nesting beach, also in Florida, loggerheads were also found to nest earlier while green turtles did not change their nesting behavior with warmer sea surface temperatures (Pike et al., 2006; 2009). Chaloupka et al. (2008) found loggerheads at foraging areas in Japan and Australia have been exposed to slowly increasing trends in mean annual sea surface temperature over the past 50 years. Evidence of warming air and water temperatures supports the female bias seen in the sex ratios produced in the foraging aggregations in the current study (along with those historic sex ratios).

Any study using hormones around developed areas should acknowledge the possible influences of endocrine disruptors (EDs). EDs (i.e. from pollutants such as plastics, insecticides, disinfectants) can contaminate bodies of water due to run-off (reviewed by Markey et al., 2002; Burkhardt-Holm, 2010). Shelby and Mendonca (2001) found 10% of male yellow-blotched map turtles had significantly lower testosterone levels in a site downstream from a pulp mill compared to levels at an unimpacted site. Perfluorinated carbons (PFCs) have also been found to, among other effects, alter hormone levels (reviewed by Olsen et al., 2003). Bioaccumulation of PFC concentration in two species of sea turtles on the central, east coast of the U.S. has been correlated with human population (O'Connell et al., 2010). This accumulation can also be influenced by species, age and habitat (Keller et al., 2005). Little is known about how much a juvenile marine turtle is susceptible to these contaminants or how much can be passed from mother to egg. However, it has been found that loggerhead eggs in southern Florida have been found to have detectable amounts of PCBs (Alava et al., 2006).

The Trident Basin is constantly flushed with ocean water and the reef site at the St. Lucie Power Plant is on the dynamic coastline of FL, with high-energy beaches. It seems unlikely EDs would impact these two sites. The IRL site is near Sebastian Inlet, and although its proximity to the inlet may allow the flushing of salt water, large discharges of agricultural runoff have been recorded (Sigua & Tweedale, 2003). Future studies examining EDs and sea turtles in these areas are needed.

The ratios from this study have various implications for the green turtle. With an overall female-biased sex ratio for juvenile green turtles at three sites on the east coast of Florida, there are significantly more females than males within these foraging aggregations. These aggregations are dynamic and there is a need for large-scale, comprehensive studies to evaluate long-term dynamics of sex ratios within a sea turtle population. Long-term studies on sex ratios have been done in certain regions (i.e. Limpus et al., 2005; Meylan et al., 2011), yet further assessment of juvenile sex ratios will provide further understanding of TSD and its relationship to temperature changes. Studies only looking at beach temperatures and hatchling ratios become more powerful when combined with information on juveniles, although there is considerably more to be understood. Although nearly 90% of loggerhead hatchlings were found to be female on the east coast of Florida (Mrosovsky & Provancha, 1992), a female bias of only 2.1:1 (female:male) was found in juvenile aggregations along the same coast, around the same time frame (Wibbels et al., 1991). Future projections of increased temperature (IPCC, 2007) may produce extreme sex ratios, but it may be difficult to untangle an already natural female bias, created by a warming climate, if ongoing studies are not continued.

APPENDIX A:
TRIDENT SUBMARINE TURNING BASIN-
JUVENILE GREEN TURTLE MORPHOMETRIC AND SEX INFORMATION

LF TAG	RF TAG	DATE	PIT Tag	SURFACE WATER TEMP (C)	SCL (cm)	TIME PASSED SINCE DRAWN	% Binding	Pg/ml	Assay No.	Predicted M/F/Unk
HA 9810	--	8/11/11	4C12750C6E	27.5	25.7	<30	83.8	4.3	5	F
HA 9825	--	8/11/11	4C12796F58	26.7	25.9	<30	83.8	4.3	5	F
HA 9885	--	8/11/11	4C12706E38	27.1	26.9	25	83.8	4.3	5	F
HA 9817	--	8/11/11	4C12742728	27.5	27.1	<30	83.8	4.3	5	F
HA 9815	--	8/11/11	4C127C7B24	27.5	27.6	<30	83.8	4.3	5	F
HA 9821	--	8/11/11	4C12710507	26.7	27.7	<30	83.8	4.3	5	F
HA 9890	--	8/11/11	4C12717E52	26.7	27.8	<30	83.8	4.3	5	F
HA 9882	--	8/11/11	4C13026C15	26.7	28.7	18	83.8	4.3	5	F
HA 9811	--	8/11/11	4C13024747	26.7	28.9	<30	83.8	4.3	5	F
HA 9823	--	8/11/11	4C12775E3A	26.7	29.3	13	83.8	4.3	5	F
HA 9819	--	8/11/11	4C12746E34	26.7	29.5	<30	83.8	4.3	5	F
HA 9880	--	8/11/11	4C1276311F	26.7	29.5	<30	83.8	4.3	5	F
HA 9877	--	8/11/11	4C12742025	26.7	29.7	<30	83.8	4.3	5	F
HA 9829	--	8/11/11	4C12771431	27.5	30.3	23	83.8	4.3	5	F
HA 9883	HA 9828	8/11/11	4C12761879	26.7	30.8	18	83.8	4.3	5	F
HA 9881	--	8/11/11	4C12723B61	26.7	32.2	<30	83.8	4.3	5	F
HA 9878	HA 9826	8/11/11	4C12730443	26.7	33.3	<30	83.8	4.3	5	F
HA 9816	--	8/11/11	4C12770930	26.7	35.9	<30	83.8	4.3	5	F
HA 9827	--	8/11/11	4C12795557	26.7	27.7	14	80.0	17.1	5	Unk
HA 9876	--	8/11/11	4C127F5126	26.7	28.3	<30	69.1	31.0	5	M
HA 9824	--	8/11/11	4C13025F7F	26.7	29.1	26	68.8	31.4	5	M
HA 9818	HA 9822	8/11/11	4C12762D3D	26.7	32.8	<30	66.9	34.4	5	M
HA 9884	--	8/11/11	4C1278670A	27.5	29.2	10	62.1	42.7	5	M
--	--	8/12/11	4B68147223	25.8	27.1	15	83.8	4.3	5	F
HA 9835		8/12/11	4C12742E4D	25.8	29.6	3	83.8	4.3	5	F
--	--	8/12/11	4C127B796F	25.8	30	7	83.8	4.3	5	F
HA 9836	--	8/12/11	4C13012354	25.8	30	9	83.8	4.3	5	F
HA 9891	--	8/12/11	4C1278170E	25.8	30.9	7	83.8	4.3	5	F
HA 9833	HB 1105	8/12/11	4C12735349	25.8	31.2	12	83.8	4.3	5	F
HA 9887	HA 9830	8/12/11	4C1302627D	25.8	32.2	<30	83.8	4.3	5	F
HA 9831	HA 9832	8/12/11	4C12747E4A	25.8	34.6	20	83.8	4.3	5	F
--	--	8/12/11	4C12723C6C	25.8	25.5	11	87.2	4.4	15	F
--	--	8/12/11	4C12754168	25.8	26.2	9	87.2	4.4	15	F

