ANALYSIS OF STRANDED LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*) IN NORTH AND SOUTH CAROLINA: GENETIC COMPOSITION AND THE EFFECTIVENESS OF NEWLY IMPLEMENTED TED REGULATIONS

A thesis submitted in partial fulfillment of the requirements for the degree

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in

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by

KRISTEN T. MAZZARELLA
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THE GRADUATE SCHOOL OF THE COLLEGE OF CHARLESTON

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ABSTRACT

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Stranded sea turtles are often used as representatives of nearshore aggregations. As of yet, no study has been conducted to validate this assumption. Therefore, haplotype frequencies of 112 stranded loggerhead sea turtles (*Caretta caretta*) from North and South Carolina were compared to nearshore live-capture data. Strandings were not significantly different from the live-capture data (Φ_{ST}=-0.0064, p=0.7986), suggesting stranded individuals are representative of the nearshore loggerhead aggregation. Additionally, South Carolina loggerhead stranding records (n=255) from May, June, and July of 2000-2003 were compared to live-capture data (n=285) from the same time period. No significant difference in size distribution was observed in 2000–2002, supporting the genetic findings. However, a significant difference in size distribution was observed in 2003 (D=0.3179, p=0.0005), necessitating further investigations to elucidate this discrepancy. As it has been shown that nearshore loggerhead aggregations are mixtures of different nesting subpopulations; the genetic origins of the sampled loggerheads were estimated using two types of mixed stock analysis. Results indicate that strandings were comprised of the Northern (NEFL-NC), South Florida (SFL), and Yucatán (MEX) nesting subpopulations, with a higher contribution than expected from the NEFL-NC with respect to rookery size. As such, coastal hazards off North and South Carolina may differentially impact the NEFL-NC nesting subpopulation. Finally, South Carolina stranding records from 2000-2005 were examined to determine the effectiveness of a 2003 change in exit opening requirements for Turtle Excluder Devices (TEDs) on U.S. shrimp trawlers, implemented to reduce adult loggerhead mortality. A significant difference was observed in size distributions of strandings before (2000-2001) and after (2004-2005) TED modification ($\chi 2$=18.087, d.f.=5, p=0.003), with a 15.3% decrease in total adult ($\geq$ 90 cm CCL) stranding numbers after new TED implementation ($\chi 2$=13.820, d.f.=1, p=0.000). These findings suggest the new TED exit openings have been successful in adult loggerhead mortality reduction.

INTRODUCTION

Loggerhead sea turtles, *Caretta caretta* (Linneas 1758), are one of six marine turtle species in the family Cheloniidae. They inhabit temperate and tropical waters worldwide in the Atlantic, Pacific, and Indian Oceans as well as bordering seas, bays, and estuaries (Dodd 1988; NRC 1990). *C. caretta* are a long-lived species, estimated to mature at approximately 30-35 years of age (Frazer & Ehrhart 1985). Adults are upwards of 92 cm straight carapace length (SCL) and 113 kg mean body weight (NRC 1990). They are opportunistic, carnivorous feeders and prefer primarily crustaceans and mollusks (Dodd 1988; Mortimer 1982).

Female loggerheads nest on temperate, sandy beaches every 2-3 years and lay multiple clutches per season with 14 day internesting intervals (Dodd 1988). They appear to display natal philopatry, returning to nest in the region from which they hatched (Bowen et al. 1993; Bowen et al. 1994; Encalada et al. 1998). Most females also display strong nest site fidelity, typically nesting within five kilometers of a previous nest (Schroeder et al. 2003) and remaining nearshore of these beaches during internesting intervals. Hatchlings emerge after 55-80 days incubation, crawl to the water and actively swim away from land (Caine 1986; Musick & Limpus 1997). They become entrained in currents and are transported into open ocean gyres in the north Atlantic, where they forage primarily within *Sargassum* rafts in the epipelagic zone (Carr 1986, 1987). Like most sea turtles, the loggerhead's habitat preferences shift with transitioning life stages

(Dodd 1988). After approximately 7-12 years or 40-60 cm SCL, "oceanic immatures" transition to coastal areas (Bjorndal et al. 2000) and switch from pelagic to benthic feeders (TEWG 2000). There is evidence that transitioning immatures return to foraging grounds off their natal regions (Bowen et al. 2004; Reece et al. 2006; Roberts et al. 2005; Sears et al. 1995). In Florida's tropical climate, immature loggerheads remain year-round residents on foraging grounds (Henwood 1987), while turtles foraging in temperate areas make fall and spring migrations. Some migrating juveniles travel along coastal corridors (Musick & Limpus 1997) while others move further offshore, following warm waters in winter; often returning to the same spring foraging ground year after year (Arendt et al. 2007). Fidelity to foraging grounds has also been observed in adult female and male loggerheads after reproductive migrations (Limpus et al. 1992; Schroeder et al. 2003), although not all females from the same nesting beach utilize the same foraging grounds (Plotkin & Spotila 2002; Schroeder et al. 2003). It appears that juveniles reaching sexual maturity imprint upon foraging grounds they will use as adults (Limpus 1994). Adult loggerheads make extensive migrations between foraging grounds and breeding areas (Limpus et al. 1992; Plotkin & Spotila 2002) with males arriving at mating grounds in advance of females (Henwood 1987). Some adult males are known to reside in breeding areas year round while others, tagged in Port Canaveral, Florida, migrate along the coast as far north as New Jersey, south to the Florida Keys or around to the Florida Panhandle (Arendt et al. 2007; Henwood 1987; SCDNR unpublished data).

Due to extreme differences both in behavior and distribution of loggerhead life stages, all life stages must be considered when developing management practices towards the maintenance and recovery of *C. caretta*. With this in mind, population models have

been developed to identify the life stages whose protection will have the greatest impact on population growth (Crouse et al. 1987; Crowder et al. 1994). These models are based primarily upon life history tables generated from demographic data collected through nesting beach surveys, strandings and in-water studies (Frazer & Ehrhart 1985). In the past, most conservation efforts focused on the protection of eggs on nesting beaches. Despite the ease of management and accessibility of this life stage, increasing survival of eggs and hatchlings without concurrent protection of other life stages will not prevent population decline (Crouse et al. 1987). Rather, protection of large juvenile and adults could have the greatest effect on conservation (Crouse et al. 1987; Crowder et al. 1994).

The southeast coast of the United States and adjacent waters are important habitat for the critical adult and juvenile loggerhead life stages (Henwood 1987; Sears et al. 1995; Teas 1993). Adult females here comprise the second largest loggerhead nesting aggregation in the world; producing 71,767 nests per year, 91% of which are laid on the east coast of Florida (NMFS & USFWS 1991; 2007; Ross 1982). Currently on these nesting beaches, loggerheads face challenges of development, loss of coastal nesting habitat, beach armoring, beach renourishment, beachfront lighting, nest predation, and global warming issues (Hawkes et al. 2007; NRC 1990; Steinitz et al. 1998). Additionally, major seasonal foraging areas have been recognized in nearshore and estuarine waters along the Canadian and U.S. Atlantic coasts and year-round in south and central Florida waters (Ehrhart et al. 2003; Hopkins-Murphy et al. 2003; Lutcavage & Musick 1985; Norrgard & Graves 1996; Roberts et al. 2005; Sears et al. 1995). Within these foraging grounds, the principal anthropogenic threat to juvenile and adult loggerheads is incidental take by commercial fisheries such as trawl fisheries, longline

fisheries and gillnets (Lewison et al. 2004; NRC 1990). Loggerheads are also subject to mortality in coastal waters via dredging, ship strikes, recreational fishing, and entanglement in or ingestion of marine debris and toxins (NRC 1990).

Given the complex life history of loggerheads, anthropogenic impacts on any life stage in waters of one area of the world can eventually effect nesting subpopulations elsewhere; therefore protection of loggerheads requires a global initiative. In the United States, loggerheads are protected by the Endangered Species Act (ESA) of 1973 where they have been listed as Threatened since 1978. Per ESA mandate, an Atlantic Loggerhead Sea Turtle Recovery Plan was published in 1984, modified in 1991 and is currently under revision to include recent findings. Internationally, the Marine Turtle Specialist Group upgraded the listing for loggerheads under the International Union for the Conservation of Nature (IUCN) from "Vulnerable" to "Endangered" throughout most of their range in 1996 (IUCN 2006). International trade of sea turtles is restricted by Appendix I in the Convention on International Trade in Endangered Species (CITES) and they are protected from international take during migrations by the Bonn Convention of 1983 (Hykle 1992).

Present management practices define loggerhead stocks by highly structured nesting beach assemblages identified using the Testudine mitochondrial DNA (mtDNA) control region (TEWG 1998; 2000). The mtDNA control region is non-coding which allows for a high rate of substitution. Since mtDNA is haploid, it has a low effective population size (N_e) and is strongly affected by genetic drift (Moritz 1994). Mitochondrial DNA is maternally inherited, consequently, low female-mediated gene flow has been observed in loggerheads due to strong female natal philopatry and nest site

fidelity (Norman et al. 1994). Studies using nuclear DNA have failed to reveal the strong structuring observed using mtDNA on nesting beaches (Encalada et al. 1998; FitzSimmons et al. 1997; Pearce 2001). This suggests that male loggerheads are not as philopatric and therefore provide gene flow between nesting subpopulations, confounding the structure observed using mtDNA (FitzSimmons et al. 1997; Pearce 2001).

Female loggerheads, eggs and hatchlings from known major nesting beaches in the Atlantic basin and Mediterranean were surveyed genetically by Bowen et al. (1993) and Encalada et al. (1998). Sampled locales included beaches in North Carolina to the Florida Panhandle in the United States; Quintana Roo, Mexico; Bahia, Brazil; and Kiparissia Bay, Greece. Ten mtDNA haplotypes were identified with two haplotypes, CC-A1 (A) and CC-A2 (B), comprising 88% of individuals sampled. In an unrooted parsimony network, these two haplotypes fell into two discrete clusters separated by 17 mutation steps with a mean sequence divergence of $p = 0.05$ (Encalada et al. 1998). The CC-A1 haplotype clustered closely with only one other haplotype, CC-A4 (D), a haplotype unique to the Brazilian nesting beaches. Other haplotypes were not unique to nesting beaches, however haplotype frequencies differed geographically (Table 1; Bowen et al. 1993; Encalada et al. 1998). Haplotype CC-A1 was observed at a frequency of 98-100% in North Carolina, South Carolina, Georgia, and northeast Florida, but occurred in only 44 % and 88% of turtles sampled in south Florida and northwest Florida, respectively. The CC-A2 haplotype was also observed in varying frequencies among northwest Florida, southwest Florida, southeast Florida, Georgia, Mexico and Greece (Encalada et al. 1998). Adjacent nesting beaches with little genetic differentiation were grouped together such that six genetically-distinct matrilineal nesting subpopulations

were identified (NMFS & USFWS 2007):

1. Northern Nesting Subpopulation: NE Florida to North Carolina, USA (NEFL-NC)
2. South Florida Nesting Subpopulation: South Florida, USA (SFL)
3. Florida Panhandle Nesting Subpopulation: NW Florida, USA (NWFL)
4. Yucatán Nesting Subpopulation: Quintana Roo, Mexico (MEX)
5. Bahia, Brazil (BRA)
6. Kiparissia Bay, Greece (GRE)

The Turtle Expert Working Group (1998) established the first four nesting subpopulations as loggerhead management units in their population assessment for loggerheads in the western North Atlantic.

The NEFL-NC produces approximately 5,151 nests per year, which constitutes a mere 7% of U.S. loggerhead nests; however, this small subpopulation plays an important role in the Atlantic loggerhead aggregation as a whole (NMFS & USFWS 2007). Sea turtles have temperature dependent sex determination by which cooler temperatures in the nest produce males and warmer temperatures generate females (Yntema & Mrosovsky 1979). Northern nesting beaches, such as those in the NEFL-NC, have the most temperate climates and provide cooler sand temperatures than those on southern nesting beaches. Thus, northern nesting beaches are important producers of male turtles. Increased global warming may increase sand temperatures such that eventually, in the lower latitudes, where few males are produced presently (Mrosovsky & Provancha 1992), beaches may not be cool enough to produce male turtles or may even be above lethal temperatures and produce no hatchlings at all. Northern beaches will therefore become increasingly important to the survival of the species (Hawkes et al. 2007), whether for male production or for future nesting beach habitat.

South Carolina beaches are part of the NEFL-NC, the status of which is currently

reported as stable or in decline (TEWG 2000). However, more recent data, from a 1983-2005 survey, shows it to be declining at 1.9% annually (NMFS & USFWS 2007). South Carolina beaches, alone, have observed a 3.1% annual decrease in nest numbers since 1980 (Hopkins-Murphy et al. 2001). Due to strong female natal philopatry, recovery from declining nest numbers through recruitment from other subpopulations is unlikely on a contemporary time scale (Avise 1995; Bowen et al. 1993).

While beaches in South Carolina produce approximately five percent of U.S. loggerhead nests annually, they are home to the largest nesting aggregation in the NEFL-NC (Hopkins-Murphy et al. 2001; NMFS & USFWS 2007). Cape Romain National Wildlife Refuge averages approximately 1,000 nests per year or 21% - 31% of South Carolina nest production and 16% - 19% of the NEFL-NC nests (Bass et al. 2004; Hopkins-Murphy et al. 2001; NMFS & USFWS 2007). Apart from nesting beaches, the nearshore and estuarine waters of South Carolina are utilized by large juvenile and adult loggerheads as foraging grounds, internesting habitat, and migratory routes between nesting and foraging areas (Hopkins-Murphy et al. 2003). Genetic data have shown that juvenile foraging grounds off the South Carolina coast are comprised of turtles from multiple nesting subpopulations (Bolten et al. 1998; Bowen et al. 2004; Bowen et al. 2005; Rankin-Baransky et al. 2001; Roberts et al. 2005; Sears et al. 1995). In addition, the NEFL-NC is thought to be overrepresented in waters off its own nesting beaches, in comparison to other subpopulations (Roberts et al. 2005). As such, anthropogenic hazards in South Carolina waters have the potential to impact large juvenile and adult life stages of loggerheads from distant subpopulations as well as the genetically-distinct Northern Nesting Subpopulation.

Live and dead sea turtles found washed ashore or floating are considered strandings. The Sea Turtle Stranding and Salvage Network (STSSN), established in 1980, documents sea turtle strandings which have been used to estimate at-sea mortality (Murphy & Hopkins-Murphy 1989). In addition, details from stranding reports used in conjunction with records of concurrent events or environmental conditions can often lead to the identification of the source of mortality. Therefore, the use of stranding records is vital to understanding the impacts of nearshore anthropogenic activities on sea turtle aggregations. Stranded sea turtles represent the subpopulations and size classes at highest risk for anthropogenically-induced mortality in coastal waters.

This study intends to look at size class distribution and genetic origin of loggerhead strandings on North and South Carolina beaches in order to infer the life stages and stocks that are negatively impacted by coastal anthropogenic factors such that appropriate management practices can be applied.

CHAPTER I:

Estimated Origin of Stranded Loggerheads in North and South Carolina

BACKGROUND

Assisting in the implementation of the loggerhead recovery plan mandated by the Endangered Species Act (1973), molecular techniques have successfully unveiled components of the population structure of loggerhead sea turtles. At present, the eastern coast of the United States is made up of three genetically-distinct nesting subpopulations, the Northern Nesting Subpopulation (NEFL-NC), South Florida Nesting Subpopulation (SFL) and the Dry Tortugas Nesting Subpopulation (DT), largely based on differing mtDNA haplotype frequencies (NMFS & USFWS 2007). Genetically differentiated subpopulations allow for the use of mixed stock analysis (MSA) to estimate the composition of a mixture of individuals (Grant et al. 1980; Pella & Milner 1987). Fisheries biologists were the first to employ MSA when they used data from known sockeye salmon (*Onchorynchus nerka*) spawning areas to estimate the origin of commercial catches (Grant et al. 1980). For sea turtles, MSA has been used to resolve the origin of loggerhead feeding aggregates (Bowen et al. 2004; Lahanas et al. 1998; Reece et al. 2006; Roberts et al. 2005) and strandings (Rankin-Baransky et al. 2001) and to identify transoceanic migrations (Bolten et al. 1998; Maffucci et al. 2006).

