CHARACTERIZATION OF INJURIES AND HEALTH OF INJURED LOGGERHEAD SEA TURTLES (*Caretta caretta*) IN COASTAL WATERS OF THE SOUTHEASTERN U.S.

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

MASTER OF SCIENCE

in

MARINE BIOLOGY

by

JESSE ELIZABETH ALDERSON
DECEMBER 2009

at

THE GRADUATE SCHOOL OF THE COLLEGE OF CHARLESTON

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ABSTRACT

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This study utilized a standardized characterization system to describe injuries observed on loggerhead sea turtles captured by the SCDNR in-water sea turtle study between 2000 and 2009. At least one injury was noted among 27.4% (*n* = 433 of 1,579) of collected loggerheads. Injury rates were higher in adults than in sub-adults and juveniles, but were comparable between males and females. Loggerheads collected from shipping channels had higher injury rates than those captured elsewhere from comparable distances from shore. Observed injuries were not evenly distributed on the body, with the carapace exhibiting the highest proportion of injury (40.4%). Types of injuries were not evenly distributed, with amputations comprising 50.9% of all injuries. Significantly more injuries were healed (87.2%) relative to other injury ages, and a significant proportion of injuries could not be attributed to any source (85.1%). These finding collectively suggest that sub-lethal injuries are common among free-swimming loggerheads, with the highest proportion of injuries affecting the posterior carapace; however, the predominantly healed state of injuries suggests low annual infliction rates and a high resiliency to injuries among loggerheads.

Analysis of several blood parameters in injured and non-injured loggerheads suggested that injuries had no detectable long-term effect on health; however, corticosterone analyses suggested that the injury healing process may affect the physiological stress response. Concentrations of circulating corticosterone were significantly different between loggerheads with healing injuries and their controls. Additionally, initial corticosterone concentrations were significantly higher in loggerheads with partially healed relative to healed injures, and the corticosterone response to capture stress was suppressed in loggerheads with partially healed injuries relative to loggerheads with healed, fresh, and stingray injuries.

ACKNOWLEDGMENTS

First and foremost, I would like to thank my committee for their extensive assistance in transforming an idea into a thesis. Thanks to my advisor, Dave Owens, who introduced me to the world of endocrinology, stimulated many interesting and helpful conversations, and provided continuous encouragement throughout this endeavor. Thanks to Mike Arendt, who graciously included me in the SCDNR in-water turtle project and provided abundant guidance at every step. Al Segars' veterinary expertise and historical knowledge of the in-water turtle project has greatly enhanced this thesis, and statistical contributions by Allan Strand proved invaluable.

I was fortunate to become involved in such an extensive project, and owe many thanks to those who have assisted in data collection over the years. In addition to committee members, these individuals include (but are not limited to) project managers J. David Whitaker, Phil Maier and Bruce Stender, as well as project staff, notably Julia Byrd, Gaëlle Blanvillain, Jeff Schwenter, Dany Burgess, Heather Cason, Michelle Pate, and Jessica Johnson. The captains, crew and supporting staff of the R/V *Georgia Bulldog* (UGA Marine Extension Service) include Lisa Liguori, Captain Lindsey Parker, Marty Higgins, Richard Puterbaugh, William McClain and Jim Dickey. Aboard the R/V *Lady Lisa* (SCDNR MRD) and the vessel operations group, I wish to thank Captains Randy Beatty, Jeff Jacobs, Bobby Shrimpf and Rob Dunlap, as well as numerous deckhands (including Brooke James, Jeremy Grigsby, Steven Czwartacki and Daryl Weldon in 2008-2009), and many volunteers. Methods were approved by NMFS Permits #1245 (2000-2005) and #1540 (2006-2010), FL Permits #140 (2000-2003) and #163 (2006-present), and GA Permits #11411 (2000-2003) and # 21303 (2008-present).

Many thanks to the numerous people who have provided additional help throughout the course of this project. April Norem not only allowed me to use the STIIS she developed for her own thesis, but also offered helpful tips for implementing the system and managing the data. Kelly Thorvalson and Shane Boylan at the SC Aquarium supplied invaluable insights to loggerhead health and injury healing. The faculty and staff of Grice Marine Lab, especially Craig Plante, Shelly Brew, Sarah Prior and Pete Meier, helped me maneuver through the logistics of graduate school with ease. Additional thesis review and suggestions were graciously provided by Gaëlle Blanvillain, Jeff Schwenter and Jared Ragland. Finally, I would like to especially thank Gaëlle Blanvillain for her careful instruction of RIA procedures and techniques; her help and patience were greatly appreciated.

The in-water trawl survey has been funded by four NMFS grants since 2000: NA97FL0375 (2000), NA07FL0499 (2001-2003), NA03NMFS4720281 (2004-2008), and NA08NMF4720502 (2009-present). Support for this thesis was provided by the SCDNR (through NMFS grant NA03NMFS4720281) and the College of Charleston.

Many thanks to my fellow graduate students, friends, and family for their shared laugher, mutual support, and sometimes necessary distraction. A special thanks to Henry DaVega; I couldn't imagine this experience without him. Finally, I would like to thank my parents, Mick and Teri Alderson, who have unconditionally supported my pursuit of this career since I was 7 years old.

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INTRODUCTION AND BACKGROUND

The loggerhead sea turtle (*Caretta caretta)*, one of six sea turtle species found in U.S. waters, has a circum-global distribution that is concentrated in the temperate and subtropical waters of the Atlantic, Pacific, and Indian Oceans (Dodd, 1988). Loggerheads have been federally protected in U.S. waters as a "threatened species" since 1978 (Endangered Species Act, ESA), and international trade of all sea turtle species has been forbidden since 1981 (Convention on International Trade in Endangered Species, CITES). In the Northwest Atlantic Ocean, five recovery units based on nesting distributions are recognized by the Loggerhead Recovery Plan, one of which (the Peninsular Florida Recovery Unit) contains one of the densest loggerhead nesting assemblages in the world (NMFS and USFWS, 2008). The five recovery units are geographically distinct, and differences in haplotype frequency (Bowen *et al.*, 1993; NMFS and USFWS, 2008) and heavy metal burdens in eggs (Stoneburner *et al.*, 1980) suggest that demes exist in the southeastern U.S. Dominance of two haplotypes (CC-A01 and CC-A02) is also pronounced among loggerheads collected from coastal foraging grounds (Sears *et al.*, 1995; Bowen *et al.*, 2005; Bowen and Karl, 2007) between Florida and Long Island, New York (Morreale and Standora, 2005).

Genetic suggestions of natal homing and site affinity and fidelity are supported by satellite-telemetry studies in the southeast U.S. Juvenile loggerheads off South Carolina generally reside within 20 km of shore between April and November, with most over-wintering on the middle and outer continental shelf off South Carolina and Georgia

(Arendt *et al.*, 2009). Acoustic, radio and satellite telemetry studies with juvenile loggerheads in the Pamlico-Albermarle Sound Complex in North Carolina demonstrate high site fidelity during the warmer months (Avens *et al.*, 2003) followed by over-wintering on the middle to outer continental shelf of southern North Carolina and/or oceanic waters over the continental slope and rise (McClellan and Reed, 2007). Adult males (Arendt *et al.*, 2009) and adult females (Plotkin and Spotila, 2002; Hawkes *et al.*, 2007) may travel long distances to spring mating and late summer post-nesting foraging areas, but even these movements keep them in close proximity to the coast. Adults may remain in these foraging grounds for extended periods of time if favorable conditions such as food availability and suitable temperatures (>17°C; Arendt *et al*., 2009) persist.

Despite widespread conservation efforts implemented in response to national and international protections, full recovery has not yet occurred. The Recovery Plan for the Northwest Atlantic Loggerhead stipulates that the following three conditions must be met in order for recovery to be declared: over a generation time of 50 years, the total number of nests per year must increase by 1-3% (depending on the recovery unit) in correlation with an increase in the number of nesting females; the estimate of relative abundance from in-water sampling sites must increase; and stranding events must not increase at a rate greater than the trends noted in in-water abundance for similar age classes. Plotkin (1995) summarized loggerhead nesting trends in the southeast U.S. and reported that species recovery had not yet occurred; a year later the International Union for the Conservation of Nature (IUCN) downgraded the status of the loggerhead from "Vulnerable" to "Endangered." However, in-water catch rates of juveniles during abundance surveys conducted from North Carolina to Florida have remained stable or have increased appreciably in the last decade (Epperly *et al.*, 2007; Erhardt *et al.*, 2007; Arendt *et al.*, 2009). Annual loggerhead stranding events declined between 1980 and 1993 in South Carolina (Crowder *et al.*, 1995), and the proportion of larger individuals stranded has declined in the Carolinas concurrent with mandatory use of Turtle Excluder Devices (TEDs) in most trawl fisheries in federal waters (Mazzarella, 2007). Although these trends are encouraging, the slow growth and late sexual maturation of the loggerhead (Dodd, 1988) may prolong the recovery period.

In addition to a *k*-selected life history and mortality risk-factors, physical injury and impaired health can further retard the already lengthy recovery process. Acute physical injury poses the most immediate harm to individuals, and may stem from a variety of sources. Anthropogenic sources of injury are considered more dire threats to loggerheads than natural injury sources, as suggested by the high priority assigned to mitigating boat strikes in the Loggerhead Recovery Plan (NMFS and USFWS, 2008). Propeller and hull injuries may result from interactions with both small boats and deep-draft vessels utilizing shipping channels and deeper coastal waters, and pose a substantial risk to loggerheads (Haines *et al.*, 1999; Mansfield *et al.*, 2002; Orós *et al.*, 2005). This risk becomes increasingly problematic as human populations gravitate to coastal areas. Additional risk of anthropogenic injury may stem from interactions with commercial and recreational fishing gear. Although protective measures such as mandated use of TEDs in trawl fisheries and the increased use of circle hooks and mackerel bait in longline fisheries appear to have reduced the incidental capture of loggerheads in several commercial fisheries (Crowder *et al.*, 1995; Watson *et al.*, 2005), fishing-related mortality has not been completely abated (Hays *et al.*, 2003), and loggerheads may still experience gear-related injuries. Because both juvenile and adult loggerheads show a great affinity for coastal areas of high human activity and development, they may be more susceptible to anthropogenic sources of injury relative to other sea turtles that occupy niches further offshore (Pritchard, 1997). As such, identifying injury and health risk factors for loggerheads provides an important data set to assist recovery efforts.

Natural sources of injury are predominately confined to predation. The primary terrestrial predators of hatchling loggerheads are birds, while predation on hatchlings in the water occurs primarily by a variety of fish species, with diving birds providing some additional predation pressure (Witherington and Salmon, 1992; Stancyk, 1995; Whelan and Wyneken, 2007). Sharks are a primary predator of both juvenile and adult loggerheads (Stancyk, 1995), and Dodd (1988) suggested that missing flippers or portions of the carapace may indicate a vulnerability of this species to shark attack. Additional sources of natural injury may result from intraspecific interactions between loggerheads; in particular, behaviors associated with breeding or competition in mating adults may result in minor injury (Miller *et al.*, 2003; Schofield *et al.*, 2006, 2007). Although such injuries do not normally affect the survival of an individual, it is important to include these to understand all aspects of injury prevalence in loggerhead sea turtles.

Several previous studies have investigated injuries caused by a specific threat within a small population of sea turtles (Poiner and Harris, 1996; Heithaus *et al.*, 2002; Schofield *et al.*, 2007), but few have comprehensively characterized injuries across a broad geographic area. Norem (2005) developed the Sea Turtle Injury Identification System (STIIS) to standardize comprehensive injury characterizations and allow for comparisons across wide spatial and temporal scales; however, prior to 2008 the STIIS had only been applied to loggerheads collected from the intake canal of the St. Lucie Nuclear Power Plant in which sea turtles are regularly entrained. Furthermore, Norem (2005) did not assess the physiological health status of injured sea turtles to determine the extent to which injuries contributed (if at all) to long-term debilitation of individual turtles. As such, additional research was needed in this important area of study.

The first objective of this study was to document injury rates and to characterize the injuries exhibited by loggerheads collected during the South Carolina Department of Natural Resources (SCDNR) in-water sea turtle project. Although injuries to sea turtles captured by the SCDNR since 2000 were reported in annual project reports (Maier *et al.*, 2004, 2005; Segars *et al.*, 2006; Arendt *et al.*, 2007, 2008), standardized recording methods were not used in the field; thus a weighted system was needed to improve comparative capabilities. As such, the first objective of this thesis was accomplished by incorporating a modified STIIS into the protocol for in-water sea turtle trawl surveys conducted by the SCDNR in coastal waters off South Carolina, Georgia, and eastern Florida. The modified STIIS was applied post-hoc to the 2000-2007 data set, and from the onset in 2008-2009.

The second objective of this study was to determine if there were differences in injury rates or characteristics among space, time, sizes and gender of loggerhead sea turtles. Previous analysis of injuries in the trawl survey emphasized frequency of occurrence and body parts affected; however, attempts to identify injury sources or to categorically evaluate injuries were disregarded. Subsequently, the second objective of this thesis enhanced the level of detail available for injuries among loggerhead sea turtles.

The third objective of this study was to compare the health and stress levels of injured and non-injured loggerheads using a suite of blood parameters. The general health of captured loggerheads was assessed using 27 standard blood parameters that were measured at sea as well as by a diagnostic laboratory. Blood chemistries and blood cell counts are routinely used to assess the health of captive and wild animals, including sea turtles. Baseline measurements of several blood parameters have been recorded in a healthy, wild population of loggerheads in the Port Canaveral, FL shipping channel (Lutz and Dunbar-Cooper, 1987; Bolten et al., 1992, 1994), and blood chemistry levels and blood cell counts have been correlated with the concentrations of mercury (Day et al., 2007) and organic contaminants (Keller et al., 2004) in the blood of loggerheads captured in the southeastern U.S. Variations in blood parameters have also been measured in wild green turtles with fibropapillomas (Aquirre and Balazs, 2000), indicating the detrimental effect of this disease on sea turtle health. Despite the widespread use of blood parameters as diagnostic tools, the health of physically injured loggerheads in the wild has received little attention, and this thesis aimed to investigate this overlooked area of study.

An additional blood analysis was performed to investigate the relationship between physical injuries and the physiological stress response. In many vertebrates, including all species of sea turtles, the stimulation of the stress response increases the secretion of the steroid hormone corticosterone by the adrenal cortex into the bloodstream (Walker *et al.*, 1986; Guillette *et al.*, 1997; Sims and Holberton, 2000). The secretion of corticosterone occurs in a predictable manner, with a short-term increase in plasma concentrations, peaking approximately three hours after the first exposure to the stressor, and then returning to basal concentrations over the next 3 to 6 hours. Norris (2007) reported basal concentrations of corticosterone as 1.12 ± 0.80 ng/mL in loggerhead sea turtles. Previous studies have observed this corticosterone response in sea turtles exposed to stressors such as extreme heat (Morris and Owens, 1982; Jessop *et al.*, 2000), capture (Schwantes, 1986; Gregory *et al.*, 1996; Snoddy *et al.*, 2009), handling (Morris, 1982), captivity (Gregory *et al.*, 1996), laparoscopy and/or ultrasound (Blanvillain *et al.,* 2008), and turning stress (during which the individual is placed on the carapace for an extended period of time; Valverde, 1996; Valverde *et al.*, 1999; Jessop *et al.*, 2004). A study with green sea turtles with fibropapillomas also revealed that the presence of this disease was correlated with an enhanced corticosterone response to capture stress (Aguirre *et al.*, 1995), which suggests that other physical factors, including injuries, may affect the corticosterone response to additional stressors.

Investigation of a potential relationship between injuries and the corticosterone response to stress has received little attention. In a study of female loggerheads nesting in Queensland, Australia, Jessop *et al.* (2004) found similar corticosterone responses to capture and turning stress in females with and without shark-inflicted injuries, regardless of the severity of the sustained injury; however this similarity was attributed to the nesting status of the females. A reduced or inhibited corticosterone response to acute stress has been noted in nesting female olive ridley sea turtles, *Lepidochelys olivacea* (Valverde *et al.*, 1999), and this inhibition phenomenon is believed to prevent the disruption of breeding activities. To investigate the effect of injuries on non-breeding individuals, this study analyzed the corticosterone response to capture stress in free-swimming juvenile loggerheads with injuries, as well as in loggerheads without injuries to provide the appropriate experimental controls.