LF TAG	RF TAG	DATE	PIT Tag	SURFACE WATER TEMP (C)	SCL (cm)	TIME PASSED SINCE DRAWN	% Binding	Pg/ml	Assay No.	Predicted M/F/Unk
HA 8621	--	8/12/11	4B627B0876	25.8	27.7	<30	87.2	4.4	15	F
HB 0729	--	8/12/11	4B6820401D	25.8	28.7	6	87.2	4.4	15	F
HA 5799	HA 9889	8/12/11	46021C6C7C	25.8	37.1	10	87.2	4.4	15	F
HA 8604	HA 8605	8/12/11	4B630F4F70	25.8	32.8	2	86.0	12.1	15	F
HA 9888	--	8/12/11	4C127A222D	25.8	32.3	11	65.5	36.8	5	M
HA 8375	--	8/12/11	4A1D763A59	25.8	31.3	11	63.2	40.7	5	M
HA 9834	--	8/12/11	4C13015B16	25.8	30	10	57.9	51.3	5	M
--	--	6/2/12	4C12790E01	27.5	23	15	88.5	7.8	9	F
HB 2269	--	6/2/12	4C12714A35	27.5	27.3	13	88.5	7.8	9	F
HB 2251	--	6/2/12	4C3D131B36	27.5	30.4	10	88.5	7.8	9	F
HB 2252	--	6/2/12	4C3D17052C	27.5	30.4	10	88.5	7.8	9	F
HB 2267	HB 2268	6/2/12	4C3B377A5F	27.5	31.2	14	88.5	7.8	9	F
HB 2264	HB 2265	6/2/12	4B627B6A38	27.5	32.8	7	88.5	7.8	9	F
HB 2259	HB 2260	6/2/12	4C12703263	27.5	34.4	10	88.5	7.8	9	F
HB 2256	HB 2257	6/2/12	4C127A3144	27.5	34.4	12	88.5	7.8	9	F
HB 2262	HB 2263	6/2/12	4C12750428	27.5	27.3	12	78.1	15.5	9	Unk
HB 2254	HB 2255	6/2/12	4C127B6074	27.5	32.2	12	80.4	34.5	9	M
--	--	6/3/12	4C12721D4C	27.1	25	11	88.5	7.8	9	F
HB 2273	--	6/3/12	4C3B3F7654	27.1	26.1	24	88.5	7.8	9	F
HB 1103	--	6/3/12	4C3B5F0412	27.1	27	10	88.5	7.8	9	F
HB 2275	--	6/3/12	4C127E682A	27.1	27.9	9	88.5	7.8	9	F
HB 1427	--	6/3/12	4C126F4851	27.1	28.9	10	88.5	7.8	9	F
HB 1107	HB 1108	6/3/12	4545541A1F	27.1	44	11	88.5	7.8	9	F
HB 1432	HB 2274	6/3/12	4C1300556A	27.1	31.5	14	85.3	25.3	9	M
HB 2271	HB 2272	6/3/12	4C12702A5B	27.1	30.3	14	81.1	33.2	9	M
HB 1101	HB 1102	6/3/12	47236F574E	27.1	37.6	14	65.0	70.0	9	M
--	--	8/18/12	4C12724043	25.6	22.6	17	85.6	4.0	13	F
EEG 906	--	8/18/12	4C3B4A4231	25.6	26.9	23	85.6	4.0	13	F
EEG 904	EEG 905	8/18/12	4C3B413211	25.6	30	12	85.6	4.0	13	F
EEG 908	--	8/18/12	4C3D1E4516	25.6	30.2	15	85.6	4.0	13	F
--	EEG 910	8/18/12	4C1279706D	25.6	33.2	19	85.6	4.0	13	F
EEG 911	--	8/18/12	4C3D235146	25.6	26.3	15	89.0	7.4	13	F
EEG 901	EEG 902	8/18/12	45345B5931	25.6	37.1	18	82.6	12.4	13	F

LF TAG	RF TAG	DATE	PIT Tag	SURFACE WATER TEMP (C)	SCL (cm)	TIME PASSED SINCE DRAWN	% Binding	Pg/ml	Assay No.	Predicted M/F/Unk
EEG 909	--	8/18/12	4C3D143B4A	25.6	25.7	19	64.2	31.7	13	M
EEG 720	--	8/19/12	4C3C5E7263	24.9	28.1	18	85.6	4.0	13	F
EEG 914	EEG 915	8/19/12	4C3B561E5D	24.9	31	10	85.6	4.0	13	F
EEG 718	EEG 719	8/19/12	4C3C5D6F11	24.9	31.1	21	85.6	4.0	13	F
EEG 715	EEG 714	8/19/12	4C3C3B0B6C	24.9	31.8	18	83.3	11.8	13	F
EEG 716	--	8/19/12	4C3B517F78	24.9	27.4	12	76.6	17.8	13	Unk

APPENDIX B:
INDIAN RIVER LAGOON-
JUVENILE GREEN TURTLE MORPHOMETRIC AND SEX INFORMATION

LF TAG	RF TAG	DATE	PIT Tag	SURFACE WATER TEMP (C)	SCL (cm)	TIME PASSED SINCE DRAWN	% Binding	Pg/ml	Assay No.	Predicted M/F/Unk
HA 9288	HA 9289	6/7/11	4B626C072E	26.6	37.6	<15	86.7	3.54	14	F
HA 9286	HA 9287	6/7/11	4B62783733	26.6	39.6	<15	87.15	4.43	15	F
HA 9284	HA 9285	6/7/11	4B62690736	26.6	51.6	<15	86.7	3.54	14	F
--	HA 9814	8/2/11	4C127B4077	29.5	36.2	<15	70.43	23.98	14	M
HA 9806	HA 9807	8/2/11	4C12785D05	29.5	43.2	<15	86.7	3.54	14	F
HB 0938	HA 9805	8/2/11	4B63085B63	29.5	46.1	<15	51.96	52.44	14	M
HB 1033	HB 1034	8/16/11	4C127D3402	28.2	32	1	71.7	27.009	15	M
HA 9842	HA 9843	8/16/11	4C1302304E	28.2	35.2	5	86.7	3.54	14	F
HA 9892	HA 9893	8/16/11	4C12720457	28.2	35.7	12	86.7	3.54	14	F
--	HA 9841	8/16/11	4C1279025D	28.2	36.7	6	37.3	94.84	14	M
HB 1026	HB 1027	8/16/11	4B63081D3C	28.2	37.9	6	87.15	4.43	15	F
HA 9894	HA 9895	8/16/11	4C126F5550	28.2	38.2	4	61.99	34.9	14	M
--	HA 9900	8/16/11	4C127B665A	28.2	38.7	6	86.7	3.54	14	F
HB 1028	HB 1029	8/16/11	4C1275116F	28.2	39.5	8	86.7	3.54	14	F
HB 0447	HB 0449	8/16/11	4B627A7020	28.2	39.9	15	86.7	3.54	14	F
HA 9846	HA 9847	8/16/11	4C12745102	28.2	43.1	8	86.7	3.54	14	F
HB 1031	HB 1032	8/16/11	4C12752326	28.2	44.4	22	87.05	11.124	15	F
HB 9850	HB 1030	8/16/11	4B62590040	28.2	45.5	7	86.7	3.54	14	F
HA 9898	HA 9899	8/16/11	4C12711925	28.2	47.7	10	85.49	9.779	14	F
HA 9896	HA 9897	8/16/11	4C12725664	28.2	55.2	<15	16.56	281.4	14	M
HB 1037	HB 1038	9/6/11	4C12727F02	27.9	39.5	15	86.7	3.54	14	F
HB 1035	HB 1036	9/6/11	50325A5F36	27.9	52.6	9	86.7	3.54	14	F
HB 1049	HB 1050	9/23/11	4C1301074D	27.9	33.1	16	79.85	14.48	14	Unk
HB 1057	HB 1056	9/23/11	4C13007B01	27.9	35	9	41.42	78.01	13	M
HB 1045	HB 1046	9/23/11	4C126F590C	27.9	35.9	14	86.7	3.54	14	F
HB 1053	--	9/23/11	4C126F7B3B	27.9	36.2	<15	86.7	3.54	14	F
HB 1051	HB 1052	9/23/11	4C127A2019	27.9	37	13	57.76	41.56	14	M
HB 1039	HB 1040	9/23/11	4C12714922	27.9	38.5	10	86.47	9.019	14	F
HB 1043	HB 1044	9/23/11	4C12782B2F	27.9	38.6	9	86.7	3.54	14	F
HB 1058	HB 1059	9/23/11	4C127B5A7B	27.9	41	11	86.7	3.54	14	F
HB 1064	HB 1065	9/23/11	4C127F2316	27.9	41	8	36.93	93.6	13	M
HB 1062	HB 1063	9/23/11	4C12725B1B	27.9	41.7	11	45.35	66.82	13	M

