ASSESSMENT OF LOGGERHEAD SEA TURTLE (*CARETTA CARETTA*) NEST MANAGEMENT TOOLS IN SOUTH CAROLINA

A Thesis
Presented to
The Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Wildlife and Fisheries Biology

---

by
Caitlynn Eileen Brig
May 2014

Accepted by:
Dr. Greg Yarrow, Committee Chair
Mr. Jamie Dozier
Dr. William Bridges

ABSTRACT

This study reassessed the use of two current management tools utilized on nesting beaches statewide as part of the protection effort for loggerhead sea turtles, with a primary focus on South Carolina barrier island nesting beaches. The nest management tools assessed in this study include 1) the relocation of all nests laid seaward of the spring high tide line (SHTL) and 2) use of a probe stick to locate the nest cavity. The relocation of nests deposited seaward of the SHTL is a common management action that is to be used only as a last resort if the nest is presumably doomed *in situ* according to nest protection guidelines provided by the U.S. Loggerhead Recovery Plan and South Carolina Department of Natural Resources (SCDNR) Marine Turtle Conservation Program. While nest relocations are increasing due to a loss of suitable nesting habitat as beaches throughout the state face increased erosion, many of these relocations are unnecessary but are conducted due to the misconception of concerned project participants that the occurrence of any tidal wash-over will negatively influence hatch success (HS), even of nests marginally landward of the SHTL. The relationship between nest location, relocation, tidal influences (wash-over and inundation), and HS were examined. A sample of nests below the SHTL (low nests) were relocated to higher grounds while remaining low nests were left to incubate *in situ*. Nests deposited above the SHTL were monitored at their *in situ* nest sites at varying distances above the SHTL. This study determined if nests laid and/or relocated above the SHTL still have the potential to wash-over and/or inundate depending on the distance of the nest above the tide line, if relocation significantly increases HS when compared to *in situ* low beach nests, if HS varies based on distance of *in situ* and relocated nests from the SHTL (i.e. zone), and whether tidal events negatively impact HS (and if so, does this relationship vary across zones). Hatch success was significantly lower for *in situ* nests below the SHTL during the 2012 season, however, no discernible differences were identified between the HS of low nests when compared to *in situ* and relocated nests above the SHTL during the 2013 season. Tidal wash-over significantly decreased the HS of low nests in 2012 and relocated nests in 2013 only. No relationship was evident between wash-over and the HS of *in situ* nests deposited above the SHTL. The ability of models to explain the relationship between wash-over frequency and HS greatly improved after addition of the predictor variable 'storm-induced inundation/wash-away'. Results of this study indicate the majority of low beach nests produce viable offspring.

During nest relocations, participants sometimes report eggs are found broken at the center or bottom of the clutch, but with no sign of direct puncture caused by the probe (i.e. yolk and/or albumen on the probe tip). Since the cause of breakage is unknown, these eggs are recorded as 'broken in nest' as opposed to the loss being attributed to probing. The goal of this study was to quantify egg loss associated with two nest location methods 1) probing and 2) hand digging the body pit to determine whether use of this tool is correlated with significantly higher loss and/ or decreased HS. Specifically, it was determined whether the number of eggs found broken inside nest cavities was significantly greater when using the probe to locate the clutch compared to an alternative method (hand digging) and whether nests found with the probe exhibit significantly lower HS. Hatch success did not vary between the methods. In addition, no eggs were found broken in nests located by hand digging during the 2012 or 2013 loggerhead nesting seasons, suggesting loss attributed to the probe is greater than previously quantified. Results of this study suggest a strong correlation exists between use of the probe as a nest location method and the presence of broken eggs in a nest upon location, however, this study does not provide evidence for causation of eggs found broken during nest relocations with no sign of direct puncture.

ACKNOWLEDGEMENTS

First off, I would like to thank my major advisor, Dr. Greg Yarrow, and Clemson University for providing me this opportunity. I would also like to thank my advisory committee, Mr. Jamie Dozier and Dr. William Bridges for their expertise and guidance during my graduate career.

I wish to thank the South Carolina Department of Natural Resources (SCDNR) and the Tom Yawkey Wildlife Center (TYWC) for providing logistical support and funding for this project. I would also like to thank the entire TYWC staff and SCDNR Marine Turtle Conservation Program staff for their patience, resources and endless support throughout the duration of this research. Many thanks also go out to the dedicated volunteers and interns who assisted me in the field throughout the muggy and buggy South Carolina summers: Curtis Walker, Liz Zinsser, John Cassell, Lauren Cruz and Alex Guyton.

Finally, I would like to thank my family for moral and monetary support over the past three years (Thanks Dad!). Your encouragement gave me the confidence and strength necessary to make it through the tunnel.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Listing

South Carolina beaches provide suitable nesting habitat for several sea turtle species, including green (*Chelonia mydas*), leatherback (*Dermochelys coriacea*), Kemp's ridley (*Lepidochelys kempii*) and most commonly loggerhead (*Caretta caretta*). Loggerhead sea turtles are made up of 9 distinct population segments (DPS); 4 are threatened and 5 are endangered (Conant et al. 2009; U.S. Department of Commerce 2010). While loggerheads are listed as endangered under the International Union for the Conservation of Nature (IUCN) Red List (MTSG 1996), the Northwest Atlantic DPS, which loggerheads nesting in South Carolina belong, is currently listed as threatened (NMFS and USFWS 1991, 2008). Five recovery units have been identified within this DPS based on genetic differences as well as geographic distribution of nesting densities, geographic separation, and geopolitical boundaries. The Northern Recovery Unit (NRU) consists of loggerhead nesting extending from southern Virginia to the Florida-Georgia border (NMFS and USFWS 1991, 2008). The majority of nests in the NRU are laid in North Carolina, South Carolina and Georgia with South Carolina nesting efforts representing approximately 66% of this recovery unit (NMFS and USFWS 1991, 2008).

Threats

Aerial surveys from the index nesting beach survey program in South Carolina have displayed a nesting decline of approximately 1.7% since 1980 (Hopkins-Murphy et al. 2001, unpublished data; NMFS and USFWS 2008). Long-lived and large-bodied organisms such as sea turtles exhibit slow levels of population recovery due to a variety of factors including 1) prolonged length of time necessary to reach sexual maturity, 2) no parental care after oviposition, and 3) high mortality rates of all life stages including eggs and hatchlings (Mortimer 1995; Davenport 1997; Heppell 1998). Loggerhead sea turtles face numerous conservation threats both natural and anthropogenic. In-water threats include incidental take by commercial fisheries, pollution, and boat collisions (Bolten et al. 1996; Witherington 2003). On nesting beaches, threats consist of the loss and degredation of nesting habitat, depredation, artificial lighting, poaching, storm surge and tidal inundation (Stancyk 1982; Lutcavage et al. 1997; Witherington 1999; Eskew 2012).

Nesting

Reproductive maturity is reached at approximately 30 - 35 years of age (Frazer and Erhardt 1985; Snover NMFS, unpublished data). The mean remigration interval (defined as the number of years between nesting) is 2 - 3 years (Richardson and Richardson 1982; Bjorndal et al. 1983). During nesting years, individuals display high nest site fidelity to their natal beaches (Carr 1975). Loggerhead nesting sites are primarily easily accessible from the ocean and characterized by open, sandy beaches backed by low dunes (Miller et al. 2003). In South Carolina, nesting occurs from 1 May through 31 October. Hatching begins in July and extends through the end of October. Throughout the nesting season, individuals lay on average 3 - 5 clutches with an internesting interval of approximately 10 - 14 days (Hopkins-Murphy et al. 1999). Each clutch contains an average of 100 - 126 eggs that incubate for approximately 60 days (Dodd 1988; USFWS and NMFS 1991, 2008). However, incubation duration varies temporally and spatially and is dependent on a combination of biotic and abiotic factors of the incubating environment such as temperature and moisture (Mrosovsky and Yntema 1980; Limpus et al. 1983; Dodd 1988).

Recovery

In 1977, the South Carolina Department of Natural Resources (SCDNR) Marine Turtle Conservation Program began conducting beach management research throughout the state. By the early 1980's, the program developed nest protection projects and stranding networks along the South Carolina coast. Today, nesting beach surveys are currently conducted on nearly all South Carolina beaches by a network of people trained by the Marine Turtle Conservation Program (SCDNR 2013). In order to examine nest count trends in South Carolina, projects conducting standardized daily ground surveys for loggerhead nests have been conducted since 1982 on six index beaches: Cape Island, Lighthouse Island, Edisto Beach State Park, Edisto Beach, Fripp Island and South Island. While annual loggerhead nest counts obtained from aerial surveys have displayed a nesting decline of approximately 1.7% since 1980 (Hopkins-Murphy et al. 2001, unpublished data; NMFS and USFWS 1991, 2008), the statewide trend includes high, medium and low nesting years (Figure 1.1) (SCDNR 2013). The SCDNR Marine Turtle Conservation Program recently announced nesting has shown an increase in the state for the past four consecutive seasons, something the nest count trend has never exhibited (SCDNR 2013) (Figure 1.1). While nest counts from the 1970's indicate recovery levels have not yet been met, the past few years including a record high nest count since 1982 of

5,194 loggerhead nests for the 2013 season, look promising for recovery of the NRU (SCDNR 2013).

The application of appropriate management techniques is essential to the conservation and recovery of the species. Research and conservation management activities should be cautiously evaluated in order to determine their potential risks and benefits. Management decisions should be based on effectiveness as demonstrated by experimentation and the systematic review of evidence from applied studies (Pullin et al. 2004). The periodic reassessment of management practices based on recent findings is essential in order to develop the most operative plan to protect species of interest in light of an ever-changing environment. This study reassessed the use of two current management tools utilized as part of the protection effort for loggerhead sea turtles in the southeastern United States, with a primary focus on South Carolina barrier island nesting beaches. The nest management tools reassessed in this study include 1) the relocation of all nests laid seaward of the spring high tide line (SHTL) and 2) the use of a probe stick to locate the nest cavity.

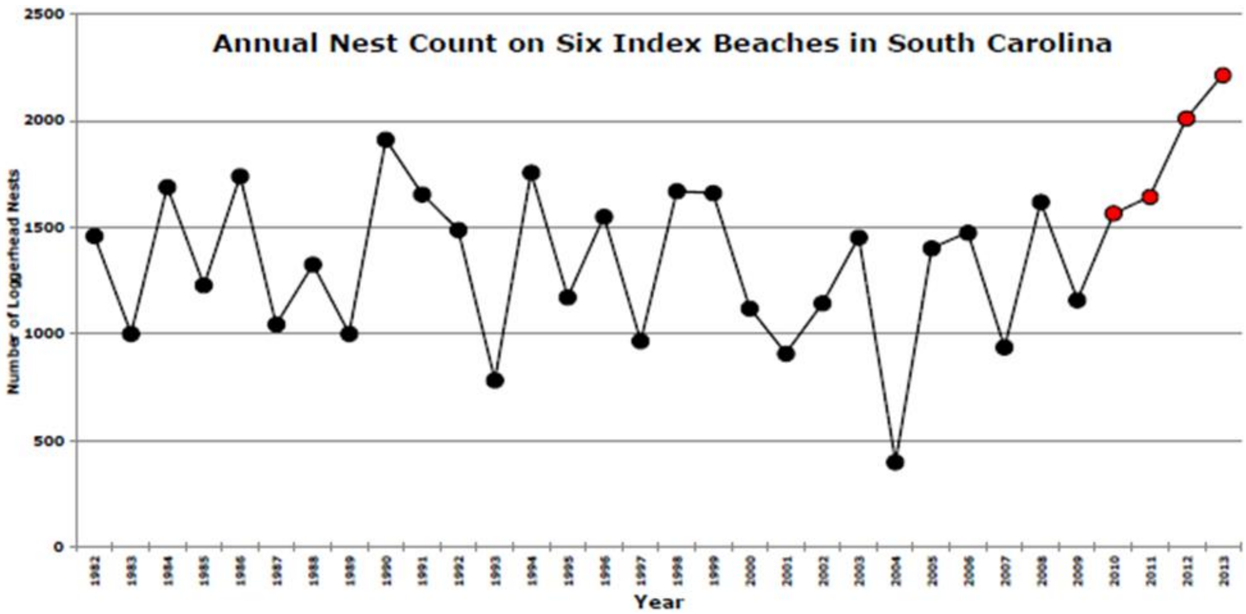


Figure 1.1: Annual loggerhead nest count trend from the 1982 - 2013 seasons on the six index nesting beaches in South Carolina: Cape Island, Lighthouse Island, Edisto Beach State Park, Edisto Beach, Fripp Island and South Island (SCDNR 2013).