Most loggerhead aggregations are mixtures of individuals from several nesting subpopulations and identification of the composition of these aggregations is necessary

for the designation of areas as critical loggerhead habitat. Three distant nesting subpopulations, NEFL-NC, SFL, and the Yucatán Nesting Subpopulation (MEX), inhabit the coastal waters of the United States, from Virginia to Massachusetts, and the inshore waters of North Carolina (Bass et al. 2004; Rankin-Baransky et al. 2001). Loggerhead aggregations adjacent to the NEFL-NC are comprised of NEFL-NC, SFL and Florida Panhandle Nesting Subpopulation (NWFL) individuals, with a small contribution from MEX (Roberts et al. 2005). A similar NEFL-NC/SFL representation occurs in the juvenile loggerhead aggregation in the Charleston Harbor Entrance Channel, South Carolina (Sears et al. 1995). In addition, the smaller NEFL-NC appears to be disproportionately represented in coastal feeding aggregates off its' natal beaches, in relation to rookery size (Rankin-Baransky et al. 2001; Roberts et al. 2005; Sears et al. 1995). Based on these observations, it is clear that anthropogenic hazards in waters off North and South Carolina have the potential to affect conservation efforts of regional as well as distant subpopulations.

The aforementioned subpopulations represented off the eastern coast of the United States appear to be in decline. The NEFL-NC has been exhibiting a 1.9 – 3.1% decline in nesting over the past few decades, while a 22.3% decline was observed in the SFL from 1989 – 2005 and appears to be even worse in recent years (NMFS & USFWS 2007). A reduction has also been detected in the NWFL, at 6.8% from 1995-2005 and in MEX since 2001 (NMFS & USFWS 2007). With this in mind, this study aims to use MSA to estimate the nesting beach origin of strandings in North and South Carolina to infer which nesting subpopulations might be differentially impacted by anthropogenic factors off the southeastern coast of the United States.

OBJECTIVES

The objectives of this study were to estimate the genetic composition of stranded loggerhead sea turtles in North and South Carolina using mixed stock analysis and to determine whether strandings are a random sampling of the nearshore loggerhead aggregation.

MATERIALS AND METHODS

Sample collection

Skin samples were collected from stranded loggerhead sea turtles in North and South Carolina from April 2003 through June 2006. Volunteers from the North and South Carolina Sea Turtle Stranding Networks removed skin from the trailing edge of the front flippers (or rear flipper if no front flipper was available) of stranded turtles using a 5 mm dermal biopsy punch (Miltex Instrument Company, Inc.) or sterile blade. Samples were preserved in 95% ethanol and stored at room temperature.

DNA Extraction

DNA was extracted from skin samples using Qiagen DNA extraction kits (Qiagen, Inc.) per manufacturer's instruction for animal tissue DNA extraction. Isolates were visualized on 1% agarose gels stained with ethidium bromide and viewed on a UV light table to confirm extraction of genomic DNA. Isolated DNA was prepared by adding 1-2 μl of sample to 5 μl GeneReleaser (Bioventures, Inc.) and run on a thermocycler following the manufacturer's protocol with times reduced by fifty percent.

DNA Amplification

A 400 base pair segment of mitochondrial DNA control region was amplified via Polymerase Chain Reaction (PCR) using previously published chelonid turtle primers CR-1/TCR5 (5' – TTG TAC ATC TAC TTA TTT ACC AC – 3') and CR-2/TCR6 (5' – GTA CGT ACA AGT AAA ACT ACC GTA TGC C – 3') (Norman et al. 1994). Amplifications were performed in 50μl reactions consisting of 1-2 μl template added to 1X PCR Buffer, 2.0 mM $MgCl_2$, 0.25 mM dNTP, 0.1mM of each primer, ddH_2O, and 1.25 units of *Taq* polymerase (Invitrogen). Amplifications were performed on an Applied Biosystems GeneAmp PCR System 9700 Series Thermocycler or a BioRad iCycler under the following conditions: initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 30 sec, annealing at 50°C for 30 sec, and template extension at 72°C for 60 sec, concluding with a final extension at 72°C for 7 min. All reactions were run with a negative control of template-free PCR reaction to test for contamination. Amplification products were visualized on 1% agarose gels stained with ethidium bromide to confirm expected amplicon size. PCR products were sequenced directly or purified by either Poly Ethylene Glycol (PEG) precipitation (http://www.uga.edu/srel/DNA_Lab/PEG_Precip'00.rtf) or Exonuclease/Shrimp Alkaline Phosphatase (EXOSAP) digestion.

Sequencing

All samples were sequenced in the forward and reverse directions using amplification primers to ensure sequence accuracy. Cycle sequencing reactions contained 1-2 μl purified product, 1.6 μl of primer (10mM), 2 μl terminators (BigDye

Terminator v3.1, Applied Biosystems) and 4.4 μl – 5.4 μl ddH_2O for a 10 μl final reaction volume. Cycle sequencing products were purified through ethanol precipitation, dried in a Savant SpeedVac DNA110 and resuspended in 10 μl formamide. Separation of cycle sequenced fragments was conducted on an ABI 377 automated sequencer for 7 hrs at 28W constant power.

Data Analysis

Sequences were compiled and edited in Sequencher (version 4.5; Gene Codes Corporation), exported into MEGA3.1 (Kumar et al. 2004) and aligned using ClustalX (Thompson et al. 1997). Haplotypes were assigned according to those maintained by the Archie Carr Center for Sea Turtle Research (ACCSTR; http://accstr.ufl.edu/ccmtdna.html) and reported to the stranding networks. New haplotype designations were submitted to GenBank and ACCSTR.

An Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) was conducted in Arlequin 3.1 (Excoffier et al. 2005) to determine whether the genetic composition of stranded individuals differed significantly between North and South Carolina and the nearshore aggregation (see Roberts et al. 2005). Pairwise F_{ST} comparisons, using conventional F-statistics, were performed in Arlequin 3.1, between all eight rookeries, the stranding data and the nearshore aggregation (see Roberts et al. 2005), in order to determine the extent of genetic differentiation between the strandings, nearshore aggregate and the rookeries. Analyses incorporated sequence data using the Tamura-Nei distance model (Tamura & Nei 1993).

Mixed stock analysis was initially carried out using a maximum likelihood (ML) algorithm implemented in the program SPAM: Statistics Program for Analyzing Mixtures (Alaska Department of Fish and Game 2003) to estimate the haplotype contributions of six known genetically-distinct rookeries (from Encalada et al. 1998) to the strandings in North and South Carolina (S1), as implemented in the analysis of the nearshore aggregation off the NEFL-NC (Roberts et al. 2005). Since the work of Roberts et al. (2005), additional samples have been collected from existing rookeries and additional rookeries have been sampled in the western Atlantic and Mediterranean (Bass et al. 2004; Bowen et al. 2004; Laurent et al. 1998; Pearce 2001). As a result, three new haplotypes and at least two new nesting subpopulations, Turkey (TUR) and DT, were added. It has been shown that increased source sampling will improve confidence in mixed stock estimates and therefore, analyses should utilize all sampled potential source rookeries (Chapman 1996; Xu et al. 1994). As such, SPAM analyses were rerun (S2) using all Atlantic loggerhead rookeries sampled to date (Encalada et al. 1998; Laurent et al. 1998; Pearce 2001). Haplotypes unique to the strandings and unassigned in source rookeries were excluded; resulting in the removal of three samples from all MSA analyses (see Results). Bootstrap resampling (n = 5000) was performed on both the baseline rookery and stranding data. Mean estimates were reported with standard deviations and 97% non-symmetric percentile bootstrap confidence intervals as they provide the best representation for skewed distributions (Pella & Masuda 2001).

A Bayesian Markov-Chain Monte Carlo (MCMC) analysis was also used due to the fact that the maximum likelihood method overestimates the contribution of stocks containing rare haplotypes (Pella & Masuda 2001). Estimates were conducted using

BAYES, a Bayesian stock-mixture analysis program that allows the input of informed priors which generate more accurate confidence intervals and avoid over-representing small rookeries and/or those with rare haplotypes (Bolker et al. 2003; Okuyama & Bolker 2005; Pella & Masuda 2001). Informed priors may include known biological information input by the user or may be generated by the program using a pseudo-Bayes method (Pella & Masuda 2001). For the initial BAYES analyses (BAYES1 and BAYES2), priors were set such that one rookery contributed 95% and the others split the remaining 5% equally. This protocol was employed for both the original Encalada et al. (1998) six rookery baseline (BAYES1) and the current eight rookery baseline (BAYES2). One chain was run per rookery, with a different rookery assigned as the major contributor for each chain. The Raftery and Lewis diagnostic (Raftery & Lewis 1996) was used to determine the MCMC chain length for each chain. Shorter chains were rerun until all were the length of the longest chain. The Gelman and Rubin shrink factor (Gelman & Rubin 1992) was calculated for each rookery and only estimates with values around 1.0 and less than 1.2 were used, to ensure that proper convergence was reached. A single 100,000 MCMC sample chain was also run with each rookery contributing equally. When initial chains were in agreement with the 100,000 MCMC sample chains, greater confidence could be placed in their combined estimates.

Juvenile foraging aggregates appear to be correlated with size of source nesting subpopulations rather than randomly dispersed along the coast (Bass et al. 2004; Bowen et al. 2004; Engstrom et al. 2002; Norrgard & Graves 1996; Rankin-Baransky et al. 2001; Witzell et al. 2002). Therefore, a third BAYES analysis (BAYES3).was run

incorporating priors weighted to reflect rookery size estimates (from NMFS & USFWS 2007).

RESULTS

A 400-base pair section of the mitochondrial DNA control region was sequenced for 115 samples while three samples failed to yield usable product. Of the 115 sequenced samples, one was identified as a Kemp's ridley (*Lepidochelys kempii*) and another was identified as a Green sea turtle (*Chelonia mydas*); both were removed from analyses. Heteroplasmy was observed in one individual at site 154, which was confirmed through repeated sequencing and clonal analysis. This individual held both haplotypes CC-A2 and CC-A7 and was also excluded from analyses. The remaining 112 individuals were used in analyses, with 73 samples from South Carolina and 39 samples from North Carolina (Table 1). Sizes ranged from 45.1 – 106.2 cm CCL, including 5 adults, 99 juveniles and 8 of unknown life stage. Genetically, thirty-five polymorphic sites defined eleven haplotypes in the analyzed samples, however when compared to the ACCSTR this number was reduced to nine, as two haplotypes were identified using a polymorphic site (bp 384) outside the range of the published region and therefore was not comparable to natal beach origin haplotypes (Table 2). Sequences were amended to the published range (380 bp) and assigned the matching haplotype. A novel haplotype (GenBank EU246539 and ACCSTR CC-A45) was discovered in one individual. This rare haplotype is most similar to CC-A13 and CC-A7 and has not been identified in any published studies of nesting beaches, foraging grounds or migratory corridors. Two haplotypes unassigned in source rookery subpopulations, CC-A13 and CC-A45, were observed in three individuals. Thus, of the 112 sampled strandings, 109 (97.3%) had haplotypes that

corresponded to ones found in known rookeries and were used in all subsequent MSA analyses. The two most common haplotypes were CC-A1 found in 58.0% of the strandings followed by CC-A2 which comprised 31.2% of strandings. These haplotypes are also the most common on source nesting beaches; with CC-A1 present in NWFL, SFL, NEFL-NC and DT while CC-A2 is found in all sampled nesting beach subpopulations except Brazil (BRA; see Table 1).

AMOVA analyses

An Analysis of Molecular Variance (Excoffier et al. 1992) was conducted to determine if North and South Carolina strandings could be pooled. No significant difference (Φ_{ST} = 0.0084, p = 0.3070±0.0139) was observed between haplotype frequencies of North and South Carolina stranded loggerheads (Table 3) and stranding data were subsequently pooled. No significant difference was observed between the pooled stranding and nearshore data (Φ_{ST} = -0.0064, p = 0.7986±0.0100; Table 4). Stranding data had eight of thirteen haplotypes in common with the nearshore aggregation (Table 5). Of the remaining five haplotypes, four were observed only in the nearshore data, while a single novel haplotype was observed in the strandings. Pairwise comparisons were then conducted between pooled stranding data, nearshore data (Roberts et al. 2005) and eight source rookeries (Encalada et al. 1998; Laurent et al. 1998; Pearce 2001). Accordingly, nearshore and stranding data were both significantly different from all rookeries ($F_{ST} \geq 0.05$; p = 0.00) except SFL (F_{ST} = 0.0012, p = 0.2703±0.0489; F_{ST} = 0.0105, p = 0.1261±0.0454, respectively; Table 6).

Mixed Stock Analyses: SPAM

Two separate runs were implemented in SPAM: S1) using the six rookeries with 10 haplotypes from Encalada et al. (1998) and S2) using the updated eight rookeries with 13 haplotypes (Encalada et al. 1998; Laurent et al. 1998; Pearce 2001). Resulting estimates for S1 indicated NEFL-NC contributed 42%, followed by SFL at 35%, MEX at 13%, Greece (GRE) at 6% and NWFL at 4% (Table 7A). Brazil was not observed as a contributor. The S2 analysis improved upon the S1 analysis by increased sampling of rookeries utilized in S1 as well as the addition of two new rookeries, DT and TUR. The estimate for NEFL-NC increased to 49%, while the SFL contribution declined to 22%. The NWFL no longer displayed a contribution, while MEX and GRE contributed 9% each, 7% contribution came from DT, and 3% from TUR (Table 7B). Standard deviations dropped and confidence intervals narrowed when increased rookery sample sizes were used. A lower limit value of a 97% non-symmetric bootstrap confidence interval (CI) greater than zero assured a rookery's presence in the mixture. Both analyses determined NEFL-NC as a definitive contributor (CI = 10-77%, 34-81% respectively). While the S1 analysis showed SFL as present (CI = 14-95%), this was not confirmed by S2 (CI = 0-43%) and GRE was present in S2 (CI = 3-35%) but not in S1.

Mixed Stock Analyses: BAYES

BAYES analysis of the stranding data (n=109) using the six Encalada et al. (1998) rookeries (BAYES1) resulted in mean estimates of 57% NEFL-NC, 14% each from SFL and MEX, 12% from NWFL and 2% from GRE (Table 8A). MCMC chain length was 24,769 and the Gelman-Rubin diagnostics ranged from 1.00 to 1.07,

indicating proper convergence of chains was reached. Standard deviations around the means were high and 97% confidence intervals were broad. The only rookery that was assuredly present was MEX, which had 97% confidence interval ranging from 3 - 32%. Using the eight current source rookeries and 13 corresponding haplotypes (BAYES2), BAYES was run to a chain length of 61,826. The Gelman-Rubin diagnostic was 1.00 - 1.01. The resulting estimates revealed that SFL and NEFL-NC each contributed 36% of the stranded individuals, followed by 13% from NWFL and 10% from MEX (Table 8B). Contribution estimates from all other rookeries were marginal (< 2%). When prior contributions were weighted to reflect rookery size (BAYES3), the contribution from MEX remained similar, while the NEFL-NC estimate decreased to 29%, SFL increased to 59%, and NWFL dropped to 3% (Table 9). The contribution from the four other rookeries remained marginal (< 1%). Chain length was 42,660 and the Gelman and Rubin diagnostics were 1.00 – 1.02. Results for BAYES1, BAYES2 and BAYES3 were in concordance with their respective 100,000 sample chain estimates (Appendix I). The observed difference between the median and the mean estimates in all BAYES analyses indicated a skewed distribution of estimates, therefore, non-symmetric percentile values were reported as they are a better fit than symmetric percentile intervals for confidence bounds (Pella & Masuda 2001).

DISCUSSION

Haplotypic composition – Strandings vs. Nearshore Aggregation

The question of whether strandings are a random sampling of the nearshore loggerhead aggregation is important to determining the validity of utilizing stranded

individuals as representatives of the nearshore aggregation (Bowen et al. 2004; Epperly et al. 1996; Rankin-Baransky et al. 2001). Therefore, this study approached the question from a genetic point of view and compared relative haplotype frequencies of loggerhead strandings in North and South Carolina to live-captured individuals from the nearshore juvenile foraging aggregation off the NEFL-NC. Genetic sampling for population assessment is a prime objective of the STSSN under the Loggerhead Recovery Plan (NMFS & USFWS 1991). No significant difference was observed between the strandings and nearshore samples. The pooled strandings had 8 haplotypes in common with the nearshore aggregation, with the three most common haplotypes, CC-A1, CC-A2 and CC-A3, observed at similar relative frequencies (Table 5). Additionally, the haplotypic diversity of strandings (h = 0.5685±0.036) was similar to that of the nearshore aggregation (h = 0.5391±0.24505) and other foraging habitat mixtures in the northwest Atlantic (Bass et al. 2004; Bowen et al. 2004; Rankin-Baransky et al. 2001). Therefore, strandings could potentially serve as an alternative to the more labor-intensive in-water sampling when determining genetic composition of nearshore aggregations.

When compared to the known rookeries, both mixtures were significantly different from all rookeries with the exception of the SFL. These results are similar to that of the juvenile foraging aggregations off Hutchinson Island, Florida and do not imply that strandings are entirely comprised of SFL individuals (Witzell et al. 2002). Rather, these results are likely due to the high haplotypic diversity of the SFL (h = 0.0648±0.0267), such that it shares 95-98% of its haplotypes with stranded and nearshore sampled individuals. More importantly, other haplotypes, present in some known rookeries, but absent from the SFL, were observed in both mixtures; suggesting the

presence of additional rookeries. Although these results may be attributable to insufficient sampling of the SFL, it is unlikely as the SFL sample size (n = 109) was larger than other sampled Atlantic loggerhead rookery (Table 1).