LF TAG	RF TAG	DATE	PIT Tag	SURFACE WATER TEMP (C)	SCL (cm)	TIME PASSED SINCE DRAWN	% Binding	Pg/ml	Assay No.	Predicted M/F/Unk
HB 1047	HB 1048	9/23/11	454C161E6D	27.9	43.5	7	86.7	3.54	14	F
HB 1060	HB 1061	9/23/11	4C127E3552	27.9	44.3	10	33.61	111.2	14	M
HB 1054	HB 1055	9/23/11	4C127C5E1E	27.9	45.7	8	86.7	3.54	14	F
HB 1378	HB 1379	10/5/11	4C1300067A	20.6	37	13	39.72	83.51	13	M
HB 1066	HB 1067	10/5/11	4C127B096B	20.6	38.2	1	66.65	28.57	13	M
HB 1376	HB 1377	10/5/11	4C12705514	20.6	42	10	85.58	4.02	13	F
HB 1068	HB 1069	10/5/11	4C12720B1C	20.6	43.6	10	85.58	4.02	13	F
--	HB 1070	10/14/11	4C127E3F20	20.6	39.1	14	55.52	45.03	13	M
HB 1354	HB 1355	11/2/11	4C12740A54	20.6	32.5	6	87.15	4.43	15	F
HB 1073	HB 1074	11/2/11	4C127F6656	20.6	34.5	6	42.2	83.498	15	M
HB 1362	HB 1363	11/2/11	4C12777E39	20.6	36.2	6	86.55	11.639	15	F
HB 1075	HB 1351	11/2/11	4C127F3839	20.6	37.3	13	87.15	4.43	15	F
HB 1352	--	11/2/11	4C12736F68	20.6	40.2	14	87.15	4.43	15	F
HB 1356	HB 1357	11/2/11	4C12785A08	20.6	41.1	7	87.15	4.43	15	F
HB 1360	HB 1361	11/2/11	4C12770758	20.6	42.1	7	87.15	4.43	15	F
HB 1358	HB 1359	11/2/11	4C127F5B54	20.6	45.3	16	87.15	4.43	15	F
HB 1353	HA 1267	11/2/11	4545192E56	20.6	45.9	8	62.9	38.83	15	M
HB 1366	HB 1367	11/18/11	4C1275316C	23	48.6	13	87.15	4.43	15	F
HB 1364	HB 1365	11/18/11	454671231B	23	55.9	14	87.15	4.43	15	F
HB 1413	HB 1414	12/9/11	4C12764038	20.2	35.7	5	87.15	4.43	15	F
HB 1409	HB 1410	12/9/11	4C12740105	20.2	36.1	8	58.3	46.317	15	M
HB 1406	HB 1407	12/9/11	4C12754D34	20.2	36.4	10	87.15	11.229	15	F
HB 1396	HB 1397	12/9/11	4C127A2714	20.2	37.2	13	87.15	4.43	15	F
--	HB 1403	12/9/11	4C12790647	20.2	37.7	9	87.15	4.43	15	F
--	HB 1408	12/9/11	4C12784B55	20.2	38.9	16	87.15	4.43	15	F
HB 1412	HB 1411	12/9/11	4C12766615	20.2	42.6	9	87.15	4.43	15	F
HB 1398	HB 1399	12/9/11	4C13004C71	20.2	50	16	69.9	29.302	15	M
HB 1394	HB 1395	12/9/11	4C1274760C	20.2	50.8	14	68.55	31.052	15	M
HB 1401	HB 1402	12/9/11	4C12760E29	20.2	54.7	8	66.65	33.464	15	M
HB 1442	HB 1443	1/25/12	4C12791C4B	21.6	31.6	16	83.2	12.64	10	F
HB 1444	HB 1445	1/25/12	4C12741058	21.6	38	10	83.2	12.64	10	F
HB 1477	HB 1478	1/25/12	4C12701054	21.6	45	13	83.2	12.64	10	F

LF TAG	RF TAG	DATE	PIT Tag	SURFACE WATER TEMP (C)	SCL (cm)	TIME PASSED SINCE DRAWN	% Binding	Pg/ml	Assay No.	Predicted M/F/Unk
HB 1446	HB 1447	1/25/12	4C1277256F	21.6	45.5	15	83.2	12.64	10	F
HB 1448	HB 1449	1/25/12	4C127F307F	21.6	45.9	14	83.2	12.64	10	F
HB 1450	HB 1476	1/25/12	4C127B0B5F	21.6	54.5	6	83.2	12.64	10	F
HB 0420	HB 1505	2/21/12	4B68135E03	20	39.6	10	83.2	12.64	10	F
HB 1503	--	2/21/12	4C13016A48	20	49.7	20	83.2	12.64	10	F
HB 1521	--	4/2/12	4C13001B7B	24.3	32.6	12	83.2	12.64	10	F
HB 1555	HB 1556	4/2/12	4C13013504	24.3	34.3	13	83.2	12.64	10	F
HB 1530	HB 1531	4/2/12	4C1270144E	24.3	36.4	15	83.2	12.64	10	F
HB 1519	HB 1520	4/2/12	4C12747864	24.3	37.5	14	83.2	12.64	10	F
HB 1532	HB 1533	4/2/12	4C12742A57	24.3	39.5	8	83.2	12.64	10	F
HB 1524	HB 1525	4/2/12	4B63071645	24.3	43.2	15	83.2	12.64	10	F
HB 1526	HB 1527	4/2/12	4C127A7F55	24.3	49.3	13	83.2	12.64	10	F
HB 1522	HB 1523	4/2/12	4C12786629	24.3	50	11	83.2	12.64	10	F
HB 1551	HB 1552	4/2/12	4A25443765	24.3	56.4	21	80.48	37.61	10	M
HB 1534	HB 1535	4/6/12	4C12716D3B	25.9	36.4	10	83.2	12.64	10	F
HB 1536	HB 1537	4/6/12	4C12776869	25.9	40	15	83.2	12.64	10	F
HB 1538	--	4/6/12	4C1278341A	25.9	41.7	13	83.2	12.64	10	F
HB 1528	HB 1529	4/6/12	4C127D0E0D	25.9	53.1	12	83.2	12.64	10	F
HB 1540	--	5/2/12	4C3C5D2334	26.6	37.1	17	87.78	2.32	11	F
HB 1541	HB 1542	5/2/12	4C3B3A7824	26.6	38.4	10	87.78	2.32	11	F
HB 1561	HB 1562	5/2/12	4C3C666C59	26.6	39.8	10	87.7	5.8411	11	F
HB 1563	HB 1564	5/22/12	4C3D1B527D	28.2	47.1	20	87.78	2.32	11	F
HB 1567	HB 1568	5/22/12	45344E7F68	28.2	50	10	87.78	2.32	11	F
HB 1569	HB 1570	5/25/12	4C3D1C1742	28.2	48	8	58.5	33.435	11	M
HB 1481	--	6/19/12	4C12751741	27	37	15	87.78	2.32	11	F
HB1112	HB 1113	6/29/12	4C12702F62	27.4	35.2	12	87.78	2.32	11	F
HB 1120	HB 1121	7/10/12	4C3B45333E	30.6	32.5	17	87	5.33	11	F
HB 1122	HB 1123	7/10/12	4C3B461443	30.6	38.3	12	87.78	2.32	11	F
HB 1124	HB 1125	7/10/12	4542550C14	30.6	48.9	14	31.95	106.65	11	M
HB 1573	HB 1574	7/27/12	4C1273505E	29.1	34.1	7	87.78	2.32	11	F
HB 1126	HB 1127	7/27/12	4CB566A43	29.1	38	6	76.9	13.149	11	Unk
HB 1180	HB 1181	8/3/12	4C3D237F2D	29.1	32.6	10	87.78	2.32	11	F

LF TAG	RF TAG	DATE	PIT Tag	SURFACE WATER TEMP (C)	SCL (cm)	TIME PASSED SINCE DRAWN	% Binding	Pg/ml	Assay No.	Predicted M/F/Unk
HB 1175	HB 1179	8/3/12	4C3C642138	29.1	43.9	18	84.4	7.8324	11	F
HB 1176	HB 1177	8/3/12	4C3C592B7E	29.1	44.1	9	84.35	7.8324	11	F
HA 3974	HA 3975	8/3/12	485E7DIE27	29.1	51.5	10	87.75	5.7829	11	F
HB 1191	HB 1192	8/13/12	4C3C626F42	29.3	28.8	17	87.78	2.32	11	F
HB 1185	HB 1186	8/13/12	4C3C5E6A5B	29.3	34.2	14	87.78	2.32	11	F
HB 1158	HB 1159	8/13/12	4C3D16253B	29.3	36	22	37.65	81.349	11	M
HB 1199	HB 1200	8/13/12	4C3B520B16	29.3	39.4	13	81.55	9.6944	11	F
HB 1195	HB 1196	8/13/12	4C1301257A	29.3	39.4	8	48.6	54.011	11	M
HB 1193	HB 1194	8/13/12	4C3D202F59	29.3	45.3	18	87.15	6.1376	11	F
HB 1163	HB 1164	8/13/12	4C3D1A7F15	29.3	48.7	11	87.78	2.32	11	F
HB 1189	HB 1190	8/13/12	4C3D263310	29.3	51.2	25	10.9	448.2	11	M
HB 1161	HB 1162	8/13/12	4C3D26283F	29.3	56.2	26	81.95	9.0142	11	F
HB 1153	HB 1154	8/13/12	4C3D183806	29.3	57.5	10	87.78	2.32	11	F
HB 1197	HB 1198	8/13/12	4C3D23064C	29.3	59	15	87.78	2.32	11	F
EEG 826	EEG 827	9/13/12	--	25.4	--	<15	87.78	2.32	11	F
EEG 828	EEG 829	9/13/12	--	25.4	--	<15	87.78	2.32	11	F
EEG 833	EEG 834	9/13/12	--	25.4	--	<15	87.78	2.32	11	F
EEG 839	EEG 840	9/13/12	--	25.4	--	<15	86.3	6.6591	11	F
EEG 830	EEG 831	9/13/12	--	25.4	--	--	61.7	28.512	11	M
EEG 839	EEG 840	9/13/12	--	25.4	--	--	57.85	33.823	11	M
EEG 844	--	9/20/12	4C3C604908	28.7	31.2	15	83.04	5.28	12	F
--	EEG 841	9/20/12	485F440C31	28.7	39.6	10	83.04	5.28	12	F
EEG 721	EEG 722	9/20/12	4C3D16307C	28.7	56.9	14	37.82	86.22	12	M
EEG 845	EEG 846	10/17/12	4C3B593527	26.6	36.7	15	55.28	45.56	12	M
EEG 848	EEG 849	10/17/12	4C3B4D0120	26.6	39	10	83.04	5.28	12	F
EEG 847	--	10/17/12	4C3C601B3B	26.6	46	10	79.15	16.6	12	Unk