LITERATURE CITED

Bolten, A.B., J.A. Wetherall, G.H. Balazs, and S.G. Pooley (compilers). 1996. Status of marine turtles in the Pacific Ocean relevant to incidental take in the Hawaii-based pelagic longline fishery.NOAA Technical Memorandum NMFS-SWFSC-230.

Carr, A.F. 1975. The Ascension Island green turtle colony. Copeia 1975: 574-555.

Conant, T.A., P.H. Dutton, T. Eguchi, S.P. Epperly, C.C. Fahy, M.H. Godfrey, S.L. MacPherson, E.E. Possardt,B.A. Schroeder, J.A. Seminoff, M.L. Snover, C.M. Upite, and B.E. Witherington. 2009. Loggerhead Sea Turtle (*Caretta caretta*) 2009 status review under the U.S. Endangered Species Act. Report to the National Marine Fisheries Service, Silver Spring, Maryland, USA. 219 p.

Davenport, J. 1997. Temperature and the life-history strategies of sea turtles. Journal of Thermal Biology 22: 479-488.

Eskew, T.S. 2012. Best Management Practices for Reducing Coyote Depredation on Loggerhead Sea Turtles in South Carolina. Thesis, Clemson University, Clemson, South Carolina, USA.

Frazer, N.B. and L.M. Ehrhart. 1985. Preliminary growth models for green, *Chelonia mydas*, and loggerhead, *Caretta caretta*, nesting at Little Cumberland Island, Georgia, USA. Herpetologica 41: 246-251.

Heppell, S.S. 1998. Application of life-history theory and population model analysis to turtle conservation. Copeia 1998: 367-375.

Hopkins-Murphy, S. R., C. P. Hope, and M. E. Hoyle. 1999. A history of research and management of the loggerhead turtle (*Caretta caretta*) on the South Carolina coast. Final report to U.S. Fish and Wildlife Service. 72 pp.

Hopkins-Murphy, S.R., T.M. Murphy, C.P. Hope, J.W. Coker and M.E. Hoyle. 2001. Population Trends and Nesting Distribution of the Loggerhead Turtle (*Caretta caretta*) in South Carolina 1980-1997. Final Report to the U.S. Fish and Wildlife Service. 41 pp.

Limpus, C.J., P. Reed, and J.D. Miller. 1983. Islands and turtles: the influence of choice of nesting beach on sex ratio. Pages 397-402 *in* Baker, J.T., R.M. Carter, P.W. Sammarco, and K.P. Stark, editors. Proceedings of the Inaugural Great BarrierReef Conference, James Cook University Press, Townsville, Queensland, Australia.

Lutcavage, M.E., P. Plotkin, B.E. Witherington and P.L. Lutz. 1997. Human impacts on sea turtle survival. 387-409. *in* Lutz, P.L. and J.A. Musick, editors. The biology of sea turtles, CRC Press, Florida.

Marine Turtle Specialist Group (MTSG) 1996. *Caretta caretta*. In: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. <www.iucnredlist.org>. Downloaded on 14 September 2013.

Miller, J.D., C.L. Limpus, and M.H. Godfrey. 2003. Nest site selection, oviposition eggs, development, hatchling, and emergence of loggerhead turtles. Pages 125-143. *in* Bolten, A.B., and B.E. Witherington, editors. Loggerhead and sea turtles. Smithsonian books, Washington, D.C., USA.

Mortimer, J.A. 1995. Factors influencing beach selection by nesting sea turtles. In: Biology and Conservation of Sea Turtles (ed. Bjorndal K.A.), pp. 45-51. Smithsonian Institute Press, Washington.

Mrosovsky, N., C.L. Yntema. 1980. Temperature dependence of sexual differentiation in sea turtles: Implications for conservation practices. Biological Conservation 18: 271-280.

National Marine Fisheries Service (NMFS) and U.S. Fish and Wildlife Service (USFWS). 1991. Recovery Plan for U.S. Population of the Loggerhead Turtle. National Marine Fisheries Service, Washington D.C.

National Marine Fisheries Service (NMFS) and U.S. Fish and Wildlife Service (USFWS). 2008. Recovery Plan for the Northwest Atlantic Population of the Loggerhead Sea Turtle (*Caretta caretta*), Second Revision. National Marine Fisheries Service, Silver Spring, Maryland.

Pullin, A.S., T.M. Knight, D.A. Stone, and K. Charman. 2004. Do conservation managers use scientific evidence to support their decision-making? Biological Conservation 119: 245-252.

Richardson, J.I. and T.H. Richardson. 1982. An experimental population model for the loggerhead sea turtle (*Caretta caretta*). In. 'Biology and Conservation of Sea Turtles', K.A. Bjorndal, ed., Smithsonian Institution, Washington, DC.

South Carolina Department of Natural Resources (SCDNR). 2013. Loggerheadlines. Available from: http://www.dnr.sc.gov/seaturtle/lhl.htm (accessed January 2014).

Stancyk, S. E. 1982. Non-human predators of sea turtles and their control. In 'Biology and Conservation of Sea Turtles'. (Ed. K. A. Bjorndal.) pp. 139–152.Smithsonian Institution Press: Washington, DC.

U.S. Department of Commerce. 2010. Endangered and threatened species; proposed listing of nine distinct population segments of Loggerhead Sea Turtles as endangered or threatened; proposed rule. Federal Register 75, 12598–12656.

Witherington, B.E. 1999. Reducing threats to nesting habitat. Pages 179-183. *in* Eckert, K.L., K.A. Bjorndal, F.A. Abreu-Grobois, and M. Donnelly, editors. Research and 36 management techniques for the conservation of sea turtles. IUCN/SSC Marine Turtle Specialist Group Publication No. 4.

Witherington, B.E. 2003. Conservation: challenges and opportunities. Pages 295-311. *in* Bolten, A.B. and B.E. Witherington, editors. Loggerhead sea turtles. Smithsonian Institution Press, Washington, D.C.

CHAPTER II

REASSESSING NEST RELOCATION AS A MANAGEMENT TOOL: EXAMINING THE EFFECTS OF NEST LOCATION, TIDAL WASH-OVER AND INUNDATION ON HATCH SUCCESS OF LOGGERHEAD SEA TURTLES NESTING WITHIN THE TOM YAWKEY WILDLIFE CENTER, GEORGETOWN COUNTY, SOUTH CAROLINA

INTRODUCTION

Sea turtles may be faced with a great loss of available nesting habitat resulting from the projected rise in sea level due to climate change (Caut et al. 2010). In the southeastern United States, average sea level has been predicted to rise between 0.20 mm - 0.34 mm per year (Rahmstorf et al. 2007; G.T. Mitchum, unpublished data). Along the northern coast of South Carolina, the sea level rise has averaged 3 - 4 mm per year during the past century (Williams et al. 2012). The intergovernmental panel on climate change (IPCC) has predicted global mean temperature could potentially increase between 1.0°C - 4.5°C and the maximum mean sea level could rise between 31 - 150 cm by the year 2100 (IPCC 2007). In addition, rainfall pattern anomalies and storm frequency and magnitude are expected to increase (Landsea 1993; Goldenberg et al. 2001; Fish et al. 2005; Webster et al. 2005; Magrin et al. 2007). An increase in the frequency and magnitude of storms may significantly impact nest success as storms may degrade and alter nesting habitat (Fish et al. 2005). Biophysical habitat alteration associated with climate change such as increased storm frequencies, coastal flooding and increased beach erosion (Klein and Nicholls 1999) may increase tidal wash-over events, nest flooding (inundation), and ultimately result in a loss of suitable sea turtle nesting habitat (Hawkes et al. 2009). Reviews discussing global research priorities for sea turtles indicate the importance of future research on how climate change may affect the physical parameters of nesting beaches that influence nest-site selection and hatch success (HS) (Hawkes et al. 2009; Hamann et al. 2010).

Relationship Between Abiotic Variables and Hatch Success

Temperature: Climate change has the potential to greatly influence cohort sex ratios and ultimately population dynamics (Janzen 1994; Mitchell et al. 2008) of thermally sensitive species exhibiting temperature-dependent sex determination (TSD) (Yntema and Mrosovsky 1980; Janzen and Paukstis 1991; Mrosovsky 1994). Sexual differentiation is based on nest temperatures during the middle third of the incubation period, the thermosensitive period (Yntema and Mrosovsky 1980). A mixed sex ratio is produced in a nest incubating within a narrow threshold range of temperatures (TRT) of a pivotal temperature (Yntema and Mrosovsky 1980). All females are produced in nests incubated at temperatures above this range and all males are produced in nests incubating at lower temperatures outside the TRT (Mrosovsky and Yntema 1980; Mrosovsky and Pieau 1991; Davenport 1997) within a thermal tolerance range of 25.0°C - 35.0 °C (Ackerman 1997). Predominantly female hatchlings are produced at most loggerhead nesting sites (Wibbels et al. 1991; Mrosovsky and Provancha 1992; Mrosovsky 1994; Marcovaldi et al. 1997; Hanson et al. 1998; Godley et al. 2001; Rees and Margaritoulis 2004; Hawkes et al. 2007) including those in Florida (Mrosovsky and Provancha 1989;

Mrosovsky 1994; Hanson et al. 1998), Georgia (Tuttle 2007; LeBlanc et al. 2012) and South Carolina (Johnston et al. 2007).

Variations in the incubation temperature of nests can be attributed to season (Matsuzawa et al. 2002), temporal variation within a season (Johnston et al. 2007), location (Davenport 1997), shading (Schmid et al. 2008; Patino-Martinez et al. 2012), moisture due to rainfall and/or storm tides (Schmid et al. 2008), and ambient temperature (Ackerman 1997). Studies have shown temperature also plays a key role in incubation duration (Bustard and Greenham 1968; McGehee 1979; Yntema and Mrosovsky 1980; Ackerman 1997).

Nest Relocations: Predicted rise in global mean temperatures and sea level may lead to an increase in tidal wash-over, nest inundations and vulnerability of nesting beaches to erosion (Fish et al. 2005). The U.S. Loggerhead Recovery Plan states nests vulnerable to erosion and with high probabilities of tidal inundation should be relocated from their original site to a more suitable site on higher grounds (NMFS and USFWS 1991, 2008). The use of relocation as a management tool is to be used only as a last resort if the nest is presumably doomed (NMFS and USFWS 1991, 2008). It has been suggested recovery plans include using some distance above the SHTL as a relocation guide instead of the SHTL itself because you cannot predict if and when storm tides will exceed the previously marked SHTL (Mrosovsky 2006).

Nest relocations are increasing due to a loss of suitable nesting habitat as beaches throughout the state face increased erosion (D.B. Griffin, personal communication). Many of these relocations are unnecessary but are conducted due to the misconception of concerned volunteers and project participants that the occurrence of any tidal wash-over will negatively influence HS, even of nests marginally landward of the SHTL (Coll 2010; D. B. Griffin and C. P. Hope, personal communication). Researchers suggest this conservation strategy is beneficial because it has shown to greatly increase productivity (Stancyk et al. 1980; Hopkins and Murphy 1983; Wyneken et al. 1988; Eckert and Eckert 1990; Tuttle 2007; Bishop and Meyer 2011). However, other studies have revealed several concerns regarding the use of nest relocations. Incorrectly performed relocations have resulted in movement-induced mortality caused by membrane detachment resulting from egg inversion after the initial twelve hours following oviposition (Limpus et al. 1979). While it has been reported in the southeastern United States that no significant differences were detected between the hatch and emergence success of *in situ* and relocated loggerhead clutches (Bimbi 2009; McElroy 2009), other studies suggest relocated sea turtle nests had significantly lower hatch and emergence success than *in situ* nests (Schulz 1975; Eckert and Eckert 1985, 1990; Herrera 2006).

Physical parameters of the incubating environment greatly influence embryonic development, HS, and ultimately fitness making nest-site selection by individual females a vital component that impacts survival of their offspring (Garmestani et al. 2000; Wood and Bjorndal 2000). Changing physical parameters from the original nest chamber has the potential to alter development and HS (Carthy et al. 2003; Mrosovsky 2006). For example, relocating nests regarded as doomed could cause them to incubate at higher temperatures than if left *in situ* due to a vertical temperature gradient between the lower and upper beach where sand temperatures are cooler closer to the water (Harrison 1987).

Incubating at higher temperatures has the potential to alter hatchling sex ratios and fitness (Ackerman 1997; Marcovaldi et al. 1997; Godley et al. 2001; Mrosovsky 2006, 2008; Pike 2008) and has been shown to decrease emergence success due to high rates of heat-related mortality of pre-emergent hatchlings that face desiccation in clutches during the latter part of the nesting season when temperatures are at their highest (Matsuzawa et al. 2002).