MATERIALS AND METHODS

Loggerhead field collection

Since 2000, SCDNR has collected juvenile and adult loggerheads as a part of an in-water sea turtle project. Sampling during the 10-year study period was conducted as four distinct sub-studies. The first sub-study was a regional survey between Winyah Bay, SC and St. Augustine, FL (2000-2003 and 2008-2009) conducted predominately between the end of May and late July to document the health and relative abundance of sea turtles at randomly selected locations in coastal waters < 40 ft deep. The second sub-study (June 2000-2003) collected loggerheads from the shipping channel and adjacent shoals near the entrance to Charleston, SC to compare catch rates and health of loggerheads collected in a non-random manner from commercial shrimping areas with randomly collected loggerheads slightly further offshore. The third sub-study (2004-2007) collected loggerheads exclusively from the shipping entrance channel for Charleston, SC between May and August to compare catch rates with a historical data set (Van Dolah and Maier, 1993) as well as to outfit a sub-set of juvenile loggerheads with satellite transmitters to evaluate seasonal distribution patterns. The fourth sub-study was conducted during April 2006 and 2007 in the Port Canaveral, FL shipping entrance channel to collect adult male loggerheads during a presumed breeding aggregation (Henwood, 1987; Wibbels *et al.*, 1987a) for reproductive studies and to satellite-tag adult male loggerheads to study their seasonal movement patterns.

In all sub-studies, sea turtles were collected using NMFS-approved "turtle nets" (20 m flat trawl, 16 cm stretch mesh in the body, and 5.1 cm stretch mesh in the tail bag) and trawl times ranged from nine to 30 minutes. Captured turtles were examined for existing tags and immediate health problems before blood samples (≤ 45 mL) were collected from the dorsal cervical sinus (Owens and Ruiz, 1980) using vacutainer tubes fitted with 21-gauge double-ended needles (Becton-Dickinson, Franklin Lakes, NJ). Blood samples were generally collected within 10 minutes of the arrival of the turtle on deck; however, due to multiple turtle captures, some blood samples were not collected until as long as 115 minutes after capture. Blood samples were collected using multiple vacutainer tubes and processed at sea for numerous research collaborators. Three blood parameters (hematocrit, blood glucose and total protein) were also measured at sea to assist with identifying any sea turtles in need of shore-based treatment.

Injury characterization

In conjunction with the standard physical examination of all collected sea turtles, a standardized injury characterization was implemented to provide comprehensive investigation of injuries encountered. A modification of the Sea Turtle Injury Identification System (STIIS) developed by Norem (2005) was incorporated at the beginning of 2008 and retro-actively applied (based on comments and photographs) to injured loggerheads collected between 2000 and 2007. The modified system records injury details including the affected body region, the type of injury, the relative age (based on the degree of healing), and the source of injury (when possible to determine). When multiple injuries of the same type were observed on an individual, professional judgment was used to determine whether the injuries were obtained in the same incident (in which case they were recorded as a single injury). This injury characterization system was applied to all collected loggerheads, including three that were captured dead. Two additional dead loggerheads were captured in the Port Canaveral, FL shipping channel, but were not included in this study due to an advanced state of decay. The data collection form to characterize injuries for this study is provided as Appendix 1.

Injury characterization was systematically completed in the following manner. Affected body position was established using one or more of 12 anatomical region designations, of which six (carapace, plastron and all flippers) included four sub-regions (a-d). The surface on which the injury occurred (dorsal vs. ventral) was also recorded.

Injury type was selected from one of nine pre-determined categories, visual examples of which are found in Appendix 2. Amputations and bites were both characterized by a missing portion of the body; however, when beak or teeth marks were present, these injuries were classified as bites. Cracks and lacerations were both near-linear injuries, but cracks were superficial and affected only hard parts, while lacerations affected soft tissue and were superficial or deep. Deep cuts on hard parts were considered lacerations due to soft-tissue involvement. Constriction injuries were characterized by symmetrical cuts on the body, and affected both hard and soft parts. Holes, punctures and depressions were all one-dimensional injuries; holes and punctures were deep injuries affecting hard and soft tissues, respectively, while depressions were superficial injuries present only on hard parts. Lastly, injuries that didn't fit in any of the aforementioned categories, or for which sufficient detail was not available to accurately characterize the injury were classified as other/unknown.

Three categories of injury age were used. Injuries that exhibited no signs of healing and closure or were still bleeding were classified as fresh. Injuries that exhibited some re-growth along the margin of the wound, indicating the healing process had begun, were classified as partially-healed. Necrotic tissue was also used to identify a partially-healed injury. Healed injuries were those characterized by full wound closure and complete re-growth of the affected scutes, scales, and integument, indicating that the healing process had finished. Loggerheads injured by stingray spines during capture represented a fourth category that was included for analyses of stress response based on circulating corticosterone concentrations.

Attempts to assign an injury source were also included. Two injury source categories (boat propeller and fishing interaction) represented anthropogenic influences, while two others (predation and intraspecific interaction) constituted natural sources. Boat propeller injuries were characterized by straight cracks or lacerations, often with a parallel arrangement. These injuries were superficial or deep, and affected both hard and soft tissues. Injuries resulting from fishing interactions were characterized by symmetrical constriction wounds resulting from line entanglement, and/or holes or punctures from embedded fish hooks. Several individuals were collected with the gear still attached, which assisted in the ability to identify this injury source. Predation injuries were characterized by crescent shaped bites with fine raking marks along the perimeter, or irregular raking marks (superficial or deep) on the body. Intraspecific interactions included beak bite marks on the neck and shoulders, lacerations from the claws of male loggerheads on the shoulders of females, and beak bite marks on the tail of males. Injury source could not be determined for amputations as well as other types of injury for which insufficient details were available. With respect to injury detail, measurements of length, width and/or depth were recorded for some injuries encountered during 2008 and 2009. Because injury measurement data were only sparsely collected, they were not analyzed statistically; however, these data are provided in Appendix 3.

Blood parameter assessment

Hematocrit (%) and blood glucose (mg/dL) were measured at sea using whole blood extracted from a non-heparinized vacutainer tube. Hematocrit was determined after ~0.1 mL of whole blood was injected into a microcapillary tube, centrifuged for five minutes (Model MB micro-capillary centrifuge, International Equipment Co., Needham Heights, MA), and then measured using a Lancer Critocap capillary tube reader. Blood glucose was determined by placing 1-2 drops of whole blood on a specialized strip which was then inserted into commercially available blood glucose meters (Eckerd Prestige Smart System Blood Glucose Meter, Home Diagnostics Inc., Ft. Lauderdale, FL used in 2000-2007; Prestige Smart System, Home Diagnostics Inc., Ft. Lauderdale, FL used in 2008-2009). Total protein (g/dL) was determined using plasma extracted from a sodium-heparin vacutainer tube. The vacutainer tube was centrifuged for five minutes (Adams Sero-fuge CT1600, Clay-Adams Co., Parsipanny, NJ) and 1-2 drops of plasma were then pipetted onto the surface of a RHC-200ATC refractometer (Westover Scientific, Woodinsville, WA). Remaining plasma was pipetted into cryogenic vials that were stored frozen in liquid nitrogen at sea until they could be transferred to a shore-based -80°C freezer to await further analysis. Stored plasma was used to measure testosterone concentrations to determine sex (Owens *et al.*, 1978) and corticosterone concentrations (detailed methods for these techniques are described elsewhere in this section).

A comprehensive reptilian profile (AE160; Antech Diagnostic Laboratories, Memphis, TN) consisting of 27 blood parameters was also determined for 369 loggerheads between 2000 and 2009, of which 116 were classified as injured. Blood samples for this analysis were collected using a designated lithium-heparin vacutainer tube. Cell type counts were determined using slide smears made from whole blood. Blood chemistries were measured from plasma that was separated at sea in one of two fashions depending on proximity to the end of the cruise. If blood sample collection occurred more than 48 hours before the cruise end date, the entire vacutainer tube was centrifuged and plasma pipetted into cryovials for storage in liquid nitrogen, otherwise ~0.1 mL of whole blood was removed via syringe, transferred to a gel-equipped microtainer tube and centrifuged for five minutes before storage at refrigeration temperatures (~10°C). Remaining whole blood was also provided to measure hematocrit. Slide smears, plasma and whole blood were shipped overnight on ice packs to the diagnostic facility in Memphis, TN upon return to shore.

Corticosterone radioimmunoassay

Corticosterone concentrations were determined by radioimmunoassay (RIA), as described by Valverde (1996). For each sample, between 100 and 500 µL of plasma was extracted with 4 mL of anhydrous diethyl ether, dried under nitrogen gas, and resuspended with 1 mL of gel buffer (pH 7.0). Two 400 µL aliquots were pipetted from the 1 mL of resuspended sample, and the tubes were incubated in a water bath for 30 minutes at 37ºC. During incubation, eight tubes with 400 µL of corticosterone standard solutions were prepared; standards started at a concentration of 0.0625 ng/mL and doubled in sequence to 8.0 ng/mL. Following incubation of the sample tubes, 100 µL of corticosterone antibody (# B3-163, lot 404A, Esoterix Laboratory Services, Inc., Calabasas Hills, CA) and 100 µL of tritiated corticosterone (~10,000 cpm; PerkinElmer Life and Analytical Sciences, Inc., Boston, MA) were added to all tubes (standards and samples), and the tubes were incubated overnight at 4ºC.

The following day, all assay tubes were placed in an ice bath and 500 µL of dextran-coated charcoal was added to each tube, except those used to determine total counts. All tubes were incubated at 4ºC for 15 minutes, and centrifuged at 2300 rpm at 4ºC for 15 minutes. The supernatant was poured into scintillation vials and 5 mL of Ecolume scintillation cocktail (MP Biomedicals, Solon, OH) was added to each vial. The radioactivity in each vial was counted for 60 seconds with a liquid scintillation counter (Wallace 1409; PerkinElmer Life and Analytical Sciences, Inc., Boston, MA). Corticosterone concentrations (ng/mL) were calculated from the counts using the standard curve in RIA MENU Software (P. Licht, University of California, Berkeley). Values were corrected by multiplying the volume extracted by the extraction efficiency and the fraction aliquoted from the reconstituted sample (40%). The extraction efficiency was calculated by adding approximately 10,000 cpm (in a 100 µL volume) of tritiated corticosterone to five 500 µL juvenile samples, five 500 µL adult male samples, and five 500 µL adult female samples. A one-way ANOVA found no significant difference in extraction efficiencies for each group ($p = 0.082$), so values were averaged to determine overall extraction efficiency (85.6%). A loggerhead control sample was extracted 4 times in each assay to evaluate intra-assay and inter-assay variability. The intra-assay and inter-assay coefficient of variations were 16.1% and 12.5%, respectively.

Corticosterone concentrations were determined for all injured loggerheads when samples were available as well as for appropriate controls. Control samples were chosen from non-injured loggerheads (approximating the same size and sex when possible) captured in the same tow as injured loggerheads in order to minimize variation in corticosterone concentrations due to spatial or temporal influences. The time each turtle spent on deck before blood samples were collected was also selected to be as similar as possible between an injured loggerhead and the corresponding control. Mean time on deck for 311 blood samples used for corticosterone analyses was 11 minutes (range = <1 to 115 min). Mean difference in elapsed time before blood collection between injured loggerheads and controls was five minutes (n = 68 paired samples, range = <1 to 20 min).

Testosterone concentrations, which were used to sex all collected loggerheads, were determined by RIA, as described by Owens *et al.* (1978). The protocol for testosterone RIA was very similar to that of the corticosterone RIA, with the substitution of testosterone standards, testosterone antibody and tritiated testosterone for the corticosterone equivalents, and the addition of 4 mL (rather than 5 mL) of Ecolume scintillation cocktail prior to radioactivity measurement. All testosterone RIAs were performed by the Owens Laboratory (Grice Marine Laboratory, College of Charleston).

Statistical analyses

Statistical testing was performed using the statistical software R, version 2.7.1 (R Development Core Team, 2008). Because data residuals were not normally distributed, non-parametric tests were employed. Two statistical tests (Fisher's Exact test and G test) were utilized for various aspects of this injury characterization study. Fisher's Exact tests have robust properties (Clarkson *et al.*, 1993) and were therefore used extensively; however, G tests were utilized in the event that computer resources were too limited to perform a Fisher's Exact test.

Injury rates (percent injured) were compared among turtle size groups, sexes, and with respect to space and time. Three size groups recognized by Arendt *et al.* (2009) were also used for this study, where juveniles were ≤ 75.0 cm SCLmin; turtles transitioning to maturity were 75.1-85.0 cm SCLmin (with maturity defined by the Turtle Expert Working Group as ≥ 82.0 cm SCLmin; TEWG, 2009), and adults were ≥ 85.1 cm SCLmin. Within the regional survey, spatial comparisons among four sub-regions recognized by Arendt *et al.* (2009) were also included with regions defined as follows: Zone 1 included loggerheads captured between St. Augustine, FL and Brunswick, GA, Zone 2 included loggerheads captured between Brunswick, GA and Savannah, GA, Zone 3 included loggerheads captured between Savannah, GA and Charleston, SC, and Zone 4 included loggerheads captured between Charleston, SC and Winyah Bay, SC.

The proportion of total injuries was also compared among body positions, injury type, injury age and injury source (when source could be identified). The proportion of injuries among left and right sides of the body were not different for the front flippers, rear flippers or eyes (Table 1), and were pooled to compare among body parts. Spatial comparisons of injury distributions among body positions and injury type (G tests) and injury age and source (Fisher's Exact tests) between the sub-study areas were included.

Blood parameter distributions were compared among non-injured and injured loggerheads. Assumptions of normality and equality of variances were tested using

Shapiro-Wilks tests and Barlett's tests, respectively. Data residuals were normally distributed and variances were equal for blood glucose (Antech data only) and phosphorus, so these parameters were evaluated using t-tests. Residual distributions for all other blood parameters were non-normal, thus Wilcoxon's Rank Sum Tests were used.

Effects of relative injury age on circulating corticosterone concentrations and the corticosterone stress response were also evaluated. Pair-wise comparisons of circulating corticosterone concentrations among injury age categories and corresponding controls were performed using t-tests (partially healed and stingray injuries) or Wilcoxon's Rank Sum Tests (healed and fresh injuries), dependent on the results of normality testing. Only injured loggerheads with a corresponding control were considered, and some controls were used more than once (Table 2). To investigate initial corticosterone levels, only loggerheads with blood drawn ≤ 10 minutes of arriving on deck were used (Table 2) to minimize confounding effects due to capture stress, which Gregory *et al.* (1996) reported sets in by 30 minutes after capture. Corticosterone data were transformed (log + 1) and tested using Analysis of Variance (ANOVA), with post-hoc comparisons performed using a Tukey HSD test. Linear model analysis was used to evaluate the corticosterone response to capture stress (time vs. corticosterone concentration) among injury age categories and controls. Linear model analysis was performed with the complete data set (Table 2), as well as repeated for juvenile loggerheads (*i.e.,* SCLmin ≤ 75.0 cm; Table 2) given that the corticosterone response to stress can be inhibited in nesting females, and the breeding status was not determined for all collected adult loggerheads.

Table 1: Descriptive statistics and significance values for comparisons of injury frequency between right and left sides of the body.

	***n* left side**	**% of injuries**	***n* right side**	**% of injuries**	**p-value**
Front Flippers	62	10.8%	70	12.2%	$p = 0.517$
Rear Flippers	43	7.5%	52	9.1%	$p = 0.392$
Eyes	3	0.5%	1	0.2%	$p = 0.624$

Table 2: Sample sizes utilized for corticosterone analyses

	Injured vs. control	**Initial circulating corticosterone**	**Corticosterone response over time**	**Corticosterone response over time: juveniles**
Control	63	47	71	57
Healed	51	164	197	144
Partial	7	26	31	26
Fresh	3	9	13	4
Stingray	7	11	19	17
TOTAL	131	257	311	248

RESULTS

Injuries

(1) Injury frequencies

Between 2000 and 2009, 1579 loggerhead sea turtles were collected of which 433 (27.4%) exhibited at least one injury. Injury rates were significantly different between life stages ($p < 0.001$), with a higher injury rate in adults (47.6%; $n = 59$ of 124) relative to juveniles (25.5%; $n = 337$ of 1321) and transitional individuals (26.0%; $n = 34$ of 131). No significant difference was noted in injury rates by sex ($p = 0.446$), with a similar proportion of males (29.0%; $n = 133$ of 458) and females (27.0%; $n = 257$ of 952) exhibiting injuries. Additionally, no significant difference in injury rate by sex was noted in adults ($p = 0.267$); however, because injuries were statistically greater among adults, the frequency of injuries among adult males (42.5%; $n = 31$ of 73) and adult females (53.2%; $n = 25$ of 47) was greater than size-independent comparisons of injury by sex.