APPENDIX C:
SAINT LUCIE NUCLEAR POWER PLANT-
JUVENILE GREEN TURTLE MORPHOMETRIC AND SEX INFORMATION

LF TAG	RF TAG	DATE	PIT Tag	SURFACE WATER TEMP (C)	SCL (cm)	TIME PASSED SINCE DRAWN	% Binding	Pg/ml	Assay No.	Predicted M/F/Unk
BBP 292	BBP 293	10/24/11	4360416842	25	51.40	14	20.15	419.9	8	M
BBP 303	BBP 304	10/29/11	4A61073F34	25	43.80	10	84.11	28.01	8	M
BBP 166	BBP 167	10/31/11	4A62796E25	24.4	50.90	5	83.2	12.64	10	F
BBP 182	BBP 183	11/1/11	4544120918	23.8	51.50	13	89.22	7.47	8	F
--	--	11/3/11	4A632C332C	23.5	27.80	7	89.22	7.47	8	F
BBP 333	BBP 334	11/7/11	4C1339171F	22.7	42.00	8	83.2	12.64	10	F
YYR 423	BBP 326	11/7/11	4A675C6E12	22.6	43.50	10	89.22	7.47	8	F
BBP 335	BBP 336	11/8/11	4C1330167F	23.4	52.50	7	85.8	24.9	8	M
BBP 337	--	11/9/11	4C13342F6B	24.2	30.60	3	89.22	7.47	8	F
--	--	11/10/11	432E2A6600	23.1	60.20	7	73.56	49.64	8	M
--	--	11/11/11	4C133D1F55	23.9	24.00	5	89.22	7.47	8	F
BBP 226	BBP 227	11/11/11	4C1334551D	23.9	39.10	9	89.22	7.47	8	F
BBP 344		11/12/11	4C13313D39	22.4	33.70	6	89.22	7.47	8	F
BBP 342	BBP 343	11/12/11	456C3E7B1D	22.4	45.70	10	66.57	66.86	8	M
BBP 353	BBP 354	11/13/11	4C13365B12	24.2	42.80	5	89.22	7.47	8	F
--	--	11/14/11	4C13331216	24.9	27.10	5	89.22	7.47	8	F
BBP 357	--	11/14/11	4C132D346B	24.9	43.10	11	89.22	7.47	8	F
BBP 361	BBP 362	11/14/11	4C132A7912	25	54.80	4	89.22	7.47	8	F
BBP 372	BBP 373	11/15/11	4C132C7632	25.4	40.50	15	52.94	111.1	8	M
BBP 376	BBP 377	11/16/11	445D17580C	25.1	60.70	14	83.2	12.64	10	F
BBP 382	BBP 383	11/17/11	4253265175	25.4	37.10	8	85.93	24.66	8	M
UUX 416	--	11/17/11	4A5E38274F	25.2	47.00	7	89.22	7.47	8	F
BBP 384	BBP 385	11/18/11	45430D265B	24.8	48.30	10	77.42	41.22	8	M
BBP 387	--	11/19/11	4B11177F4D	24.5	28.80	5	89.22	7.47	8	F
BBP 277	BBP 278	11/22/11	4B7B445E44	24.5	38.70	6	89.22	7.47	8	F
BBP 394	BBP 395	11/23/11	4537492F03	25	51.80	10	57.62	93.89	8	M
BBR 006	BBR 007	11/28/11	4B0D3E305B	24	51.60	4	88.58	19.94	8	M
YYR 307	--	12/6/11	433B572979	22	42.40	12	89.22	7.47	8	F
UUX 310	--	12/9/11	4326311F23	23.2	60.90	10	89.22	7.47	8	F

LF TAG	RF TAG	DATE	PIT Tag	SURFACE WATER TEMP (C)	SCL (cm)	TIME PASSED SINCE DRAWN	% Binding	Pg/ml	Assay No.	Predicted M/F/Unk
--	--	12/23/11	4B113C261C	24.7	28.90	8	89.22	7.47	8	F
--	--	12/23/11	4A6A292033	24.7	29.20	15	89.22	7.47	8	F
BBR 027	BBR 028	12/23/11	4A67625A32	24.6	45.70	9	89.22	7.47	8	F
BBR 080	BBR 081	4/1/12	4A0A68214F	22.2	36.60	3	82.89	13.33	12	Unk
BBR 090	*BBR 091*	4/2/12	4A0B6E2D1C	22.2	43.60	6	83.04	5.28	12	F
BBP 199	BBR 134	4/26/12	4C132D2C53	22.9	50.50	15	83.04	5.28	12	F
BBR 326	BBR 327	7/19/12	4C1333643C	26	41.70	5	44.31	67.71	12	M
BBR 112	BBR 113	7/21/12	4A0B032757	23.8	44.70	15	65.77	30.63	12	M
BBP 273	BBP 274	7/21/12	441464071E	23.8	44.80	10	78.99	16.47	12	Unk
BBR 373	BBR 374	8/7/12	982000167841305/ 3D6000A010E19	26.2	40.60	11	72.42	23.13	12	M
BBR 386	BBR 387	8/9/12	982000167799856/ 3D6000A006C30	26.1	54.90	5	83.04	5.28	12	F
BBR 390	BBR 391	8/16/12	982000167821061/ 3D6000A00BF05	26.4	43.60	9	83.55	12.78	12	F
--	--	8/27/12	982000167840723/ 3D6000A010BD3	25.5	27.20	5	83.04	5.28	12	F
--	--	10/28/12	982000167776632/ 3D6000A001178	24.8	27.90	9	83.04	5.28	12	F
BBR 423	--	10/28/12	982000167826986/ 3D6000A00D62A	24.8	44.80	13	83.04	5.28	12	F
BBR 426	BBR427	10/29/12	4A5F163F38	24.5	41.30	15	83.04	5.28	12	F

LF TAG	RF TAG	DATE	PIT Tag	SURFACE WATER TEMP (C)	SCL (cm)	TIME PASSED SINCE DRAWN	% Binding	Pg/ml	Assay No.	Predicted M/F/Unk
BBR 036	--	10/31/12	466B06687E/ 4A6B646A72	23.7	46.60	12	83.04	5.28	12	F
BBR 440	BBR441	11/1/12	982000167827292/ 3D6000A00D75C	23.7	35.90	8	65.77	30.63	12	M
BBR 442	--	11/2/12	982000167826699/ 3D6000A00D50B	26	31.30	4	83.04	5.28	12	F
BBR 447	--	11/3/12	982000167841371/ 3D6000A010E5B	26.5	31.20	5	83.04	5.28	12	F

APPENDIX D:
LITERATURE SEARCH RESULTS OF JUVENILE MARINE TURTLE SEX RATIO STUDIES

The following four tables are literature search results for loggerhead (*Caretta caretta*, Table 1), green turtle (*Chelonia mydas*, Table 2), Kemp's ridley (*Lepidochelys kempii*, Table 3), and hawksbill (*Eretmochelys imbricata*, Table 4) marine turtles.

Table D1

Juvenile loggerhead (*Caretta caretta*) sex ratio studies found in the literature (gray and published). * Denotes significance from 1:1 (Female:Male), RIA= Radioimmunoassay, SCL= straight carapace length, CCL= curved carapace length, unk= unknown, est. indicates estimated size range from figures in the source.