Tidal wash-over and inundation events: To date, numerous studies have previously investigated the effects of tidal wash-over and inundation on nest temperatures (Schmid et al. 2008), developmental stage of embryonic arrest (Whitmore and Dutton 1985; Limpus 1985; Eckert and Eckert 1990; Foley et al. 2006; Caut et al. 2010), hatch success (Mrosovsky et al. 1983; Whitmore and Dutton 1985; Hilterman and Goverse 2003; Foley et al. 2006; Pike and Stiner 2007; Caut et al. 2009, Coll 2010) and emergence success (Hilterman and Goverse 2003; Coll 2010) of multiple species with varying results. Research suggests that not only the occurrence of a wash-over event has the potential to decrease HS, but the timing, frequency and level of the wash-over/ inundation event(s) are also important (Foley *et al.* 2006; Caut *et al.* 2010; Coll 2010).

Tidal wash-over events have the ability to cool the incubation temperature of nests left *in situ* that are laid at or below the SHTL. Cooling events that lower sand temperatures such as tidal wash-over and brief inundation may not be as harmful to nest success as previously thought. For example, these events could minimize mortality caused by extreme heat and may also lead to a higher proportion of male hatchlings (Carthy et al. 2003; Margaritoulis and Rees 2003).While several studies have concluded the occurrence of wash-over events can significantly decrease HS in loggerheads (Foley et al. 2006; Pike and Stiner 2007; Coll 2010), some hatchlings are still produced in these nests (Whitmore and Dutton 1985; Hilterman 2001; Mrosovsky 2006; Pike and Stiner 2007; Caut et al. 2010; Coll 2010; Shaw 2013). This calls for the reassessment of relocating nests that are vulnerable to any tidal wash-over since it has been shown not all nests deposited close to or below the tide line are actually doomed.

While a negative relationship exists between tidal influences (i.e. wash-over and inundation) and HS (Whitmore and Dutton 1985; Hilterman and Goverse 2004; Caut et al. 2010; Coll 2010), the impact of the frequency of tidal events and nest location (including whether a nest was relocated) on HS needs further quantification. Recovery plans suggest further research evaluating the tolerance of eggs to tidal threats should be conducted to develop operative nest management guidelines relative to such threats. An evaluation regarding the appropriateness of manipulative nest management tools such as relocation is also recommended (NMFS and USFWS 1991, 2008). Because of the differences reported between study sites and species, there is a need to further examine species-specific HS as it relates to tidal wash-over and inundation, which may negatively impact the HS of loggerhead sea turtles in South Carolina.

RESEARCH GOALS AND HYPOTHESES

The purpose of this study was to assess the current use of relocation as a management tool in South Carolina by 1) comparing the HS of relocated nests with the HS of *in situ* nests at varying distances above and below the SHTL and 2) examining the effects of tidal wash-over and inundation on the HS of relocated and *in situ* nests located at various distances above and below the SHTL. This study builds on previous graduate work that determined it takes frequent wash-over throughout the incubation period to significantly lower HS (Coll 2010). Specifically, investigations were conducted on the effects of 1) zone (defined as nest location in terms of distance above or below the SHTL) on HS, 2) relocation of nests moved to areas above the SHTL on HS, 3) wash-over frequency during normal high tide events on HS and 4) inundation and wash-away during storm tide events on HS. By comparing the HS of a sample of nests left *in situ* at various beach zones, a sample of nests that were relocated above the SHTL, and the effect of wash-over and inundation on HS among zones, this study was able to determine if the relationship between tidal impacts and HS varied based on distance from the SHTL and whether nests experienced relocation. Furthermore, it was determined if nests deposited marginally landward of the SHTL are negatively impacted by storm surge as a guide for relocations. The relationship between egg loss and HS was also evaluated to determine if nests that experienced loss should be excluded from analyses. The effects of interest were examined with the following hypotheses.

Hypotheses:

H_O 1: Mean HS of nests that experience egg loss does not significantly differ from mean HS of nests that do not experience loss.

H_A 1: Mean HS of nests that experience egg loss is significantly lower than mean HS of nests that do not experience loss.

H_O 2: Mean HS does not significantly vary between zones.

H_A 2: Mean HS significantly varies between zones.

H_O 3: Mean HS does not significantly vary based on wash-over frequency.

H_A 3: There is a negative relationship between wash-over frequency and HS.

H_O 4: The relationship between mean HS and wash-over does not vary among zones.

H_A 4: The relationship between mean HS and wash-over differs between zones.

H_O 5: Mean HS does not vary based on the occurrence of storm-induced inundation/ wash-away events.

H_A 5: Mean HS of nests that experience storm-induced inundation/wash-away is significantly lower than mean HS of nests not impacted by storm tides.

MATERIALS AND METHODS

Study Site: Loggerhead sea turtle nesting data were collected 11 May - 14 October 2012 and 11 May -11 October 2013 at the Tom Yawkey Wildlife Center (TYWC), a publically managed wildlife center located near Georgetown, South Carolina (33.2°N, -79.2°W). The South Carolina Department of Natural Resources (SCDNR) manages the TYWC. It is separated from the mainland by the Intracoastal Waterway and consists of Cat Island, North Island, Sand Island, and South Island (Figure 2.1). The property is managed as a wildlife center with severely limited public access and is composed of approximately

9,700 hectares of managed wetlands surrounded by tidal marsh, longleaf pine (*Pinus palustris*) forest, ocean beach and maritime forest (SCDNR 2014). Nesting beach surveys have been annually conducted on South Island since 1977. This site has averaged 175 nests per season since annual surveys began and is considered a high density nesting beach for loggerheads in the state of South Carolina (SCDNR 2010).

Loggerhead nesting surveys used in this study were conducted on South Island beach. Sea turtles had access to the full length and width of beach since no structures such as seawalls exist. South Island consists of 6.08 km of undisturbed, beach managed for sea turtle and shorebird nesting. The dominant flora include sea oats (*Uniola paniculata*), seacoast marsh elder (*Iva imbricata*), and seaside panicum (*Panicum amarum*) which contribute to the establishment and maintenance of coastal dunes that provide suitable loggerhead nesting habitat. The maritime forest behind the dunes is characterized by a variety of salt-tolerant evergreens such as wax myrtle (*Myrica cerifera*), yaupon (*Ilex vomatoria*), live oak (*Quercus virginiana*), red bay (*Persea borbonia*), Southern magnolia (*Magnolia grandiflora*), cabbage palmetto (*Sabal palmetto*), saw palmetto (*Serenoa repens*) and loblolly pine (*Pinus taeda*). Erosional forces that occurred between the 2011 and 2012 nesting seasons created foredunes (defined as the side of sand dunes nearest to the sea) that are steeply scarped beginning slightly south of the beach entrance (33.149°N, -79.224°W) and extending north of the entrance to approximately (33.168°N, -79.199°W) leaving the beach with what appears to be less suitable nesting habitat than in prior years (i.e. a narrower beach with steeper dunes). These scarped dunes prevent most sea turtles from crawling to higher dune elevations or into vegetated areas of the dunes to lay eggs (personal observation). The south end of the beach consists of a flat wash-out section that experiences flooding during spring tides and storm tides making this area less suitable for nesting. While the north end of the beach past the scarped dunes does consist of well-established dunes exceeding 2 m tall, the path to reach dunes at the far north end extends a great distance beyond the tide line and is covered with dense wrack (defined as vegetation, largely *Spartina*, cast on the shore), debris and often trash. Dunes along the entirety of South Island began to re-establish prior to the end of the 2013 season.

Nest location and identification: Nest surveys on South Island were conducted by project participants at sunrise seven days a week throughout the nesting season which ranged from 11 May - 18 August 2012 and 11 May - 11 August 2013. The nesting beach was patrolled by use of ATV, 4WD truck, or by foot. Nests were located by following crawls to the body pit constructed by the female during the previous night's nesting attempt. A line was drawn through all tracks so data were not collected more than once per nesting attempt. To determine whether a clutch was deposited or if the crawl was false, meaning a non-nesting emergence where no eggs were deposited (SCDNR 2014) a probe stick was carefully inserted into the sea turtle nest body pit. All emergences were recorded as a nest or false crawl. Clutches were found when a depression was felt when probing the sand. The nest was then dug into by hand. If eggs were located, one was excavated and stored in a 50 mL vial containing 95% ethanol for use in the NRU loggerhead DNA genetic fingerprinting study (Shamblin et al. 2011). Any eggs broken by the probe stick were removed so not to attract predators or cause microbial contamination that could spread to the rest of the incubating clutch (Wyneken et al. 1988). If an egg was broken by the probe, that egg was used as the genetic sample. In the absence of broken eggs, one egg was collected from the nest for genetic testing. An alternate method of clutch location that consisted of digging the body pit by hand was used to locate the nest cavity for 50% of nests determined to need relocation in 2012 and 50% of relocated nests during the 2013 season. This was done as part of a separate investigation to determine if a correlation exists between probing and the presence of broken eggs in the bottom or middle of a clutch during a nest relocation with no sign of direct puncture (which may suggest indirect pressure created in the nest when the probe is inserted into the substrate causes egg breakage) (see Chapter III).

All nests were protected with approximately 1.2 m X 1.2 m plastic or metal screens and staked at the four corners to deter predators such as raccoons (*Procyon lotor*) and coyotes (*Canis latrans*). Several markers were used to identify nests: brightly colored flagging tape was tied to two stakes, a flag was inserted into the center of the nest, and a numbered stake was inserted into the dune directly behind the nests laid below the spring high tide line (SHTL). In this study, the SHTL refers to the highest spring tide occurring in March/April (an equinoctial spring tide). During this time around the equinox, extreme tidal forces are prevalent causing the highest spring tides. Nests laid above the line marking the SHTL (often characterized by the wrack line and/or scarped dunes) are considered ‘safe’ from tidal influences since subsequent spring tides do not typically exceed equinoctial springs in the absence of storm surge. An extra marker and metal screens that could be more efficiently located with a metal detector were used for these nests in case other markers were washed away or buried by sand accretion or dune collapse. All markers were labeled with the date the nest was laid and the nest number. Coordinates were taken for each nest so they could be easily located using a Garmin GPSMAP 60CSx.Throughout the incubation period; nests were monitored daily for losses, signs of depredation and/or disturbance (Table 2.1).

Nest relocation: Nests partially depredated by coyotes on the night of oviposition were relocated in 2012 and 2013. Nests laid below the SHTL by > 3 m were relocated to higher grounds > 3 m above the SHTL due to threats caused by vehicular traffic and the high probability of repeated wash-over and/or inundation at this distance below the SHTL. Nests were relocated to areas > 3 m above SHTL due to the low probability of inundation at this distance. The criteria used for selection of artificial nest locations (i.e. relocations) were based primarily on the distance above the SHTL. However, the site for relocation was also based on dune height and vegetation. If a well-established dune without dense vegetation was located directly inland of the original nest site, the nest was relocated to this dune. If this type of dune was not located directly inland of the original nest site, the closest suitable site to the original location was chosen as the relocation site (SCDNR 2014). This was done in order to most accurately recreate the conditions of the original site and to minimize disturbance. Once an appropriate site was determined, an egg chamber approximately 8-10" in diameter and the same depth as the initial nest was constructed using a shovel, hands, or shells. Clutches were excavated from their *in situ* location and transferred using a plastic bucket to the new site where the eggs were carefully placed into the newly constructed chamber in the same layer as they were laid in the original chamber. Nests were covered with damp, cool sand from the original chamber, protected with screening and marked as previously stated.

Hatching: Nest inventories were conducted 21 July - 9 October 2012 and 25 July - 11 October 2013. All nests laid on South Island were inventoried with the exception of undetected (wild) nests and two nests laid on a separate beach on Winyah Bay behind the manager’s house. Undetected nests were defined as nests not located after laying but discovered after predation or signs of emergence. Nests were checked daily for signs of emergence beginning on day 45 of the incubation period (SCDNR 2014). Field signs used to determine emergence activity included a crater in the center of the nest or the presence of hatchling tracks. Nests were excavated 3 days after the first sign of emergence. Nests where emergence signs were not evident were inventoried 75 days after the date they were laid, with an exception being nests laid in May 2012. These nests were inventoried 80 days after being laid if emergence signs were not observed due to a potentially extended incubation period due to unseasonably cool and rainy conditions (D. B. Griffin, personal communication). Nests were excavated, contents of the egg chamber were counted and clutch size was determined. Eggs were recounted during inventories even if they had been previously counted during relocation. The clutch count determined during excavation was used in analyses. The number of unhatched eggs, hatched eggs (defined as an intact shell greater than or equal to 50%), pipped eggs (defined as an egg broken by a hatchling that dies before it is able to fully emerge from the egg), live hatchlings and dead hatchlings were also counted. All contents which included unhatched eggs, shells from hatched eggs, and dead hatchlings were discarded into the ocean so they did not attract predators.