Injury rates varied significantly by sub-study ($p < 0.001$), with lower injury rates among loggerheads collected in the regional survey (24.3%, $n = 286$ of 1175) and the Charleston area survey (25.8%, $n = 28$ of 108) relative to loggerheads collected from either the Charleston (38.4%, $n = 84$ of 219) or the Port Canaveral (45.5%, $n = 35$ of 77) shipping channel surveys. Within the regional survey area, injury rates were not significantly different between zones ($p = 0.112$; Figure 1).

Injury rates varied significantly among years ($p < 0.001$) within the regional survey. The lowest injury rate (10.1%, $n = 18$ of 178) occurred in 2000 (Figure 2) and was different from all other years of the regional survey. Injury rates in the regional survey were also statistically greater during 2001-2003 than 2008-2009 ($p = 0.027$); however, injury rates were not significantly different between 2001 and 2003 ($p = 0.530$), nor between 2008 and 2009 ($p = 0.240$). Significant differences ($p = 0.003$) in injury rates among years were also noted in the Charleston area survey, with the lowest injury rate (3.3%, $n = 1$ of 30) in 2000 (Figure 2); when this year was removed, injury rates were not significantly different among years ($p = 0.907$). Injury rates were not significantly different among years in the Charleston ($p = 0.635$; Figure 3) or the Port Canaveral shipping channels ($p = 0.806$; Figure 3).

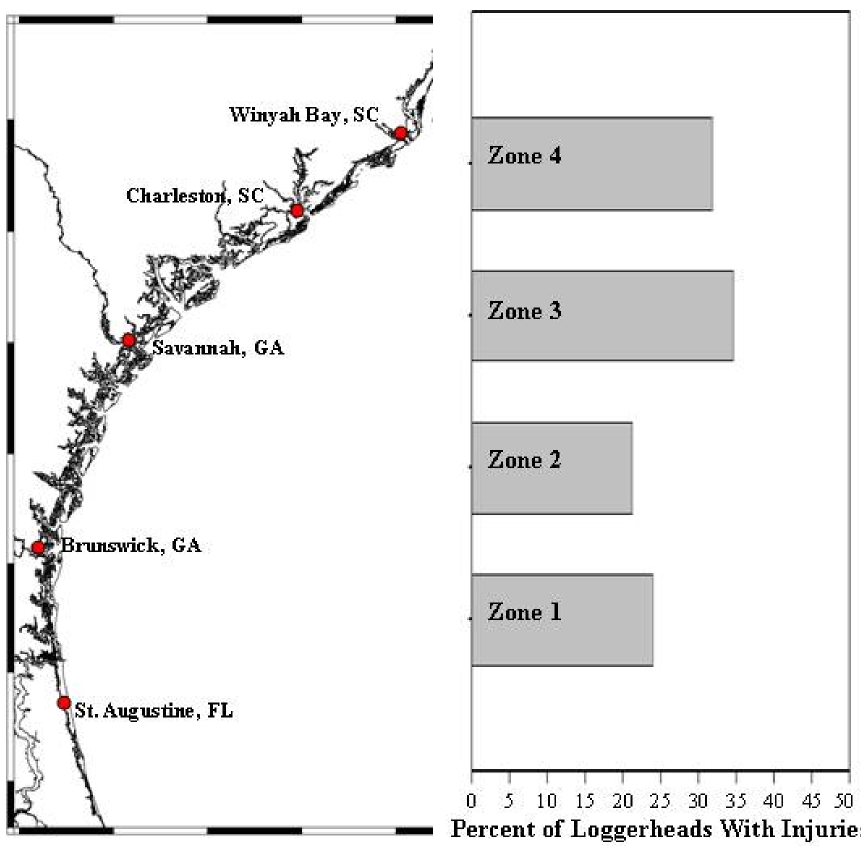

Figure 1: Injury rates among four latitudinal zones within the regional survey area. Zone 1: St. Augustine, FL to Brunswick, GA; Zone 2: Brunswick, GA to Savannah, GA; Zone 3: Savannah, GA to Charleston, SC; Zone 4: Charleston, SC to Winyah Bay, SC.

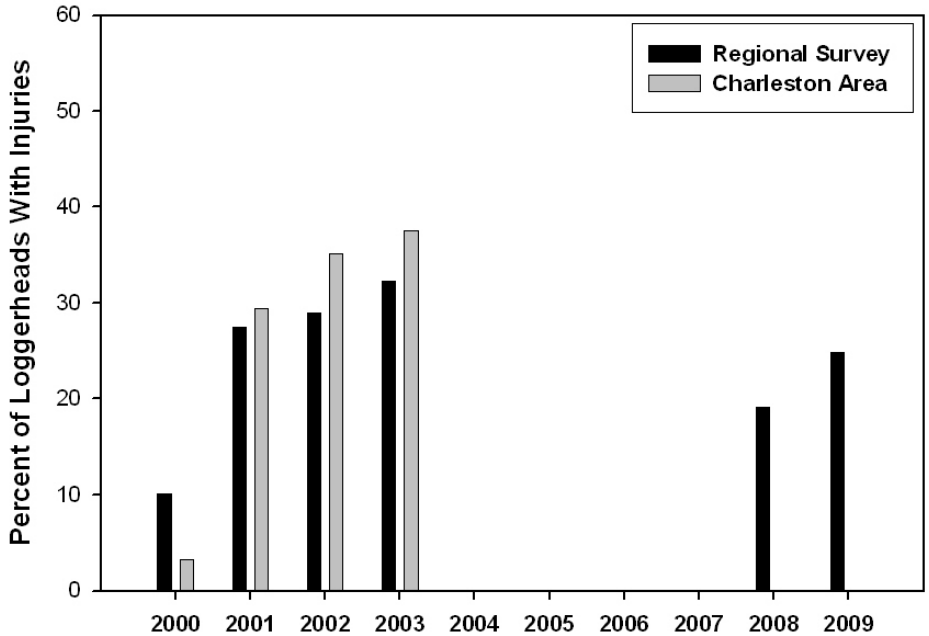

Figure 2: Annual injury rates in the regional and Charleston sub-study areas.

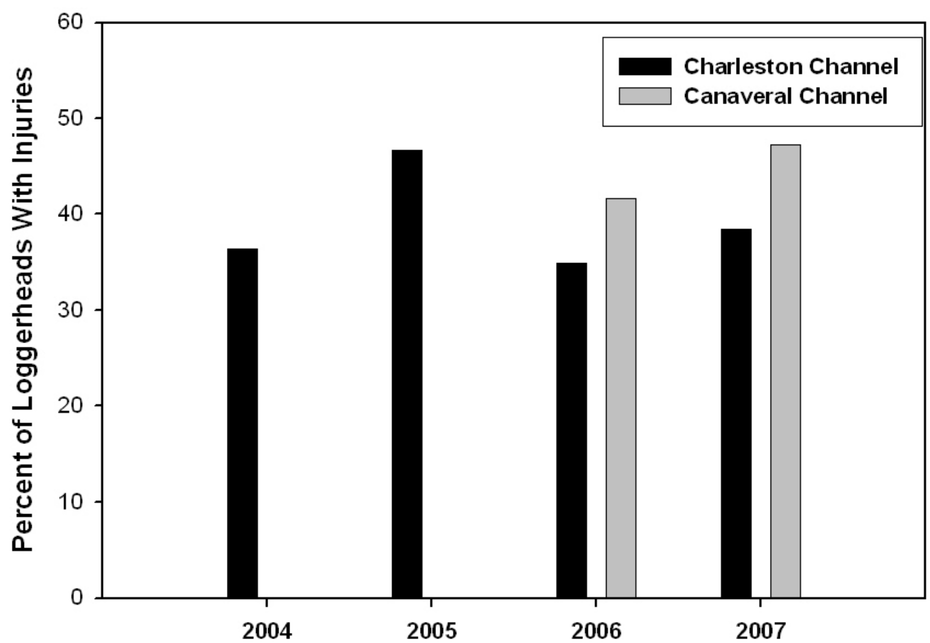

Figure 3: Annual injury rates in the Charleston, SC and Port Canaveral, FL shipping channels.

(2) Injuries by position on the body

Twenty-four percent of injured loggerheads (*n* = 106 of 433) exhibited multiple injuries, with up to four distinct injuries noted per turtle (Table 3). A grand total of 572 unique injuries were noted among 433 injured loggerheads, and the following analyses refer to the characteristics of the 572 unique injuries.

A significant difference was observed between the proportion of injuries on each region of the body ($p < 0.001$), with injuries to the carapace (40.4%, *n* = 231) occurring most often (Figure 4). The rear carapace (sub-regions a and b) exhibited significantly more injuries ($p < 0.001$) than the front portion (sub-regions c and d; Figure 5). The front flippers and rear flippers were the next most frequently injured body parts, with significantly more injuries ($p = 0.008$) on the front flippers (23.1%, *n* = 132) than the rear flippers (16.6%, *n* = 95). Injuries to all remaining body regions were uncommon and frequency of occurrence ranged from 0.7% (*n* = 4) for the eyes to 8.6% (*n* = 49) for the mouth/beak (Figure 4). The proportion of injuries in each category of injury position on the body did not vary significantly between sub-study areas ($p = 0.237$; Figure 6).

Table 3: Frequency of single versus multiple injuries among loggerhead sea turtles.

	# Loggerheads	**# Injuries**
1-Injury	327	327
2-Injuries	79	158
3-Injuries	21	63
4-Injuries	6	24
TOTAL	433	572

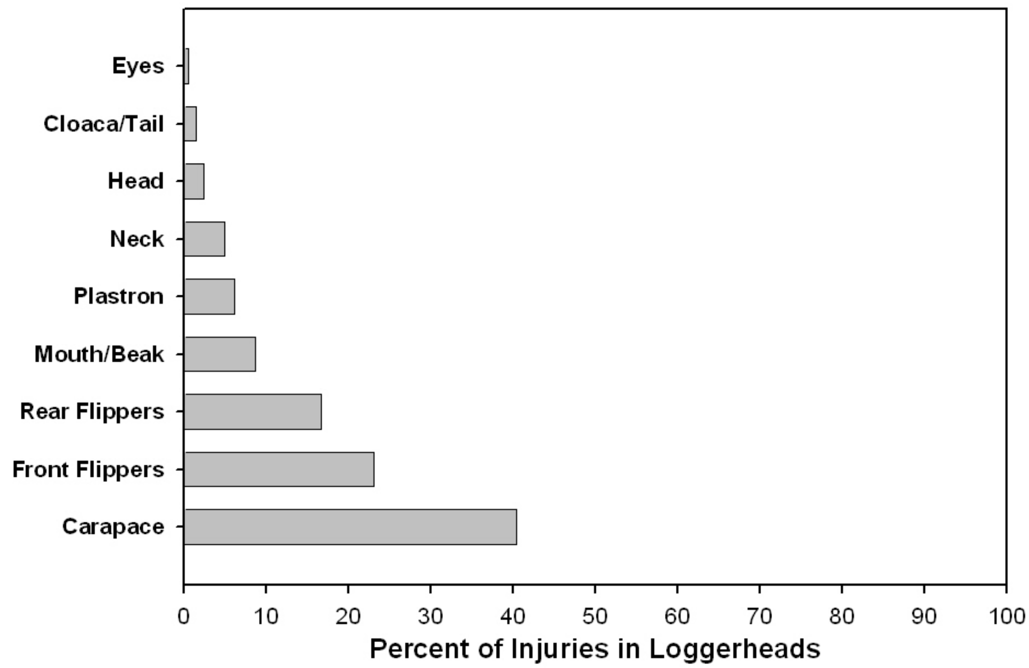

Figure 4: Proportion of observed injuries among body positions.

Figure 5: Proportion of injuries among four sub-regions of the carapace.

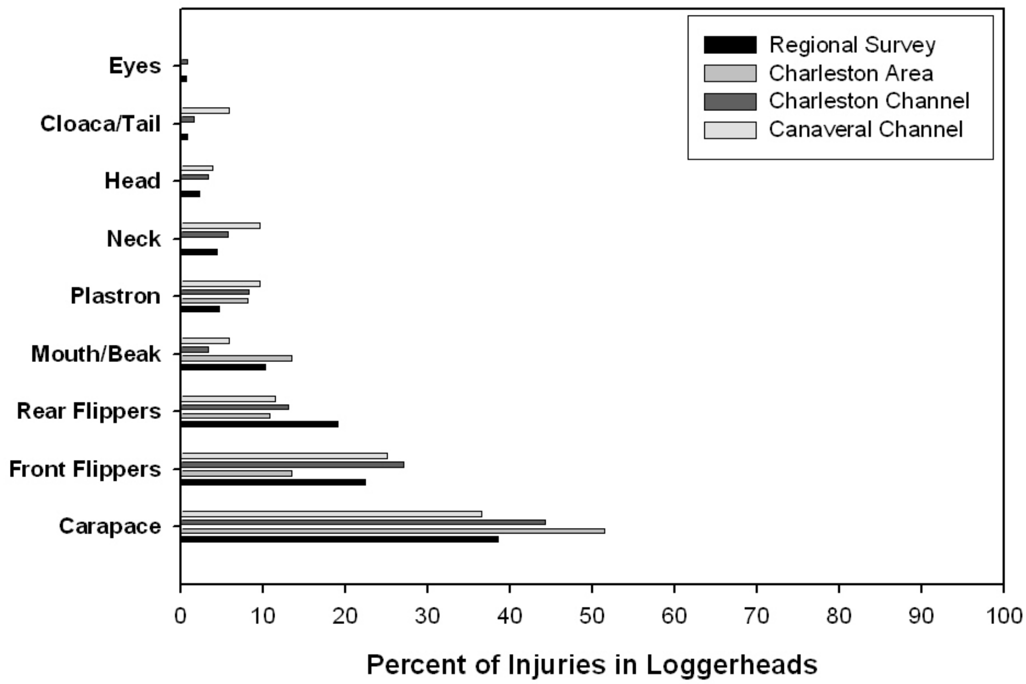

Figure 6: Proportion of injuries among body positions and sub-study areas.

(3) Injuries by type

Injury types were not equally distributed (p < 0.001). Among nine injury types, amputations comprised 50.9% (*n* = 291) of all observed injuries (Figure 7). The remaining injury types accounted for between 14.9% (lacerations: *n* = 85) and 1.0% (constrictions: *n* = 6) of all injuries (Figure 7). The proportion of injuries by type did not vary significantly between sub-study areas (p = 0.767; Figure 8).

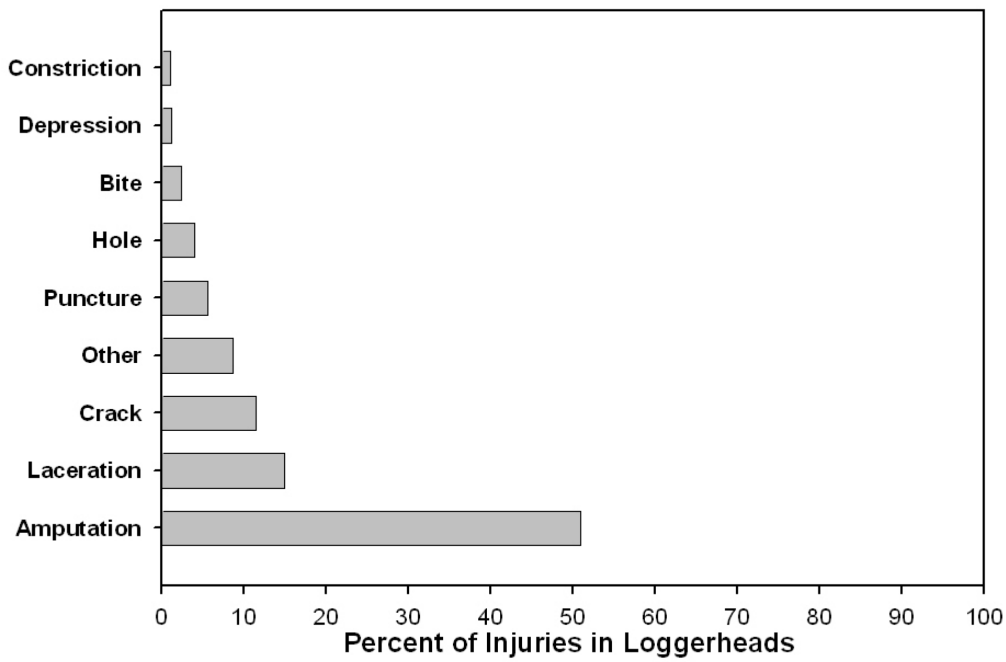

Figure 7: Proportion of observed injuries among injury types.