Caution must be used when employing stranding data as they may be biased by fisheries interactions. Examination of size class data implies that the sampled strandings were not biased in this way. Roberts et al. (2005) samples had an overall mean of 64.8 cm SCL which translates to 72.0 cm CCL using the conversion formula from Byrd et al. (2005). The average size of stranded individuals was 73.1 cm CCL suggesting stranded individuals were within the same size class as the nearshore aggregation. Temporal variation between samples, however, may have affected results. Stranding collections took place year-round from April 2003 through June 2006 (although few strandings occurred in the winter months), while nearshore samples were collected solely during May, June and July of 2000. These biases may be eliminated by collecting biopsies from all loggerhead strandings concurrent with nearshore live-capture events, as would year-round in-water sampling. Regardless of time of year, strandings may be biased by season as winds and currents determine whether turtle carcasses make it to shore. It may also be advisable to focus comparisons on in-water data off the states from which strandings are collected, to determine whether there is a difference in stranding composition along the east coast of the United States. If strandings are truly representative of what is offshore and juveniles and adult females home to their natal region, then we should observe a cline of decreasing CC-A1 haplotypes and increasing CC-A2 as you move from north to south along the east coast of the U.S (Bowen et al. 2004).

Mixed Stock Analysis

Mixed stock analysis of sea turtle aggregations using mtDNA carries a number of significant caveats. Loggerhead nesting subpopulations have been defined by differences observed in mtDNA haplotype frequencies on nesting beaches. Mitochondrial DNA is usually characterized by few common and many scarce haplotypes (Xu et al. 1994). Not only is this the case for loggerhead mtDNA, but common haplotypes are shared widely amongst nesting subpopulations and rare haplotypes are often not unique to one rookery. Therefore, although differentiation between relative haplotype frequencies of source rookeries is notable, the haplotypes themselves overlap, making origin estimation difficult; as a result, wide confidence intervals are observed around estimates (Xu et al. 1994).

A major assumption of MSA is that all existing rookeries have been sampled. In order to address this caveat, these analyses were run with all known, sampled rookeries to date; which increased both the number of baseline subpopulations from six to eight and increased the sample size of existing SFL, NWFL and GRE rookery data. There is still a possibility that not all baseline subpopulations have been sampled as substantial rookeries have been observed around Cuba, Cape Verde and in the Bahamas (SWOT 2006). In addition, current rookery sampling may not have been sufficient to identify all haplotypes from each region. To address this, BAYES uses MCMC which accounts for 'missed haplotypes' or haplotypes which exist in more than one rookery but were not sampled in some; while SPAM assumes they are from an unidentified source, rather than the result of a sampling error (Pella & Masuda 2001). In addition, neither program is capable of incorporating 'orphan' haplotypes, those found in mixtures but not observed in any

source rookery, and they must be excluded from analyses. To this end, an examination of 'orphan' haplotypes in this study revealed less than 3% of haplotypes observed in strandings fit this category; suggesting, qualitatively, that rookeries have been sampled sufficiently (Bowen et al. 2004).

With the aforementioned caveats notwithstanding, two major findings have resulted from the MSA analyses. No matter which analysis was conducted, or which source data were used, all estimates of stranding origins indicated the NEFL-NC and SFL rookeries as the main contributors, although exact proportions of each differed. This is likely a reflection of the close proximity of these rookeries to the North and South Carolina coasts; as well as the large SFL rookery size, potentially providing more individuals to the Atlantic waters. Other studies of nearshore foraging aggregations found similar correlations of source subpopulation contributions with rookery size or proximity to rookery (Bass et al. 2004; Bowen et al. 2004; Rankin-Baransky et al. 2001; Roberts et al. 2005; Sears et al. 1995). In addition, the proportional contribution of the NEFL-NC was much higher than expected considering its small rookery size. Similar to the nearshore aggregation off the NEFL-NC (Roberts et al. 2005), it appears that the NEFL-NC is overrepresented in strandings on its' own nesting beaches.

SPAM

The SPAM analysis, regardless of whether six or eight rookeries were utilized, elicited similar source rookery contribution estimations. The highest percentage of loggerhead strandings (42-49%) was estimated to originate in the NEFL-NC nesting subpopulation. As North and South Carolina beaches are part of the NEFL-NC, these results are in concordance with previous findings that juveniles home to foraging grounds

adjacent to their natal region (Bass et al. 2004; Bowen et al. 2004; Sears et al. 1995). The addition of the new rookeries, DT and TUR, did not affect the estimated NEFL-NC contribution; as both new rookeries lacked the CC-A1 haplotype, for which the NEFL-NC is nearly fixed. Although considered the major contributor to several foraging grounds along the eastern coast of the United States (Bass et al. 2004; Bowen et al. 2004; Rankin-Baransky et al. 2001; Roberts et al. 2005; Witzell et al. 2002), the SFL, over 10 times larger and containing six more haplotypes than the NEFL-NC, was estimated as comprising a smaller percentage of strandings (22-35%) than the NEFL-NC. Nevertheless, attention must be paid to the high variances, as when incorporated, it is possible that the reverse would be true.

A few noteworthy differences occurred with the addition of new rookery information. First, a decline was observed in the SFL contribution. The CC-A2 haplotype, previously attributed to SFL and GRE, was present in both new rookeries; therefore, their addition caused the haplotype's contribution to be further divided amongst the new rookeries. A similar trend was observed with the MEX contribution. In the first analysis, the CC-A8 and CC-A10 haplotypes were unique to MEX; therefore, the presence of these haplotypes in the strandings could only be accounted for by MEX. However, the CC-A10 haplotype was found in additional samples from GRE and in DT. As a result, MEX was no longer necessarily the sole CC-A10 contributor. As for GRE, initially providing only the CC-A2 haplotype; additional sampling revealed the CC-A10 haplotype and consequently increased the GRE estimated contribution to the strandings.

BAYES

In the BAYES analyses, the three highest contributors were NEFL-NC, SFL and MEX. In addition, NWFL displayed a considerable contribution in the first two analyses but its estimated presence dropped to 3% when prior information, regarding rookery size, was employed. This change was likely due to the small rookery size of the NWFL subpopulation; with 910 nests per year, it is the second smallest of the rookeries employed. Small rookeries are often overestimated in MSA, however, the use of ecologically informed priors, such as rookery size, in Bayesian analyses has been shown to prevent such a shortfall (Okuyama & Bolker 2005).

The NEFL-NC, SFL and MEX contribution estimates varied across tests. Variations observed between BAYES1 and BAYES2 analyses are likely attributable to the further sampling of the SFL and GRE rookeries. The additional CC-A1 haplotypes from the SFL and CC-A2 haplotypes from both SFL and GRE may have diluted the contributions of these haplotypes from the NEFL-NC and MEX respectively, while subsequently increasing the SFL contribution. A correlation appears to exist between rookery size and that rookery's contribution to mixed aggregations (Bolten et al. 1998; Bowen et al. 2004; Lahanas et al. 1998; Reece et al. 2006), therefore estimates were weighted to reflect rookery size (BAYES3). The resulting estimates indicated the SFL was the highest contributor at 59%, followed by the NEFL-NC at 29% and MEX at 9%. All other rookery contributions were estimated at less than 3%. A similar hierarchy of contributions was observed in studies of strandings in the northeast United States, neritic loggerhead foraging aggregations from northeast Florida up the United States eastern seaboard, and the pelagic juvenile aggregation in the northeast Atlantic, although exact

point estimates varied widely (Bass et al. 2004; Bolten et al. 1998; Bowen et al. 2004; Rankin-Baransky et al. 2001; Witzell et al. 2002).

MSA Summary

In summary, mixed stock analysis can produce dramatically different results depending on the method used to determine stock contribution estimates. Results from SPAM and BAYES were varied and the existence of wide variances around their estimates, inherent in loggerhead subpopulations with overlapping haplotypes amongst rookeries, made MSA comparisons problematic. While mean estimates taken at face value appear to be different, considering the wide variances around these MSA estimates, observed differences are likely not as great as they appear.

Rare and missing haplotypes appear to be the biggest hurdle for MSA and provide a 'double-edged sword'. On one hand, they are a necessary addition; as the frequencies of the more common haplotypes alone cannot define contributions from existing rookeries. In addition, increasing baseline sample size, a necessity for enhancing estimates and decreasing 'missed' haplotypes, will amplify the number of rare haplotypes. On the other hand, the inclusion of rare and missing haplotypes complicates the estimation of turtle origins, as no program has yet been developed which can process that type of information and provide accurate and precise estimates.

Despite this, the estimates provided by each program had distinctive merits. SPAM provided estimates with the narrowest confidence intervals and lowest standard deviations, however, considering prevalence of rare haplotypes and small rookery sample sizes, the range of confidence may have been underestimated (Bolker et al. 2003). The

wider confidence intervals assigned by BAYES may be more accurate, but less precise. The estimates resulting from the incorporation of rookery size in BAYES appeared to make the most biological sense. BAYES analysis with the use of informed priors still has the same shortfalls as when BAYES assigns priors, but appears to improve upon estimates. BAYES also has test statistics that verify whether models fit and provide information on the reliability of the estimates it produces (Pella & Masuda 2001).

In the future, the development of new statistical measures to handle the rare and missing haplotypes is necessary to provide more accurate and precise estimates. Increased and equal sampling of rookeries, especially those yet to be sampled will provide for better estimates for small rookeries. Large baseline sampling prevents contribution estimates from being based on the distribution of the most common haplotypes, which often incorrectly allocates contributions (Pella & Masuda 2001).

In addition, the more differentiated the baseline subpopulations are, the easier it will be to assign origin to stranded individuals. Currently, longer mtDNA control region sequences have been developed in hopes of providing better delineations between nesting subpopulations, especially those which share haplotypes (Abreu-Grobois et al. 2006). Once rookeries are resampled for these longer sequences, mixed stock analysis will likely be more accurate as haplotypes will be better associated with source rookeries rather than overlapping. Finally, as rookery size appears to provide the best estimate in the BAYES analyses in relation to confidence intervals, it is important to keep updated rookery size information as different subpopulations may change over time.

CONCLUSION

In conclusion, the results from this study indicate that the genetic composition of loggerhead strandings, in North and South Carolina, is a random sampling of the nearshore aggregation. As of yet, support cannot be provided for this statement by mixed stock analysis as the wide confidence intervals around origin estimates makes comparisons problematic. The findings in this study support the theory that juveniles home to foraging grounds in their natal region. Strandings in North and South Carolina appear to be a mixture of NEFL-NC, SFL and MEX, with the NEFL-NC present in higher percentages than expected based on rookery size, even when rookery size is used to weight the analysis. Contribution from the NWFL subpopulation is unclear. Although the other small rookeries and those with rare haplotypes have the widest confidence intervals around their estimations; knowledge of their presence in the strandings is essential to management of the habitat. Continued incorporation of necropsies to determine causes of death, in conjunction with genetic studies of stranded individuals, will aid in establishing management strategies for the protection of loggerheads while in coastal waters. Until primary mortality sources are identified, it is important to try to mitigate all coastal anthropogenic hazards that may affect these aggregations. Conservation efforts geared towards this habitat will provide protection not only for regional loggerheads, but also for turtles from distant subpopulations.

CHAPTER II:

Effectiveness of Newly Implemented TED Regulations

BACKGROUND

Incidental capture in shrimp trawls has historically been a primary cause of sea turtle mortality (NRC 1990; Talbert et al. 1980; Weber et al. 1995). A conservative estimate, using data from 1977 – 1984, suggests approximately 47,000 turtles are incidentally captured per year; 11,000 of which are mortalities (Henwood and Stuntz 1987). On the Atlantic coast, shrimpers in North Carolina, South Carolina, Georgia and Florida waters generally operate within 5 km of shore (NRC 1990), overlapping with primary habitat for large juvenile and adults sea turtles (NRC 1990; Weber et al. 1995). Sea turtle encounters with trawl nets may result in injury, distress or death by drowning. In an effort to reduce sea turtle mortality, modifications to shrimp trawl gear were developed, and the Turtle Excluder Device (TED) was unveiled in 1980 (Weber et al. 1995). A TED, a modification of a by-catch device originally developed in the 1970s, consists of a grid of bars fitted into the trawl net, which allows turtles and other megafauna to escape while retaining target organisms (Figure 2). Shrimp pass through the bars while turtles, other megafauna and debris hit the bars and are ejected through a mesh-covered opening in the net.

TED development and implementation has been fraught with controversy since its' conception. Despite its' ability to maintain shrimp catch volumes (< 10% catch loss)

while reducing bycatch and debris by up to 40% (Clark & Griffin 1991), the original NMFS TED was not well received by shrimpers because of its large size, weight and three-dimensional design (Sally Murphy, personal communication). However, the lighter, two-dimensional flat-grid TEDs, designed by fishermen, were better accepted. In spite of this, several years of legislative disputes over TED implementation ensued (SCDNR 2005; Weber et al. 1995). During this time, annual shrimp trawl-related mortality of loggerhead sea turtles in the Atlantic and Gulf of Mexico was estimated at 6,800 by Henwood and Stuntz (1987) and corrected to 27,200 by the National Research Council (1990). Additionally, a significant increase in turtle strandings was correlated with the onset of the commercial shrimping season in South Carolina and Texas (Murphy & Hopkins-Murphy 1989; NRC 1990; Talbert et al. 1980). Final TED regulations were published in 1987 which required TEDs to be 97% effective in reducing turtle bycatch (Federal Register 1987, 52 FR 24244). Although the final rule was in place, full TED implementation and adherence to regulations was not enforced at that time (SCDNR 2005). A strong inverse correlation between TED use and strandings was observed over the course of the next few years, while challenges to the rule caused legislation to repeatedly halt and reinstate TED use (Crowder et al. 1995; SCDNR 2005). In 1990, when federal enforcement of TED regulations began, a substantial reduction in stranding numbers occurred across the North Carolina, South Carolina, Georgia and Texas coasts (Crowder et al. 1994). At this time, TEDs were only required between May 1st and August 31st; hence, in September 1990, a sharp rise in strandings occurred when TEDs were no longer employed (Weber et al. 1995). These strandings provided the impetus for

the interim final rule in 1991 and the 1992 final rule, which instituted year round TED use in inshore and offshore waters of the Atlantic (Federal Register 1992, 57 FR 57348).

The 1992 final rule also introduced federal size regulations for TED opening dimensions (Federal Register 1992, 57 FR 57348). Openings were to be ≥ 35 inches (88.9 cm) horizontal length by ≥ 12 inches (30.5 cm) height for trawlers along the Atlantic coast. Height is measured simultaneously with width and is measured at the midpoint of the horizontal taut length (width). Concern over the minimum TED opening dimensions abounded in the mid 1990s when a rise in strandings indicated adult turtles (≥ 90 cm CCL) were not being excluded (Sally Murphy, personal communication). TED testing for 97% effectiveness had utilized small juvenile turtles that averaged 34.4 cm SCL (Epperly & Teas 2002) and had not determined TED effectiveness at excluding larger turtles. Epperly and Teas (1999) published a report challenging the escape opening size as did the results of a morphometrics study conducted in South Carolina by South Carolina Department of Natural Resources (SCDNR) and published by Byrd et al. (2005). Byrd et al. (2005) determined the body depth of nesting loggerheads on Cape and Pritchard's Islands in South Carolina to be larger than the required 12 inch height of the TED escape opening. The estimated maximum size of loggerheads that could fit through an opening with the minimum height requirements was approximately 80 cm SCL (Byrd et al. 2005; Epperly & Teas 1999; Epperly & Teas 2002), thus leaving the two most critical life stages, large juveniles and adults, vulnerable to being trapped in a trawl net. Stoneburner (1980) reported a gradient of decreasing loggerhead body depth of adult females from north to south along the Atlantic coast while Maier et al. (2004) found a similar gradient for mean turtle length of loggerheads live-caught in nearshore Atlantic

waters. Therefore, loggerheads in South Carolina waters are longer and have greater body depths than turtles at lower latitudes and thus are at a higher risk of being caught in trawl nets fitted with TEDs of the regulation size. The state of South Carolina consequently passed regulations that increased the TED escape opening size in 2002 to 35 inches wide by 20 inches high to allow for the exclusion of even the largest loggerheads (South Carolina Code of Laws 50-5-765; SCDNR 2005). Federal regulations, however, were not amended until 2003 (Federal Register 2003, 68 FR 8456). In addition to accounting for large loggerheads, the federal amendment further increased TED openings to allow for the escape of endangered leatherback turtles (*Dermochelys coriacea*), the largest sea turtle species, which have been observed in waters off the coast of the southeastern United States in increasing abundance since 1989 (Murphy et al. 2006). Concern for the incidental capture of leatherbacks by trawl fisheries reinforced the need for a TED size increase. With the amended federal TED size regulations, any size loggerhead should be able to escape from trawl nets with TEDs installed.