Ratio (F:M)	Time period collected	N	No. of Unk	Methods	Location	Size range (cm)	Reference
2.9:1*	Sep-Dec 1995 to 1997 June-Dec 1998-2002	1106	2	RIA Validated with 89 within the study	Core and Pamlico Sounds, NC	41.4- 75.7 SCL	Braun-McNeill et al. 2007
54.2% female	1986- 2005	310	unk	Stranded- direct examination of gonads	Mediterranean Sea (4 different areas)	5- 75 CCL (est.)	Casale et al. 2006
2:1*	2000-2006	224	0	Laparoscopy and histology For juv pelagic stage 2:1* For benthic immatures 2:1	Eastern North Atlantic, offshore Madeira Island	171- 687 mm SCL	Delgado et al. 2010
1.56:1*	2000-2011	218	0	Necropsy	Mediterranean Sea	unk	Maffucci et al. 2013
1.74:1*	18 mo, Sep 1980-April 1983	166	10	RIA Validated with 22 laparoscopied Cc's within the study	Cape Canaveral	45- 76 SCL (est.)	Wibbels et al. 1987
3.21:1*	September 1982- April 1984	61	2		Hutchinson Island	40- 76 SCL (est.)	
1.40:1	2-3 days/week, 2 mo May 1983- July 1983	24	0		Indian River	44- 75 SCL (est.)	
1.96:1	May 1983- Nov 1983	21	4		Chesapeake Bay	40- 75 SCL (est.)	
3.6:1*	6 months	60	0	RIA All were laparoscopied	Heron Island Reef, Australia	unk	Wibbels et al. 1989

Table D2

Juvenile green turtle (*Chelonia mydas*) sex ratio studies found in the literature (gray and published). * Denotes significance from 1:1 (Female:Male), IRL= Indian River Lagoon, RIA= Radioimmunoassay, SCL= straight carapace length, CCL= curved carapace length, unk= unknown.

Ratio (F:M)	Time period collected	N	No. of Unk	Methods	Location	Size range (cm)	Reference
5.27:1*	1995-1997	100	6	RIA	IRL	25.8- 73 SCL	Bagley 2003
5.71:1*	1995-1997	100	6	RIA	Trident Basin	24.2- 45.4 SCL	Bagley 2003
2.62:1*	1995-1998	100	6	RIA	Reef location in Indian River county, FL	26.2- 70.8 SCL	Bagley 2003
7:1*	March- December 1996	8	0	RIA	Deadman Bay, FL, Big Bend	27.9- 70.7 SCL	Barichivich 2006
5.57:1*	1995- 1996	46	0	RIA	North Carolina	unk	Bass et al. 1998
1.4:1.0	11-22 April 1988	120	9	RIA	Great Inagua, Bahamas	25- 70 SCL (est.)	Bolten et al. 1992
3.25*1	After a major cold stun Dec 2000/ Jan 2001	51	0	Necropsy	Saint Joseph Bay, FL	61% < 35 SCL, 15% >45 SCL	Foley et al. 2007
0.9:1	1987	37	0	RIA	IRL	32.1 – 75.0 CCL	Leupschen 1987
1.2:1.0	6 mo	145		Laparoscopy	Heron Reef, Australia	unk	Limpus & Reed 1985
CCL>64.99 2000- 3.77:1 2001- 2.72:1 2002- 3.3:1 2003- 3.15:1 2004- 3.35:1	2000-2004, each winter	166 134 116 108 113	0	Laparoscopy	Shoalwater Bay, Australia	> 64.99 CCL	Limpus et al. 2005
CCL< 65.0 2000- 2.26:1 2001- 1.68:1 2002- 1.92:1 2003- 1.29:1	2000- 2004, each winter	75 99 184 151	0	Laparoscopy	Shoalwater Bay, Australia	< 65.0 cm CCL	Limpus et al. 2005

Ratio (F:M)	Time period collected	N	No. of Unk	Methods	Location	Size range (cm)	Reference
2004- 1.57:1		229					
Not sig.		56	0	Laparoscopy	Bermuda	30.5- 75.5 SCL	Meylan et al. 1992
1.75:1*	1989, after a cold-stun event	66	0	66 necropsied	Mosquito Lagoon	26.6- 77 SCL	Schroeder & Owens 1994
2:1	January 1986- December 1992	6	unk	Necropsy- did other species also, only small amount Cm	Texas coast	unk	Stabenau et al. 1996
2:1*	6 months	200	1, but verified	RIA All laparoscopied	Heron Island, Australia	unk	Wibbels et al. 1989
0.96:1 (pooled ratio)	Dec 1983- Aug 1984	66	3	RIA	Hawaiian Archipelago	39.6- 75.3 cm SCL	Wibbels et al. 1993

Table D3

Juvenile hawksbill (*Eretmochlys imbricata*) sex ratio studies found in the literature (gray and published). Original table found in Blanvillain et al. (2007) and added to. * Denotes significance from 1:1, RIA= Radioimmunoassay, SCL= straight carapace length, CCL= curved carapace length, unk= unknown.

Ratio	Time period collected	N	No. of Unk	Methods	Location	Size range (cm)	Reference
2.37:1*	2005-2007	69	5	RIA Validated with 17 laparoscopied Ei	South FL reef, Palm Beach county	35.7- 68.4 SCL	Blanvillain et al. 2007
0.8:1	1993-1996	120	1	RIA Validated with 14 laparoscopied Ei within the study	Mona Island, Puerto Rico	20- 65 SCL	Diez & Van Dam 2003
2.6* to 5:1*	April 1995- October 1999	72	8	RIA Validated with 18 laparoscopied Lk from different studies done at Mona Island, Puerto Rico	Buck Island Reef, US Virgin Islands	22.5- 75.7 SCL	Geis et al. 2003
4.4-7.7:1*		62	9 ?	RIA Validated with levels from laparoscopied Ei used in previous studies in the same region	Anegada, Greater Antilles	unk	Hawkes et al. 2013
2.71:1*	April 1996- April 1998	143	4	RIA	Dominican Republic	19.5- 69.7 SCL	León & Diez 1999
2.57:1*	May 1969- April 1988	109	2	Laparoscopy (107) Necropsy (2) Includes one adult male	Southern Great Barrier Reef, Australia	35.0- 87.5 CCL	Limpus 1992
4.2:1*	6 months	26		RIA All were laparoscopied	Heron Island Reef, Australia	unk	Wibbels et al. 1989

Table D4

Juvenile Kemp's ridley (*Lepidochelys kempii*) sex ratio studies found in the literature (gray and published). Original table found in Geis et al. (2005) and added to. * Indicates significance from 1:1, RIA= Radioimmunoassay, SCL= straight carapace length, CCL= curved carapace length, MSCL= minimum straight line carapace, unk= unknown.

Ratio	Time period collected	N	No. of Unk	Methods	Location	Size range (cm)	Reference
1.0:1.2	1994	88	unk	Necropsy	Upper TX and LA coast	unk	Cannon 1998
1.3:1.0* wild, 1.5:1* with headstarted turtles	1992-1997	247	10	RIA 74 were laparoscopied within the study	Upper TX and LA coast	21.8- 62.1 SCL	Coyne 2000
1.4:1.0	1977-1987	48	unk	Necropsy	Cape Cod, MA	unk	Danton & Prescott 1988
3.7:1*	1998-2000	42	0	RIA Validated with 48 laparoscopied Lk from previous study in western Gulf of Mexico	Steinhatchee, FL	22.2- 48.2 SCL	Geis et al. 2005
1.8:1.0*	May- October 1992	39	3	RIA Done in same area as Geis et al. (2005), 6-8 yrs earlier	Cedar Keys, FL	25.0- 60.0 MSLC	Gregory & Schmidt 2001
3.2:1.0*	1985-1987	30	0	Necropsy There were 50 stranded, and only 30 were looked at for sexing	Long Island Sound NY	unk	Morreale et al. 1992
1.0:1.0	1983-1989	81	unk	Necropsy	South TX coast	unk	Shaver 1991
76.4% female	1986-1992	144	0	Necropsy Adults included	Upper TX coast	10.0- 60.0 SCL	Stabenau et al. 1996
1.9:1.0*	2000-2001	100	13	RIA	Gullivan Bay, Ten Thousand Islands, FL	27- 52 MSCL (est.)	Witzell et al. 2004

APPENDIX E:
PRELIMINARY EXAMINATION OF INCUBATION DURATION AND AIR TEMPERATURE ON THE ARCHIE CARR NATIONAL WILDLIFE REFUGE

INTRODUCTION

For green turtles, pivotal temperature has been found/estimated to be near 29°C (Standora & Spotila, 1985; Godfrey et al., 1996; Kaska et al., 1998; Broderick et al., 2000; Booth & Astill, 2001; Godley et al., 2002; Godfrey & Mrosovsky, 2006) with successful incubation approximately between 25 and 35°C (reviewed by Hawkes et al., 2009). With sex being determined by temperature and possessing a narrow transitional range, these animals may become more susceptible to changes in the environment, producing increasing amounts of females (Hawkes et al., 2007; 2009). Surprisingly there is a lack of long-term information for green turtle nest temperatures. This information would allow conclusions to be made about whether nesting beaches are warming. Hays et al. (2003) attempted to reconstruct previous nest temperatures for green turtles on Ascension Island and suggested a general warming trend of sand over the past 150 years. Fuentes et al. (2010) found no significant change in beach temperatures in Australia over the past 18 years, but models predicted a complete feminization of hatchlings by 2070.