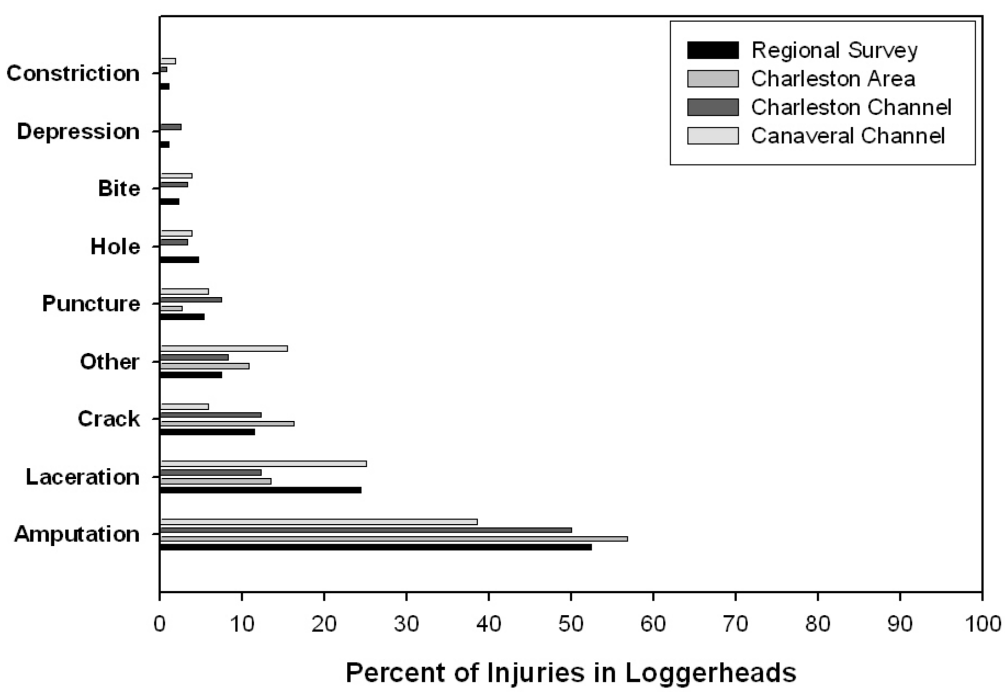

Figure 8: Proportion of injury types among sub-study areas.

(4) Injuries by recency (age)

Significant differences in relative injury age were observed ($p < 0.001$). Eighty-seven percent (n = 499) of injuries were classified as fully healed at the time of capture. Injuries classified as partially healed (8.2%, n = 47) were twice as common as injuries classified as fresh (4.4%, n = 26; Figure 9). A significant difference in injury age among sub-studies was also noted ($p < 0.001$; Figure 10) such that healed injuries dominated among all sub-studies. Partially healed injuries accounted for >10% of all injuries in the Charleston shipping channel (15.6%, n = 19 of 122), and fresh injuries accounted for >10% of all injuries in the Port Canaveral shipping channel (13.5%, n = 7 of 52).

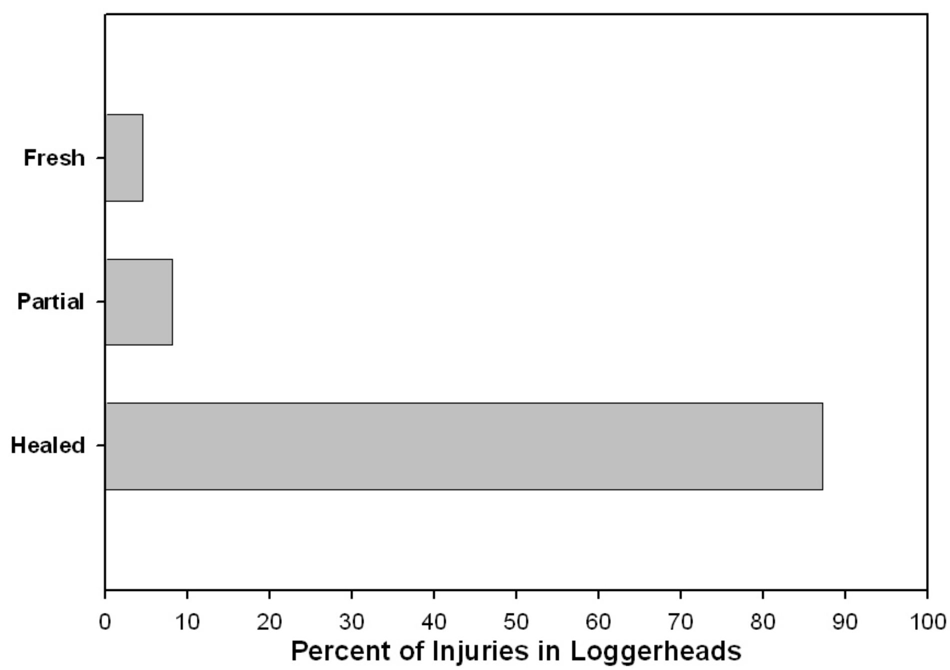

Figure 9: Proportion of observed injuries among injury ages.

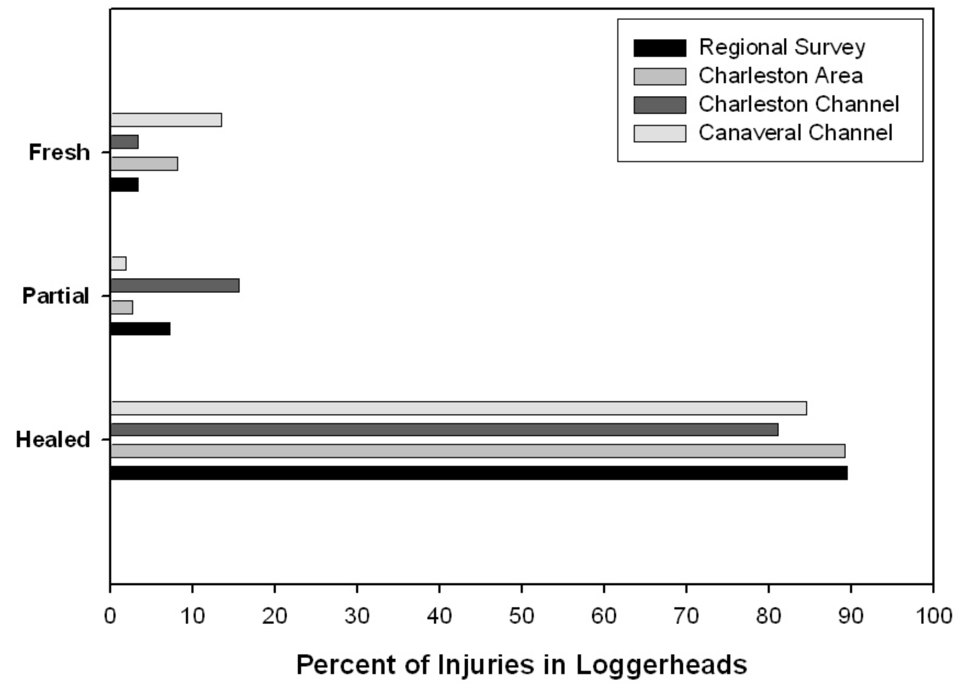

Figure 10: Proportion of injury ages among sub-study areas.

(5) Injuries by source

Eighty-five percent of observed injuries ($n = 487$) could not be attributed to a source. When injuries with an unknown source were removed, significant differences were noted in the proportion of injuries attributed to each source ($p < 0.001$). Injuries attributed to boat propellers were most common (6.6%, $n = 38$), followed by intraspecific interactions (3.3%, $n = 19$), predation injuries (2.8%, $n = 16$) and injuries associated with fishing gear (2.1%, $n = 12$; Figure 11). Excluding injuries with an unknown source, the proportion of injuries attributed to each source was also significantly different between sub-study areas ($p = 0.008$). In particular, there was a relatively high proportion of injuries attributed to intraspecific interactions (17.3%, $n = 9$ of 52) in the loggerheads captured in the Port Canaveral shipping channel relative to loggerheads captured in other sub-study areas (Figure 12). Predation injuries were rarely documented, and were seen in only 5.7% (*n* = 7 of 122) of the loggerheads captured in the Charleston shipping channel, 2.5% (*n* = 9 of 361) of loggerheads captured in the regional survey, and were absent among loggerheads collected from both the Charleston area and the Port Canaveral shipping channel surveys (Figure 12).

The proportion of injuries attributed to a particular source varied with regard to the type of injury. Amputations, by definition, have no attributable source; therefore, 100% (*n* = 291) of amputations fell into the category of unknown source. Relatively large proportions of lacerations and cracks were attributed to boat propeller strikes (34.1%, *n* = 29 of 85 and 13.8%, *n* = 9 of 65, respectively). Social interactions contributed to 50.0% (*n* = 7 of 14) and predation contributed to 42.9% (*n* = 6 of 14) of bite injuries. Fishing interactions contributed to 25.0% (*n* = 8 of 32) of puncture injuries and 66.7% (*n* = 4 of 6) constriction injuries (Table 4).

The proportion of injuries attributed to a particular source also varied with regard to the loggerhead life stage. Injures to loggerheads with no identified life stage (*i.e.,* dead captures with no measurements) were all attributed to boat strikes (*n* = 3). Intraspecific interactions contributed to a relatively high proportion of injuries in adult loggerheads (16.1%, *n* = 14 of 87) relative to juveniles and transitional individuals (0.5%, *n* = 2 of 433 and 6.1%, *n* = 3 of 49, respectively). Adults and transitional individuals exhibited no predation injuries, and transitional individuals exhibited no fishing injuries (Table 5).

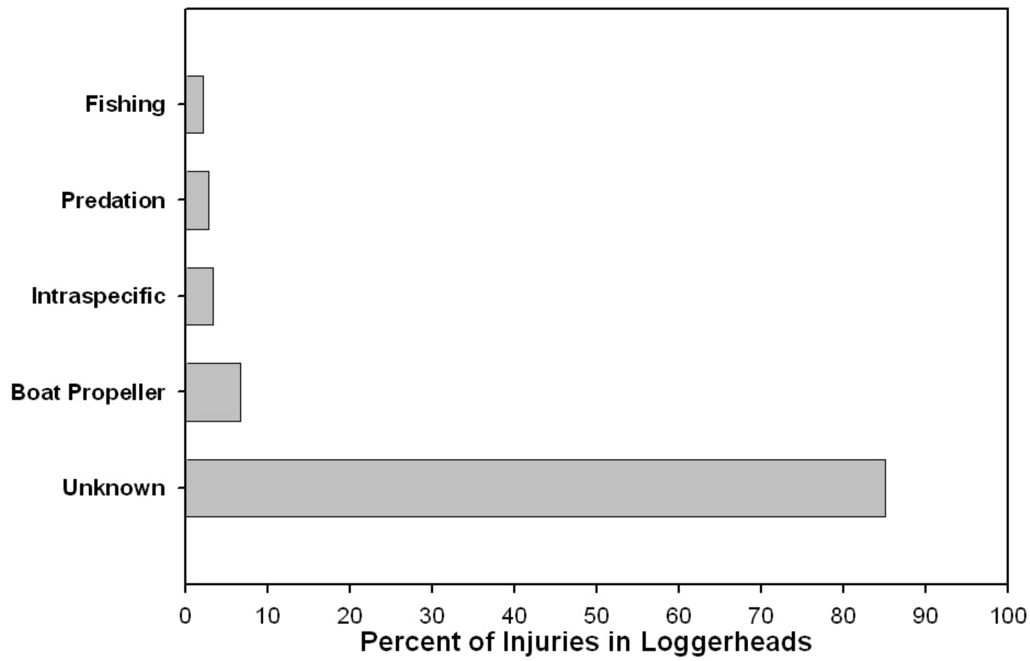

Figure 11: Proportion of observed injuries among injury sources.

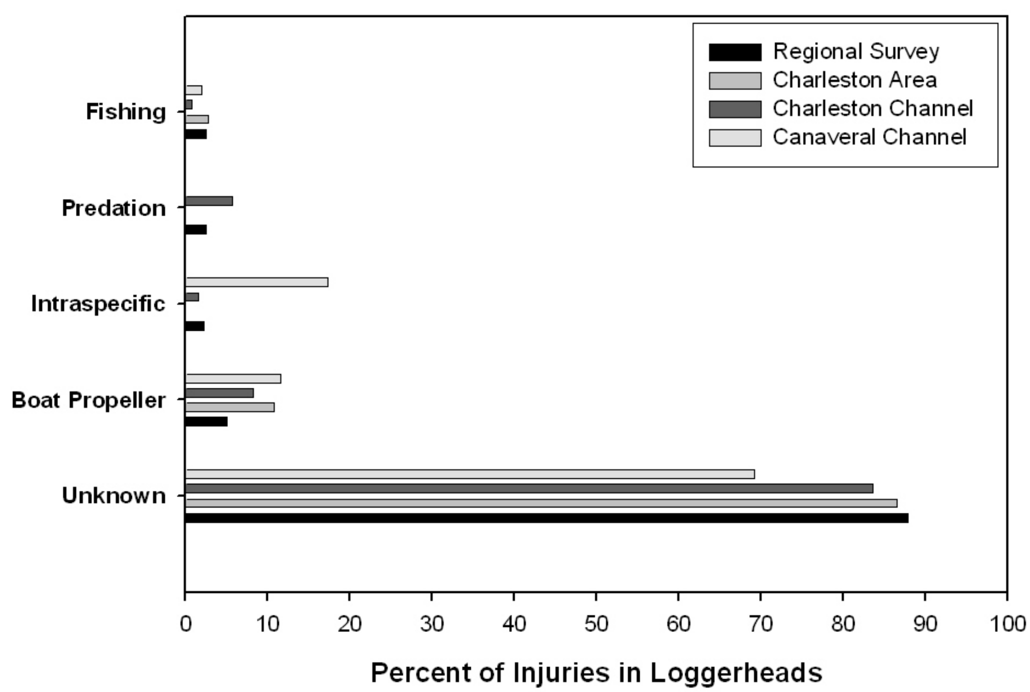

Figure 12: Proportion of injury sources among sub-study areas.

Table 4: Distribution of injury sources by type of injury. Shading represents injury sources that were not recorded for an injury type.

	n	Unknown	Boat	Intraspecific	Predation	Fishing
Amputation	291	100.0%				
Laceration	85	50.6%	34.1%	10.6%	4.7%	
Crack	65	76.9%	13.8%		9.2%	
Other	49	95.9%		4.1%		
Puncture	32	71.9%		3.1%		25.0%
Hole	23	100.0%				
Bite	14	7.1%		50.0%	42.9%	
Depression	7	100.0%				
Constriction	6	33.3%				66.7%
TOTAL	572	85.1%	6.6%	3.3%	2.8%	2.1%

Table 5: Distribution of injury sources by loggerhead life stage. Shading represents injury sources that were not recorded for an injury type.

	n	Unknown	Boat	Intraspecific	Predation	Fishing
Juvenile	433	86.8%	6.7%	0.5%	3.7%	2.3%
Transitional	49	89.8%	4.1%	6.1%		
Adult	87	77.0%	4.6%	16.1%		2.3%
Unknown	3		100.0%			
TOTAL	572	85.1%	6.6%	3.3%	2.8%	2.1%

Injuries and loggerhead health

(1) Blood parameters

Among 27 blood parameter comparisons between injured and non-injured loggerheads (all sizes and sexes combined), only five showed significant differences (Table 6). Hematocrit ($p < 0.001$) values measured at sea were significantly lower for injured loggerheads. Blood urea nitrogen (BUN; $p < 0.001$), uric acid ($p < 0.001$) and phosphorus ($p = 0.009$) values measured by Antech were significantly lower for injured loggerheads, but creatine phosphokinase (CPK; $p = 0.026$) values were significantly higher among injured loggerheads.

Table 6: Descriptive statistics of blood parameters in non-injured vs. injured loggerheads. Significant differences between groups are highlighted.