Experimental Design: In order to test the effects of tidal wash-over and inundation on the HS of *in situ* and relocated nests, a sample of all nests were relocated based on *a priori* categorization scheme. The remaining nests were monitored at their *in situ* nest sites at varying distances above and below the SHTL. This experimental design was meant to test whether or not nests laid and/or relocated above the SHTL still have the potential to wash-over depending on the distance of the nest above the tide line, if HS varies based on distance of *in situ* and relocated nests from the SHTL (i.e. zone), and whether tidal events negatively impact HS on South Island (and if so, does this relationship vary across zones) (Figure 2.2). Nests laid > 3 m below the SHTL were all relocated to higher grounds due to 1) a high probability of failure due to erosion and repeated inundation and 2) vehicular use below this distance for management activities such as trapping (personal observations). Suitable areas 3 m above the SHTL were designated as the demarcation to relocate nests because this zone (zone 3-R) has the lowest probability of inundation. During the 2012 season, all nests laid at or below the SHTL by ≤ 3 m (zone 1) were left to incubate *in situ* in order to monitor the effects of wash-over and inundation on the HS of low beach nests. During the 2013 season, 33% of nests laid in zone 1 (1 out of every 3 nests laid at this distance) were left *in situ* to continue this study. However, the other 66% of nests laid in zone 1 were relocated as part of a separate investigation (see chapter III). There was interest in examining *in situ* nests deposited slightly above the SHTL (≤ 3m; zone 2) to compare the vulnerability of these nests to tidal events caused by storm surge, storm-induced inundation and/or decreased HS with the vulnerability of nests incubating below the SHTL (zone 1) and both *in situ* and relocated nests incubating higher than nests in zone 2 (≥ 3 m above SHTL). These zones were chosen to ultimately determine 1) if distance above the SHTL is an appropriate guide for nest relocations and 2) if relocation significantly increases HS when compared to vulnerable nests left to incubate below the SHTL.

High Tides: During the 2012 season, the spring tide in May was higher than average due to strong northeast winds during this tidal event, although it varied by location at the site. These tidal fluctuations lead to the decision to make the potential wash-over zone (zone 2) begin just above April's SHTL and extend 3 m above the SHTL. There was potential these nests will experience one or more wash-over and/or inundation events depending on the occurrence of storm surge (defined as abnormally high tides and waves associated with storm activity), wind speed and direction (personal observations). Nests located ≥ 3 m above April's SHTL did not experience inundation, making zone 3 the least vulnerable to tidal influences, and therefore leading to the decision to move the sample of relocated nests to this zone.

The number of nests deposited in each zone was counted throughout the season. Nests were monitored using wash-over cups twice daily throughout the entire incubation period; once after each high tide event. The design for wash-over cups was based on field methods reported by Collins (2012). Wash-over cups were constructed using 350 mL plastic containers. Each cup was glued to a square wooden base that had a 15 cm nail inserted into the bottom to help anchor the cup into the substrate. Holes were made around the cup just below the rim in order to collect seawater during tidal wash-over events. Lids were placed on the cups to prevent the entry of rainwater. Wash-over cups were placed to the right of the nest screening parallel to the nest cavity and were buried to the bottom of the holes (Figure 2.3). The occurrence of a wash-over event was determined by any fluid found in the cup during wash-over checks and/or the tide line reaching > 50% of the nest chamber in the center of the screen (Figure 2.4). The number of wash-over events and dates these events occurred were recorded for each nest (Table 2.2, Table 2.3). Inundation events, defined as nest flooding, were also recorded for each nest. For the 2013 season, eggs that failed to hatch were staged based on revised criteria of Whitmore and Dutton (1985) in order to determine what stage of development embryonic mortality occurred (early, middle, or late stage) (Table 2.4). This data was used in combination with wash-over data to more accurately determine the number of tidal wash-over events it took to cause embryonic mortality within a given nest.

At the start of the 2012 season, volume of seawater was measured in the wash-over cups with the assumption all water contained in the cups was from wash-over events and lids on the wash-over containers kept out rainwater. Due to sand filling the cups and making measurements inaccurate, volume of seawater was not determined. Nests were considered washed away if the entire clutch was washed out into the ocean or if sand was removed from the nest during a wash-over event exposing any of the clutch.

Statistical Analyses: Undetected (wild) nests, nests partially depredated by coyotes, and nests laid on the side beach of South Island on Winyah Bay were excluded from all analyses examining loggerhead HS. The level of significance was $\alpha = 0.10$ for all comparisons in order to increase power and decrease the probability of committing a Type II error (i.e. false negatives). A one-way analysis of variance (ANOVA), regression, and t-tests were used to test hypotheses. These methods require certain assumptions for the hypothesis test results to be valid. These assumptions were evaluated in each of the following hypothesis test analyses. The assumption of normality was tested using the Shapiro Wilk W statistic and graphically using normal quantile plots and histograms. In some cases the normality assumption was found to be violated. The assumption of equal variance was tested with Levene's Test. In some cases the equal variance assumption was found to be violated. Fortunately, hypothesis test results using methods that allow for violation of assumptions (i.e. transformations, nonparametrics) yielded results similar to the original ANOVA, regression and t-test results. This was most likely due to the violations not being too severe and the large sample sizes resulting in somewhat robust ANOVA, regression and t-tests (Box 1953). Therefore the standard (i.e. parametric) ANOVA, regression and t-test results were used because these tests are more statistically powerful and the mean was the measure of central tendency of greatest interest for this study. Also, non-parametric tests can be less efficient, less powerful and do not always control the probability of Type II error (Freidlin and Gastwirth 2000). All statistical calculations were performed with JMP software (V.9, SAS). Hatch success was calculated for each nest as

([# hatched eggs / clutch size] * 100) for all investigations.

HS vs. Loss

The analysis for hypothesis 1 (H_O 1: Mean HS of nests that experience egg loss does not significantly differ from HS of nests that do not experience loss) was based on a one factor CRD, where egg loss in a nest due to probing and/or depredation was the treatment. The model for the investigation was: $y = \mu + \tau + \varepsilon$, where y = HS, μ = overall mean, τ = treatment (loss) and ε = error. Nests were monitored daily throughout the incubation period for loss events.

One-tailed t-tests assuming equal variance were used to analyze the model and test the hypothesis. When examining the relationship between HS and whether a nest experienced loss during the incubation period, data from the 2012 and 2013 seasons were analyzed separately since the relationship was inconsistent between years (Table 2.7). Due to nests that experienced loss showing a significantly lower mean HS than nests with no loss in 2013, nests experiencing loss were eliminated from analyses for the 2013 season. However, nests that experienced loss were included in analyses for the 2012 season since loss did not negatively impact HS.

HS vs. Zone

The analysis for hypothesis 2 (H_O 2: Mean HS does not significantly vary between zones) was based on a one factor completely randomized design (CRD). However, the study design actually was observational and opportunistic. Beach zone was the treatment factor, defined by 1) distance above or below the SHTL and 2) whether a nest was *in situ* or relocated. Treatment was only manipulated by the experimenter for one zone (zone 3-R). All nests in the other three treatments were laid in a given zone by the female loggerhead. Sample size of each zone was opportunistic and all nests laid in each zone throughout the 2012 nesting season were used in this study. However, during the 2013 season, 66% of nests laid in zone 1 were relocated to higher grounds in order to increase the sample size of relocated nests for a separate investigation (see Chapter III). The model for this investigation was: $y = \mu + \tau + \varepsilon$, where y = HS, μ = overall mean, τ = treatment (zone) and ε = error.

The average (± SD) HS was calculated across zones. ANOVA was used to analyze the model and test the hypothesis. Since the relationship between mean HS and zone was not consistent between years, data from the 2012 and 2013 seasons were examined separately in analysis.

Results of ANOVA provided evidence to reject the null hypothesis (H_O 2: Mean HS does not significantly vary between zones), and several follow-up tests were conducted. To answer specific *a priori* hypotheses about the treatment mean, a series of linear combinations (i.e. contrasts) was defined to examine the relationship between nest location and relocation (i.e. zone) on HS (Table 2.5). Fisher's Least Significant Difference (LSD) was used for *a posteriori* multiple mean comparison test in order to identify pairwise significance between HS in different zones to further substantiate results suggested by the above-mentioned contrasts (Table 2.6). This multiple comparison procedure was chosen to control Type II errors. Correction methods such as Bonferroni were not applied to the pairwise comparisons or contrasts to be consistent with the objective of reducing Type II error in this study.

HS vs. Wash-over and Inundation

All wash-over analyses were conducted for each nesting season separately since year to year variation was evident. Nests were monitored twice daily (after each high tide event) for the occurrence of wash-over and/or inundation. For the 2013 season, all nests that experienced a loss event due to probing and/or depredation during the incubation period were excluded from analyses since loss negatively impacted HS. To guard against counting wash-overs after nest emergence, nests with no evidence of emergence that experienced wash-over after day 60 of the incubation period were also excluded from analyses of wash-over impacts (Coll 2010).

A two-way ANOVA was used to examine the simple effects and interaction effects of the factors wash-over and zone on HS. The analysis for hypothesis 4 (H_O 4: The relationship between mean HS and wash-over does not vary among zones) was based on a 4 x 2 factorial CRD, where zone (Z), wash-over (W) and wash-over*zone interaction (W*Z) were the factorial effects. The model for the investigation was: $y = \mu + \tau + \varepsilon$, where y = HS, μ = overall mean, τ = treatments and ε = error. The treatment effect was partitioned into the factorial effects as follows: $y = \mu + W + Z + W*Z + \varepsilon$. Data from the 2012 and 2013 seasons were analyzed separately since the relationship between HS and zone differed between years. After ANOVA results provided evidence to reject the null hypothesis for the 2012 season (H_{O}: There is no interaction between the two factors), follow-up tests were conducted. A series of linear combinations (i.e. contrasts) was defined to examine the interaction effect between zone and wash-over on HS. Correction methods such as Bonferroni were not applied to contrasts to be consistent with the objective of reducing Type II error.

In order to determine whether temporal variation existed between HS and the week each nest was laid, the relationship between HS and week laid was examined by zone. This analysis was based on a one factor CRD, where week laid was the treatment factor. The model for this investigation was: $y = \mu + \tau + \varepsilon$ where μ = overall mean, τ = treatment (week laid (L)), and ε = error. Zone was not used as a block in the model since only zone 1 nests from the 2012 season displayed an evident relationship between week laid and HS. Therefore, the remaining zones were not further examined. The best fit model for describing the week laid effect was a 2nd order polynomial model: $y = \beta_0 + \beta_1 L + (\beta_2 L)^2 + \varepsilon$. The model was applied to each zone. Model terms were defined as: β_0 = intercept, β_1 = initial change in HS per week, β_2= change in the HS change per week and ε = error.

When the relationship between HS and wash-over frequency was examined, data from the 2012 and 2013 seasons were analyzed separately since the relationship between HS and wash-over frequency was inconsistent between years. The analysis for hypothesis 3 (H_O 3: Mean HS does not significantly vary based on wash-over frequency) was based on a one factor CRD where wash-over frequency was the treatment. The model for this investigation was: $y = \mu + \tau + \varepsilon$ where μ = overall mean, τ = treatment (wash-over frequency) and ε = error. The best fit regression model for this investigation was a first order linear model: $y = \beta_0 + \beta_1 W + \varepsilon$, where β_0= intercept, $\beta_1 W$ = initial change in HS per wash-over event and ε = error.

Multiple regression models were used to further investigate the relationship between 1) week laid and HS and 2) wash-over frequency and HS for nests in zone 1 during the 2012 season since this was the only zone to exhibit a discernible relationship between these variables and HS. These relationships were further examined with addition of the term 'storm induced inundation and/or wash away' (S) to the above models. This additional treatment factor was added to models after rejection of hypothesis 5 ($H_{O\ 5}$: Mean HS does not vary based on the occurrence of storm-induced inundation/ wash-away events) since a one-tailed t-test assuming equal variance suggested a negative relationship existed between the occurrence of inundation/wash-away during storm tides and HS. Multiple regression models were able to more accurately assess 1) the nonlinear relationship between week laid and storm-induced inundation/wash-away on HS and 2) the linear relationship between wash-over frequency during normal high tide events and inundation/wash-away during storm tide events on HS.

Inverse prediction was used to determine the number of wash-overs required to drop HS below a specified 60% (defined as a nest failure) recommended on nesting beaches by the U.S. Loggerhead Recovery Plan (NMFS and USFWS 1991, 2008). This estimate was made for nests that experienced wash-over but did not experience inundation since all nests to become flooded during storm events exhibited HS below 60%.