Strandings documented by the STSSN have been critical to the understanding and management of sea turtle/trawl fishery interactions. For example, increased stranding numbers coinciding with the beginning of commercial shrimping season demonstrated a clear interaction between the fishery and sea turtles that was crucial to the development and implementation of TED regulations (Lewison et al. 2003; Weber et al. 1995). As informative and accessible as strandings are to investigations into anthropogenic impacts on sea turtles, it is important to mention a few caveats.

To begin with, not all sick or dead sea turtles strand. When a sea turtle dies, its body initially sinks to the bottom, where decomposition occurs, causing a build up of gas

that floats the animal to the surface where it drifts and washes ashore or eventually sinks again (Epperly et al. 1996). Winds and currents transport injured or sick turtles and turtle carcasses to coastal waters and beaches. However, marine scavengers and seasonal variations in currents and wind direction control the number of sea turtle mortalities that actually reach the shore as strandings. In two studies of loggerhead carcass recovery in the United States, only 6 of 22 tagged carcasses released in nearshore waters eventually stranded on shore (Murphy & Hopkins-Murphy 1989). Epperly et al. (1996) reported a mere 7-13% of fishery-related mortalities ever come ashore in winter months in North Carolina. Therefore, stranding documentation must be considered an underestimate of true at-sea turtle mortality (Murphy & Hopkins-Murphy 1989); however, combined with information on the at-sea environment and loggerhead aggregation, strandings can increase our knowledge of the risks loggerheads face off our coast.

It is also important to consider size of the turtle when attempting to determine anthropogenic impacts. Larger turtles have a better chance of surviving hazards such as boat strikes, debris ingestion and toxins as their size dampens the effect of the injury or harmful intake. Risk factors may also only impact specific sizes of turtles; for example, a small TED exit opening would still trap large turtles, but allow small turtles to escape. Additionally, the size of the turtle may dictate its' location in coastal waters. Juvenile turtles on foraging grounds are more likely to be found in ship channels, bays, and sounds (Hopkins-Murphy et al. 2003; Lutcavage & Musick 1985; Maier et al. 2004; Sears et al. 1995), which may be major areas of outflow of land based debris and contaminants (Day et al. 2005; Keller et al. 2005). These highly productive areas are also favored by both commercial and recreational fishermen. Alternately, adult females are found in areas of

high relief (Epperly et al. 1995; Lutcavage & Musick 1985; Maier et al. 2004) which trawlers find difficult to trawl in and often avoid (Hopkins-Murphy et al. 2003).

OBJECTIVES

This study intends to compare size distributions of stranding data to data from studies of in-water loggerhead aggregations to determine the size classes of loggerheads at risk off the coast of South Carolina and whether loggerhead mortality is biased towards a specific life stage. Additionally, size class information will be examined before and after the implementation of larger TEDs in order to infer the effectiveness of new TED regulations at reducing large juvenile and adult loggerhead mortality.

MATERIALS AND METHODS

The South Carolina Sea Turtle Stranding and Salvage Network (SCSTSSN) records information on the date, location, size, species and condition of each stranded individual sea turtle (NRC 1990). A juvenile turtle's gender is often undetermined externally and should be verified with an internal examination of the gonads. An external examination is usually sufficient for mature individuals ($\geq$ 90 cm CCL). If conditions are favorable, necropsies are conducted, however, this is uncommon and hence, information on sex and cause of death is usually unresolved.

This study utilized two sets of loggerhead stranding data. Year-round records of dead *C. caretta* from 2000 through 2005 were furnished by SCDNR. Additionally, SCDNR supplied (courtesy of Mike Arendt) in-water data from an abundance study of the nearshore sea turtle aggregation from Winyah Bay, South Carolina to St. Augustine,

Florida (Maier et al. 2004). Details of in-water data collection can be found in Maier et al. (2004). Size and location information were compiled for each dataset. For the purpose of this study, size was defined as curved carapace length (CCL), a lengthwise measurement made from the nuchal notch to the posteriormost tip of the carapace using a flexible tape measure.

Strandings vs. In-water Aggregation

Stranding records (n = 255) of dead *C. caretta* collected from May, June and July of 2000 - 2003 and live, in-water loggerhead data (n = 285) from Maier et al. (2004) collected off South Carolina during the same time period were employed. Data encompassed time periods when the two datasets overlapped to reduce the effect of effort and seasonal variations.

Kolmogorov-Smirnov two-sample tests were applied to determine if the size distribution of stranded individuals was representative of the nearshore aggregation. First, pairwise tests were performed to determine if distributions differed among years within each dataset (stranding and nearshore). Next, tests compared size distributions by year between the two datasets.

Strandings – Before and After TED implementation

These analyses utilized year-round loggerhead stranding records from South Carolina for 2000 - 2001 (prior to larger TED implementation) and 2004 - 2005 (post-larger TED regulations). Data were assembled by size, and individuals were categorized as either adults or juveniles. Individuals ≥ 90 cm CCL were considered adults, while

juveniles were those under 90 cm CCL (TEWG 1998). Individuals with estimated or no measurements were excluded, as a size class could not be assigned.

A chi-square test of homogeneity was conducted to test whether the size distribution of stranded loggerheads differed before and after larger TED implementation. Data were divided into six categories: < 60 cm, 60.0-69.9 cm, 70.0-79.9 cm, 80.0-89.9 cm, 90.0-99.9 cm and ≥ 100 cm. A two-sample Kolmogorov-Smirnov test of equal distributions was also conducted as added support. To further elucidate the effect of larger TEDs, a chi-square test was employed to determine if relative adult/juvenile proportions of strandings were dependent upon the implementation of larger TEDs. Chi-square tests were conducted in Minitab 14 (Minitab, Inc.) and Kolmogorov-Smirnov tests were conducted in R version 2.2.0 (R Development Core Team 2005).

Stranding records from 2002 and 2003 were excluded from analyses as they likely confound the stranding data for the following reasons: 1) In South Carolina, TED escape opening size was increased on two separate occasions. The 2002 larger TED regulations required TED exit openings of an intermediate size and would complicate analyses. 2) The 2002 larger TED regulations were only applicable in South Carolina waters; therefore, strandings close to the North Carolina or Georgia borders may not reflect South Carolina regulations. 3) In 2003, an unusually high number of debilitated turtle strandings and boat strike mortalities occurred (Murphy et al. 2006; SCDNR 2003). The compromised condition of such turtles could not be attributed to incidental capture, but may have increased the turtle's susceptibility to being caught in a trawl.

RESULTS

Strandings vs. In-water Aggregation

Size distributions for stranding (n = 255) and in-water (n = 285) data were bimodal, with the exception of 2000 stranding data. The distributions displayed a major peak in the juvenile range around 70 cm CCL for strandings and between 65 cm and 80 cm CCL for in-water data. A minor peak was observed in the bimodal distributions around the adult 100 cm CCL sizes, while 2000 stranding data exhibited a plateau around 90 cm CCL before dropping off again. Within the stranding data, size distributions were not significantly different among years (Table 10). However, a visual examination of the distributions showed a decline in the number of adult strandings and an increase in the abundance of stranded juveniles (Figure 3). A significant difference was found in the nearshore aggregation between 2000 and 2003 size distributions ($D = 0.3149$, $p = 0.0013$) but not among other years (Table 11, Figure 4). As a result, yearly comparisons of size distributions of stranded loggerheads to the nearshore loggerhead aggregation were conducted. No significant differences were observed for the years 2000 through 2002, however, the null hypothesis of equal size distributions was rejected ($D = 0.3179$, $p = 0.0005$) for the 2003 comparison (Table 12, Figure 5). In 2003, the strong juvenile peaks were at 75 cm – 80 cm CCL for the nearshore aggregation and around 70 cm CCL for strandings. Both data sets had a smaller adult peak around 100 cm CCL in 2003.

Strandings – Before and After Large TED implementation

Size distributions of stranded loggerheads from 2000 – 2005 ranged from 30.5 cm to 119.4 cm. Chi-square tests of homogeneity were conducted to determine if size distributions differed between 2000 and 2001 or between 2004 and 2005. No significant

difference was observed within either pair of years (χ^2 = 7.557, d.f. = 5, p = 0.182 & χ^2 = 2.636, d.f. = 5, p = 0.756 respectively), and data for the pairs were subsequently pooled into two categories, before larger TEDs (n = 174) and after larger TEDs (n = 179), respectively. Pooled size distributions were not equal before and after larger TED implementation (χ^2 = 18.087, d.f. = 5, p = 0.003, Table 13) and the 90.0 – 99.9 cm size class contributed most to the χ^2 value, followed by the 70.0 – 79.9 cm size class (Table 13). These results were also supported by a Kolmogorov-Smirnov two sample test of equal distributions (D = 0.1661, p = 0.015, Figure 6). Adult/juvenile proportions were significantly different before and after larger TED implementation to the 95% confidence level (χ^2 = 13.820, d.f. = 1, p=0.00) with adults contributing the most to the χ^2 value (Table 14). Relative adult proportions were reduced from 25.9% prior to 2002 to 10.6% after larger TED implementation in 2003 (Figure 7).

DISCUSSION

Stranding data have been used to determine life history, distribution, population trends, information on mass mortality events and fisheries impacts on sea turtles and other marine species (Maldini et al. 2005; McFee & Hopkins-Murphy 2002; Nieri et al. 1999; Work & Rameyer 1999). Although stranding data are incomplete, and often underestimate true mortality at sea (McFee & Hopkins-Murphy 2002; Murphy & Hopkins-Murphy 1989), combined with other information, strandings can shed light on how anthropogenic impacts that occur at sea, and are otherwise difficult to study, are affecting loggerhead aggregations.

It is presumed that stranded turtles represent the individuals at risk for natural and anthropogenically-induced mortality in coastal waters. Principal anthropogenic threats in nearshore South Carolina waters include incidental take in commercial fisheries (trawls and longlines), dredging, boat strikes, recreational fishing and entanglement in or ingestion of marine debris and toxins (NRC 1990). Natural mortality sources, such as disease and predation, also have a strong impact on abundance and survival of sea turtles in nearshore waters. Risk of mortality may increase for certain classes (size, sex, or species) of sea turtles when they are present in high abundance or sources of mortality may be targeting certain classes over others. By comparing strandings to in-water aggregations, we can determine whether strandings are a random sampling of the nearshore aggregation or if mortality sources are biased towards certain size classes regardless of their abundance.

Strandings as representation of nearshore aggregation

Yearly comparisons of size distributions resulted in no difference between stranded individuals and the nearshore aggregation from 2000 through 2002. These results suggest that, in these years, strandings were representative of the nearshore loggerhead aggregation and mortality risks were correlated with size class abundance in nearshore waters. However, in 2003, the size distribution of South Carolina strandings was significantly different from that of the nearshore aggregation. Stranded loggerheads were primarily juveniles around 70 cm CCL, which represented less than 20% of the nearshore aggregation that year. Alternatively, the most abundant nearshore size class, 80 cm CCL, was present in only 27% of strandings. Due to the high number of DTS

strandings in 2003, the two-sample Kolmogorov-Smirnov test between stranded and in-water data was rerun with the DTS turtles removed. When DTS data were excluded, the size distribution of strandings was still significantly different from the nearshore aggregation ($D = 0.2945$, $p = 0.0067$). Thus, in 2003, it appears strandings were not correlated with size class abundance in the nearshore aggregation.

Comparisons among years revealed no difference in the size distribution of strandings; with the most frequently stranded size class consistently around 70 cm CCL. The nearshore aggregation size distributions, however, differed significantly between 2000 and 2003. In the midsummer of 2003, an unusual cold-water upwelling event occurred along the Mid-Atlantic coast of the United States. Beginning in July, coastal waters were pushed offshore by persistent southerly winds and multiple heavy rains and were replaced by cold deep waters. This anomaly of cold continental shelf waters spread north from Florida and west from the Gulf Stream. It has been suggested that a temperature boundary caused by the upwelling may have concentrated sea turtles in warm nearshore waters and thus resulted in an unusual in-water size distribution (Maier et al. 2004). Further examination of the nearshore size distributions revealed a gradual increase in the most common size class from 65 cm CCL to 80 cm CCL over the four years, suggesting a trend of increasing mean turtle length in the most common size class. Maier et al. (2004) proposed that this trend may be accounted for by growth of a size cohort and further supported their theory by a comparison of the observed 2003 in-water distribution to one projected for 2003 with a growth model using measurements taken in 2000 (Maier et al. 2004). If the most common nearshore size class is truly increasing with the growth of individuals in a cohort, the significant difference that appeared only in

comparisons between 2000 and 2003 was appropriate, as the most time had elapsed between these two years allowing the detection of a difference that would have been too small to perceive in comparisons of previous years. This growth theory implies that the strandings and in-water size distributions are gradually drifting into dissimilarity.

In addition to the observed shift in size, the most common size class in the nearshore aggregation displayed a 1.6-fold increase in abundance from 2000 to 2003. This abundance increase may be attributed to the upwelling event concentrating more juveniles in the South Carolina foraging grounds (Maier et al. 2004). Another explanation may be that turtles recruited into the area. Epperly et al. (1995) indicated a size gradient of smaller loggerheads in Long Island Sound and larger turtles to the south, from Chesapeake Bay to Indian River. Juveniles which had foraged in northern locales may have moved to South Carolina foraging grounds as they graduated into a new size class.

Results remain inconclusive as to whether the size distributions of strandings are a random sampling of the nearshore aggregation. The conflicting results of 2000 - 2002 and 2003 stranding to in-water size distribution data comparisons require additional years of study to resolve. If strandings continue to match the nearshore aggregation as they did in 2000 – 2002, it can be presumed that 2003 was an anomalous year and strandings are a good representation of the nearshore aggregation. However, if the in-water data continues to show a growth trend in the most common size class and strandings do not reflect this trend, then strandings are neither abundance-based, nor a random sampling of the nearshore population, but rather mortalities are biased towards the 70 cm CCL size class regardless of their abundance.

It is important to note that, although the nearshore waters appear to be showing an increase in juvenile abundance (Maier et al. 2004); without a reduction in juvenile strandings, this increase may be negated. Increased abundance in the large juvenile life stage (57.1 cm – 87.0 cm) is essential to the recovery of the loggerhead species (Crouse et al. 1987), therefore conservation efforts should be directed towards identification and reduction of the source of the bias towards 70 cm CCL loggerheads, such that they receive sufficient protection resulting in a reduction of strandings.

Effectiveness of larger TED implementation

TED exit opening sizes were increased in 2002 and 2003 in South Carolina to allow for the escape of large loggerheads and leatherbacks. Before these regulations were in place, the TED escape openings were only large enough to release turtles < 79.8 cm SCL (Epperly & Teas 1999; 2002) and adults comprised as much as 31% of yearly strandings (SCDNR, unpublished data). As sampling for the in-water study was switched to only the Charleston Shipping Channel in 2004; data were not available for nearshore waters off the coast of South Carolina for the years following the 2003 TED regulations (Segars et al. 2006); therefore, stranding data were queried to determine if increases to TED exit opening size had an effect on adult mortality. Stranding totals were similar (n = 174 and n = 179, respectively), however, distributions varied significantly before (2000 - 2001) and after (2004 - 2005) larger TED implementation. The chi-square analysis revealed that the reduction in the number of stranded adults contributed most to the significant difference in distributions. More specifically, a 15.3% decline in relative adult proportions was observed after the implementation of larger TEDs.

Before delving further into the discussion, it is important to note that all strandings with size information were included in these analyses, and some were not solely trawl-related mortalities. The only individuals that were eliminated were partial or divided carcasses and highly decomposed individuals with missing size information. In such cases, life stage was unable to be determined. Obvious boat strikes and illness-related deaths were not removed since prior capture in a trawl may have compromised the individual and secondarily contributed to their death. Definitive trawl-related mortalities – those collected aboard trawl boats - were rare and drowning, primarily associated with sea turtle/trawl fishery interactions, was infrequently diagnosed. Although a drowning death can be presumed when a healthy turtle strands with no wounds or abnormalities and food in its' stomach, the compromised condition of most carcasses may have reduced the ability to identify this condition through necropsy. In South Carolina, nearly all turtles in good condition are necropsied, representing 25% of all strandings (Charlotte Hope, pers. comm.). Despite this, exclusion of any individuals with known, unrelated cause of death would have been problematic as adult strandings constituted 18% of total strandings from 2000 – 2005. Any reduction in sample size would create such a loss of power that no statistical test would have been applicable to the analyses. Therefore factors, other than larger TED implementation, may have also contributed to the observed results.