	Non-Injured Loggerheads					Injured Loggerheads					
	n	Mean	StDev	Min	Max	*n*	Mean	StDev	Min	Max	p-value
Hematocrit (%)	1135	34.5	4.9	12	57	419	33.5	5.0	13	46	**<0.001**
Glucose (mg/dL)	932	92.1	25.2	40	254	400	91.4	23.9	27	177	0.868
Total Protein (g/dL)	1136	4.7	1.2	0.8	9.2	424	4.7	1.3	1	8.2	0.675
Blood Chemistry	***n***	**Mean**	**StDev**	**Min**	**Max**	***n***	**Mean**	**StDev**	**Min**	**Max**	**p-value**
Albumin (g/dL)	246	1.1	0.3	0.4	2.8	116	1.1	0.2	0.4	1.6	0.290
AST (U/L)	246	208.0	80.1	73.0	630.0	116	193.7	72.9	72.0	512.0	0.064
BUN (mg/dL)	245	79.8	29.2	16.0	174.0	116	61.4	30.3	16.0	143.0	**<0.001**
Calcium (mg/dL)	246	7.7	1.6	1.0	11.9	116	7.5	1.2	1.6	10.8	0.238
Chloride (mEq/mL)	246	118.0	6.6	78.0	141.0	116	116.6	5.8	92.0	132.0	0.072
CPK (U/L)	246	1420.8	4590.5	126.0	70740	116	1372.2	1639.8	352.0	15041	**0.026**
Globulin (g/dL)	246	3.5	1.1	1.2	6.5	116	3.4	1.1	0.9	6.7	0.238
Glucose (mg/dL)	246	99.3	31.3	7.0	198.0	116	94.8	28.7	10.0	202.0	0.172
Phosphorus (mg/dL)	246	7.5	1.2	3.0	11.4	116	7.8	1.2	5.3	10.9	**0.009**
Potassium (mEq/L)	246	4.8	1.2	2.7	19.9	116	4.7	0.7	3.3	7.3	0.396
Sodium (mEq/L)	246	156.7	6.2	102.0	186.0	116	156.6	5.4	138.0	171.0	0.833
Total Protein (g/dL)	246	4.6	1.2	1.6	7.8	116	4.5	1.2	1.9	7.9	0.334
Uric Acid (mg/dL)	246	1.3	0.9	0.1	9.2	116	1.0	0.9	0.1	7.6	**<0.001**
Complete Blood Count	***n***	**Mean**	**StDev**	**Min**	**Max**	***n***	**Mean**	**StDev**	**Min**	**Max**	**p-value**
WBC ($x10^3$)	247	10.8	3.9	3	25	113	10.3	4.0	3	25	0.233
Basophils (%)	247	0.4	1.6	0	22	113	0.3	0.6	0	2	0.839
Eosinophils (%)	247	4.2	6.3	0	30	113	4.2	6.4	0	42	0.418
Heterophils (%)	230	39.9	18.0	7	82	105	38.8	18.9	0	86	0.579
Lymphocytes (%)	247	53.2	20.1	12	93	113	53.9	19.7	13	92	0.751
Monocytes (%)	247	1.3	1.7	0	8	113	1.3	2.2	0	13	0.245
Az Monocytes	140	1.0	1.9	0	12	82	1.2	2.3	0	12	0.816
Absolute Basophils	247	53.1	356.5	0	5500	113	23.7	51.8	0	240	0.886
Absolute Eosinophils	247	429.5	733.7	0	4080	113	467.8	953.6	0	5880	0.622
Absolute Heterophils	247	4353.0	2777.2	480	22880	113	3942.1	2439.1	0	14520	0.155
Absolute Lymphocytes	247	5797.5	3421.7	960	18480	113	5639.6	3537.1	1410	21000	0.476
Absolute Monocytes	247	151.2	228.7	0	1750	113	139.6	237.9	0	1190	0.262
Absolute Az Monocytes	140	95.0	183.6	0	1080	82	95.4	169.1	0	700	0.772
Hematocrit (%)	197	33.7	6.7	7.0	80.0	90	32.8	5.0	9.0	42.0	0.082

(2) Corticosterone

Circulating plasma corticosterone concentrations measured at various times after capture were not significantly different between control loggerheads and loggerheads with healed ($p = 0.768$), fresh ($p = 1.00$) or stingray ($p = 0.183$) injuries; however, concentrations were significantly different between loggerheads with partially healed injuries and their selected controls ($p < 0.001$). Log-transformed (log + 1) initial corticosterone concentrations were also significantly higher in loggerheads with partially healed relative to loggerheads with healed injuries ($p = 0.042$); however, no other significant differences were noted among injury ages (Tables 7 and 8).

Linear model analysis was used to compare the rate of increase of corticosterone concentrations with respect to the amount of time each loggerhead spent on deck before blood was drawn (Table 9; Figures 13a and 13b). A significant difference was revealed in the corticosterone response between loggerheads with partially healed injuries relative to loggerheads with healed, fresh and stingray injuries (Table 10). No significant difference in the corticosterone response between loggerheads with partially healed injuries and control turtles was noted (Table 10). When considering juvenile loggerheads only, the linear model analysis showed a significant difference in the corticosterone response (Table 11; Figures 14a and 14b) between loggerheads with partially healed injuries and those with stingray injuries and no injuries (Table 12).

Table 7: Descriptive statistics for initial corticosterone (ng/mL) for each injury age.

	n	Mean	StDev	Min	Max
Control	47	2.85	3.07	0.27	13.44
Healed	164	2.89	2.89	0.00	14.27
Partial	26	4.20	3.03	0.62	12.08
Fresh	9	3.17	6.63	0.07	20.70
Stingray	11	2.34	1.65	0.39	6.07

Table 8: Significance values for comparison of initial corticosterone concentration using ANOVA. Statistically significant values are highlighted in yellow.

	Control	Healed	Partial	Fresh	Stingray
Control					
Healed	$p = 1.00$				
Partial	$p = 0.126$	**$p = 0.042$**			
Fresh	$p = 0.807$	$p = 0.737$	$p = 0.087$		
Stingray	$p = 1.00$	$p = 1.00$	$p = 0.391$	$p = 0.927$	

Table 9: Rates of increase in corticosterone levels with capture stress by injury age.

	n	Linear Equation
Control	71	$y = 0.0919x + 2.2467$
Healed	197	$y = 0.1191x + 2.3477$
Partial	31	$y = 0.0332x + 3.9446$
Fresh	13	$y = 0.2457x + 1.1759$
Stingray	19	$y = 0.1305x + 2.1246$

Table 10: Significance values for comparison of line slopes using linear model analysis of corticosterone response. Statistically significant values are highlighted in yellow.

	Control	Healed	Partial	Fresh	Stingray
Control					
Healed	$p = 0.992$				
Partial	$p = 0.056$	**$p = 0.034$**			
Fresh	$p = 0.215$	$p = 0.198$	**$p = 0.022$**		
Stingray	$p = 0.382$	$p = 0.364$	**$p = 0.048$**	$p = 0.769$	

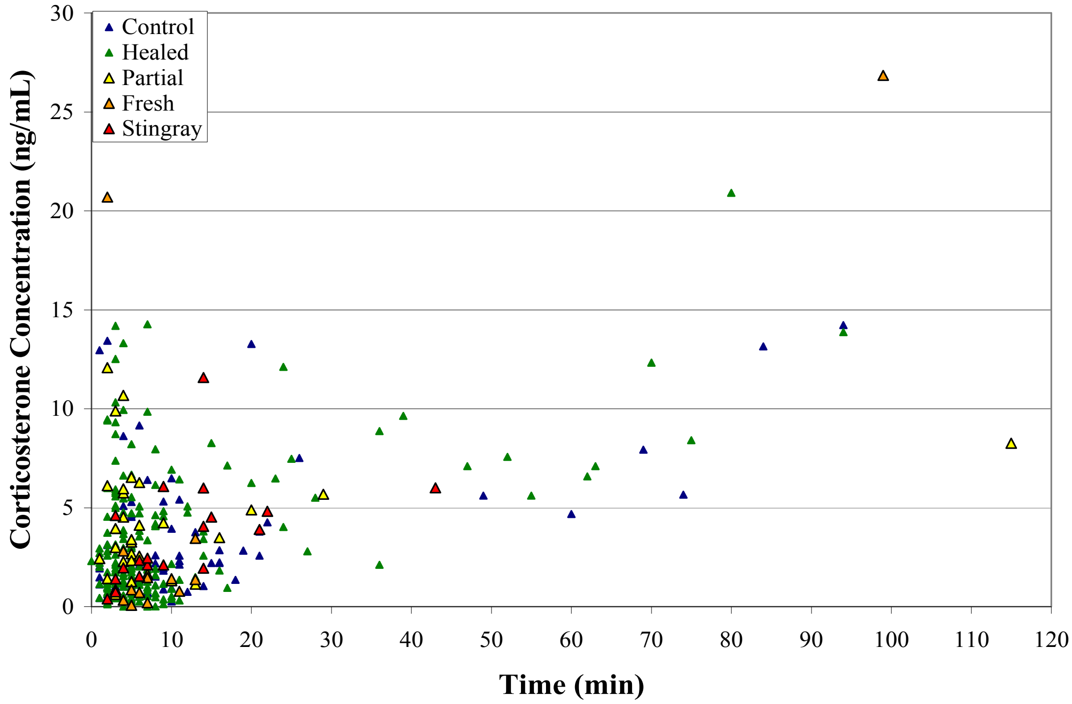

Figure 13a: Corticosterone concentrations versus time on deck before bleeding for control and injured loggerheads (n = 331) collected by trawling between 2000 and 2009.

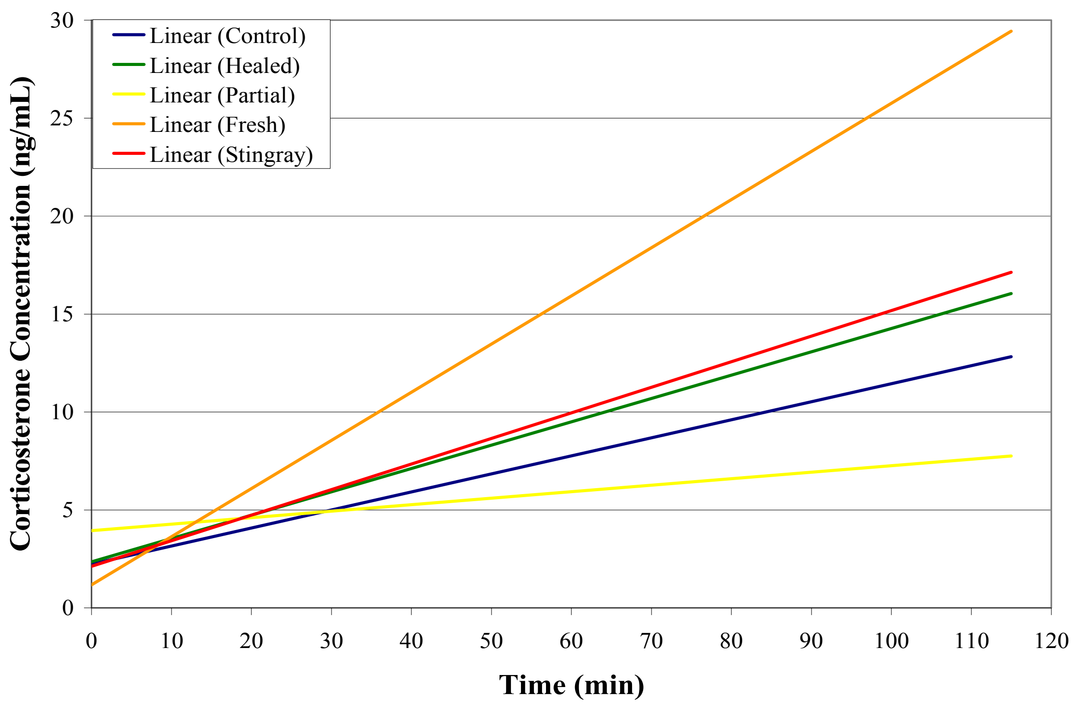

Figure 13b: Rates of increase in corticosterone concentrations versus time on deck before bleeding among control and injured loggerheads (n = 331) collected by trawling between 2000 and 2009.

Table 11: Rates of increase in corticosterone levels with capture stress by injury age, juvenile loggerheads only.

	n	**Linear Equation**
Control	57	y = 0.0945x + 2.1917
Healed	144	y = 0.0783x + 2.9464
Partial	26	y = 0.0292x + 4.2600
Fresh	4	y = 0.1897x + 7.1212
Stingray	17	y = 0.1268x + 2.1744

Table 12: Significance values for comparison of line slopes using linear model analysis of corticosterone response in juveniles only. Statistically significant values are highlighted in yellow.

	Control	**Healed**	**Partial**	**Fresh**	**Stingray**
Control					
Healed	p = 0.214				
Partial	**p = 0.026**	p = 0.119			
Fresh	p = 0.525	p = 0.834	p = 0.596		
Stingray	p = 0.412	p = 0.151	**p = 0.031**	p = 0.289	

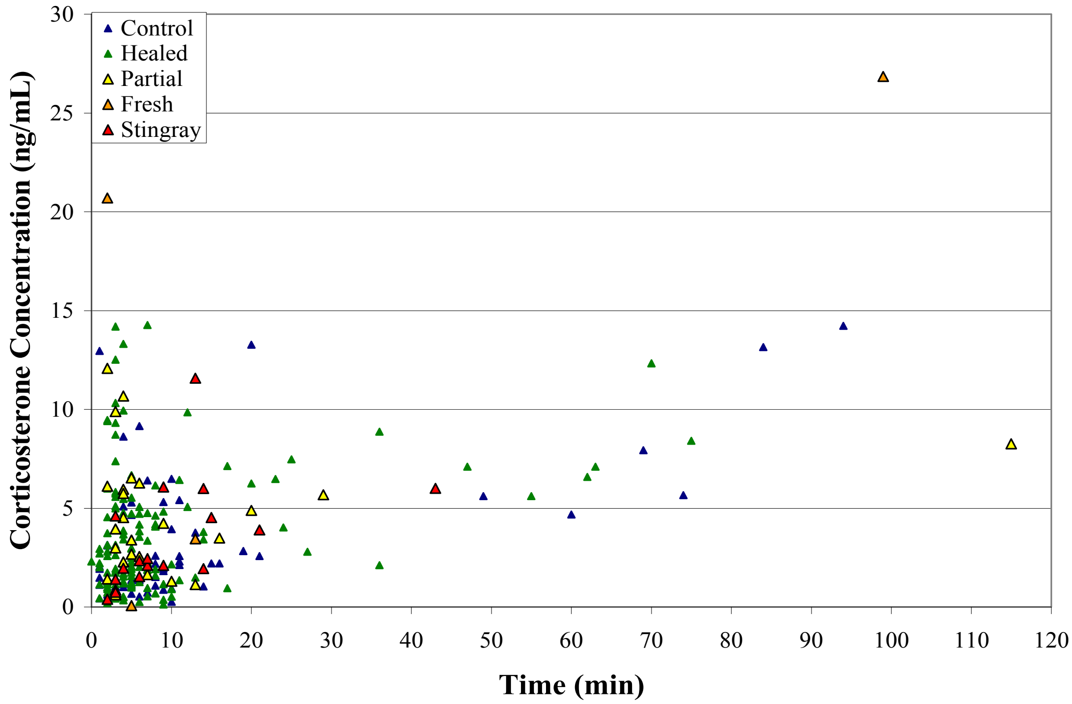

Figure 14a: Corticosterone concentrations versus time on deck before bleeding for control and injured juvenile loggerheads (n = 248) collected by trawling between 2000 and 2009.