Another way of looking for changes in beach temperatures has been done by examining changes in incubation duration (reviewed Wibbels, 2003). In order to make some sense as to what we were seeing (female-biased juvenile green turtle aggregations), this preliminary study examined air temperatures near Melbourne Beach, FL, for the years 2001-2010 and 1985-1994, to account for when juvenile green turtles may have been incubating on the beach for the current study and in the previous study (Bagley, 2003). The Archie Carr National Wildlife Refuge (ACNWR) has been monitored for decades, and incubation temperatures were examined for both green turtles and loggerheads over a ten-year period to see if incubation durations have decreased, which would be indicative of a warming beach.

METHODS

Air Temperature

Monthly mean maximum air temperatures were obtained from NOAA National Climatic Data Center station USW00012838 (2012) near the ACNWR in Brevard County for the time periods of 1985-1994, to presumably account for air temperatures in Melbourne Beach FL when Bagley (2003) juvenile green turtles may have been incubating, and 2001-2010, when juveniles obtained in the current study may have been incubating. Temperatures in Brevard County were chosen as this area accounts for nearly half of all nests laid in FL each year (FWCC, 2012). An ANOVA was run by month (July-November) comparing the two time periods. Welch's ANOVA was run when variances were not equal.

Incubation Duration

Incubation time in days was determined for loggerhead and green turtles from 2001-2011 for nests on the ACNWR in Brevard County (21 km stretch of beach). Researchers from the University of Central Florida Marine Turtle Research Group (UCF MTRG) marked nests within 12 hours of deposition. Nests were checked daily and emergence date was recorded when seen, and the number of days of incubation was calculated. It is important to note that incubation days may be overestimated by a few days since once a hatchling sheds its shell, it may remain under the sand for a few days before emerging (reviewed by Godfrey & Mrosovsky, 1997). Monthly averages were calculated for incubation duration based on the month they were deposited. Monthly averages were not calculated if there were 5 or fewer nests with available incubation durations. Nests that incubated less than 40 days or over 80 days were excluded. Correlations were run for each month including all years, using Spearman's rank test.

RESULTS

Air Temperature

November was the only significant month when comparing mean maximum air temperatures for the two time periods (Table 1), and a trend of warmer temperatures in the latter time period from June through October with a lower temperature in November was seen (Fig. 1).

Table E1

ANOVA results for monthly mean maximum air temperatures by time period (1985-1994 and 2001-2010) for the months June-November.

Month	Analysis	*P*-value	*F* ratio
June	Welch's ANOVA	0.1246	2.73
July	ANOVA	0.194	1.83
August	Welch's ANOVA	0.0543	4.38
September	ANOVA	0.1554	2.20
October	ANOVA	0.5425	0.39
November	Welch's ANOVA	0.0428	4.76

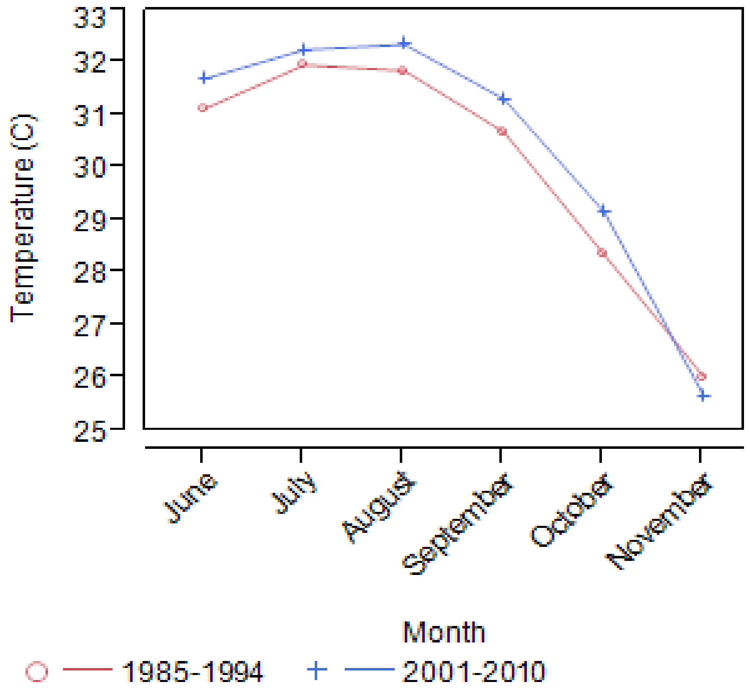


Fig. E1

Monthly mean maximum temperature for June-November for two time periods.

Incubation Duration

May through July was used for loggerheads and June through August was used for green turtles based on the available data. Overall 14 loggerhead and 6 green turtle nests were excluded resulting in 1206 loggerhead and 886 green turtle nests (Table 2, 3). Not all months had calculated incubation durations due to small sample sizes. All months analyzed for loggerheads (May-June) were significant, and all three months (June-August) analyzed for green turtles were significant (Table 4) and considerable variation was seen (Fig. 2, 3).

Table E2

Loggerhead incubation duration (days) from 2001-2011 by month. The number of nests used to calculate the average incubation duration is in parenthesis.

Year	May	June	July
2001	--	57 (8)	--
2002	60 (6)	58 (5)	--
2003	56 (41)	54 (69)	53 (15)
2004	53 (35)	51 (41)	51 (9)
2005	59 (43)	56 (46)	55 (55)
2006	56 (59)	53 (116)	52 (66)
2007	56 (24)	52 (61)	52 (18)
2008	55 (45)	55 (48)	56 (17)
2009	57 (30)	53 (73)	53 (24)
2010	55 (33)	50 (59)	49 (37)
2011	54 (29)	52 (68)	50 (26)

Table E3

Green turtle incubation duration (days) from 2001-2011 by month. The number of nests used to calculate the average incubation duration is in parenthesis.

Year	June	July	August
2001	--	55 (13)	--
2002	62 (15)	57 (24)	--
2003	54 (37)	54 (36)	56 (17)
2004	53 (10)	52 (15)	--
2005	56 (32)	52 (88)	54 (42)
2006	55 (28)	53 (54)	56 (45)
2007	55 (20)	52 (47)	53 (10)
2008	57 (18)	57 (20)	61 (7)
2009	53 (18)	53 (50)	54 (28)
2010	51 (20)	50 (56)	54 (31)
2011	52 (48)	51 (57)	--

Table E4

Results for regressions run by month for 2001-2011, with loggerhead (Cc) months of May-June on the left and green turtle (Cm) months of June-August on the right.

	Cc			Cm	
	Spearman's ρ	P		Spearman's ρ	P
May	-0.19	0.0003	June	-0.38	0.0001
June	-0.25	0.0001	July	-0.34	0.0001
July	-0.30	0.0001	August	-0.017	0.0165

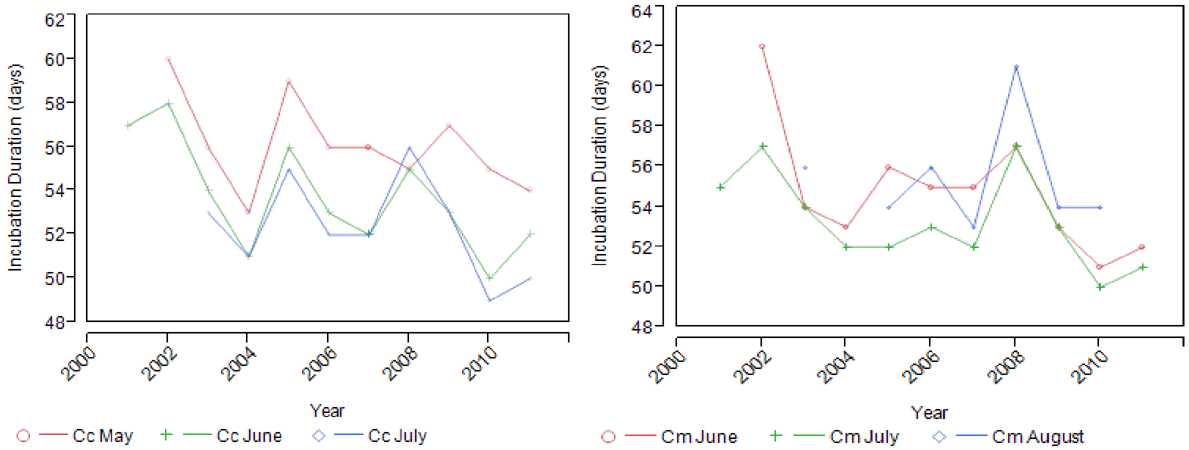


Fig. E2

Overlay of incubation durations for loggerhead (left) and green turtles (right) for 2001-2011.