Reduction in stranded adult proportions

Nest numbers were investigated as an estimate of the abundance of adults present in the nearshore aggregation in order to determine if in-water adult female abundance may have contributed to our findings. A reduction in average nest numbers from

approximately 5,200 to 3,000 was observed on South Carolina beaches over 20 years (SCDNR, unpublished data), revealing a 3.1% annual decline (Ehrhart et al. 2003; NMFS & USFWS 2007). A similar 1.9% annual decrease was also observed in standardized ground surveys of nests from North Carolina to Georgia, providing evidence of a decline in adult female abundance likely attributed to pre-TED incidental capture deaths (Ehrhart et al. 2003). Therefore, if the number of nesting females is representative of the adult nearshore aggregation, the decline in adult strandings may be explained by the fact that many adult females had been killed in previous years leaving fewer adults returning to the nearshore aggregation.

Shrimp trawling, named the major threat to sea turtles by the National Research Council (1990), is itself a declining fishery. Notably, there has been a 40% reduction in commercial trawl licenses purchased in South Carolina since 2000 (SCDNR, unpublished data). Commercial imports of farm-raised shrimp have dropped the market value of shrimp such that many shrimpers have had to look to other occupations. The smaller remaining fleet spends less time on the water due to rising gas prices and puts efforts in only when the conditions are highly productive. Therefore, fewer trawlers are on the water making fewer trips and thus decreasing the risk of sea turtle/trawl fishery interactions. A reduction in encounter rate caused by such changes in shrimping effort may have contributed to the decline in adult strandings, especially when coupled with the use of the new larger TEDs.

Increase in stranded juvenile proportions

The reduction in relative adult proportions was coupled with an alarming increase in the proportion of juvenile strandings, which bears investigation. The increase is not likely a result of the modifications to the TED opening size, as TED testing, using juvenile turtles, has been conducted on the new larger TEDs (Federal Register 2003, 68 FR 8456).

Debilitated turtle syndrome (DTS) has been observed in increasing prevalence since 1999 and is often fatal (SCDNR 2003). Sea turtles with DTS are usually emaciated and covered with small barnacles indicating the turtles have been inactive for some time and therefore are very ill. Data were queried for DTS mortalities and of the 55 suspected cases in the combined study years, 91% were juveniles. The average size of stranded DTS individuals in South Carolina was 74 cm CCL, within the 70 cm CCL size class most commonly stranded. Furthermore, suspected DTS-related juvenile mortalities showed a 15% increase over the time periods before and after larger TED implementation, coinciding with the 15.3% increase in juvenile strandings (Figure 7). Thus, the rise in juvenile DTS mortalities may have added to the increase in juvenile strandings in recent years.

Finally, the increased juvenile strandings may simply be due to an increase in juvenile abundance in nearshore waters. Maier et al. (2004) found a nearly ten-fold increase in juvenile loggerhead abundance off the southeast coast of the United States in 2000 - 2003 compared with CPUE values from the 1970s and 1980s (Henwood & Stuntz 1987; Ulrich 1978). As there is no data available on the in-water abundance of juveniles along the entire South Carolina coast for years after larger TED implementation, a future study to this effect may provide additional support to this theory.

In conclusion, the proportion of stranded adult loggerheads differed significantly with the implementation of larger TED exit openings; therefore, their proportional decline in mortality may be partially explained by the implementation of new TED regulations. It is also possible that the decline is a reflection of decreasing adult abundance in nearshore waters. The increasing juvenile strandings in 2003 is alarming, yet not likely attributable to shrimp trawling but possibly due to increasing juvenile abundance coupled with a rising prevalence of Debilitated Turtle Syndrome and boat strikes. Continued investigations into the abundance and size distribution of the nearshore loggerhead aggregation would aid in further understanding of the impact larger TED openings have had on adult loggerheads, especially if the nesting population increases.

LITERATURE CITED

Abreu-Grobois, A., J. A. Horrocks, A. Formia, P. H. Dutton, R. LeRoux, X. Velez-Zuazo, L. Soares, and A. B. Meylan. 2006. New mtDNA D-loop primers which work for a variety of marine turtle species may increase the resolution of mixed stock analyses. Page 179 in M. Frick, A. Panagopoulou, A. Rees, and K. Williams, editors. Book of Abstracts from theTwenty-Sixth Annual Symposium on Sea Turtle Biology and Conservation International Sea Turtle Society, Athens, Greece. 376pp

Alaska Department of Fish and Game. 2003. SPAM Version 3.7: Statistics Program for Analyzing Mixtures. Alaska Department of Fish and Game, Commercial Fisheries Division, Gene Conservation Lab.

Arendt, M. D., A. L. Segars, J. Byrd, J. D. Whitaker, D. Owens, and G. Blanvillain. 2007. Examination of local movements and migratory behavior of sea turtles during spring and summer along the Atlantic coast off the southeastern United States. South Carolina Department of Natural Resources (SCDNR) and College of Charleston. Annual Report to the Office of Protected Resources, NOAA Fisheries. Grant Number NA03NMF4720281. 45 pp.

Avise, J. C. 1995. Mitochondrial DNA polymorphism and a connection between genetics and demography of relevance to conservation. Conservation Biology **9**:686-690.

Bass, A. L., S. P. Epperly, and J. Braun-McNeill. 2004. Multi-year analysis of stock composition of a loggerhead turtle (*Caretta caretta*) foraging habitat using maximum likelihood and Bayesian methods. Conservation Genetics **5**:783-796.

Bjorndal, K. A., A. B. Bolten, and H. R. Martins. 2000. Somatic growth of juvenile loggerhead sea turtles *Caretta caretta*: duration of pelagic stage. Marine Ecology Progress Series **202**:265-272.

Bolker, B. M., T. Okuyama, K. A. Bjorndal, and A. B. Bolten. 2003. Sea turtle stock estimation using genetic markers: accounting for sampling error of rare genotypes. Ecological Applications **13**:773-775.

Bolten, A. B., K. A. Bjorndal, H. R. Martins, T. Dellinger, M. J. Biscoito, S. E. Encalada, and B. W. Bowen. 1998. Transatlantic developmental migrations of loggerhead sea turtles demonstrated by mtDNA sequence analysis. Ecological Applications **8**:1-7.

Bowen, B. W., J. C. Avise, J. I. Richardson, A. B. Meylan, D. Margaritoulis, and S. R. Hopkins-Murphy. 1993. Population structure of loggerhead turtles (*Caretta caretta*) in the northwestern Atlantic Ocean and Mediterranean Sea. Conservation Biology **7**:834-844.

Bowen, B. W., A. L. Bass, S. Chow, B. M., K. A. Bjorndal, A. B. Bolten, T. Okuyama, B. M. Bolker, S. P. Epperly, E. Lacasella, D. Shaver, M. Dodd, S. R. Hopkins-Murphy, J. A. Musick, M. Swingle, R.-B. K., W. Teas, W. N. Witzell, and P. H. Dutton. 2004. Natal homing in juvenile loggerhead turtles (*Caretta caretta*). Molecular Ecology **13**:3797-3808.

Bowen, B. W., A. L. Bass, L. Soares, and R. J. Toonen. 2005. Conservation implications of complex population structure: lessons from the loggerhead turtle (*Caretta caretta*). Molecular Ecology **14**:2389-2402.

Bowen, B. W., N. Kamezaki, C. J. Limpus, G. R. Hughes, A. B. Meylan, and J. C. Avise. 1994. Global phylogeography of the loggerhead turtle (*Caretta caretta*) as indicated by mitochondrial DNA haplotypes. Evolution **48**:1820-1828.

Byrd, J., S. Murphy, and A. Von Harten. 2005. Morphometric analysis of the Northern Subpopulation of *Caretta caretta* in South Carolina, USA. Marine Turtle Newsletter **107**:1-4.

Caine, E. A. 1986. Carapace epibionts of nesting loggerhead sea turtles: Atlantic coast of U.S.A. Journal of Experimental Marine Biology and Ecology **95**:15-26.

Carr, A. 1986. Rips, FADS, and little loggerheads. BioScience **36**:92-100.

Carr, A. 1987. New perspectives on the pelagic stage of sea turtle development. Conservation Biology **1**:103-121.

Chapman, R. W. 1996. A mixed stock analysis of the green turtle: the need for null hypotheses. Pages 137-146 in B. W. Bowen, and W. N. Witzell, editors. Proceedings of the International Symposium on Sea Turtle Conservation Genetics. NOAA Technical Memorandum NMFS-SEFSC-490. National Technical Information Services, Springfield, VA.

Clark, J., and W. Griffin. 1991. Simulated economic impact of TED regulations on selected vessels in the Texas shrimp fishery. Marine Fisheries Review **53**:1-8.

Crouse, D. T., L. B. Crowder, and H. Caswell. 1987. A stage-based population model for loggerhead sea turtles and implications for conservation. Ecology **68**:1412-1423.

Crowder, L. B., D. T. Crouse, S. S. Heppell, and T. H. Martin. 1994. Predicting the impact of turtle excluder devices on loggerhead sea turtle populations. Ecological Applications **4**:437-445.

Crowder, L. B., S. R. Hopkins-Murphy, and J. A. Royle. 1995. Effects of Turtle Excluder Devices (TEDs) on loggerhead sea turtle strandings with implications for conservation. Copeia **1995**:773-779.

Day, R. D., S. J. Christopher, P. R. Becker, and J. D. Whitaker. 2005. Monitoring mercury in the loggerhead sea turtle, *Caretta caretta*. Environmental Science and Technology **39**:437-446.

Dodd, K. C. 1988. Synopsis of the biological data on the loggerhead sea turtle. Biological Report **88**:110.

Ehrhart, L. M., D. A. Bagley, and W. E. Redfoot. 2003. Loggerhead turtles in the Atlantic Ocean: geographic distribution, abundance and population status. Pages 157-174 in A. B. Bolten, and A. B. Witherington, editors. Loggerhead Sea Turtles. Smithsonian Books, Washington, DC.

Encalada, S. E., K. A. Bjorndal, A. B. Bolten, J. C. Zurita, B. Schroeder, E. Possardt, C. J. Sears, and B. W. Bowen. 1998. Population structure of loggerhead turtle (*Caretta caretta*) nesting colonies in the Atlantic and Mediterranean as inferred from mitochondrial DNA control region sequences. Marine Biology **130**:567-575.

Engstrom, T. N., P. A. Meylan, and A. B. Meyan. 2002. Origin of juvenile loggerhead turtles (*Caretta caretta*) in a tropical developmental habitat in Caribbean Panama. Animal Conservation **5**:125-133.

Epperly, S. P., J. Braun, and A. Chester. 1996. Beach strandings as an indicator of at-sea mortality of sea turtles. Bulletin of Marine Science **59**:289-297.

Epperly, S. P., J. Braun, and A. Veishlow. 1995. Sea turtles in North Carolina waters. Conservation Biology **9**:384-394.

Epperly, S. P., and W. G. Teas. 1999. Evaluation of TED opening dimensions relative to size of turtles stranding in the western North Atlantic. U.S. Department of Commerce, National Marine Fisheries Service. SEFSC Contribution PRD-98/99-08. 31 pp.

Epperly, S. P., and W. G. Teas. 2002. Turtle excluder devices - Are the escape openings large enough? Fisheries Bulletin **100**:466-474.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online **1**:47-50.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application of human mitochondrial DNA restriction data. Genetics **131**:479-491.

Federal Register. 1987. 52 FR 24244, June 29, 1987.

Federal Register. 1992. 57 FR 57348, December 4, 1992.

Federal Register. 2003. 68 FR 8456, February 21, 2003.

FitzSimmons, N. N., C. Moritz, C. J. Limpus, L. Pope, and R. Prince. 1997. Geographic structure of mitochondrial and nuclear gene polymorphisms in Australian green turtle populations and male-biased gene flow. Genetics **147**:1843-1854.

Frazer, N. B., and L. M. Ehrhart. 1985. Preliminary growth models for green, *Chelonia mydas*, and loggerhead, *Caretta caretta*, turtles in the wild. Copeia **1985**:73-70.

Gelman, A., and D. B. Rubin. 1992. Inference from iterative simulation using multiple sequences Statistical Science **7**:457-511.

Grant, W. S., G. B. Milner, P. Krasnowski, and F. M. Utter. 1980. Use of biochemical genetic variants for identification of sockeye salmon (*Oncorhynchus nerka*) stocks in Cook Inlet, Alaska. Canadian Journal of Fisheries and Aquatic Sciences **37**:1236-1247.

Hawkes, L. A., A. C. Broderick, M. H. Godfrey, and B. J. Godley. 2007. Investigating the potential impacts of climate change on a marine turtle population. Global Change Biology **13**:1-10.

Henwood, T. A. 1987. Movements and seasonal changes in loggerhead turtle, *Caretta caretta*, aggregations in the vicinity of Cape Canaveral, Florida (1978-84). Biological Conservation **40**:191-202.

Henwood, T. A., and W. E. Stuntz. 1987. Analysis of sea turtle captures and mortalities during commercial shrimp trawling. Fisheries Bulletin **85**:813-817.

Hopkins-Murphy, S. R., T. M. Murphy, C. P. Hope, J. W. Coker, and M. E. Hoyle. 2001. Population trends and nesting distribution of the loggerhead turtle (*Caretta caretta*) in South Carolina 1980-1997. South Carolina Department of Natural Resources Final Report to the U.S. Fish and Wildlife Service. 41 pp.

Hopkins-Murphy, S. R., D. W. Owens, and T. M. Murphy. 2003. Ecology of immature loggerheads on foraging grounds and adults in internesting habitat in the eastern

United States. Pages 79-92 in A. B. Bolten, and A. B. Witherington, editors. Loggerhead Sea Turtles. Smithsonian Books, Washington, D.C.

Hykle, D. 1992. The migratory species (Bonn) convention and marine turtle conservation. Pages 61-63 in M. Salmon, and J. Wyneken, editors. Proceedings of the Eleventh Annual Memorandum on Sea Turtle Biology and Conservation NOAA Technical Memorandum NMFS-SEFC-302.

International Union for the Conservation of Nature (IUCN). 2006. 2006 IUCN Red List of Threatened Species. www.iucnredlist.org.

Keller, J. M., K. Kannan, S. Taniyasu, R. D. Day, M. D. Arendt, A. L. Segars, and J. R. Kucklick. 2005. Perflorinated compounds in the plasma of loggerhead and Kemp's ridley sea turtles from the southeastern coast of the United States. Environmental Science and Technology **39**:9101-9108.

Kumar, S., K. Tamura, and M. Nei. 2004. MEGA 3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Pages 150-163. Briefings in Bioinformatics.

Lahanas , P. N., K. A. Bjorndal, A. B. Bolten, S. E. Encalada, M. M. Miyamoto, R. A. Valverde, and B. W. Bowen. 1998. Genetic composition of a green turtle (*Chelonia mydas*) feeding ground population: evidence for multiple origins. Marine Biology **130**:345-352.

Laurent, L., P. Casale, M. N. Bradai, B. J. Godley, G. Gerosa, A. C. Broderick, W. Schroth, B. Shierwater, A. M. Levy, D. Freggi, E. M. Abd El-Mawla, D. A. Hadoud, H. E. Gomati, M. Domingo, M. Hadjichristophorou, L. Kornaraky, F. Demirayak, and C. Gautier. 1998. Molecular resolution of marine turtle stock

composition in fishery bycatch: a case study in the Mediterranean. Molecular Ecology **7**:1529-1542.

Lewison, R. L., L. B. Crowder, A. J. Read, and S. A. Freeman. 2004. Understanding impacts of fisheries bycatch on marine megafauna. TRENDS in Ecology and Evolution **19**:598-604.

Lewison, R. L., L. B. Crowder, and D. Shaver. 2003. The impact of turtle excluder devices and fisheries closures on loggerhead and Kemp's ridley strandings in the western Gulf of Mexico. Conservation Biology **17**:1089-1097.

Limpus, C. J. 1994. The loggerhead turtle, *Caretta caretta*, in Queensland: feeding ground selection following her first nesting season. Pages 78-81 in K. A. Bjorndal, A. B. Bolten, D. A. Johnson, and P. J. Eliazar, editors. Proceedings of the 14th annual workshop on sea turtle biology and conservation. NOAA Technical Memorandum NMFS-SEFSC-351.

Limpus, C. J., J. D. Miller, C. J. Parmenter, R. D, N. McLachlan, and R. Webb. 1992. Migration of green (*Chelonia mydas*) and loggerhead (*Caretta caretta*) turtles to and from eastern Australian rookeries. Wildlife Research **19**:347-357.

Lutcavage, M., and J. A. Musick. 1985. Aspects of the biology of sea turtles in Virginia. Copeia **1985**:449-456.