RESULTS

HS vs. Loss

When examining the relationship between HS and whether a nest experienced loss during the incubation period, data from the 2012 and 2013 were analyzed separately since the relationship between loss and HS differed between seasons. A one tailed t-test assuming equal variance (F = 0.44, p = 0.51) indicated no significant difference between mean HS of nests that did not experience loss (n = 107, mean HS = 71.2% ± 27.9%, 90% C.I. = [66.8%, 75.8%]) with mean HS of nests that experienced loss (y = 43, mean HS = 69.2% ± 25.9%, 90% C.I. = [62.6%, 75.9%]) (t = -0.41, p = 0.34) during the 2012 season. However, in 2013 a one-tailed t-test assuming equal variance (F = 1.07, p = 0.30) indicated mean HS of nests that did not experience loss (n = 99, mean HS = 74.9% ± 24.2%, 90% C.I. = [70.9%, 79.0%]) was significantly higher than mean HS of nests that experienced loss (n = 30, mean HS = 64.5% ± 28.4%, 90% C.I = [55.7%, 73.3%]) (t = -1.99, p = 0.02). In analyses for the 2013 season, all nests that experienced a loss event(s) were excluded from analyses since I did not want this variable (loss) to influence the validity of the investigation of treatment effects (zone, wash-over, and zone*wash-over interaction effects) on HS.

HS vs. Zone

During the 2012 season, ANOVA indicated the mean HS significantly differed between the zones (F = 5.30, p < 0.01). Fisher's LSD all pairwise comparisons indicated that mean HS of nests laid in zone 1 (n = 90, mean HS = 58.5% ± 34.2%, 90% C.I. = [53.3%, 63.7%]) was significantly lower than nests laid in zone 2 (n = 25, mean HS =

77.4% ± 22.3%, 90% C.I. = [67.5%, 87.4%]) ($t = -2.79$, $p < 0.01$), nests laid in zone 3-I ($n = 30$, mean HS = 75.8% ± 26.0%, 90% C.I. = [66.8%, 84.8%]) ($t = -2.74$, $p < 0.01$), and nests relocated to zone 3-R ($n = 14$, mean HS = 80.9% ± 15.9%, 90% C.I. = [67.7%, 94.1%]) ($t = -2.61$, $p = 0.01$). During the 2013 season, ANOVA suggested mean HS did not significantly differ between zones ($F = 0.68$, $p = 0.57$). However, the descriptive relationship between HS and zone was similar to that of 2012. Mean HS of nests laid in zone 1 ($n = 15$, mean HS = 69.4% ± 30.2%, 90% C.I. = [58.9%, 79.8%]) was lower than the mean HS of nests laid in zone 2 ($n = 24$, mean HS = 78.8% ± 16.7%, 90% C.I. = [70.6%, 87.1%]) ($t = -1.27$, $p = 0.21$), nests laid in zone 3-I ($n = 25$, mean HS = 71.8% ± 24.0%, 90% C.I. = [63.8%, 79.9%]) ($t = -0.78$, $p = 0.44$) and nests relocated to zone 3-R ($n = 50$, mean HS = 73.5% ± 28%, 90% C.I. = [70.1%, 83.7%]) ($t = 1.10$, $p = 0.27$) (Figure 2.5, Table 2.6).

To answer a series of specific hypotheses, mean comparisons of interest were made using a set of linear contrasts. Combinations were examined separately by year since the relationship between zone and HS was not significantly consistent between years. To determine whether mean HS of nests laid just above the SHTL (zone 2), and therefore still vulnerable to tidal wash-over events during storm tides and other extreme tide events, significantly differed from mean HS of nests left to incubate *in situ* below the SHTL (zone 1), mean HS of zone 2 was compared to mean HS of zone 1. During the 2012 season, mean HS of nests in zone 1 significantly differed from mean HS of nests in zone 2 ($F = 7.8$, $p < 0.01$). No significant difference between HS of nests in zones 1 and 2 was detected for the 2013 season ($F = 1.59$, $p = 0.20$). To determine if mean HS of nests relocated to higher grounds that were laid below the SHTL was significantly higher when compared to nests left *in situ* below the SHTL, a contrast comparing mean HS in zone 3-R to mean HS in zone 1 was examined. During the 2012 season, mean HS of nests relocated to zone 3-R was significantly higher than mean HS of nests left to incubate *in situ* below the SHTL (zone 1) ($F = 6.8$, $p = 0.01$). However, no significant difference between HS of nests in zones 1 and 3-R was detected for the 2013 season ($F = 1.18$, $p = 0.58$). Finally, in order to determine if there is an optimal distance (i.e. a distance where nests are 'safe' from storm tides) above the SHTL where nests should be moved as a guide for relocations, mean HS between zones 2 and 3-I was compared. The results of this contrast for the 2012 and 2013 seasons indicated that mean HS did not significantly differ between nests laid ≤ 3 m above the SHTL (zone 2) and nests laid > 3 m above the SHTL (zone 3-I) ($F = 0.04$, $p = 0.80$, 2012; $F = 0.31$, $p = 0.58$, 2013).

HS vs. Wash-over and Inundation

The interaction effects of zone and wash-over occurrence were also examined by year. During the 2012 season, two-way ANOVA suggested significant treatment effects ($F = 2.21$, $p = 0.05$). I was unable to examine the significance of each main effect and interaction effect on HS since no relocated nests (zone 3-R) experienced wash-over throughout the season. However, profile plots were examined to further determine the relationship between the treatments wash-over, zone, and wash-over*zone interaction on HS (Figure 2.6). Linear contrasts were used as follow-up tests to determine which means significantly differed. The LS mean HS of low nests in zone 1 that experienced wash-over (LS mean = 61.2%, standard error (S.E.) = 3.8%) significantly differed from the LS mean HS of nests in zone 1 that did not experience wash-over (LS mean = 90.6%, S.E. = 15.9%) ($F = 3.20$, $p = 0.07$). The LS mean HS of nests in zone 2 that experienced wash-over (LS mean = 78.6%, S.E. = 9.2%) did not significantly differ from the LS mean HS of nests in zone 2 that did not experience wash-over (LS mean = 75.6%, S.E. = 8.7%) ($F = 0.05$, $p = 0.81$). The LS mean HS of nests in zone 3-I that experienced wash-over (LS mean = 78.5%, S.E. = 19.6%) did not significantly differ from the LS mean HS of nests in zone 3-I that did not experience wash-over (LS mean = 81.4%, S.E. = 5.8%) ($F = 0.02$, $p = 0.89$). Mean HS of nests that did not experience wash-over did not vary between zones.

During the 2013 season, two-way ANOVA also suggested significant treatment effects ($F = 4.51$, $p < 0.01$). Effect tests indicated significance of the treatments wash-over ($F = 11.94$, $p < 0.01$), zone ($F = 6.20$, $p < 0.01$) and wash-over*zone interaction ($F = 7.99$, $p < 0.01$). Profile plots were again examined to further determine the relationship between the treatments wash-over, zone, and wash-over*zone interaction on HS (Figure 3). Linear contrasts were used as follow-up tests to determine which means significantly differed. The LS mean HS of nests in zone 1 that experienced wash-over (LS mean = 65.1%, S.E. = 6.8%) did not significantly differ from the LS mean HS of nests in zone 1 that did not experience wash-over (LS mean = 77.9%, S.E. = 9.7%) ($F = 1.16$, $p = 0.28$). The LS mean HS of nests in zone 2 that experienced wash-over (LS mean = 85.0%, S.E. = 8.2%) did not significantly differ from the LS mean HS of nests in zone 2 that did not experience wash-over (LS mean = 76.3%, S.E. = 5.3%) ($F = 0.81$, $p = 0.37$). The LS mean HS of nests in zone 3-I that experienced wash-over (LS mean = 64.2%, S.E. =

15.3%) did not significantly differ from the LS mean HS of nests in zone 3-I that did not experience wash-over (LS mean = 72.5%, S.E. = 4.5%) ($F = 0.27$, $p = 0.60$). However, the LS mean HS of relocated nests in zone 3-R that experienced wash-over (LS mean = 1.59 e-16 %, S.E. = 15.3%) significantly differed from the LS mean HS of nests in zone 3-R that did not experience wash-over (LS mean = 81.6%, S.E. = 3.8%) ($F = 26.74$, $p < 0.01$).

The only zone that showed a relationship between week laid (L) and HS was zone 1 during the 2012 season ($r = 0.46$, $p < 0.01$). The best fit model was a 2nd degree polynomial: y (HS) = 0.2512 + 0.0500*L – 0.0064*(L – 7.9)², where β_0 = 0.2512 (S.E. = 0.0917), β_1 = 0.0500 (S.E. = 0.0102), β_2 = -0.0064 (S.E. = 0.0030), and $\bar{L}$ = 7.9 (R^2 = 0.25, RMSE = 0.30) (Figure 2.7). The significant terms in this model were week laid (t = 4.91, $p < 0.01$) and (week laid – 7.9)² (t = -2.15, p = 0.03). There was no relationship between week laid and HS for any zones in 2013. After examining the nonlinear relationship evident between week laid and zone 1 in 2012, I observed the decreased HS in nests laid early in season could be attributed to lower than average temperatures and higher than average precipitation levels experienced in the latter half of May and early June of 2012 (NOAA 2012), and the occurrence of several tropical storms and a category I hurricane (Tropical Storm Beryl, Hurricane Chris, and Tropical Storm Debby) that caused storm surge throughout the southeastern U.S. between late May and late June of the nesting season. This storm activity caused not only wash-over to many incubating nests, but also inundation and complete or partial wash-away in several nests in zone 1. Therefore, I added the term 'storm induced inundation/wash away' (S_N = no storm tide effects, S_Y = storm tide effects) to the model. After adding this term, the 2nd order polynomial multiple regression model greatly improved ($R^2 = 0.68$, RMSE = 0.19, n = 90) (Figure 2.8): y (HS) = 0.3318 + 0.0243*L + 0.2554*S – (0.0036*(L – 7.9))², with parameter estimates for the terms β_0 (t = 5.15, $p < 0.01$), β_1 (t = 3.15, $p < 0.01$), β_2 (t = 10.17, $p < 0.01$) and β_3 (t = -1.69, p = 0.09) being significant predictor variables of HS in zone 1 during the 2012 season. The intercepts between nests that experienced storm-induced inundation/wash-away (β_0 S_Y= 0.208, S.E. = 0.085) and nests that did not ($\beta_0 S_N$ = 0.790, S.E. = 0.086) differed. This regression model suggests that HS is a nonlinear function of both week laid and whether nests experienced storm-induced inundation/wash-away during the 2012 season in zone 1.

There was a negative linear relationship between wash-over frequency and HS in zone 1 during the 2012 season (r = - 0.43, $p < 0.01$) (Figure 2.9). In the best fitting linear model: y (HS) = 0.808 – 0.037*W, where β_0 = 0.808 and β_1W = -0.037 ($R^2 = 0.22$, RMSE = 0.29, n = 86), the term wash-over frequency was significant (t = -4.91, $p < 0.01$). The term storm-induced inundation/wash-away was added to the model as a predictor variable of HS since a t-test assuming equal variances indicated mean HS of nests that experienced inundation/wash-away during storm tides (mean HS = 8.1%, SD ± 15.2%, n = 16, 90% C.I. = [1.4%, 14.8%]) was significantly lower than mean HS of nests that did not (mean HS = 76.9%, SD ± 21.9%, n = 70, 90% C.I. = [73.3% ,80.7%]) (Table 2.9). The model greatly improved after adding this term ($R^2 = 0.60$, RMSE = 0.21, n = 86) (Figure 2.10). This regression model suggests HS was a linear function of wash-over frequency and storm tide influences (inundation/wash-away) during the 2012 season.

However, the term wash-over frequency significantly differed in its ability to explain HS during the 2012 season based on whether nests were inundated/washed away by storm tides or not. The best fit linear model for S_N: y (HS) = 0.790 - 0.014*W, where β_0 = 0.790 and β_1W = - 0.014, β_0 (t = 18.49, p < 0.01) and β_1W (t = -1.82, p = 0.07) were both significant terms. Wash-over frequency was a weakly significant term in this model. The best fit linear model for S_Y: y (HS) = 0.208 - 0.012*W, where β_0 = 0.208 and β_1W = 0.012. While β_0 was a significant term for the S_Y model (t = 2.42, p = 0.03), β_1W was not a significant model term (t = -1.41, p = 0.18). Although wash-over frequency was a significant explanatory variable of HS for nests that did not experience storm-induced inundation/wash-away, wash-over frequency was not a significant predictor of HS for nests that experienced inundation/wash-away by storm tides during the 2012 season.

For the 2013 season, no linear models fit the data using only the term wash-over frequency as a predictor of HS. After adding the term inundation/wash-away to the model, all linear models still lacked fit. No relationship was evident between these predictor variables and HS since nonlinear curves also lacked fit.

It was predicted to take 16 (n = 70, 90% C.I. = [10.69, 47.98]) wash-over events to decrease HS below a predetermined value of 60% excluding nests that experienced storm-induced inundations and wash-away. The predicted number of wash-overs to decrease HS below the expected response could not be predicted for nests that experienced flooding or wash-away since all exhibited HS below 60% HS (Figure 2.11).