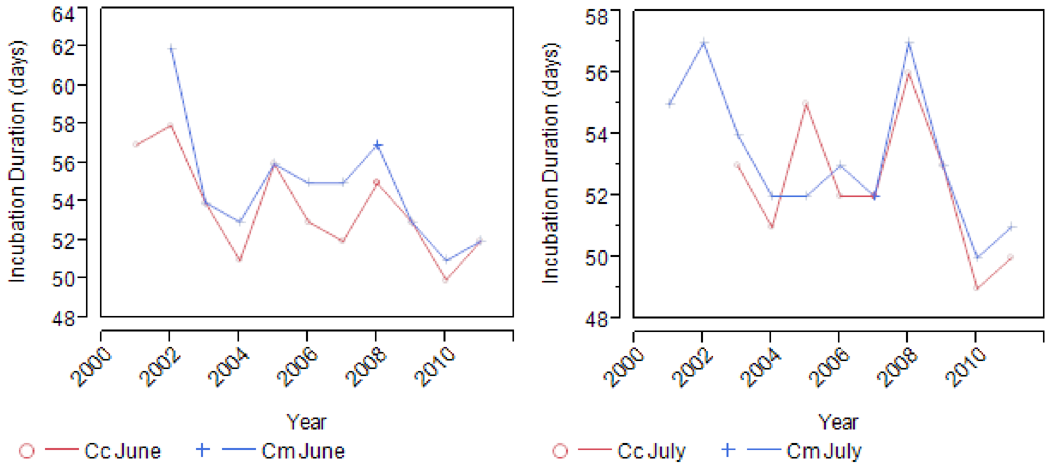


Fig. E3

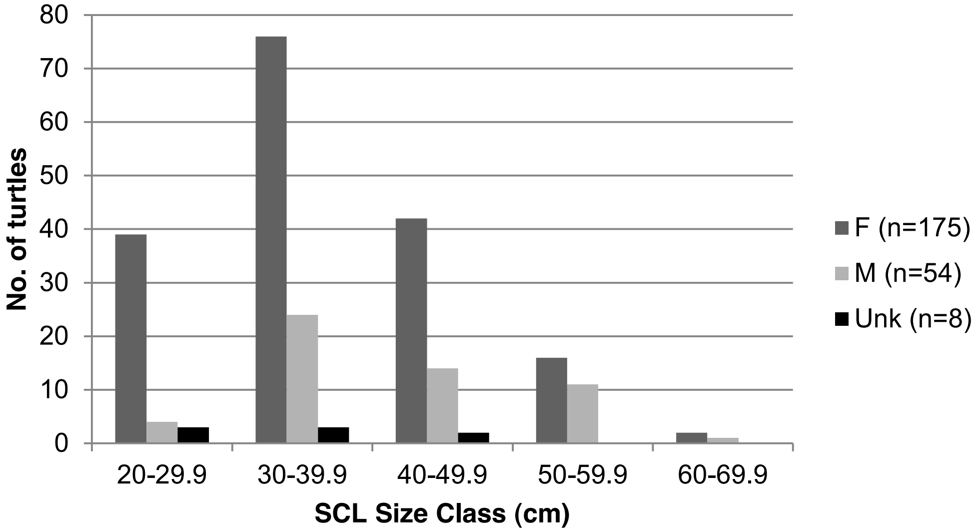
Overlay of incubation durations for loggerhead and green turtles for the months of June (left) and July (right).

DISCUSSION

Trends for average monthly temperatures for 2001-2010 were slightly higher than averages for 1985-1996 for June-September and were slightly lower in November (Table 1, Fig. 2). These temperatures are similar to what has been described in a recent study on flowering plants for FL with overall warmer summer and fall months and lower minimum temperatures in the winter months (Von Holle et al., 2010). Either there has not been a significant change in temperature on the beach or the two time periods are too close together to pick up significance. This is confirmed with the current juvenile sex ratio study results when compared to those found from samples collected in the late 1990s (Bagley, 2003), where sex ratios were significantly female-biased but not significantly different from each other.

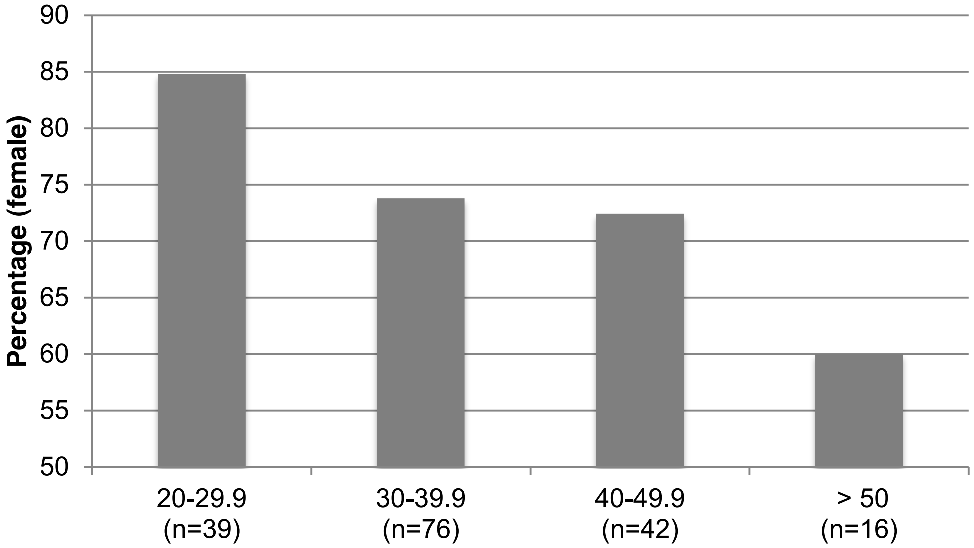
For incubation duration, a lot of variation can be seen (Fig. 2, 3) as was expected, since many factors can contribute to incubation duration (reviewed by Hawkes et al., 2009) including the physical features of the nesting beach (Hays et al., 2003), the climatic events during a season (Godfrey et al., 2006), the albedo (Hays et al., 1995) or even whether the nest is shaded by vegetation (Janzen, 1994). However, monthly incubation durations did significantly decrease for both species (except for green turtles in August). The trend of slightly warmer temperatures for 2001-2010 and the decrease in incubation duration for 2001-2011 points to a possibly warming atmosphere for incubating clutches. The surprising result of the significant decrease in incubation temperature over just a 10 year period without a significance in air temperature could suggest that even small changes in temperature can affect sea turtles, as been shown in painted turtles (*Chrysemys picta*) (Janzen 1994). However, determining the pivotal temperature for green turtle nests from the east coast of FL in a laboratory will allow estimating overall sex ratio (ex. Godfrey et al., 1999; Mrosovsky et al., 1999; Godley et al., 2001).

APPENDIX F:
ADDITIONAL TABLES AND FIGURES



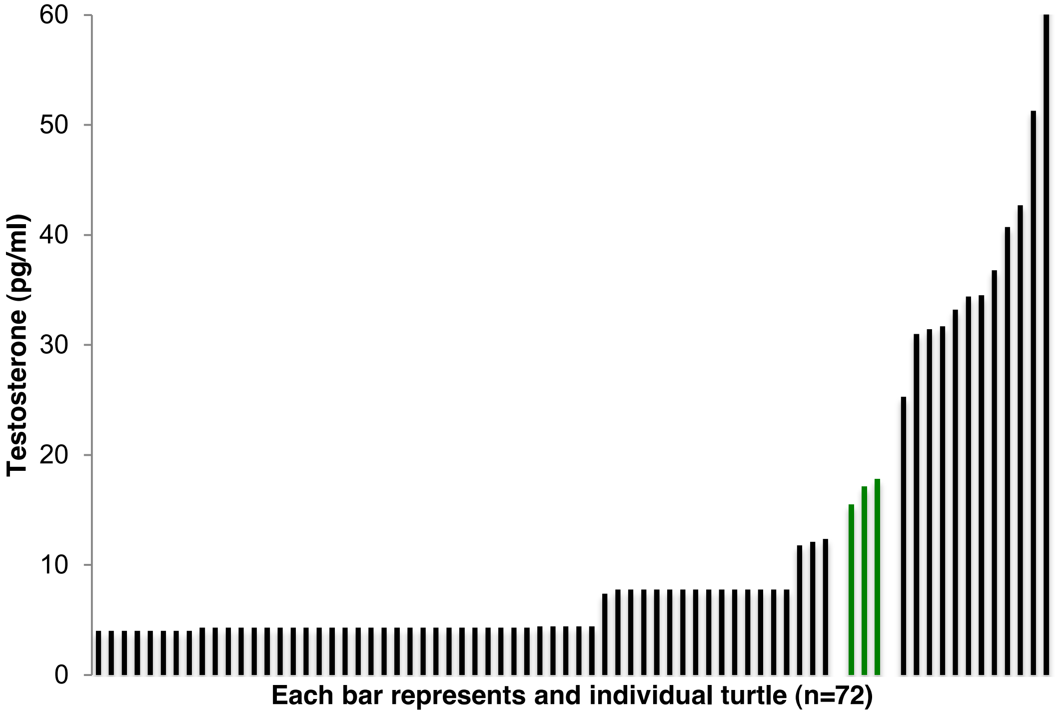
F1

Size class distribution by sex including all three sampling sites.