Maffucci, F., W. H. C. F. Kooistra, and F. Bentivegna. 2006. Natal origin of loggerhead turtles, *Caretta caretta*, in the neritic habitat off the Italian coasts, Central Mediterranean. Biological Conservation **127**:183-189.

Maier, P. P., A. L. Segars, M. D. Arendt, J. D. Whitaker, B. W. Stender, L. Parker, R. Vendetti, D. Owens, J. M. Quattro, and S. R. Murphy. 2004. Development of an

index of sea turtle abundance based upon in-water sampling with trawl gear. South Carolina Department of Natural Resources (SCDNR). Final Project Report to National Marine Fisheries Service and National Oceanic and Atmospheric Administration. Grant Number NA07FL0499. 84 pp.

Maldini, D., L. Mazzuca, and S. Atkinson. 2005. Odontocete stranding patterns in the main Hawaiian Islands (1937-2002): How do they compare with live animal surveys? Pacific Science **59**:55-67.

McFee, W. E., and S. R. Hopkins-Murphy. 2002. Bottlenose dolphin (*Tursiops truncatus*) strandings in South Carolina, 1992-1996. Fisheries Bulletin **100**:258-265.

Mitchell, J. F., J. W. Watson, D. G. Foster, and R. E. Caylor. 1995. The Turtle Excluder Device (TED): a guide to better performance. NOAA Technical Memorandum. NMFS-SEFSC-336. 35 pp.

Moritz, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. Molecular Ecology **3**:401-411.

Mortimer, J. A. 1982. Feeding ecology of sea turtles. Pages 102-109 in K. A. Bjorndal, editor. Biology and Conservation of Sea Turtles. Smithsonian Institution Press, Washington, D.C.

Mrosovsky, N., and J. Provancha. 1992. Sex ratio of hatchling loggerhead sea turtles: data and estimates from a 5-year survey. Canadian Journal of Zoology **70**:530-538.

Murphy, T. M., and S. R. Hopkins-Murphy 1989. Sea turtle & shrimp fishing interactions: a summary and critique of relevant information. Center for Marine Conservation, Washington, D.C.

Murphy, T. M., S. R. Murphy, D. B. Griffin, and C. P. Hope. 2006. Recent occurrence, spatial distribution, and temporal variability of leatherback turtles (*Dermochelys coriacea*) in nearshore waters of South Carolina, USA. Chelonian Conservation and Biology **5**:216-224.

Musick, J. A., and C. J. Limpus. 1997. Habitat utilization and migration in juvenile sea turtles. Pages 137-163 in J. A. Musick, and P. L. Lutz, editors. The Biology of Sea Turtles Volume I. CRC Press, Washington, D.C.

National Marine Fisheries Service (NMFS) and U.S. Fish and Wildlife Service (USFWS). 1991. Recovery plan for U.S. population of loggerhead turtle. 64 pp.

National Marine Fisheries Service (NMFS) and U.S. Fish and Wildlife Service (USFWS). 2007. Loggerhead sea turtle (*Caretta caretta*) 5-year review: summary and evaluation. Silver Spring, Maryland. 65 pp.

National Research Council (NRC) 1990. Decline of the sea turtles: causes and prevention. The National Academies Press, Washington, D.C.

Nieri, M., E. Grau, B. Lamarche, and A. Aguilar. 1999. Mass mortality of Atlantic spotted dolphins (*Stenella frontalis*) caused by a fishing interaction in Mauritania. Marine Mammal Science **15**:847-854.

Norman, J. A., C. Moritz, and C. J. Limpus. 1994. Mitochondrial DNA control region polymorphisms: genetic markers for ecological studies of marine turtles. Molecular Ecology **3**:363-373.

Norrgard, J. W., and J. E. Graves. 1996. Determination of the natal origin of a juvenile loggerhead turtle (*Caretta caretta*) population in Chesapeake Bay using mitochondrial DNA analysis. Pages 129-136 in B. W. Bowen, and W. N. Witzell,

editors. Proceedings of the International Symposium on Sea Turtle Conservation Genetics. NOAA Tech. Memo NMFS-SEFSC-396.

Okuyama, T., and B. M. Bolker. 2005. Combining genetic and ecological data to estimate sea turtle origins. Ecological Applications **151**:315-325.

Pearce, A. F. 2001. Contrasting population structure of the loggerhead turtle (*Caretta caretta*) using mitochondrial and nuclear DNA markers. MS Thesis, University of Florida, Gainesville, Florida. 72 pp.

Pella, J. J., and M. Masuda. 2001. Bayesian methods for analysis of stock mixtures from genetic characters. Fisheries Bulletin **99**:151-167.

Pella, J. J., and G. B. Milner. 1987. Use of genetic marks in stock composition analysis. Pages 247-276 in N. Ryman, and F. W. Utter, editors. Population genetics and fishery management. University of Washington Press, Seattle, WA, U.S.A.

Plotkin, P. T., and J. R. Spotila. 2002. Post-nesting migrations of loggerhead turtles *Caretta caretta* from Georgia, USA: conservation implications for a genetically distinct subpopulation. Oryx **36**:396-399.

R Development Core Team. 2005. R: A language and environment for statistical computing. ISBN 3-900051-07-0, URL http://www.R-project.org. R Foundation for Statistical Computing, Vienna, Austria.

Raftery, A. E., and S. M. Lewis. 1996. Implementing MCMC. Pages 115-130 in W. R. Gilks, S. Richardson, and D. J. Spiegelhalter, editors. Markov Chain Monte Carlo in Practice. Chapman and Hall, London.

Rankin-Baransky, K., C. J. Williams, A. L. Bass, B. W. Bowen, and J. R. Spotila. 2001. Origin of loggerhead turtles stranded in the northeastern United States as determined by mitochondrial DNA analysis. Journal of Herpetology **35**:638-646.

Reece, J. S., L. M. Ehrhart, and C. L. Parkinson. 2006. Mixed stock analysis of juvenile loggerheads (*Caretta caretta*) in Indian River Lagoon, Florida: implications for conservation planning. Conservation Genetics **7**:345-352.

Roberts, M. A., C. J. Anderson, B. Stender, A. Segars, J. D. Whittaker, J. M. Grady, and J. M. Quattro. 2005. Estimated contribution of Atlantic coastal loggerhead turtle nesting populations to offshore feeding aggregations. Conservation Genetics **6**:133-139.

Ross, J. P. 1982. Historical decline of loggerhead, ridley, and leatherback sea turtles. Pages 189-195 in K. A. Bjorndal, editor. Biology and Conservation of Sea Turtles. Smithsonian Institution Press, Washington, D.C.

Schroeder, B., A. Foley, and D. A. Bagley. 2003. Nesting patterns, reproductive migrations, and adult foraging areas of loggerhead turtles. Pages 114-124 in A. B. Bolten, and A. B. Witherington, editors. Loggerhead Sea Turtles. Smithsonian Books, Washington, DC.

Sears, C., B. Bowen, R. Chapman, S. Galloway, S. Hopkins-Murphy, and C. Woodley. 1995. Demographic composition of the feeding population of juvenile loggerhead sea turtles (*Caretta caretta*) off Charleston, South Carolina: evidence from mitochondrial DNA markers. Marine Biology **123**:869-874.

Segars, A. L., M. D. Arendt, and J. D. Whitaker. 2006. Examination of local movement and migratory behavior of sea turtles during spring and summer along the Atlantic

coast off the southeastern United States. South Carolina Department of Natural Resources (SCDNR). Annual Report to National Marine Fisheries Service for Grant Number NA03NMF4720281. 39 pp.

South Carolina Code of Laws 50-5-765. South Carolina Marine Resources Act of 2000.

South Carolina Department of Natural Resources (SCDNR). 2003. Loggerheadlines: sea turtle news from South Carolina. Aug-Nov 2003. 6 pp.

South Carolina Department of Natural Resources (SCDNR). 2005. Turtle Excluder Device (TED) chronology Page http://www.dnr.state.sc.us/marine/turtles/teds.html. Accessed 4/2005.

Steinitz, M. J., M. Salmon, and J. Wyneken. 1998. Beach renourishment and loggerhead sea turtle reproduction: A seven year study at Jupiter Island, Florida. Journal of Coastal Research **14**:1000-1013.

Stoneburner, D. L. 1980. Body depth: an indicator of morphological variation among nesting groups of adult loggerhead sea turtles (*Caretta caretta*) Journal of Herpetology **14**:205-206.

SWOT. 2006. State of the World's Sea Turtles: Volume II. Conservation International. 52 pp.

Talbert, O. R., Jr, S. E. Stancyk, J. M. Dean, and J. M. Will. 1980. Nesting activity of the loggerhead sea turtle (*Caretta caretta*) in South Carolina: a rookery in transition. Copeia **1980**:709-719.

Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution **10**:512-526.

Teas, W. G. 1993. Species composition and size class distribution of marine turtle strandings on the Gulf of Mexico and southeast United States coasts, 1985-1991. NMFS-SEFSC-315. 43 pp.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research **24**:4876-4882.

Turtle Expert Working Group (TEWG). 1998. An assessment of the Kemp's ridley (*Lepidochelys kempii*) and loggerhead (*Caretta caretta*) sea turtle populations in the western North Atlantic. U.S. Department of Commerce. NOAA Technical Memorandum NMFS-SEFSC-409. 96 pp.

Turtle Expert Working Group (TEWG). 2000. Assessment update for the Kemp's ridley and loggerhead sea turtle populations in the western North Atlantic. U.S. Department of Commerce. NOAA Technical Memorandum NMFS-SEFSC-444. 115 pp.

Ulrich, G. F. 1978. Incidental catch of loggerhead turtles by South Carolina commercial fisheries. Report to National Marine Fisheries Service. Contract No's 03-7-042-35151 and 03-7-042-35121. 36 pp.

Weber, M., D. Crouse, R. Irvin, and S. Iudicello 1995. Delay and denial: a political history of sea turtles and shrimp fishing. Center for Marine Conservation, Washington, D.C.

Witzell, W. N., A. L. Bass, M. J. Bresette, D. A. Singewald, and J. C. Gorham. 2002. Origin of immature loggerhead sea turtles (*Caretta caretta*) at Hutchinson Island, Florida: evidence from mtDNA markers. Fisheries Bulletin **100**:624-631.

Work, T. M., and R. A. Rameyer. 1999. Mass stranding of wedge-tailed shearwater chicks in Hawaii. Journal of Wildlife Disease **35**:487-495.

Xu, S., C. J. Koback, and P. E. Smouse. 1994. Constrained least squares estimation of mixed population stock composition from mtDNA haplotype frequency data. Canadian Journal of Fisheries and Aquatic Sciences **51**:417-425.

Yntema, C. L., and N. Mrosovsky. 1979. Incubation temperature and sex ratio in hatchling loggerhead turtles: A preliminary report. Marine Turtle Newsletter **11**:9-10.

TABLES

Table 1: Relative mtDNA haplotype frequencies observed on nesting beaches (Encalada et al. 1998; Laurent et al. 1998; Pearce 2001), the nearshore juvenile foraging aggregation from Winyah Bay, SC to St. Augustine, FL (Roberts et al. 2005) and stranded individuals in this study. Rookery size information taken from NMFS & USFWS (2007). Abbreviations for Nesting Subpopulations and Mixtures: Northern (NEFL-NC), South Florida (SFL), Florida Panhandle (NWFL), Dry Tortugas (DT), Yucatán (MEX), Bahia, Brazil (BRA), Greece/Cyprus/Libya/Israel/Italy (GRE), Turkey (TUR) and North Carolina (NC), South Carolina (SC).

Haplotypes		Nesting Beaches								Nearshore Aggregation (Roberts et al. 2005)	Strandings		
ACCSTR	Roberts et al. 2005	NEFL-NC	SFL	NWFL	DT	MEX	BRA	GRE	TUR		NC	SC	NC/SC Combined
CC-A1	A	104	52	38	4					117	20	45	65
CC-A14	A2		2							7		1	1
CC-A37	A3									1			
CC-A2	B	1	45	7	50	11		78	19	74	15	20	35
CC-A13	B2									1	1	1	2
CC-A20	B3		1							1	1		1
CC-A40	B4									1			
CC-A3	C		4	2		2			13	7	2	1	3
CC-A4	D						11						
CC-A5	E		1										
CC-A6	F							2					
CC-A7	G		3	2						4			
CC-A8	H					1				1		1	1
CC-A9	I				2	1				2			
CC-A10	J				2	5		1		1		3	3
CC-A11	N/A		1										
CC-A45	N/A											1	1
	Total	105	109	49	58	20	11	81	32	217	39	73	112
	Rookery Size	5151	65460	910	246	1617	4837	3050	2000				

Table 2: Mitochondrial DNA control region haplotypes observed on nesting beaches, the nearshore juvenile foraging aggregation from Winyah Bay, SC to St. Augustine, FL and stranded individuals in this study. Variable sites are relative to haplotype CC-A1 (A). Amended from full sequences available on the ACCSTR website (http://accstr.ufl.edu/ccmtdna.html)

Haplotype		
ACCSTR	Roberts et al. (2005)	Variable Sites by base position
		111112222223333333333333
		223445789915558023358000111244455555
		580466190721451337927578028078901 26
CC-A1	A	TGTTTAGAGAACCGGCCGCA-AATAACC------A
CC-A14	A2	......A......A...A..-..............-.
CC-A37	A3	......A...G..A...A..-..............-.
CC-A2	B	CACC-G...GG.TA.TTATGG.GCG..TTGCAAG.
CC-A13	B2	CACC-GA..G..TA.TTATGG.GCG..TTGCAAG.
CC-A20	B3	.ACC-G...G..TA.TTATGG.GCG..TTGCAAG.
CC-A40	B4	CACC-G...GG.TA.TTATGG.GCG.ATTGCAAG.
CC-A3	C	CACC-G...G..TAATTATGG.GCG..TTGCAAG.
CC-A4	D	............A...A..-..............-.
CC-A5	E	CACC-G...G..TA.TTATGG.GCG..TTGCAAG-
CC-A6	F	CACC-G...G..TA.TTATGG.GCG-.TTGCAAG.
CC-A7	G	CACC-G...G..TA.TTATGGGGCG..TTGCAAG.
CC-A8	H	CACC-G...G...A.TTATGG.GCG..TTGCAAG.
CC-A9	I	CAC.-G.G.G..TA.TTATGG.GCG..TTGCAAG.
CC-A10	J	CACC-G...G..TA.TTATGG.GCG..TTGCAGG.
CC-A11	N/A	............A.....-.....-------.
CC-A45	N/A	CACC-GA..G..TA.TTATGGGGCG..TTGCAAG.

Table 3: Population differentiation results from AMOVA run using Tamura-Nei distances (Tamura & Nei 1993) from North and South Carolina strandings. Fixation index and p-value are presented.

Source of variation	d.f.	Sum of squares	Variance Components	Percentage of Variation
Among populations	1	62.4180	0.3706	0.8400
Within populations	110	4793.6270	43.5784	99.1600
Total	111	4856.0450	43.9490	

Fixation Index (Φ_{ST}) = 0.0084

p-value = 0.3070±0.0139

Table 4: Population differentiation results from AMOVA using Tamura-Nei distances (Tamura & Nei 1993) from pooled North and South Carolina strandings and nearshore data from Roberts et al. (2005). Fixation index and p-value are presented.

Source of variation	d.f.	Sum of squares	Variance Components	Percentage of Variation
Among populations	1	4.0840	-0.4471	-0.6400
Within populations	327	22938.1790	70.1473	100.6400
Total	328	22942.2630	69.7002	

Fixation Index (Φ_{ST}) = -0.0064

p-value = 0.7986±0.0100

Table 5: Relative haplotype frequencies for stranded loggerheads in North and South Carolina and the nearshore aggregation (Roberts et al. 2005). Shaded cells indicate haplotypes which are shared.

Haplotype		Mixture	
ACCSTR	Roberts et al. 2005	Nearshore (n = 217)	Stranded (n = 112)
CC-A1	A	0.5390	0.5800
CC-A14	A2	0.0323	0.0089
CC-A37	A3	0.0046	0.0000
CC-A2	B	0.3410	0.3120
CC-A13	B2	0.0046	0.0179
CC-A20	B3	0.0046	0.0089
CC-A40	B4	0.0046	0.0000
CC-A3	C	0.0323	0.0268
CC-A7	G	0.0184	0.0000
CC-A8	H	0.0046	0.0089
CC-A9	I	0.0092	0.0000
CC-A10	J	0.0046	0.0268
CC-A45	N/A	0.0000	0.0089

Table 6: Pairwise F_{ST} values for comparisons between pooled North and South Carolina stranding data (Stranded), Nearshore data (Roberts et al. 2005), and nesting beach data (Laurent et al. 1998; Pearce 2001; Roberts et al. 2005). Abbreviations for Nesting Subpopulations: Northern (NEFL-NC), South Florida (SFL), Florida Panhandle (NWFL), Dry Tortugas (DT), Yucatán (MEX), Bahia, Brazil (BRA), Greece/Cyprus/Libya/Israel/Italy (GRE), Turkey (TUR). Bolded figures indicate no significant difference observed.