DISCUSSION

In this study, HS of loggerheads on South Island at the TYWC was not highly variable among years. During the 2012 nesting season, average HS for all nests laid on South Island beach was 65.2%, while average HS for the 2013 nesting season was 71.7%. When compared to other years at this site, HS was similar to that reported during the 2010 season (mean HS = 64.5%) and higher than HS reported for the 2011 season (mean HS = 55.1%) on South Island (Eskew 2012).

Loggerhead HS ranges from 53.1% to 83.8% with HS averaging approximately 54% in the Northwest Atlantic (Dodd 1988; Conant et al. 2009).When compared to data from the Northwest Atlantic population segment, as well as statewide HS data, loggerhead HS on South Island follows a similar pattern. Statewide nesting data shows average HS throughout South Carolina during the 2012 (69.9%) and 2013 seasons (65.0%) was similar to South Island. The HS reported in this study also appeared similar to results reported by project managers on the approximately 50 South Carolina beaches included in the nesting beach survey program over the past five loggerhead nesting seasons (mean HS 2009 - 2013 = 65.5%). This comparison is significant because South Island was the only nest protection project to leave a sample of nests to incubate *in situ* below the SHTL, while other nesting projects relocated all nests laid seaward of the SHTL in accordance with the guidelines stated by SCDNR and the U.S. Loggerhead Recovery Plan (NMFS and USFWS 1991, 2008; SCDNR 2014).

While nests vulnerable to tidal influences have been regarded as ‘doomed’ by numerous management and recovery plans throughout South Carolina and the southeastern U.S. (NMFS AND USFWS 1991, 2008; Bishop and Meyer 2011), the importance of the findings of the present study is substantial, since not all of the nests that experienced wash-over and/or storm-induced flooding failed. Not only do sea turtle nests left to incubate *in situ* in areas vulnerable to tidal wash-over and inundation often produce viable offspring as demonstrated by this study and several others (Whitmore and Dutton 1985; Mrosovsky 2006; Pike and Stiner 2007; Caut et al. 2010; Coll 2010; Shaw 2013), when included in the calculation of mean HS for two consecutive nesting seasons, HS on South Island was not highly variable from statewide data (SCDNR 2013; seaturtle.org database). During my study, HS on South Island (71.7%) was higher than the state average (65.0%) by nearly 7% during the 2013 season. The impacts of nest location and tidal influences on the HS of loggerheads nesting on South Island during the 2012 and 2013 seasons are discussed in detail in the following sections.

HS vs. Zone

The relationship between HS and zone (i.e. nest location defined as distance above or below the SHTL and whether a nest was relocated) was inconsistent between years. ANOVA indicated significant difference of mean HS between zones during the 2012 season, but not during the 2013 season. However, ANOVA included all nests in each zone, those that were impacted by tides and those that were not. Mean HS of nests that did not experience wash-over did not vary between zones, suggesting zone itself did not significantly impact HS.

During the 2012 season, mean HS of nests in zone 1 (58.5%) significantly differed from mean HS of nests in zone 2 (77.4%), suggesting nests located slightly above the SHTL, although still vulnerable to tidal influences, exhibited higher HS in 2012 than nests incubating below the SHTL that have the highest probability of wash-over and/or inundation. No significant difference between HS of nests in zones 1 (69.4%) and 2 (75.5%) was detected for the 2013 season. During the 2012 season, mean HS of nests relocated to zone 3-R (80.9%) was significantly higher than mean HS of nests left to incubate *in situ* below the SHTL in zone 1 (58.5%), suggesting nest relocation has the ability to significantly increase HS as reported in previous investigations throughout the southeastern U.S. (Stancyk et al. 1980; Hopkins and Murphy 1983; Wyneken et al. 1988; Eckert and Eckert 1990; Tuttle 2007; Bishop and Meyer 2011). However, no significant difference between HS of nests in zones 1 (69.4%) and 3-R (73.5%) was detected for the 2013 season. The observed difference among seasons regarding the relationship between HS of low nests in zone 1 with other *in situ* and relocated nests could be attributed to numerous wash-over and inundation events caused by an increase in storm activity and consequently storm-induced flooding associated with storm surge throughout the 2012 season that was not evident during the 2013 season (Figure 2.12; Table 2.9).The impacts of wash-over and inundation caused by storm surge and/or spring tides is further discussed in the following section.

Mean HS did not significantly differ between nests laid ≤ 3 m above the SHTL (zone 2) and nests laid > 3 m above the SHTL (zone 3-I), a relationship that was consistent between years. Although previous studies have suggested recovery plans include using some distance above the SHTL as a relocation guide instead of the SHTL itself, since it is difficult to predict if and when storm tides will exceed the previously marked SHTL (Mrosovsky 2006), results of this study suggest distance above the SHTL should not be used alone as a guide for nest relocations. However, using distance in combination with additional variables could be an effective strategy. For example, Wood and Bjorndal (2000) reported that slope had the greatest influence on nest-site selection by female loggerheads, most likely due to its association with nest elevation. Provancha and Erhart (1987) also reported loggerheads prefer steeply sloped beaches. Nests deposited at an increased slope and elevation are less likely to experience tidal wash-over or become inundated if they do wash-over (Mortimer 1982; Wood and Bjorndal 2000). Incorporating slope and elevation in combination with distance above the SHTL could enable participants to choose the most successful relocation sites.

A potential source of error in this investigation is that HS of *in situ* nests at two varying categorical distances (either ≤ 3 m above SHTL (zone 2) or > 3 m above SHTL (zone 3-I)) were compared to determine whether distance above the SHTL should be used as a relocation guide if one zone yielded higher HS than the other. In actuality, nests should have been relocated to two varying distance categories (either ≤ 3 m above SHTL or > 3 m above SHTL) and only HS of relocated nests compared. However, all nests in this study were relocated > 3 m above SHTL, the distance least vulnerable to tidal influences.

HS vs. Wash-over and Inundation

Although ANOVA indicated significant difference of mean HS between zones during the 2012 season, all nests in each zone (those that were impacted by tides and those that were not) were included in ANOVA. Mean HS of nests that did not experience wash-over did not vary between zones, suggesting zone itself did not significantly impact HS.

Results from two-way ANOVA indicated an interaction effect was present between the factors zone and wash-over. The relationship between wash-over and HS was inconsistent among zones and years. During the 2012 season, the only zone to be negatively impacted by tidal wash-over (in terms of a significant decrease in HS) was zone 1. However, this relationship was not evident during the 2013 season. Only relocated nests (zone 3-R) that experienced wash-over exhibited significantly lower HS in 2013, while wash-over did not impact HS in other zones, including zone 1 where the largest number of nests experienced wash-over ($n = 10$). The relationship between relocated nests and wash-over could not be determined for the 2012 season since no relocated nests experienced wash-over. A possible explanation for the failure of relocated nests that experienced wash-over in 2013 is alteration of the physical parameters of the original nest chamber at the relocation site. Changing physical parameters from the original nest chamber could alter embryonic development, HS, and ultimately hatchling survival (Carthy et al. 2003; Mrosovsky 2006; Foley 1998; Garmestani et al. 2000; Wood and Bjorndal 2000). The dimensions of the nest cavity reportedly influence the incubating environment (Carthy 1996) and if not reconstructed properly, the relocated chamber may not allow for sufficient drainage of heavy rain and seawater. Saturated sand around the nest has the ability to impede gas exchange which can lead to embryonic asphyxiation (Ackerman 1997; Foley et al. 2006). A potential source of error for this comparison is the low sample size of relocated nests to experience wash-over (n = 2) compared to those that did not (n = 48).

The only zone that showed a relationship between week laid and HS was zone 1 during the 2012 season. At the onset of the 2012 nesting season, South Carolina was faced with lower than average temperatures in the latter half of May and early June (NOAA 2012) that may have adversely impacted HS if incubation temperatures fell below a thermal tolerance of 25.0°C (Ackerman 1997). In addition, storm surge from early season hurricanes and tropical storms caused many incubating nests to wash-over, inundate, and/or wash-away early in the nesting season and led to a significant decrease in HS. The addition of storm-induced flooding as a predictor variable greatly improved the ability of the regression model to explain HS during the 2012 season. This improved model suggested HS was a nonlinear function of both week laid and whether nests experienced storm-induced inundation/wash-away during the 2012 season in zone 1. Although other zones experienced wash-over during storm tides, no other zones experienced inundation, most likely due to an increase in slope and elevation that is evident at greater distances from the SHTL on South Island (personal observations) that allows for better drainage. In this study, nests were only inundated during storm events during the 2012 season. No typical high tides or spring tides caused inundation during the 2012 season. However, beach erosion was high from several extreme spring tide events (as well as tropical storm activity) that led to many scarped foredunes, leveled dunes altogether, and flooded areas not typically exposed to seawater.

Wash-over frequency significantly differed in its ability to explain HS during the 2012 season based on whether nests experienced storm-induced inundation or wash-away. Although wash-over frequency was a significant explanatory variable of HS for nests that did not experience inundation, wash-over frequency was not a significant predictor of HS for nests that experienced storm-induced inundation during the 2012 season. Once a nest experienced inundation, HS decreased drastically (mean HS = 8.1%, n = 16) during the 2012 season when compared to nests that experienced wash-over (s) throughout the season but not inundation or wash-away (mean HS = 76.9%, n = 97). All nests that experienced one or more inundation events exhibited HS below 60%, considered nest failures (Table 2.9). However, viable offspring were produced in approximately 55% of nests that experienced inundation that did not wash-away during the course of the incubation period (n = 9). While the sample size of inundated nests was too small to make statistical comparisons regarding the effect of inundation frequency on HS, average HS of nests that experienced only one inundation event (mean HS = 18.4%; n = 5) was not substantially higher than the average HS of nests that experienced multiple inundation events (mean HS = 9.5%; n = 4) during the 2012 season.

For the 2013 season, no models fit the data using only the terms wash-over frequency and storm-induced inundation as predictors of HS. This can be explained by the decreased sample size of low nests in zone 1, the location most vulnerable to tidal influences, included in wash-over analyses during the 2013 season (n = 15) compared to the 2012 season (n = 56). Also, there was less wash-over, inundation, and storm activity in 2013 (Table 2.3; Table 2.9). However, several relocated nests (zone 3-R, n = 2) and nests located *in situ* above the SHTL (zone 2, n = 5; zone 3-I, n = 1) experienced wash-over, but not inundation, during Tropical Storm Andrea in early June 2013. The only 2 nest inundations that occurred in 2013 were caused by extreme spring tides that were accompanied by high northeasterly winds. Both of these nests produced viable offspring (Table 2.9).

Results from the 2012 season suggest that the occurrence of even one inundation event by storm surge may have more adverse effects on loggerhead HS than numerous wash-overs experienced during normal high tide events. These inundation events coincided with tropical storms and hurricanes throughout 2012. All nests inundated during tropical storm events had a HS below 60%. Average HS for nests inundated by tropical storms in Georgia has also been reported to be less than 60%, with inundated nests averaging 45% HS in 2008 (Dodd and Mackinnon 2008). These storm events were characterized by storm surge, sea levels above the normal mean high level, and often heavy rainfall. Storm surge causes mortality by flooding the egg chamber, consequently drowning developing embryos, and causing partial and/or complete wash-away of incubating nests (Milton et al. 1994; Caut et al. 2010).

While other studies have reported only loggerhead nests laid late in the nesting season experienced storm-induced inundation due to the latter part of the nesting season coinciding with peak hurricane season (Pike and Stiner 2007), inundations and wash-away events associated with storm surge were more common during the beginning of the

2012 season in this study. It is also important to note that all inundated nests during the 2012 season experienced inundation during the 1st third of their incubation period. The lower HS reported in these nests may be correlated to the timing of the inundation event(s) since embryonic asphyxiation caused by inundation is reportedly more prone during the early stage of embryonic development for various species of sea turtles (Whitmore and Dutton 1985; Limpus 1985; Foley et al. 2006; Trullas and Paladino 2007).