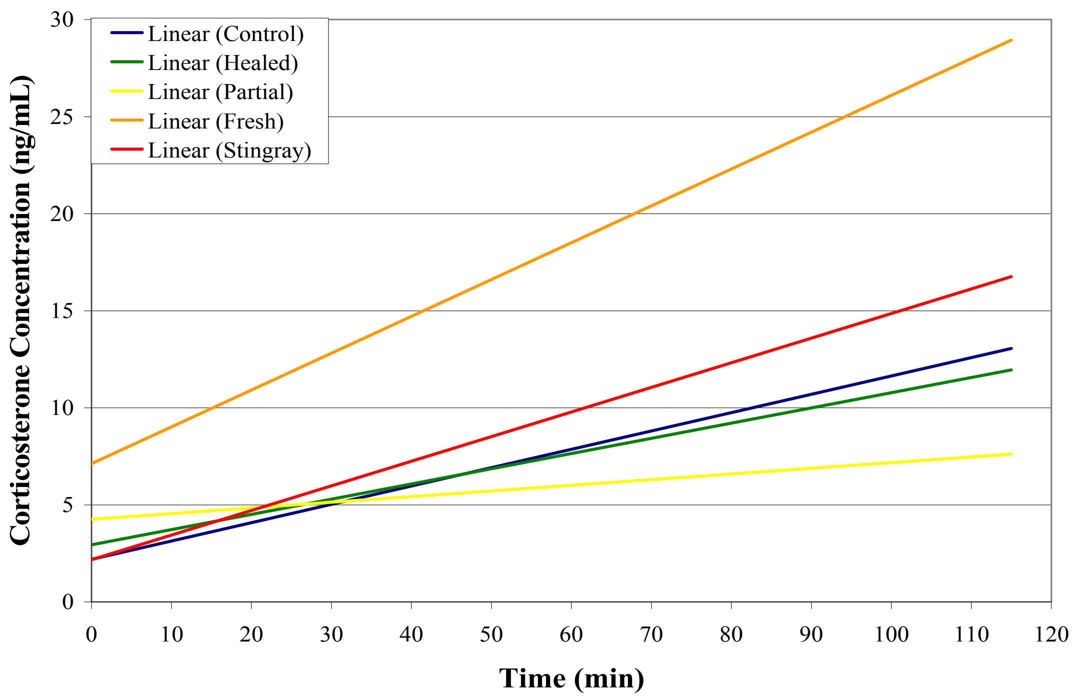

Figure 14b: Rates of increase in corticosterone concentrations versus time on deck before bleeding among control and injured juvenile loggerheads (n = 248) collected by trawling between 2000 and 2009.

DISCUSSION

Injuries

More than one in four of nearly 1600 collected loggerheads exhibited injuries, affirming that free-swimming loggerheads in the southeast U.S. are routinely injured. Injury rates reported in the present study were substantially less than the 58.4% injury rate (*n* = 142 of 243) for loggerheads collected from the St. Lucie Power Plant in 2000 (Norem, 2005). In contrast, injury rates reported in the present study were greater than injury rates noted among loggerheads captured as bycatch in a prawn fishery in northern Australia (0%, *n* = 0 of 6; Poiner and Harris, 1996), or among loggerheads collected as longline bycatch in the Ionian Sea (4.2%, *n* = 8 of 190; Deflorio *et al.*, 2005). Although some of this variation may be due to differences in geographic location and loggerhead size among studies, differences in sample size and injury classification methodology render comparisons with these latter studies difficult. However, because of similar methodologies between this study and Norem (2005), reliable spatial and temporal comparisons of injury rates and types within and between these studies are possible.

Injury rates varied significantly by area of capture, with the highest occurrence of injuries in loggerheads collected from the two shipping channels. Due to the high proportion of injuries with an unknown source in all sub-study areas, it was difficult to confidently identify major injury-causing agents in any area. Additionally, the high prevalence of healed injuries indicated that the injury-causing event occurred well before the time of capture, therefore making it impossible to determine the geographic location in which the injury occurred. Although the precise location of injury acquisition cannot be identified due to the mobile nature of loggerheads, satellite telemetry and tag-recapture studies indicate high site fidelity among loggerheads; thus, it is probable that fresh injuries occurred in the general vicinity of where individuals were collected.

Higher prevalence of injuries in shipping channels may not necessarily suggest that loggerheads are injured more frequently in shipping channels, but rather that loggerheads with injuries (both recent and old) may favor shipping channels. The deeper bathymetry and muddy sediments within the channels may provide an injured loggerhead protection that is not available in offshore areas. Mudding behavior (*i.e.,* burrowing into muddy sediments) has been observed in loggerheads collected in the Port Canaveral shipping channel during periods of cold water temperatures (< 15 °C; Carr *et al*., 1980; Ogren and McVea, 1995), presumably for protection during hibernation. Muddy, anoxic sediments may provide additional adjuvant qualities to injured loggerheads. In addition to sediment structure, loggerheads may seek refuge in shipping channels to avoid rough seas, which would be especially beneficial to an injured loggerhead. Maier *et al.* (2004) suggested refuge qualities for the Charleston, SC shipping channel; this was corroborated by Arendt *et al.* (2009) who found an association between higher wave heights and southeast winds and the prevalence of loggerheads in the Charleston shipping channel. Satellite telemetry also showed that loggerheads captured and tagged in the Charleston shipping channel did not remain in the channel long after release, but did return to the channel episodically between spring and fall, as well as made highly directional movements to return to the channel the following spring (Arendt *et al.*, 2009). However, the individuals chosen for the telemetry study were generally healthy and exhibited few injuries. Continuous monitoring of shipping channels using automated acoustic receivers to permit fine-scale study of site utilization patterns has been recommended by Arendt *et al.* (2009), and this approach would also enable evaluation of potential differences in usage patterns among injured and non-injured loggerheads.

Annual injury rates were stable in the Charleston and Port Canaveral shipping channels, but varied significantly in the regional and Charleston area surveys. The variation in annual injury rates in these later two areas was largely due to a significantly lower injury rate in 2000 (10.1% and 3.3%, respectively) relative to injury rates in other years (19.2%-32.3% in the regional survey, 29.4%-37.5% in the Charleston area survey). These exceptionally low injury rates may be a result of the high frequency of smaller loggerheads collected in 2000 relative to later years (Arendt *et al.*, 2009). Strong Gulf Stream intrusion across the continental shelf in the South Atlantic Bight occurred in 2000, and Arendt *et al.* (2009) suggested that associated changes in circulation patterns and/or changes in food distribution may have concentrated smaller juveniles in the nearshore sampling areas. Given that juvenile loggerheads exhibit significantly lower injury rates than adults, a higher proportion of smaller loggerheads in 2000 may have effectively 'diluted' the rate of injury that year.

Post-hoc application of the STIIS to data collection between 2000 and 2007 may have also contributed to reduced documentation of injuries in 2000. All comments on data forms suggesting that loggerheads exhibited injuries resulted in a default classification of 'injured' for the purpose of this study, with photographs used to provide greater detail for injury characterization. However, in the course of examining photographs, it became apparent that not all present injuries (particularly those that appeared to be less severe) were appropriately recorded on the data form. Thus, given that photographs were missing for 30% (n = 53 of 178) of loggerheads captured in the regional survey and 77% (n = 23 of 30) of loggerheads captured in the Charleston area sub-study in 2000, it is possible that injuries may have been under-reported that year. A similar number of photographs were missing for loggerheads collected during the regional survey in 2001 (35%, n = 64 of 182); however, in contrast to 2000, numerous injury-related comments were made on the data form in that year. For all other sub-studies between 2001 and 2007, photographs were missing for <10% of all loggerheads collected. The continued use of the STIIS in the in-water turtle study (particularly when applied in the field as opposed to post-hoc) should improve confidence for future injury characterization efforts.

Injury rates were higher in adult loggerheads than in juvenile loggerheads. Given a similarity in seasonal distribution patterns and overlap in foraging habitats, greater injury frequency rates among adults most likely reflects a greater period of opportunity to become injured during a lifetime. Additionally, adults may be more likely to survive injuries relative to juveniles; thus, mortality may lead to underestimation of injury rates in smaller turtles. Intraspecific injuries obtained during courtship and mating were restricted to adults, and accounted for nearly one-fifth of injuries noted for adult loggerheads in this study. Norem (2005) also reported increasing injury rates for flipper amputations among three turtle size groupings (<71, 71-84 and >85 cm SCLmin), such that flipper amputations were significantly higher in adults (6.3%, n = 20 of 316) relative to loggerheads <71 cm SCLmin (1.63%, n = 18 of 1107). Although size-related trends in injury rates were similar, even the greatest flipper injury rates reported by Norem (2005) were substantially lower than the 17-23% flipper injury rate reported by the present study, despite overall injury rates being much higher in Norem (2005). Thus, the distribution of injuries sustained by turtles in the St. Lucie Power Plant intake canal appears to be markedly different than turtles collected in coastal waters from South Carolina through central Florida, particularly given that the distribution of injury characteristics were similar among the sub-study areas of the present study.

In contrast to male-biased injury rates reported in previous studies, male and female loggerheads examined in this study appear to be equally susceptible to injury. Norem (2005) found a significantly higher rate of flipper amputation in male loggerheads (11.4%, n = 4 of 35) relative to female loggerheads (6.2%, n = 18 of 292). Heithaus *et al.* (2002) also found a higher proportion of adult male loggerheads (58.3%, n = 21 of 36) with shark-inflicted injuries relative to probable adult female loggerheads (12.9%, n = 4 of 31). Male-biased injury rates reported by Norem (2005) and Heithaus *et al.* (2002) may actually be an artifact of small sample size, especially given that Wirsing *et al.* (2008) reported comparable escape speeds and maneuverability among male and female loggerheads in response to simulated shark attack using motor craft, supporting equal susceptibility to injury in both sexes.

Small sample sizes in the aforementioned studies may have partially stemmed from sexing methods. Both Norem (2005) and Heithaus *et al.* (2002) utilized tail lengths to determine the sex of adult turtles, where individuals with longer tails (>25 cm) were defined as male and turtles with shorter tails as female (Heithaus *et al.*, 2002). Because tail length is only an indicator of sex in adult sea turtles, juveniles can not be included in these analyses. It is unlikely that excluding juveniles completely altered the outcome, as the adult male and adult female loggerheads collected by SCDNR exhibited comparable injury rates. However, the ability to include juveniles in the analyses greatly increases sample size, which may more accurately represent sex-specific trends. Further difficulty arises in identifying turtle sex by tail length alone, as this method may result in some misidentification due to the inability to distinguishing large immature males and small mature females. Utilizing concentrations of testosterone in the plasma is a widely used and relatively reliable method of accurately sexing juvenile and adult sea turtles (Wibbels *et al.*, 1987b; Wibbels *et al.*, 2000). Some caution should be taken, as plasma concentrations exhibit some seasonal variation (Braun-McNeill *et al.*, 2007; Blanvillain *et al.*, 2008); however, loggerheads in the present study were collected in late spring to mid-summer when water temperatures were above 20ºC, and blood samples were usually collected fairly quickly after capture, thus reducing testosterone variation due to water temperature and capture stress.

Carapace injuries comprised almost half of all injuries observed in this study. Norem (2005) noted similar results: 41.1% of all observed injuries (including data from loggerhead, green, and hawksbill, *Eretmochelys imbricata*, sea turtles) occurred on the carapace, with all other regions of the body exhibiting very low frequencies of injury. The results from both the present study and Norem's study suggest that the carapace, particularly the posterior carapace, is the part of the body most susceptible to injury. This trend may be due to the large size of this body region, as a larger surface area increases the probability of injury. The high injury rate to the carapace may also be related to the relatively poor escape abilities observed in loggerheads. Heithaus *et al.* (2002) found loggerheads had slower escape speeds and poorer maneuverability relative to green turtles inhabiting the same area. The posterior portion of the carapace is left highly exposed as a loggerhead swims away from a source of injury; as such, if the speed and maneuverability are not sufficient to escape an injury-causing threat, the posterior portion of the carapace would be the most likely to receive the resulting injury. A higher frequency of injury on the posterior region of the carapace may also reflect injury survivability. Injuries to the front end of the carapace may more often be lethal, given that most vital organs are located in the anterior portion of the body. As this study primarily focused on living individuals, incorporating data from stranding events may be useful to investigate mortality rates from injuries to various regions of the body.

In contrast to the carapace, injuries were more common on the front flippers than on the rear flippers. The higher injury rate in the front flippers may be related to larger flipper size which increases the probability of interaction with an injury-causing source. In addition to greater surface area, the insertion point for the front flippers reduces the capacity to 'shield' the front flippers from attack. In contrast, the rear flippers may be protected by 'tucking' them up underneath the carapace; indeed, only one observed injury to the rear portion of the carapace also affected the rear flippers, suggesting sufficient protection was usually provided by the carapace. The differential function of the two sets of flippers may also increase the probability of injury to the front flippers. Front flippers are used mainly for propulsion, while rear flippers are primarily for steering. An attempt to escape an injury-causing event (a task requiring high propulsion), or to defend against injury may leave the front flippers highly exposed. Indeed, wild and captive sea turtles have been known to defend themselves against predation by beating their flippers on the surface of the water to make loud noises (Rudloe, 1979), thus increasing the exposure of the front flippers to injury. Further behavioral studies during escape or defense events may elucidate the degree of front flipper vulnerability during such activities.

Almost 90% of all observed injuries were fully healed at the time of capture. This is not unexpected, as an injury spends significantly more time in a healed state than a healing state, especially if obtained early in the turtle's life. The healing process may last from several weeks to approximately one year, depending on the severity of the injury (K. Thorvalson, SC Aquarium, pers. comm.). Once an injury is fully healed, it remains unchanged for the remainder of the turtle's life. As such, healed injuries mask historical frequencies and sources of injury, which is particularly cumbersome given that injury sources may not remain constant over time. The inability to determine a numerical age for healed injuries limited the ability to distinguish changes in the magnitude of any injury-causing source over time. However, younger injury age categories were useful in evaluating the biological effects during the healing process, particularly with respect to corticosterone, which is discussed elsewhere.

The vast majority of observed injuries (85.1%) could not be attributed to any source, which limited the ability to isolate the vulnerability of loggerheads to specific threats. This was partly due to the high frequency of amputation injuries, which may result from a variety of sources including shark predation, severe boat propeller interactions and constrictions that dissect associated tissue. Furthermore, as injuries of different natures heal, many distinguishing characteristics may be lost, which further reduces the ability to determine the injury source. However, injury source determination was possible for approximately one in five injures, and trends among these sources may be representative of injury-causing threats in coastal waters.

More injuries were attributed to boat propeller interactions than any other source, particularly in the regional survey and the Charleston shipping channel. All sampling locations occurred in areas of high boating activity, which increased the likelihood of encountering a boat-related injury. Although loggerheads occupy open-ocean habitats where boat traffic is less concentrated, considerably more time is spent in coastal foraging areas where the risk of a boat strike event is probably greater. Norem (2005) reported that only 1% of all observed injuries were caused by boat propellers, which may have been partially attributed to collection of loggerheads with a high affinity for a sampling area relatively sheltered from high-speed boat traffic. Additionally, injury source could not be determined for 45% of injuries reported by Norem (2005); therefore it is possible that boat-strike injuries in that study were underestimated.

Further underestimation of the interaction between sea turtles and boats likely exists due to the limited documentation of lethal injuries in these in-water studies. A total of five dead loggerheads have been collected (all from shipping channels) by this project since 2000, and all five deaths were attributed to severe interactions with boat propellers. Additionally, a healthy juvenile loggerhead collected near Jacksonville, FL in June 2009 was found dead two months later approximately six kilometers away, with amputation and laceration injuries on the carapace consistent with boat-strike interaction. It is likely that many more fatal interactions between boats and loggerheads have occurred but have gone unreported, as suggested by the high priority threat level assigned to vessel strikes in the Loggerhead Recovery Plan (NMFS and MSFWS, 2008).

Boat-related injuries have also been observed in high proportions of stranded sea turtles worldwide (Haines *et al.*, 1999; Mansfield *et al.*, 2002; Orós *et al.*, 2005).

Although some injuries may have been received post-mortem, many were likely obtained while alive and may have been the cause of death. Mitigating the interactions between sea turtles and boats may simply require slower vessel speeds, particularly in areas of large sea turtle aggregations. Hazel *et al.* (2007) found the likelihood of collision with green sea turtles increased with increasing boat speed, and the proportion of individuals avoiding an approaching boat decreased significantly with speed. Decreasing vessel speeds will have less effect than removing boats from the water altogether, but the latter suggestion is not feasible. Vessel speed limits (idle speeds within refuge areas; Laist and Shaw, 2006) have apparently reduced the number of injuries and mortalities associated with boat strikes in the Florida manatee, *Trichechus manatus latirostris* (Laist and Shaw, 2006; Calleson and Frohlich, 2007). Similar speed regulations in other states may also successfully reduce the number of negative interactions between sea turtles and boats.

Predation injuries were infrequently (< 6%) noted in both the Charleston shipping channel and regional survey sub-studies, but were absent in the other two sub-studies, perhaps due in part to smaller sample sizes. As with the injuries attributed to boat strikes, the proportion of injuries attributed to predation by this study was likely underestimated. Some of the observed amputation injuries may have resulted from the bite of a predator removing a portion of the body, but if raking or teeth marks were not observed, the injury could not be attributed to predation. Additional under-representation of predatory attacks likely exists due to attacks leading to mortality. However, defense behaviors such as beating the front flippers on the surface of the water or using their carapace as a shield (Rudloe, 1979) may reduce mortality due to predation, but may subsequently increase the proportion of non-lethal injuries resulting from interactions with predators. The complex relationship between loggerheads and sharks (or other predators) is not well understood, and more research is needed in this area, as evidenced by the inclusion of predation as a recovery threat in the Loggerhead Recovery Plan (NMFS and MSFWS, 2008).