Mixture/Nesting Subpopulation	Nearshore	Stranded	NEFL-NC	SFL	NWFL	DT	MEX	BRA	GRE	TUR
Nearshore		+	-	**+**	-	-	-	-	-	-
Stranded	**-0.0031**		-	**+**	-	-	-	-	-	-
NEFL-NC	0.2763	0.2976		-	-	-	-	-	-	-
SFL	**0.0012**	**0.0105**	0.3999		-	-	-	-	-	-
NWFL	0.0697	0.0510	0.1913	0.1204		-	-	-	-	-
DT	0.3172	0.3749	0.8851	0.2716	0.6176		-	-	-	-
MEX	0.2371	0.2651	0.8518	0.1878	0.4679	0.1628		-	-	-
BRA	0.5445	0.5703	0.9828	0.5486	0.7151	0.7998	0.6068		-	-
GRE	0.4265	0.5110	0.9570	0.4098	0.7709	0.0415	0.3857	0.9369		-
TUR	0.2902	0.3276	0.8517	0.2439	0.5175	0.2508	0.0958	0.6615	0.4340	

Table 7: SPAM Results: Mean estimates of contributions from known rookeries to loggerhead strandings in North and South Carolina. (A) S1 analyses used only Encalada et al. (1998) rookeries and (B) S2 analyses used all currently sampled rookeries (Encalada et al. 1998; Laurent et al. 1998; Roberts et al. 2005). Standard deviations and 97% non-symmetric bootstrap confidence intervals are reported. Lower CI > 0 indicate definitive inclusion of rookery in mixture. The Pella-Masuda model of baseline allele frequency distributions was implemented.

(A) S1

Nesting Subpopulation	Mean Estimate	S.D.	97% Nonsymmetric Confidence Intervals	
			Lower	Upper
NEFL-NC	0.4215	0.1583	0.1046	0.7788
SFL	0.3494	0.2248	0.1417	0.9500
NWFL	0.0404	0.1105	0.0000	0.0000
MEX	0.1272	0.0786	0.0000	0.2495
BRA	0.0000	0.0000	0.0000	0.0000
GRE	0.0616	0.0982	0.0000	0.0004

(B) S2

Nesting Subpopulation	Mean Estimate	S.D.	97% Nonsymmetric Confidence Intervals	
			Lower	Upper
NEFL-NC	0.4925	0.1082	0.3401	0.8159
SFL	0.2239	0.1697	0.0000	0.4279
NWFL	0.0046	0.0225	0.0000	0.0000
DT	0.0674	0.0971	0.0000	0.0021
MEX	0.0942	0.0664	0.0000	0.2029
BRA	0.0000	0.0005	0.0000	0.0000
GRE	0.0907	0.0939	0.0343	0.3458
TUR	0.0267	0.0382	0.0000	0.0000

Table 8: BAYES Results: Mean estimates of contributions from known rookeries to loggerhead strandings in North and South Carolina using combined MCMC chains. BAYES1 used only Encalada et al. (1998) rookeries and BAYES2 used all currently sampled rookeries (Encalada et al. 1998; Laurent et al. 1998; Roberts et al. 2005). Standard deviations, 97% non-symmetric confidence intervals, and median estimates are reported. Lower CI > 0 indicate definitive inclusion of rookery in mixture.

(A) BAYES1
Chain length: 24,769

Nesting Subpopulation	Mean Estimate	S.D.	97% Nonsymmetric Confidence Intervals		Median Estimate
			Lower	Upper	
NEFL-NC	0.5732	0.2796	0.0000	0.9082	0.6626
SFL	0.1447	0.2126	0.0000	0.7745	0.0301
NWFL	0.1164	0.1989	0.0000	0.6977	0.0102
MEX	0.1412	0.0765	0.0275	0.3224	0.1282
BRA	0.0017	0.0041	0.0000	0.0136	0.0001
GRE	0.0229	0.0473	0.0000	0.1785	0.0016

(B) BAYES2
Chain length: 61,826

Nesting Subpopulation	Mean Estimate	S.D.	97% Nonsymmetric Confidence Intervals		Median Estimate
			Lower	Upper	
NEFL-NC	0.3609	0.2543	0.0000	0.7746	0.4051
SFL	0.3625	0.2159	0.0507	0.8809	0.3263
NWFL	0.1330	0.2039	0.0000	0.6556	0.0093
DT	0.0183	0.0415	0.0000	0.1535	0.0005
MEX	0.0981	0.0568	0.0112	0.2334	0.0890
BRA	0.0012	0.0035	0.0000	0.0111	0.0000
GRE	0.0100	0.0224	0.0000	0.0790	0.0003
TUR	0.0161	0.0363	0.0000	0.1336	0.0004

Table 9: BAYES3 Results: Mean estimates of contributions from known rookeries to loggerhead strandings in North and South Carolina using priors were weighted to reflect rookery sizes according to NMFS & USFWS (2007)(see Table 1). Analyses used all currently sampled rookeries (Encalada et al. 1998; Laurent et al. 1998; Roberts et al. 2005). Results of combined MCMC chains are reported with standard deviations, 97% non-symmetric confidence intervals, and median estimates. Lower CI > 0 indicate definitive inclusion of rookery in mixture.

Chain length: 42,660

Nesting Subpopulation	Mean Estimate	S.D.	97% Nonsymmetric Confidence Intervals		Median Estimate
			Lower	Upper	
NEFL-NC	0.2888	0.2535	0.0000	0.7519	0.2989
SFL	0.5902	0.2653	0.1260	0.9688	0.5707
NWFL	0.0286	0.1082	0.0000	0.4551	0.0000
DT	0.0003	0.0047	0.0000	0.0000	0.0000
MEX	0.0862	0.0543	0.0000	0.2142	0.0787
BRA	0.0006	0.0024	0.0000	0.0065	0.0000
GRE	0.0028	0.0123	0.0000	0.0350	0.0000
TUR	0.0024	0.0137	0.0000	0.0281	0.0000

Table 10: P-values for pairwise Kolmogorov-Smirnov two-sample tests conducted on size distributions of South Carolina loggerhead stranding data for May, June and July of 2000 - 2003.

Year	2000	2001	2002	2003
2000		-	-	-
2001	0.5473		-	-
2002	0.5930	0.2370		-
2003	0.5634	0.0791	0.9596	

Table 11: P-values for pairwise Kolmogorov-Smirnov two-sample tests conducted on size distributions of the nearshore aggregation off the NEFL-NC collected during an in-water study by Maier et al. (2004) for May, June and July of 2000 - 2003. Significant values ($p < 0.05$) are indicated in bold.

Year	2000	2001	2002	2003
2000		-	-	**+**
2001	0.1158		-	-
2002	0.0887	0.7752		-
2003	**0.0013**	0.2016	0.4690	

Table 12: Kolmogorov-Smirnov two-sample test results for comparisons of size distributions of the South Carolina loggerhead stranding data and the nearshore aggregation (Maier et al. 2004) during May, June and July of 2000 - 2003. Significant values ($p < 0.05$) are indicated in bold.

Year	D	p-value
2000	0.1396	0.5184
2001	0.2173	0.1072
2002	0.1898	0.2907
2003	0.3179	**0.0005**

Table 13: Chi-square test of homogeneity of size distributions of stranded loggerheads before larger TED implementation (2000 - 2001) and after larger TED implementation (2004 - 2005). Observed counts are on top, expected counts are below the observed and chi-square contributions are below expected counts. Shaded cells indicate highest contributors to the chi-square statistic.

Size (cm)	<60.0	60.0-69.9	70.0-79.9	80.0-89.9	90.0-99.9	≥ 100.0
Before Large TEDs (2000-2001)	16	47	49	17	22	23
	14.29	49.29	61.12	17.75	13.80	17.75
	0.20	0.11	2.40	0.03	4.87	1.56
After Large TEDs (2004-2005)	13	53	75	19	6	13
	14.71	50.71	62.85	18.25	14.20	18.25
	0.20	0.10	2.34	0.03	4.73	1.51

$\chi^2 = 18.087$, d.f. = 5, p = 0.003

Table 14: Chi-square test of homogeneity of adult/juvenile proportions of stranded loggerheads before larger TED implementation (2000 - 2001) and after larger TED implementation (2004 - 2005). Observed counts are on top, expected counts are below the observed and chi-square contributions are below expected counts. Shaded cells indicate highest contributors to the chi square statistic.

Life Stage	Adult (≥ 90 cm CCL)	Juvenile (<90 cm CCL)
Before Large TEDs (2000-2001)	45	129
	31.55	142.45
	5.74	1.27
After Large TEDs (2004-2005)	19	160
	32.45	146.55
	5.58	1.24

χ^2 = 13.820, d.f. = 1, p=0.00

FIGURES

Figure 1: Neighbor-joining tree of 17 *C. caretta* mtDNA control region haplotypes from the Atlantic and Mediterranean constructed using Tamura and Nei's (1993) model of evolution. Scale indicates genetic distance. Common haplotypes are indicated in bold.

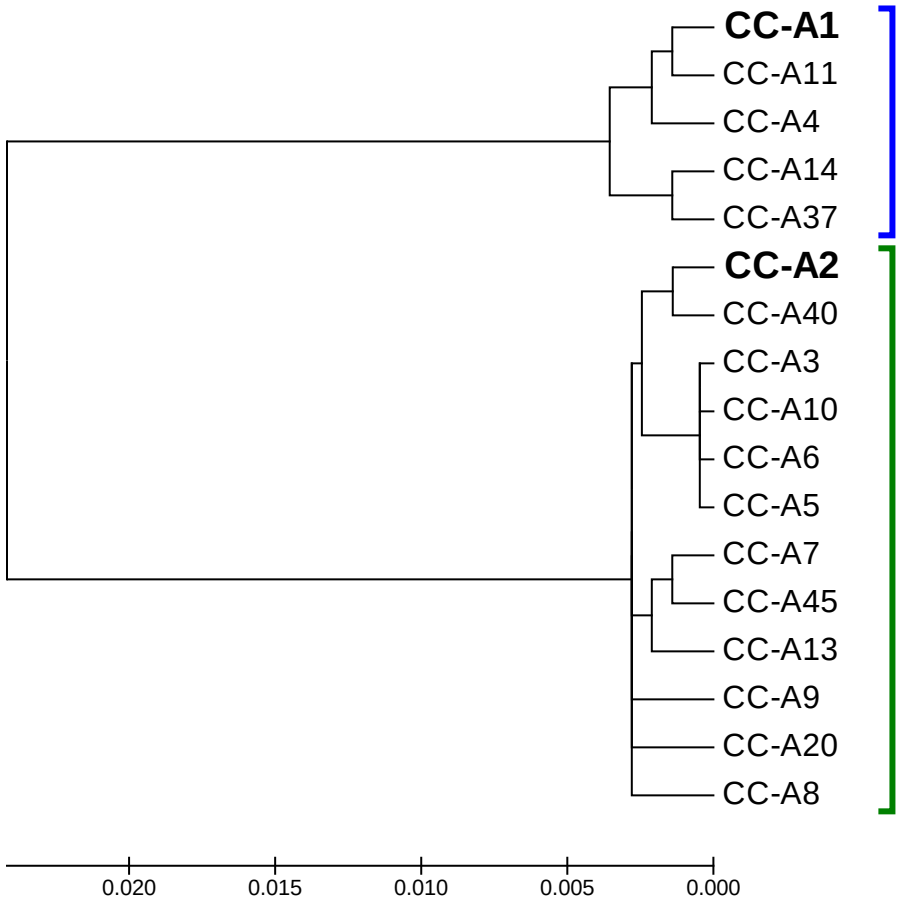

CC-A1
CC-A11
CC-A4
CC-A14
CC-A37
CC-A2
CC-A40
CC-A3
CC-A10
CC-A6
CC-A5
CC-A7
CC-A45
CC-A13
CC-A9
CC-A20
CC-A8
0.020
0.015
0.010
0.005
0.000

Figure 2: Diagram of an example TED design (Mitchell et al. 1995)

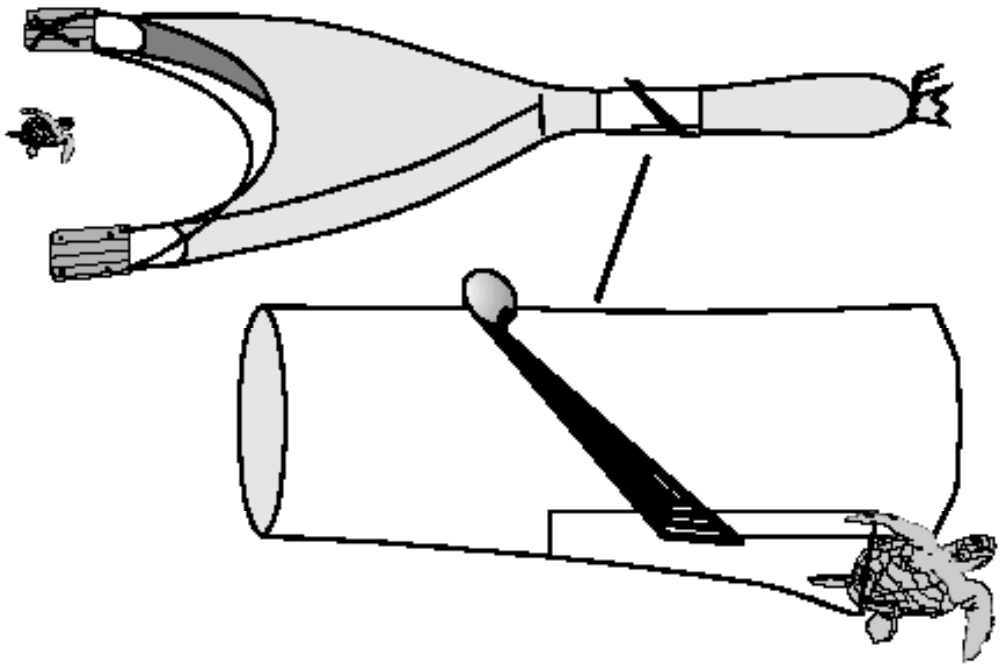


Figure 3: Size distributions of stranded loggerheads in South Carolina from 2000 – 2005. Distributions were not significantly different (see Table 10); however, they appear to show the start of a trend towards increasing juvenile strandings and decreasing adult strandings.

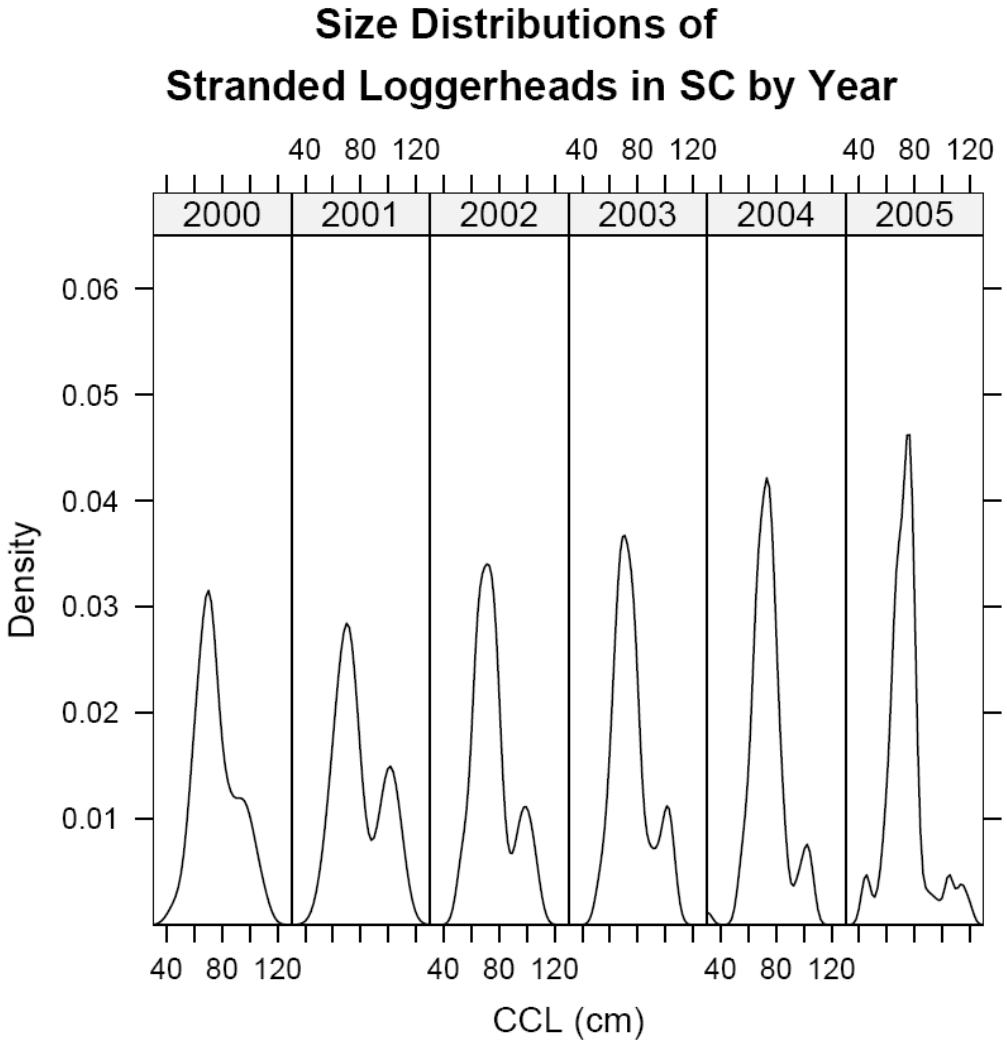

Size Distributions of
Stranded Loggerheads in SC by Year
2000
2001
2002
2003
2004
2005
Density
0.06
0.05
0.04
0.03
0.02
0.01
40 80 120
CCL (cm)

Figure 4: Size distributions of loggerheads in the nearshore aggregation off South Carolina for 2000 – 2003. A significant difference ($p < 0.001$) was observed between 2000 and 2003 (see Table 11).