A potential source of error in this study was not determining the stage of development in which embryonic arrest occurred during the 2012 season. Staging unhatched eggs to determine when embryonic mortality occurred can allow adjustment of the number of wash-overs it took to cause nest failure (or decrease HS). For example, a nest reported to have washed over 15 times in analyses examining the effects of wash-over frequency on HS may have actually failed after only 5 wash-overs. To correct for this error, eggs that failed to hatch were staged based on revised criteria of Whitmore and Dutton (1985) during the 2013 season in order to determine what stage of development embryonic mortality occurred (early, middle, or late stage) (Table 2.4). This data was used in combination with wash-over data as an attempt to adjust the number of tidal wash-over events it took to cause embryonic mortality within a given nest. Unfortunately, staging data did not allow for the correction of any wash-over frequencies for the 2013 season because 1) all wash-overs on a given nest either occurred during the same stage or 2) the egg contents of washed over nests were unable to be determined. For example, if a nest experienced 4 wash-overs all during the early stage of incubation, which event caused mortality was unable to be determined. However, if a nest had experienced 4 wash-overs, 1 of which occurred during the early stage and 3 of which occurred during the late stage, and all unhatched eggs contained embryos that fell into the early stage category, the assumption that it only took the 1 wash-over early in development to cause failure could have been made, thus being able to adjust the number of wash-overs from 4 to 1. More detailed criteria consisting of 30 + stages has been published (Dodd 1988), but requires detailed training and laboratory work to pinpoint the day of mortality for each individual embryo. Time and resources were not available for this advanced procedure during the course of this study.

An additional source of error in this study is the method used to detect inundation. Inundation was defined as a nest in standing water, or the occurrence of flooding evident at the top of the nest. However, nests that have not been washed by tides may have also experienced the adverse effects of inundation because of immersion due to the water table below the surface of the beach (Caut et al. 2010). The height of the water table was not assessed and it is therefore likely that the number of nests affected by immersion below the sand surface was underestimated. Foley et al. (2006) addressed this problem by burying PVC pipes with holes drilled at various depths to monitor groundwater inundation while assessing the effects of sand characteristics and inundation on HS of loggerhead clutches in southwest Florida. Shaw (2013) also determined the maximum amount of inundation inside the nest cavity by installing piezometers in a sample of incubating nests in southwest Florida.

Comparison of This Study to Previous Work in South Carolina

It was predicted to take approximately 16 wash-over events (n = 70, 90% C.I. = [10.69, 47.98]) wash-over events to decrease HS below a predetermined value of 60% excluding nests that experienced storm-induced inundations and wash-away. The predicted number of wash-overs to decrease HS below the expected response could not be predicted for nests that experienced storm-induced inundation because all nests that experienced flooding or wash-away were below 60% HS. Width of the confidence interval shows the variability in the number of wash-overs it would take to decrease HS of low nests below a successful level. This variability could be attributed to the contribution of other factors reported to influence loggerhead HS that were not measured in this study such as slope, elevation, temperature, nest depth, proximity to vegetation, sand grain size, sand water salinity and groundwater inundation on the HS of a given nest impacted by tidal influences (Wood and Bjorndal 2000; Miller et al. 2003; Foley et al. 2006).

Since this study is a continuation of an investigation conducted by Coll (2010) on loggerhead nesting beaches in South Carolina (Edisto Beach State Park, Folly Beach, and Fripp Island), it is important to make comparisons between methods and findings. Coll's study predicted it would take 4 or more wash-overs (confidence interval not reported) to significantly decrease HS below a predicted 60%. However, nests included in her study consisted only of relocated nests and *in situ* nests above the SHTL; none of the nests included in analyses incubated below the SHTL. Instead of leaving nests below the SHTL and monitoring their fate throughout the season, participants relocated all nests seaward of the SHTL and placed wooden stakes near the original *in situ* nest site (initial sites). Project participants monitored the initial nest site location for wash-overs or wash-away events (defined as a stake falling over, becoming exposed, or washing away) during daily nest surveys. The difference in methodology between studies is substantial since this investigation included nests incubating below the SHTL and was able to accurately determine 1) the number of wash-overs it would take nests incubating at this location to decrease HS, 2) if a nest actually washed away during a tidal event (opposed to a stake washing away) and 3) determine the HS of nests left to incubate below the SHTL. Based upon the results of this study, a stake washing away during a tidal event at a given site is not an accurate assessment of whether a nest would have washed away at that site. Throughout the 2012 and 2013 nesting seasons, stakes and screens of nests in zone 1 occasionally washed away during high tides, but the eggs remained inside the nest cavity. Some of these nests still exhibited very high HS. For example, nest 80 during the 2013 season experienced 8 wash-over events, had stakes wash away 3 times, the screen wash away once, and yielded a HS of 99% (the highest of any nest during the 2013 season). It is also important to reiterate that the relationship between wash-over and location differed within and between years. *In situ* nests at higher beach zones, even those marginally above the SHTL, were not negatively impacted by wash-overs in this study, but were impacted in Coll's study and were reported to exhibit reduced HS when compared to nests that did not experience wash-over above the SHTL by Whitmore and Dutton (1985) in green and leatherback clutches.

CONCLUSIONS

While the results of other studies support the conclusion that the occurrence of wash-over and inundation can significantly decrease HS in loggerheads (Foley et al. 2006; Pike and Stiner 2007; Coll 2010; Shaw 2013) and other species of sea turtles (Whitmore and Dutton 1985; Caut et al. 2010), it is important to remember that some hatchlings are still produced in these nests (Whitmore and Dutton 1985; Hilterman 2001; Foley et al. 2006; Mrosovsky 2006; Pike and Stiner 2007; Caut et al. 2010; Coll 2010; Shaw 2013). Throughout the course of this study, many nests survived tidal events remarkably well. Results from the 2012 season suggest that the occurrence of inundation caused by storm surge may have more adverse effects on loggerhead HS than numerous wash-overs experienced during normal high tide events. Ernest and Martin (1993) similarly reported that frequent or prolonged inundation significantly decreased nest success while occasional wash-over events did not negatively impact loggerhead nests in Florida. In the present study, inundation events coincided with tropical storms and hurricanes throughout 2012. Storm surge caused mortality during this study by flooding the egg chamber, consequently drowning developing embryos, and causing partial and/or complete wash-away of incubating nests, which has also been reported in other investigations (Milton et al. 1994; Caut et al. 2010).

While HS of nests left below the SHTL may have been significantly lower than HS reported in other zones during the 2012 season, it was only slightly lower than the recommended 60% (58.5%). Also, in 2013 the HS of nests below the SHTL did not differ from that of other locations. Due to the concerns regarding the use of nest relocations, the results of this study indicate that relocations should be performed conservatively since the majority of low beach nests produced viable offspring. In this study, monitoring a sample of nests left to incubate *in situ* below the SHTL in order to assess whether they would be 'doomed' without relocation as a nest management tool, lead to the finding that these nests are far from 'doomed'. Further conservation and management implications of nest relocations and the impacts of wash-over are discussed in Chapter IV.

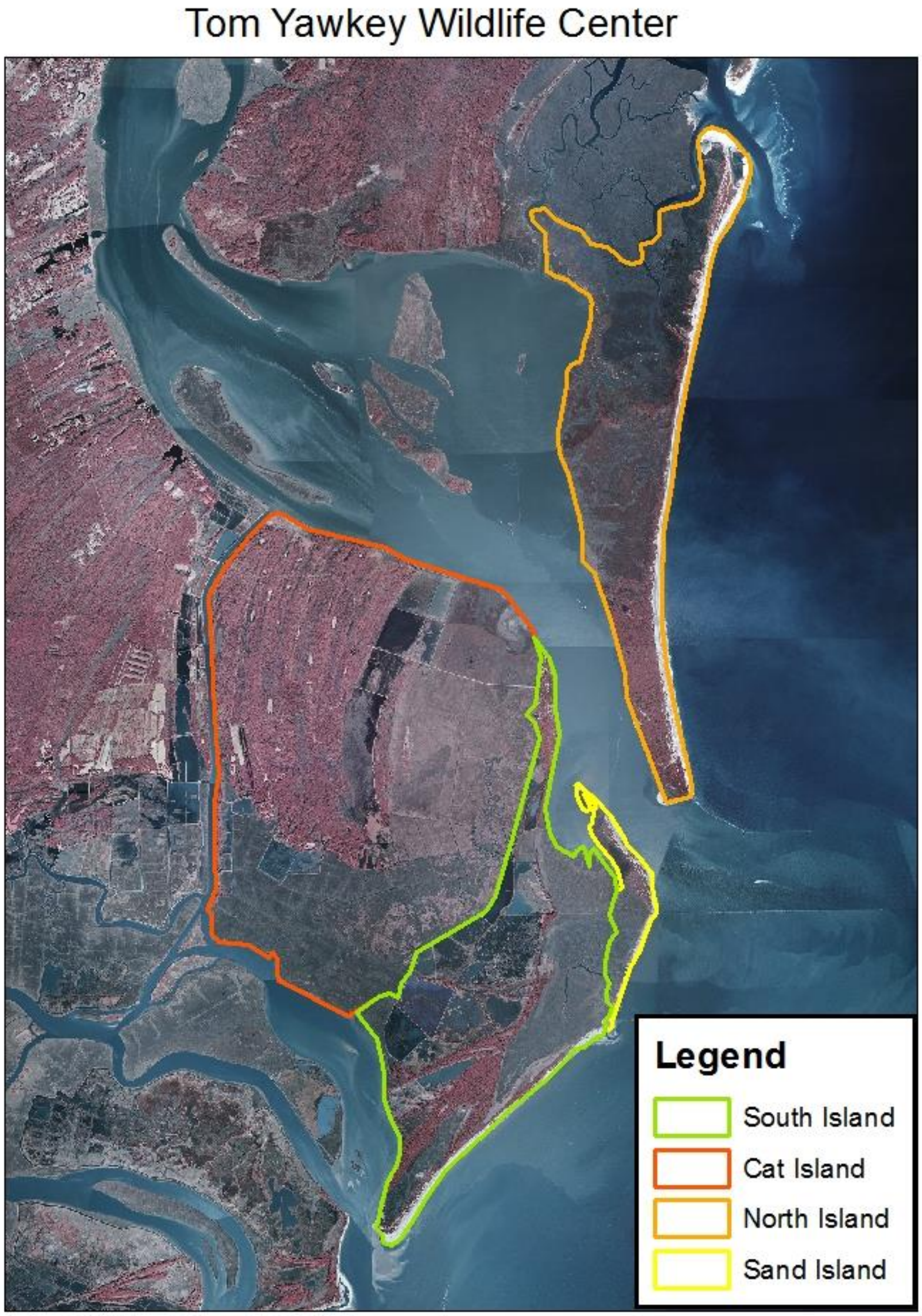


Figure 2.1: Study area within the Tom Yawkey Wildlife Center in Georgetown County, South Carolina. Loggerhead nests in this study were sampled along the Atlantic coast of South Island (outlined in green) (image Eskew 2012).

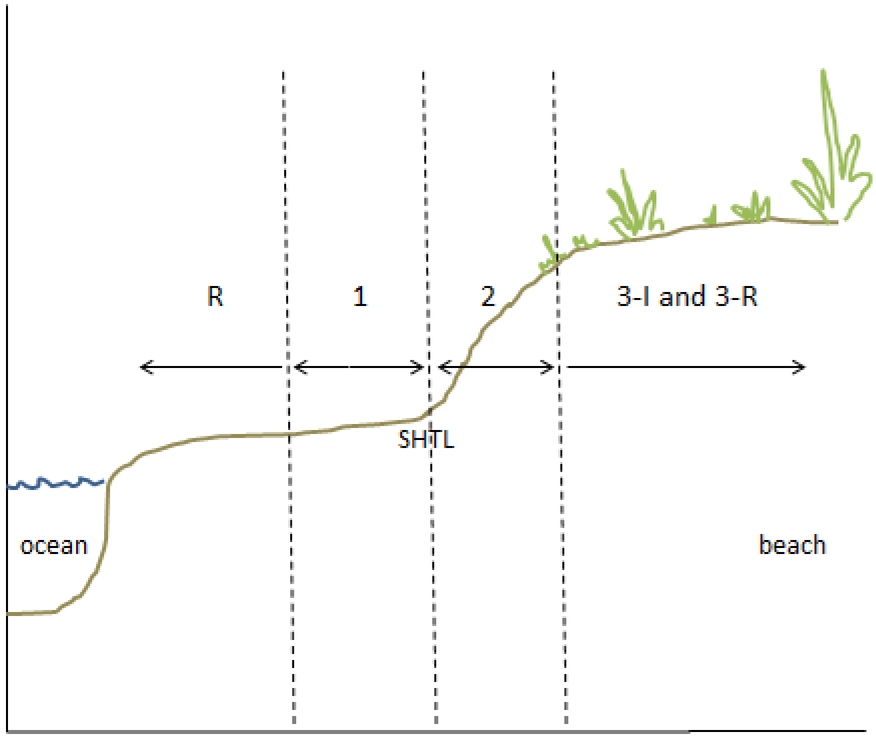
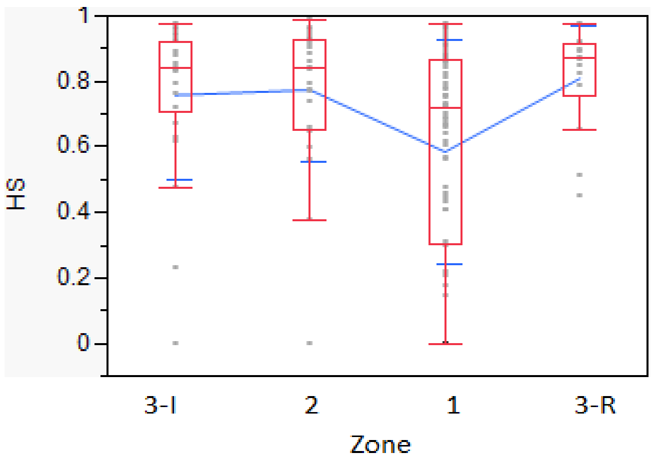


Figure 2.2: Schematic representation of South Island beach profile at the Tom Yawkey Wildlife Center, Georgetown County, South Carolina displaying zones as defined by distance from the SHTL, *in situ* or relocated, and probability of inundation. Legend: **R** = nests laid below the SHTL by > 3 m, highest probability of inundation, relocate all nests to 3-R; **1** = zone 1: nests laid at the SHTL or below the SHTL by ≤ 3 m (low nests), high probability of inundation, *in situ* all (2012), *in situ* 33% and relocate 66% to 3-R (2013); **2**= zone 2: nests laid above the SHTL by ≤ 3 m, medium probability of inundation, *in situ* all; **3-I** = zone 3 *in situ*: nests laid above the SHTL by > 3 m and left to incubate *in situ*, lowest probability of inundation, *in situ* all; **3-R** = zone 3 relocated: all nests laid below the SHTL by > 3 m (2012 and 2013) and 66% of nests laid ≤ 3 m below the SHTL in 2013 were relocated to this zone > 3 m above the SHTL, lowest probability of inundation.