Injuries associated with interactions with fishing gear were less common than predation injuries, though, similar to other injury sources, may have been underreported given that some constriction injuries due to line entanglement may have been classified as amputation injuries. Nonetheless, the infrequent occurrence of punctures or holes from hooks may indicate the effectiveness of regulations to reduce sea turtle mortality and injuries associated with fisheries. Watson *et al.* (2005) demonstrated a reduction in loggerhead bycatch in longline fisheries equipped with circle hooks and mackerel bait. Additionally, use of the circle hooks appeared to reduce hook ingestion by loggerheads, thus also reducing mortality and internal injury. However, Hays *et al.* (2003) compiled data from several satellite telemetry studies and found evidence of continued mortality for sea turtles caught as bycatch in fisheries worldwide. Specifically, the abrupt termination of telemetry tracks for six of 50 (12%) loggerheads suggested that they had interacted with fishing gear, and death was confirmed by direct observation of three of these individuals. Considering the great number of commercial and recreational vessels actively fishing the world's oceans at any given time, it is difficult to estimate the annual mortality of sea turtles as a result of fishing activity. Additionally, as the number of loggerheads in the water increases (Ehrhart *et al.*, 2007; Epperly *et al.*, 2007; Arendt *et al.*, 2009), the number of interactions between fishing gear and turtles may also rise. Increased fishery observer coverage would enhance understanding of the extent to which fishery activity negatively affects loggerheads under different management strategies.

Intraspecific injuries were highest among loggerheads collected from the Port Canaveral shipping channel. This trend was not unexpected, as sampling at this location was timed to increase the probability of collecting adult male loggerheads during a presumed breeding aggregation (Henwood, 1987). Indeed, most adult male loggerheads collected from the Port Canaveral shipping channel were breeding adults (Blanvillain *et al.*, 2008), and most intraspecific injuries likely resulted from the courtship and mating which has been documented there. Injuries associated with mating were also seen in the loggerheads captured in the regional survey area and the Charleston shipping channel; however, adult captures were much less common in these areas; thus, the proportion of mating-related injuries from these other locations was understandably smaller.

In summary, approximately one in four loggerheads collected from coastal foraging habitats off SC, GA and FL between 2000 and 2009 exhibited a wide range of predominately healed injuries, suggesting that relatively few turtles may actually become injured on an annual basis. Injury distributions were generally similar among space, time and turtle sex; however, size-related and body-specific differences were noted. Injury types reported in this study encompass those reported by Norem (2005); however, results from this study may be more representative of coastal foraging habitats due to site-specific injuries reported by Norem (2005). In both studies, two distinct limitations precluded quantitative assessment of the contribution of specific injury sources. First, amputations comprised the majority of injury types which prevented definitive assignment of injury source. And second, the extent to which turtle injuries lead to mortality remains poorly documented. However, infrequent collection of dead loggerheads from shipping channels where sea turtles are known to aggregate may indicate that mortality is relatively low. Alternatively, some carcasses may sink to the sea floor and/or be scavenged rather than wash ashore, effectively making their probability of collection or detection quite low. Nonetheless, in addition to implementing the STIIS to record data from studies conducted in other foraging grounds, implementing the STIIS to stranding networks could also compliment data from living individuals, so long as the ability to distinguish between cause of death and port-mortem injuries was possible.

Injuries and loggerhead health

Health of injured loggerheads was minimally different from non-injured loggerheads based on a suite of standardized blood parameters used for health assessments. Significant differences between injured and non-injured loggerheads were detected for five parameters, but logical explanations exist for each. Slightly lower hematocrit levels (as measured at sea) for injured loggerheads (mean = 33.5%) were statistically different than hematocrit levels (mean = 34.5%) for non-injured loggerheads; however, the biological impact of this difference may be negligible. Additionally, hematocrit levels measured by Antech Laboratories showed no significant difference between injured and non-injured loggerheads, which further suggests that differences in this parameter as measured at sea were null. Significant differences in the distribution of phosphorus, BUN, uric acid and CPK concentrations between injured and non-injured loggerheads most likely represent an artifact of combining all sizes and sexes for health comparisons between injured and non-injured loggerheads, specifically the inclusion of reproductively-active adult male loggerheads (Blanvillain *et al.*, 2008). Adult male loggerhead captured in the Port Canaveral shipping channel (Arendt *et al.*, 2008) exhibited higher mean levels of phosphorus (9.0 ng/dL) and CPK (1998 U/L) as well as lower mean levels of BUN (33.0 mg/dL) and uric acid (0.5 mg/dL) relative to levels in both injured and non-injured loggerheads reported in the current study. Because adult males collected in the Port Canaveral shipping channel were often injured, their inclusion in the injured category skewed blood parameter distributions.

Differences in blood levels attributed to adult males likely signal important physiological changes that occur during reproductive activities, but the utility of using these parameters for physiological studies was underemphasized previously (Arendt *et al.*, 2008; Blanvillain *et al.*, 2008). Increased levels of phosphorus in the blood is often associated with changes in bone tissue such as the softening of the plastron in adult males prior to periods of mating (S. Boylan, SC Aquarium, pers. comm.), a phenomenon that was documented among adult males collected in this study (Blanvillain *et al.*, 2008). Decreases in the levels of BUN and uric acid in adult males may be due to temporary starvation (S. Boylan, SC Aquarium, pers. comm.) associated with migration to and interim residence in the Port Canaveral shipping channel for presumed mating (Blanvillain *et al.*, 2008). Prior studies have suggested seasonal variation in BUN levels of loggerheads collected in the Port Canaveral shipping channel, but consistent trends were not observed (Lutz and Dunbar-Cooper, 1987; Bolten *et al*., 1994). Furthermore, observations from Lutz and Dunbar-Cooper (1987) were based on small sample sizes (n = 4 to 11 samples per month), so reported seasonal trends must be approached with caution. Elevated levels of CPK are often associated with changes in muscle tissue, including changes resulting from a physical injury. However, CPK is a very dynamic parameter, and levels above 1,000 U/L can also result from the stress of capture or the stick of a needle during blood collection (S. Boylan, SC Aquarium, pers. comm.). Nonetheless, these observations collectively shed light on the importance of performing diagnostic blood screenings in conjunction with reproductive physiology studies, as well as the importance of examining a suite of (rather than a few) blood parameters when assessing the health of an individual.

Corticosterone was less useful for evaluating long-term health effects associated with injuries given the ephemeral nature of steroid hormones; however, corticosterone analyses did suggest that the injury healing process may have affected the physiological response to stress. Pair-wise comparisons between loggerheads of each injury age and their corresponding controls revealed that only loggerheads with healing injuries showed any variation in corticosterone levels relative to non-injured loggerheads. Analyses of initial corticosterone levels and the corticosterone response to capture stress further support this trend, as loggerheads with partially healed injuries showed the only significant variations. However, because sample sizes for all injury age categories other than healed were quite small, further evaluation of these trends is needed before the results presented here should be considered definitive.

Additional caveats regarding the use of initial concentration as an indicator of basal concentrations also deserve mention. First, is it highly probable that capture stress had begun to influence corticosterone concentrations even within the first 10 minutes a loggerhead spent on deck; indeed, all categories of injury age showed higher mean initial corticosterone concentrations (2.34 – 4.20 ng/mL) relative to the basal concentrations of 1.12 ± 0.8 ng/mL reported by Norris (2007). Second, the methods and gear used for capture and the species of sea turtle can greatly influence initial corticosterone measurements (Table 13). Gregory *et al*. (1996) reported significantly higher initial corticosterone concentrations in loggerheads captured by trawl net versus tangle net; however, average initial corticosterone concentrations were not significantly different between loggerheads captured in 10 minute trawls relative to 25-30 minute trawls. Loggerheads captured by longline (Southwood *et al*., unpublished) showed higher mean initial corticosterone concentrations relative to those noted by Gregory *et al.* (1996) and the current study. The mean initial corticosterone concentration recorded for Kemp's ridley turtles captured by tangle net (Gregory and Schmid, 2001) was much higher than the mean initial concentration of corticosterone in loggerheads captured by the same methods (Gregory *et al.*, 1996). Snoddy *et al*. (2009) noted high mean concentrations of initial corticosterone in both green and Kemp's ridley turtles captured by gillnet, although measurements varied greatly with respect to collection water depth and the length of time the turtle spent entangled. As such, measurements of initial corticosterone must be approached with caution, as they may include the early influence of capture stress, and therefore any attempt to elucidate the effect of injuries must include appropriately selected controls, as utilized in the current study.

With these caveats in mind, the initial corticosterone concentrations were used to propose a basic timeline for circulating corticosterone concentrations throughout the healing process. No corticosterone concentrations were available for injured individuals prior to receiving the injury (*i.e.,* baseline measurements). However, the initial corticosterone concentrations of loggerheads with fresh injuries and stingray injuries were not significantly higher than control individuals, suggesting that corticosterone release may be suppressed immediately following an injury-causing event. There was one exception of a high initial concentration of corticosterone in a loggerhead with a fresh injury (20.7 ng/mL), which may indicate that circulating corticosterone peaks before the healing process begins. The significantly higher concentrations of initial corticosterone in loggerheads with partially healed injuries (relative to individuals with healed injuries) may suggest that circulating corticosterone remains elevated throughout the healing process. Initial corticosterone was not significantly different between loggerheads with healed injuries and control loggerheads, suggesting that circulating corticosterone may return to pre-injury levels when the healing process is finished. This proposed timeline has not been tested, and it should be validated in a controlled laboratory setting by repeatedly measuring corticosterone concentrations in an injured loggerhead for the duration of the healing process. Although it is impossible to eliminate all stress due to capture or captivity, repeatedly sampling the same individuals should reduce individual variations in response to these stressors.

Linear increases in circulating corticosterone concentrations as a function of time on deck prior to blood sample collection (*i.e.,* the corticosterone stress response) were attributed to stress associated with capture and handling. This stress response was noted for non-injured (controls) and injured (all age categories) loggerheads; however, loggerheads with partially healed injuries exhibited a greatly reduced corticosterone response relative to loggerheads with stingray, fresh and healed injuries. Although sample sizes in several categories of injury age were small, this trend suggests that the injury healing process may correlate with a suppression of the corticosterone stress response; this suppression may stem from reduced activity of the adrenal gland and warrants further discussion.

Reduced activity of the adrenal gland may have contributed to a reduced stress response in loggerheads with partially healed injuries by one of two pathways: adrenal fatigue and/or regulation by the central nervous system. The first of these pathways results from the inability of the adrenal gland to sustain a long-term elevation of corticosterone secretion. Initial corticosterone concentrations suggest that loggerheads may maintain elevated concentrations of circulating corticosterone throughout the healing process; however, in order to maintain these elevated concentrations, the adrenal gland would need to continually secrete corticosterone at a relatively high rate for an extended period of time (*i.e.,* until the injury fully heals). If the injury is extensive and the healing process takes a long time to complete, the adrenal gland may be physically unable to maintain this high rate of secretion. This, in turn, could prevent the injured individual from responding to an additional stressor in the same physiological manner as an uninjured loggerhead. Alternatively, inhibited corticosterone secretion may have originated with the central nervous system (*i.e.,* adrenal inhibition) which would halt or desensitize the response before corticosterone could be secreted by the adrenal gland.

Adrenal inhibition may occur in loggerheads with partially healed injuries as a means of "ignoring" additional stressors. It is widely accepted that stress slows the healing process in humans and other animals (Marucha *et al.*, 1998; Padget *et al.*, 1998; Ebrecht *et al.*, 2004); thus, the disruption of the stress response through the prevention of corticosterone secretion may allow healing to continue at optimum rates. This adrenal inhibition phenomenon has been observed in several sea turtle species during periods of reproductive activity. In a typical stress response, reproductive behavior is suppressed in order to speed the rate at which homeostasis is regained (Wingfield *et al.*, 1998).

However, adult female olive ridleys are unresponsive to turning stress during *arribadas* (Valverde *et al.*, 1999), and it is presumed that the lack of a stress response in these individuals allows them to continue breeding and nesting, despite the presence of outside stressors. Adrenal inhibition has also been noted in the presence of injuries. Shark-injured female loggerheads (n = 5) and non-injured female loggerheads (n = 5) nesting in eastern Australia exhibited similar corticosterone responses to capture and turning stress (Jessop *et al.*, 2004); however, it is likely that the lack of a corticosterone response was due to the nesting status of these loggerheads, as non-injured adult females captured outside the breeding season (n = 7) exhibited a significantly higher response relative to both groups of nesting individuals.

Due to the possibility of adrenal inhibition in reproductively active adults, a second corticosterone stress response analysis was performed using only juvenile loggerheads. This analysis showed a significant difference in the corticosterone response to capture stress between loggerheads with partially healed injuries and loggerheads with stingray injuries and control loggerheads. Although these results are not identical to the analysis of all available samples, both analyses showed significant differences only between loggerheads with partially healed injuries and other injury age categories; thus, supporting the possible impact of the injury healing process on the physiological stress response. The difference in the results of the two analyses may be due to corticosterone inhibition in the reproductively active adults; however, samples sizes may have also played a role. In the juvenile-only analysis, sample sizes were smaller (*i.e.,* four freshly injured loggerheads) and the available corticosterone samples were almost exclusively distributed in the early part of the response curve, which may have skewed the data or contributed to ambiguous results. Because of the need to minimize the effect of stress on blood parameters measured by collaborating researchers, and the need to quickly process loggerheads (~20 minutes) in order to complete as many replicate trawling events as possible, blood samples collected past 10 minutes on deck were not frequently available to assess a long-term response. Increasing sample size and collecting additional blood samples at intervals greater than within 10 minutes of capture should strengthen confidence in the results reported here.

In summary, the presence of injuries among loggerheads did not appear to pose chronic health problems. Examination of a suite of blood parameters provides an excellent means for diagnosing physiological changes that occur during reproductive activity and the injury healing process; however, without suitable sample size and the ability to block for confounding variables, interpretation of the results of such diagnostic exercises are subject to great debate. The physiological effects of sub-lethal injuries have not been well studied in loggerhead sea turtles; thus, the preliminary results presented in this portion of this study provide a novel base for future research efforts. Given the need to block for confounding variables, and a necessary accessibility to sea turtles with fresh and/or healing injuries, rehabilitation facilities present an ideal location to conduct such studies. Because sea turtle patients at rehabilitation facilities are generally held for at least a month before being discharged due to the administration of antibiotics (which also must be taken into account), multiple blood samples could be collected at various progressions during the healing process. This scenario provides an ideal research situation to further evaluate the role of corticosterone in the process of healing injuries, which may prove beneficial by leading to more expeditious treatment regimens.

Table 13: Initial corticosterone concentrations following capture using different gears. The length of time the gear soaked (trawl) or the period of time the turtle spent entangled (tangle net and gillnet) are noted under capture method.

Study	**Species**	**Life Stage**	**Capture Gear**	***n***	**Mean**	**StDev**	**Min**	**Max**
Current	Loggerhead	All	Trawl (9-30min)	257	3.00	3.09	0.00	20.7
Gregory *et al.*, 1996	Loggerhead	All	Trawl (10-30min)	51	2.07	0.35		
Gregory *et al.*, 1996	Loggerhead	All	Tangle net (< 15min)	11	0.55	0.15		
Southwood *et al.*, unpub*	Loggerhead	Juvenile	Longline	9	4.34	3.63	0.39	11.61
Gregory and Schmid, 2001	Kemp's	Juvenile	Tangle net (< 15min)	39	6.16	2.31	0.03	
Snoddy *et al.*, 2009	Green	Juvenile	Gillnet (20-240min)	12	20.8	16.5	0.29	51.8
Snoddy *et al.*, 2009	Kemp's	Juvenile	Gillnet (20-240min)	4	7.8	7.7	3.50	19.3

*Data are unpublished and used with the permission of Amanda Southwood, Dept. of Biology & Marine Biology, UNCW, 601 South College Rd., Wilmington, NC 28403.