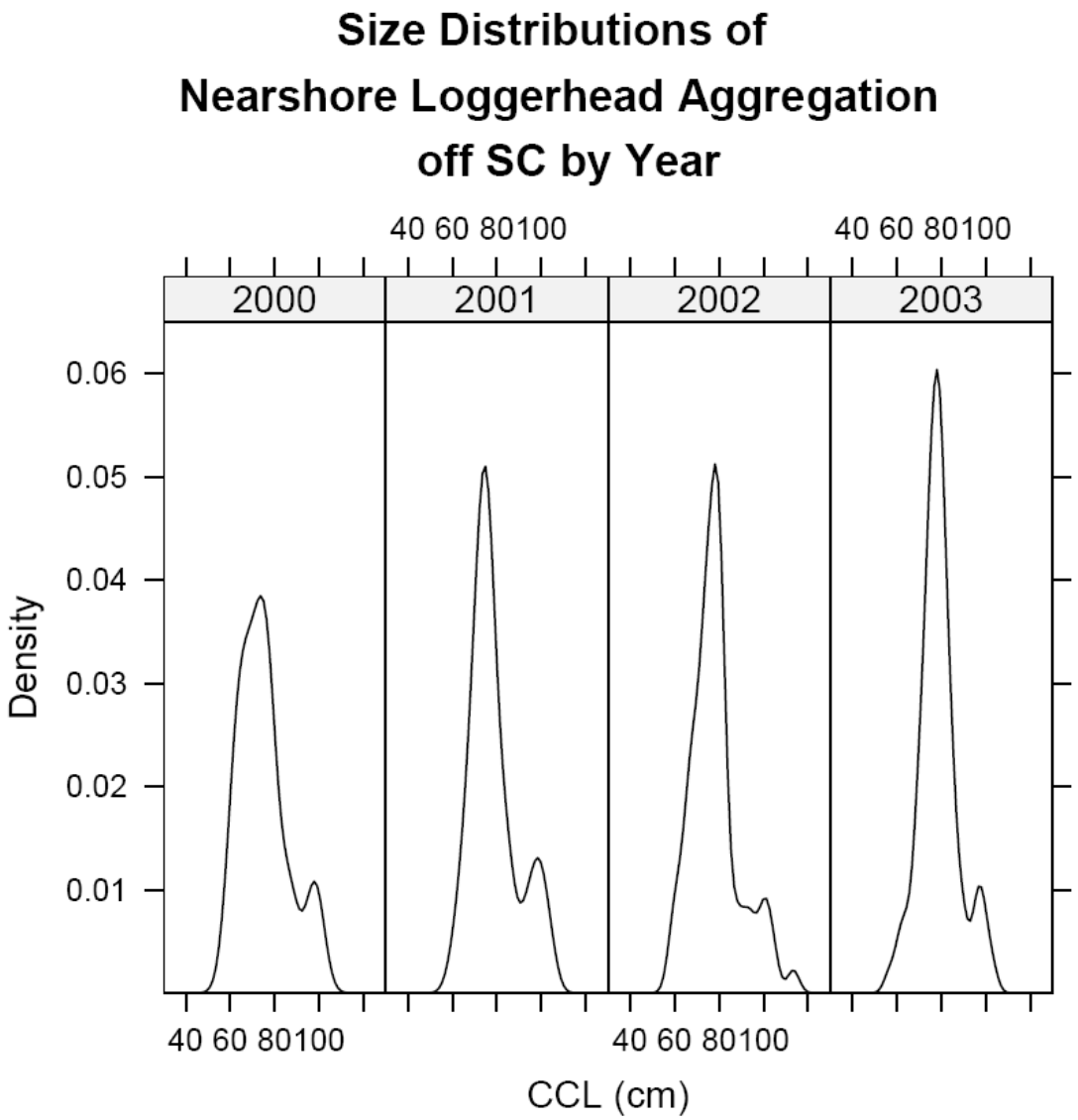
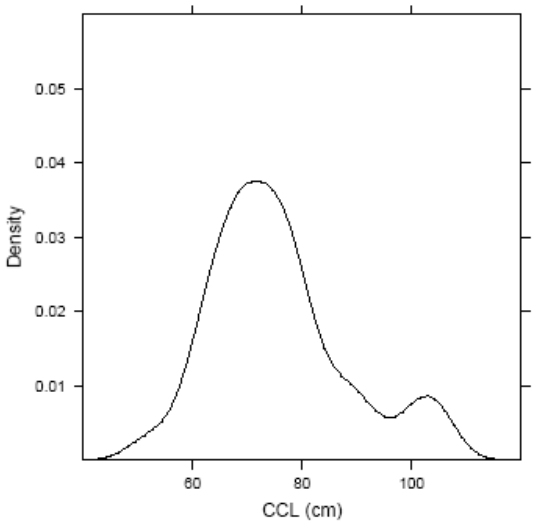

Size Distributions of
Nearshore Loggerhead Aggregation
off SC by Year
40 60 80100
40 60 80100
2000
2001
2002
2003
0.06
0.05
0.04
0.03
0.02
0.01
Density
40 60 80100
40 60 80100
CCL (cm)

Figure 5: Size distributions in May, June and July of 2003 for (A) stranded loggerheads (n = 74) and (B) the nearshore loggerhead aggregation (n = 92) in South Carolina (Maier et al. 2004). Distributions were significantly different (p = 0.0005; Table 12).

A. Stranded Loggerheads



B. Nearshore Loggerheads

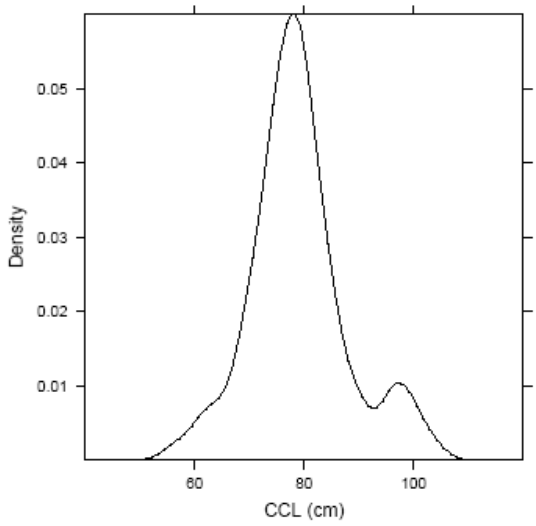


Figure 6: Size distributions of stranded loggerheads (A) before larger TEDs (2000 - 2001) and (B) after larger TEDs (2004 - 2005). Distributions are significantly different (D = 0.1661, p = 0.0154).

A. Before Large TEDs (2000-2001) **B. After Large TEDs (2004-2005)**

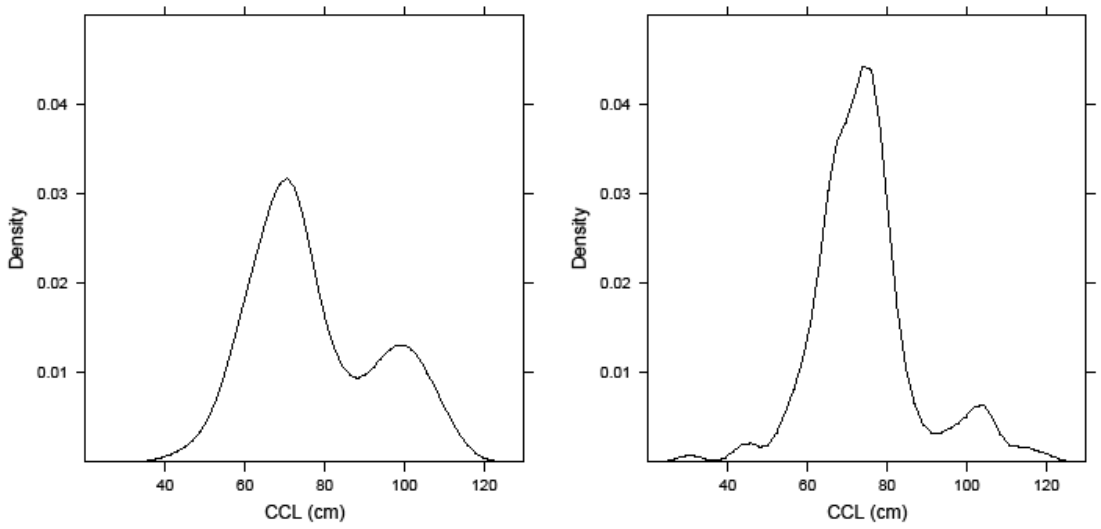
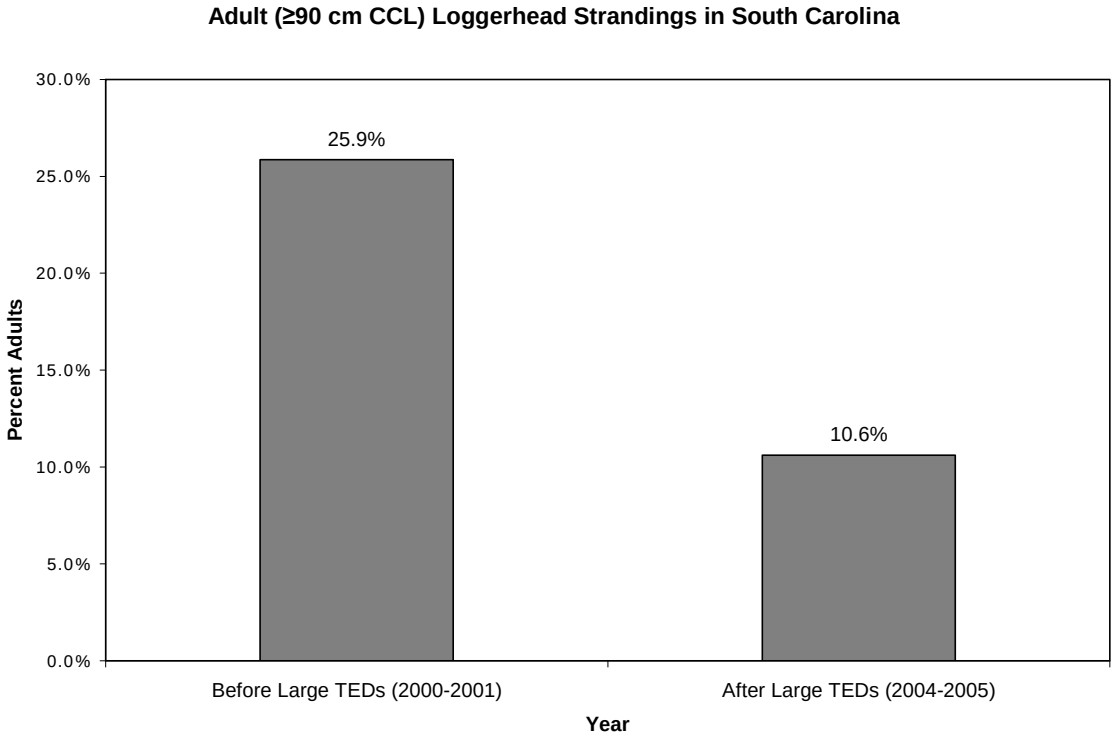


Figure 7: Proportions of adults (≥ 90 cm CCL) compared to total numbers (n = 174) of stranded loggerheads in South Carolina for 2000 - 2001 (before larger TEDs) and compared to total strandings (n = 179) for 2004 - 2005 (after larger TEDs). Proportions are significantly different (χ^2 = 13.820, d.f. = 1, p = 0.00, Table 14)


Adult (≥90 cm CCL) Loggerhead Strandings in South Carolina
30.0%
25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
Percent Adults
25.9%
10.6%
Before Large TEDs (2000-2001)
After Large TEDs (2004-2005)
Year

APPENDIX I: 100,000 MCMC sample chains for BAYES estimates.

BAYES 1

Nesting Subpopulation	Mean Estimate	S.D.	97% Nonsymmetric Confidence Intervals		Median Estimate
			Lower	Upper	
NEFL-NC	0.5636	0.2776	0.0000	0.9076	0.6492
SFL	0.1353	0.1975	0.0000	0.6735	0.0270
NWFL	0.1362	0.2079	0.0000	0.7057	0.0200
MEX	0.1398	0.0749	0.0298	0.3193	0.1269
BRA	0.0016	0.0040	0.0000	0.0132	0.0001
GRE	0.0234	0.0476	0.0000	0.1795	0.0016

BAYES 2

Nesting Subpopulation	Mean Estimate	S.D.	97% Nonsymmetric Confidence Intervals		Median Estimate
			Lower	Upper	
NEFL-NC	0.3637	0.2561	0.0000	0.7797	0.4130
SFL	0.3544	0.2140	0.0499	0.8819	0.3172
NWFL	0.1358	0.2094	0.0000	0.6645	0.0090
DT	0.0188	0.0430	0.0000	0.1631	0.0005
MEX	0.0989	0.0573	0.0119	0.2350	0.0899
BRA	0.0012	0.0034	0.0000	0.0112	0.0000
GRE	0.0100	0.0222	0.0000	0.0783	0.0003
TUR	0.0173	0.0378	0.0000	0.1397	0.0005

BAYES 3

Nesting Subpopulation	Mean Estimate	S.D.	97% Nonsymmetric Confidence Intervals		Median Estimate
			Lower	Upper	
NEFL-NC	0.2898	0.2498	0.0000	0.7466	0.2976
SFL	0.6091	0.2643	0.1351	0.9721	0.6020
NWFL	0.0123	0.0682	0.0000	0.2022	0.0000
DT	0.0004	0.0059	0.0000	0.0000	0.0000
MEX	0.0828	0.0538	0.0000	0.2097	0.0757
BRA	0.0006	0.0023	0.0000	0.0063	0.0000
GRE	0.0026	0.0115	0.0000	0.0323	0.0000
TUR	0.0024	0.0137	0.0000	0.0262	0.0000

ABBREVIATIONS

AMOVA	Analysis of Molecular Variance
BAYES1	BAYES run with 6 rookeries
BAYES2	BAYES run with 8 rookeries
BAYES3	BAYES run with 8 rookeries incorporating rookery size
BRA	Bahia, Brazil rookery
CCL	Curved Carapace Length (using flexible tape measure)
CITES	Convention on International Trade in Endangered Species
DT	Dry Tortugas Nesting Subpopulation
ESA	Endangered Species Act
EXOSAP	Exonuclease/Shrimp Alakaline Phosphatase
GRE	Greece/Cyprus/Libya/Israel/Italy rookeries
IUCN	International Union for the Conservation of Nature
MCMC	Markov Chain Monte Carlo
MEX	Yucatán Nesting Subpopulation
MSA	Mixed Stock Analysis
mtDNA	Mitochondrial DNA
NEFL-NC	Northern Nesting Subpopulation
NMFS	National Marine Fisheries Service
NRC	National Research Council
NWFL	Florida Panhandle Nesting Subpopulation
PEG	Poly Ethylene Glycol
S1	SPAM run with 6 rookeries
S2	SPAM run with 8 rookeries
SCDNR	South Carolina Department of Natural Resources
SCL	Straight Carapace Length (using calipers)
SFL	South Florida Nesting Subpopulation
STSSN	Sea Turtle Stranding and Salvage Network
TED	Turtle Excluder Device
TEWG	Turtle Expert Working Group
TUR	Turkey rookeries
USFWS	United States Fish and Wildlife Service