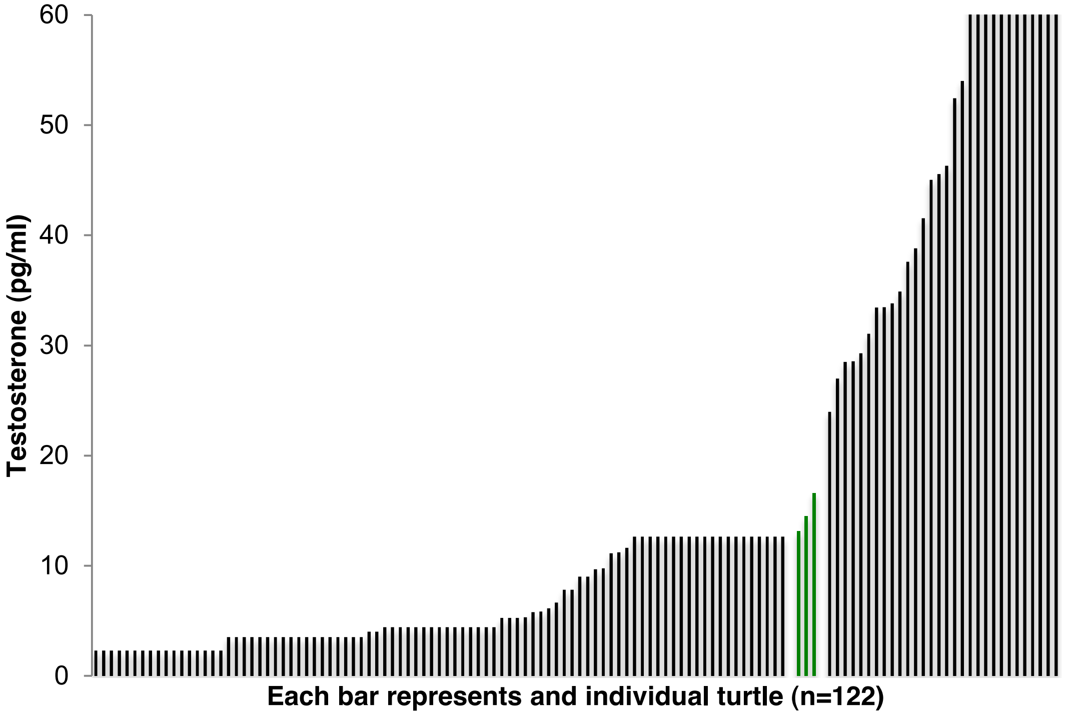
F2

Percent female of all samples within the different size classes.



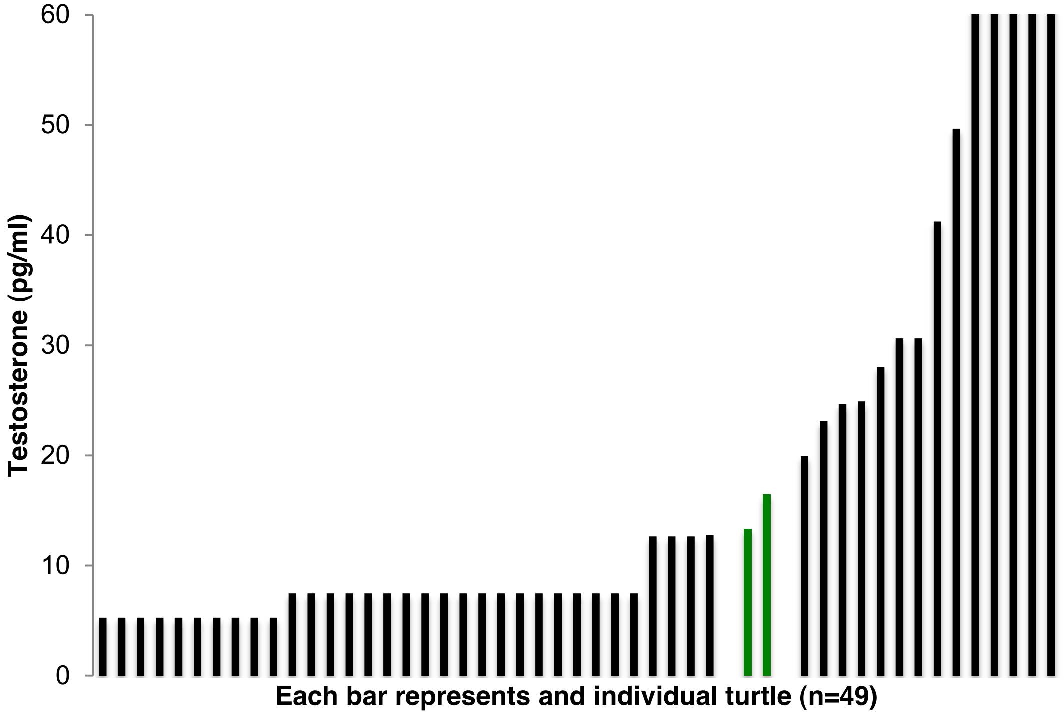
F3

Testosterone levels of green turtles from Trident Basin. Green bars represent the unknown samples (n=3), left of the green bars are females (n=57), right of the green bars are males (n=12).



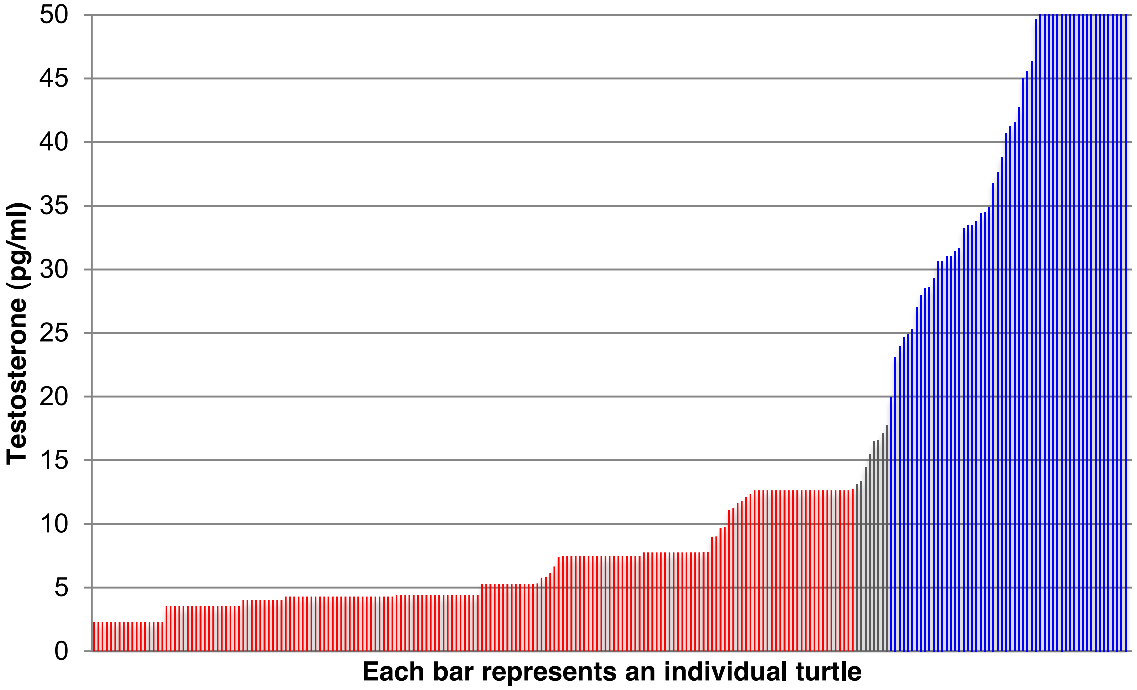
F4

Testosterone levels of green turtles from the IRL. Green bars represent the unknown samples (n=3), left of the green bars are females (n=89), right of the green bars are males (n=30).



F5

Testosterone levels of green turtles from the St. Lucie Power Plant. Green bars represent the unknown samples (n=2), left of the green bars are females (n=33), right of the green bars are males (n=14).



F6

Testosterone levels of green turtles from 3 different sites. Red bars represent females (n=179), gray bars represent unknowns (n=8), and blue bars represent males (n=56).

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