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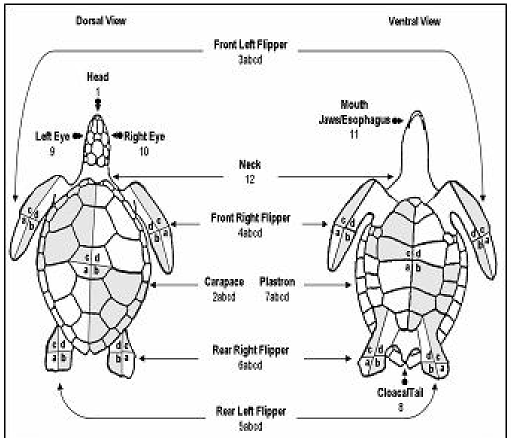
APPENDIX 1

Injury	Body Region	View	Injury Region	Amputation	Bite	Cut or Constriction	Other (type and shape)	Injury Depth	Injury Recency	Injury Source	Comments
1	1 2 3 4 5 6 7 8 9 10 11 12	d v l r	a b c d	< or > 50% Crescent U-shape V-shape Other	Crescent U-shape V-shape Scallop	Crack Laceration Depression Puncture		W: L: D:	f p h	Boat Shark Fishing Social Tagging Unknown	
2	1 2 3 4 5 6 7 8 9 10 11 12	d v l r	a b c d	< or > 50% Crescent U-shape V-shape Other	Crescent U-shape V-shape Scallop	Crack Laceration Puncture Depression		W: L: D:	f p h	Boat Shark Fishing Social Tagging Unknown	
3	1 2 3 4 5 6 7 8 9 10 11 12	d v l r	a b c d	< or > 50% Crescent U-shape V-shape Other	Crescent U-shape V-shape Scallop	Crack Laceration Puncture Depression		W: L: D:	f p h	Boat Shark Fishing Social Tagging Unknown	
4	1 2 3 4 5 6 7 8 9 10 11 12	d v l r	a b c d	< or > 50% Crescent U-shape V-shape Other	Crescent U-shape V-shape Scallop	Crack Laceration Puncture Depression		W: L: D:	f p h	Boat Shark Fishing Social Tagging Unknown	
5	1 2 3 4 5 6 7 8 9 10 11 12	d v l r	a b c d	< or > 50% Crescent U-shape V-shape Other	Crescent U-shape V-shape Scallop	Crack Laceration Puncture Depression		W: L: D:	f p h	Boat Shark Fishing Social Tagging Unknown	



Sea Turtle Injury Identification System (STIIS)
©Norem 2005

Cut/Constriction Types

1) Crack: near linear; superficial; hard part only
2) Laceration: near linear; severe (soft tissue exposed); hard or soft parts affected
3) Depression: non-linear; superficial; hard part only
4) Puncture - non-linear; severe (soft or sub-dermal tissue exposed); hard or soft parts affected

Injury Recency

1) Healed (h): complete scute/scale/integument re-growth, full wound closure
2) Partially healed (p): partial scute/scale/integument re-growth around wound, fibrin deposition
3) Fresh (f): no signs of wound closure or fibrin deposition, fleshy appearance

Injury Sources

1) Boat prop: cracks or lacerations (or series of either); may be shallow or deep
2) Shark bite: curved shaped wound with fine marks/rakings near perimeter of wound
3) Fishing: symmetrical constriction wounds; active line entanglement; embedded fish hooks
4) Social: 'nipping' bite marks on neck/shoulder area or tips of flippers
5) Tagging: symmetrical scars on trailing edge of flippers
6) Uknown: amputations; other wounds with no distinguishable traits

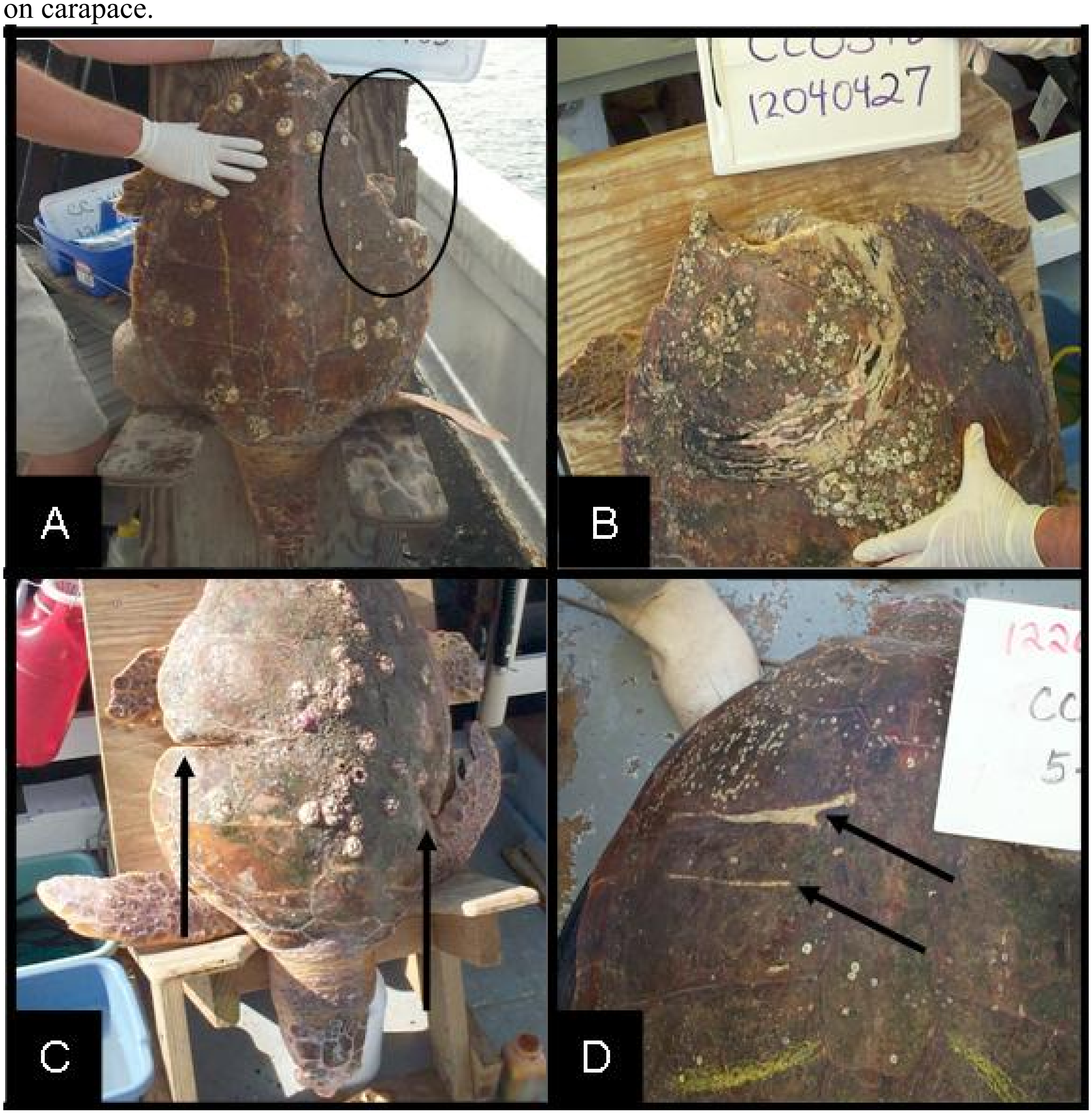
Body Parts

1 – Head	2 – Carapace	3 – FL flipper	4 – FR flipper
5 – RL flipper	6 – RR flipper	7 – Plastron	8 – Cloaca/Tail
9 – Left eye	10 – Right eye	11 – Mouth/Jaw	12 – Neck

Additional Injury Comments

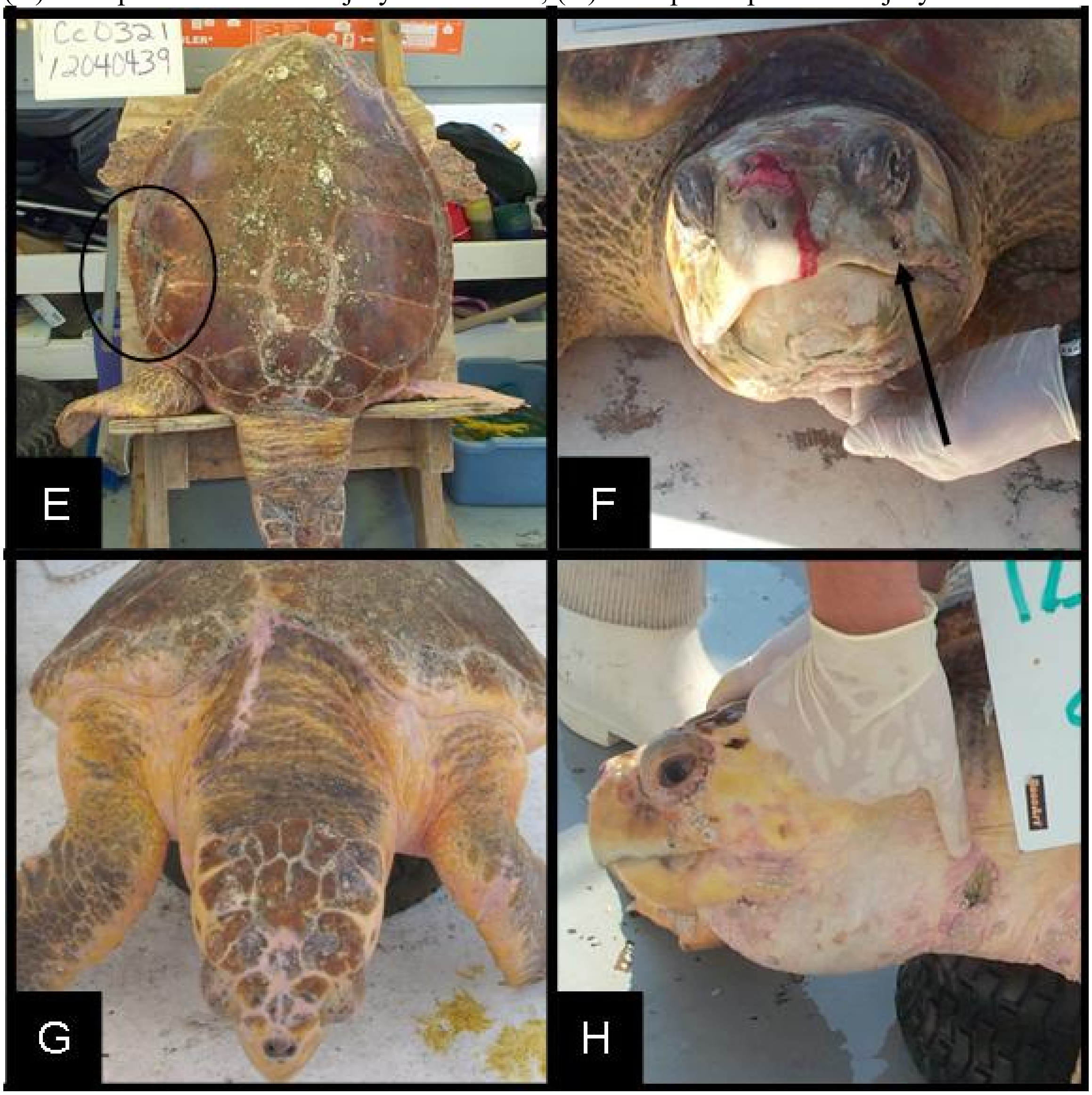
APPENDIX 2

(A) example of amputation injury on the carapace; (B) example of bite injury on the carapace; (C) example of constriction injury on the carapace; (D) example of crack injury on carapace.



APPENDIX 2, CONTINUED

(E) example of depression injury on the carapace; (F) example of hole injury on the beak; (G) example of laceration injury on the neck; (H) example of puncture injury on neck.



APPENDIX 3

Turtle Number	Injury Position	Injury Type	Injury Age	Injury Source	Measure-ment number	Injury Width (cm)	Injury Length (cm)	Injury Depth (cm)
CC0455	Carapace	Punct.	Partial	Unk.	1	9.0		
CC0455	FLF	Amp.	Partial	Unk.	1	2.5	3.9	
CC0456	Carapace	Amp.	Healed	Unk.	1	8.0		
CC0456	Carapace	Amp.	Healed	Unk.	1	2.0	4.0	
CC0457	Carapace	Amp.	Healed	Unk.	1	9.0	29.0	Full
CC0457	Carapace	Crack	Healed	Unk.	1	3	7.0	Full
CC0457	RLF	Amp.	Healed	Unk.	1	3.5	7.3	
CC0464	Carapace	Amp.	Partial	Unk.	1	0.9	7.0	Full
CC0464	Carapace	Lac.	Partial	Unk.	1	3.7	1.5	
CC0478	RLF	Amp.	Healed	Unk.	1	1.5		1.5
CC0482	Carapace	Lac.	Healed	Boat	1	5.0	5.4	2.6
CC0482	Carapace	Lac.	Healed	Boat	2		8.5	
CC0501	Beak	Crack	Partial	Unk.	1		3.5	
CC0503	RLF	Amp.	Healed	Unk.	1		12.5	
CC0503	Carapace	Depress.	Partial	Unk.	1	12.0	10.8	
CC0504	Carapace	Amp.	Healed	Unk.	1		7.0	2.2
CC0507	FLF	Amp.	Healed	Unk.	1	3.0	9.0	
CC2517	Neck	Lac.	Healed	Unk.	1		11.5	
CC2522	FRF	Punct.	Fresh	Unk.	1	5.0	2.0	
CC2522	FRF	Punct.	Fresh	Unk.	2	3.5		
CC2532	Carapace	Lac.	Partial	Boat	1		12.8	
CC2532	Plastron	Lac.	Partial	Boat	1		10.9	
CC2532	RRF	Lac.	Partial	Boat	1		18.4	8.3
CC2533	Carapace	Amp.	Healed	Unk.	1	13.3		5.9
CC2542	FRF	Lac.	Healed	Unk.	1	2.5	11.0	Full
CC2548	RRF	Punct.	Healed	Unk.	1	3.2	5.5	Full
CC2551	FLF	Punct.	Partial	Fish.	1	2.8	6.0	Full
CC2557	Carapace	Depress.	Partial	Unk.	1	26.0	10.0	1.0
CC2557	Carapace	Lac.	Partial	Unk.	1	11.0	6.5	Full
CC2174R	Carapace	Amp.	Healed	Unk.	1	29.0		
CC2174R	Plastron	Lac.	Partial	Unk.	1	4.1	5.1	1.0
CC2565	Carapace	Amp.	Healed	Unk.	1	3.0	8.5	Full
CC2596	Carapace	Amp.	Healed	Unk.	1		3.3	1.5
CC2596	Carapace	Amp.	Healed	Unk.	1		2.7	1.5
CC2604	Carapace	Amp.	Healed	Unk.	1	1.5	6.0	
CC2610	RLF	Amp.	Healed	Unk.	1	4.5		6.0
CC2613	FLF	Amp.	Healed	Unk.	1	5.5	6.5	
CC2613	RLF	Amp.	Healed	Unk.	1	8.0	12.5	
CC2637	Carapace	Amp.	Healed	Unk.	1	7.1	10.2	2.5
CC2637	Carapace	Amp.	Healed	Unk.	2	3.5		1.0

APPENDIX 3, CONTINUED

Turtle Number	Injury Position	Injury Type	Injury Age	Injury Source	Measure-ment number	Injury Width (cm)	Injury Length (cm)	Injury Depth (cm)
CC2638	FRF	Lac.	Partial	Unk.	1	3.5	5.5	
CC2638	Carapace	Hole	Healed	Unk.	1	1.0	2.5	0.5
CC2643	Carapace	Bite	Healed	Shark	1	3.0	14.0	Full
CC2643	FRF	Amp.	Healed	Unk.	1		19.0	
CC2643	FLF	Amp.	Healed	Unk.	1	2.0	5.0	3.0
CC0520	FRF	Amp.	Healed	Unk.	1		12.5	3.0
CC0520	Carapace	Amp.	Healed	Unk.	1		8.4	4.0
CC0522	Carapace	Amp.	Healed	Unk.	1	5.0	21.8	
CC2664	FLF	Lac.	Partial	Shark	1		7.6	
CC2671	FLF	Amp.	Healed	Unk.	1		9.0	
CC2708	Carapace	Punct.	Healed	Unk.	1	2.9	1.5	Full
CC2718	Plastron	Lac.	Healed	Unk.	1		3.8	
CC2729	Neck	Lac.	Healed	Unk.	1		4.8	
CC2745	Plastron	Lac.	Healed	Unk.	1		3.8	
CC2752	RLF	Amp.	Healed	Unk.	1	7.0	9.3	