Figure 2.3: Pictures of nest set up with wash-over cups used to determine whether a tidal wash-over event occurred at a nest during the previous high tide throughout the 2012 and 2013 loggerhead nesting seasons on South Island beach at Tom Yawkey Wildlife Center, Georgetown County, South Carolina.

Figure 2.4: Illustration of wash-over criteria in the absence of seawater filling the wash-over cup of a given nest. The occurrence of a tidal wash-over event was then determined by observing whether the previous high tide line covered > 50% of the clutch centered beneath the screen. The blue line indicates the previous high tide.

a. 2012



b. 2013

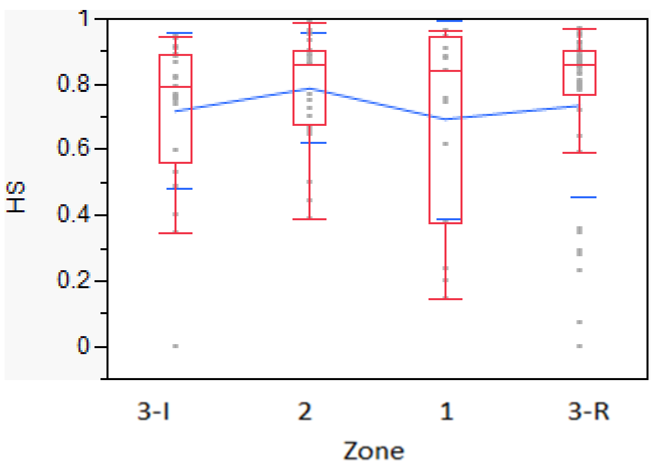
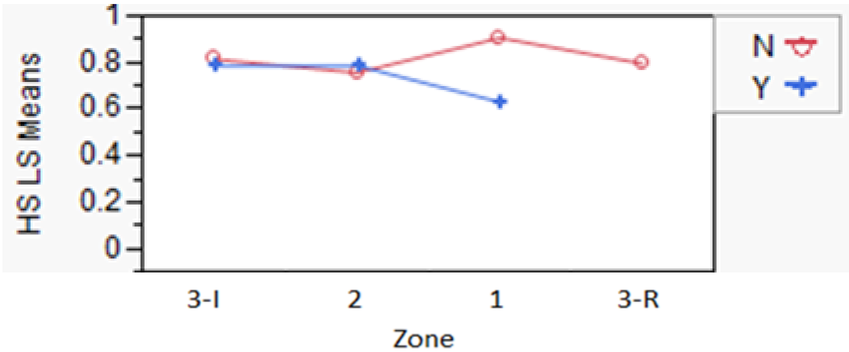


Figure 2.5: The relationship between zone and mean HS of loggerhead nests on South Island at Tom Yawkey Wildlife Center, Georgetown County, South Carolina during a) the 2012 nesting season and b) 2013 nesting season, displayed in outlier box plots. Blue lines are connecting means for comparison. The horizontal line within each box indicates the sample median. Lines extend from each end of the box to the highest and lowest value within 1.5 IQR of the upper and lower quartile, respectively. Horizontal blue lines within each plot represent one standard deviation above and below the mean. ANOVA indicated mean HS differed among the zones ($F = 5.30$, $p < 0.01$, $\alpha = 0.10$) during the 2012 season. Fisher's LSD indicated nests in zone 1 had a significantly lower mean HS than nests in other zones in 2012 ($t = -2.79$, $p < 0.01$, $\alpha = 0.10$). ANOVA indicated no significant differences in mean HS between the zones during the 2013 season ($F = 0.68$, $p = 0.57$, $\alpha = 0.10$).

a. 2012



b. 2013

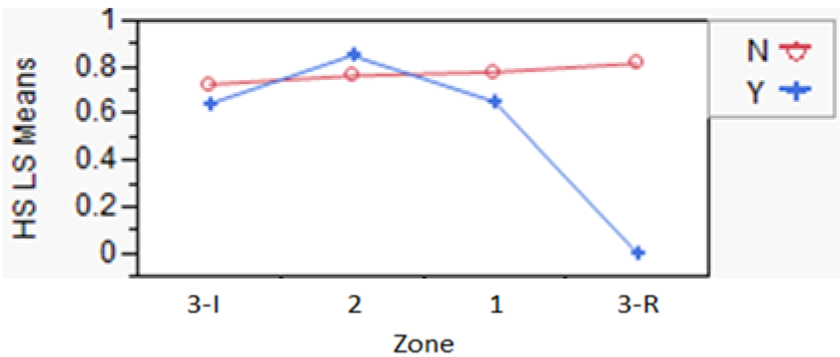


Figure 2.6: Profile plots representing the simple effects and interaction effects of zone and wash-over during a) the 2012 loggerhead nesting season and b) the 2013 loggerhead nesting season on South Island at Tom Yawkey Wildlife Center in Georgetown County, South Carolina. LS mean HS of nests that did not experience wash-over (N) are denoted by the red lines and LS mean HS of nests that experienced one or more wash-over events throughout the incubation period (Y) are denoted by blue lines.

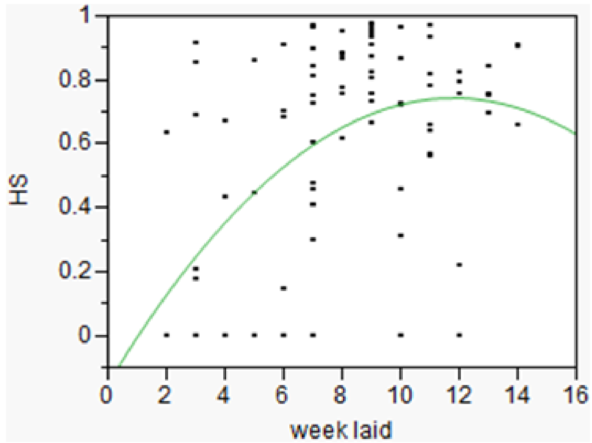


Figure 2.7: Nonlinear relationship between week laid and HS for zone 1 during the 2012 loggerhead nesting season on South Island at Tom Yawkey Wildlife Center, Georgetown County, South Carolina. The best fit model (R^2 = 0.25, RMSE = 0.30, n = 90) was a 2nd degree polynomial: y (HS) = 0.2512 + 0.0500*L – 0.0064*(L – 7.9)².

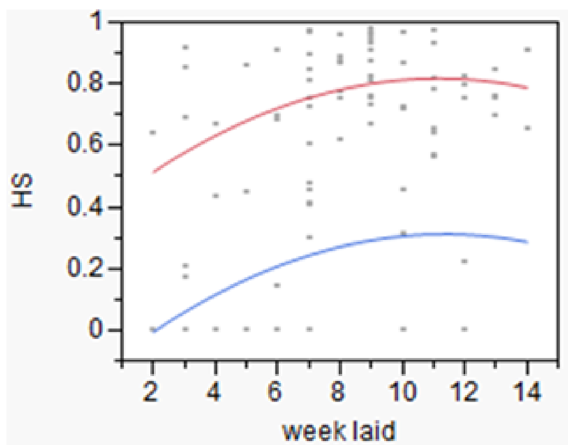


Figure 2.8: Nonlinear relationship between week of nest initiation and HS in zone 1 during the 2012 loggerhead nesting season on South Island at Tom Yawkey Wildlife Center, Georgetown County, South Carolina after adding the model term 'storm-induced inundation/wash-away'. The red line represents the nonlinear relationship between week laid and HS for nests that did not experience inundation/wash-away during storm tides. The blue line represents the nonlinear relationship between week laid and HS of nests impacted by storm tides. After adding this term, the 2nd order polynomial regression model greatly improved (R^2 = 0.68, RMSE = 0.19, n = 90): y (HS) = 0.3318 + 0.0243*L + 0.2554*S – (0.0036*((L – 7.9)*(L – 7.9)).

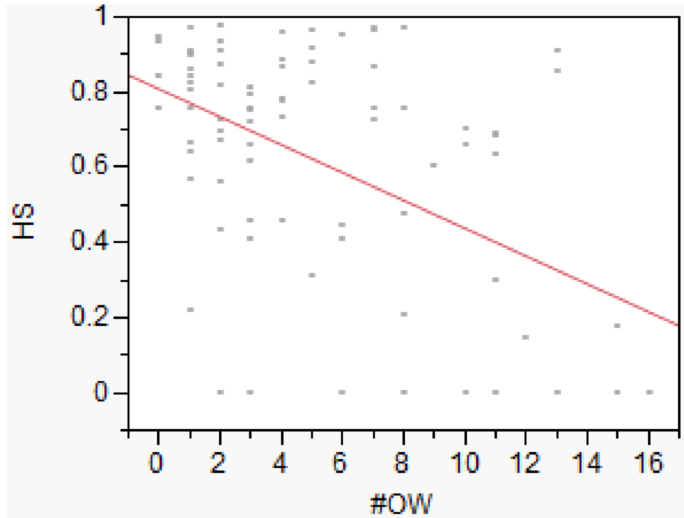


Figure 2.9: Linear relationship between wash-over frequency and HS in zone 1 during the 2012 nesting season on South Island at Tom Yawkey Wildlife Center, Georgetown County, South Carolina. In the best fit linear model: y (HS) = 0.808 – 0.037*W (R^2 = 0.22, RMSE = 0.29, n = 86), the term wash-over frequency was significant (t = -4.91, $p < 0.01$).

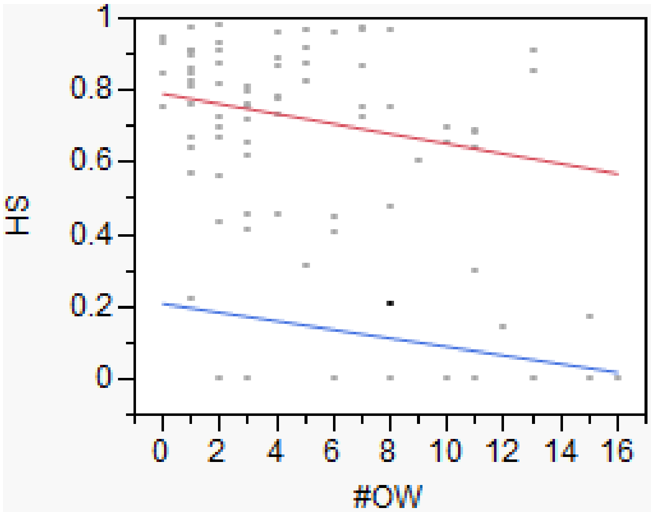


Figure 2.10: Linear relationship between wash-over frequency and HS across all zones after adding the model term ‘storm-induced inundation/wash-away’ during the 2012 season on South Island at Tom Yawkey Wildlife Center, Georgetown County, SC (R^2 = 0.60, RMSE = 0.21, n = 86). Wash-over frequency was a significant predictor of HS (t = -1.82, p = 0.07) for the best fit linear model of S_N: y (HS) = 0.790 - 0.014*W. Wash-over frequency was not a significant predictor of HS (t = -1.41, p = 0.18) for the best fit linear model of S_Y: y (HS) = 0.208 - 0.012*W.

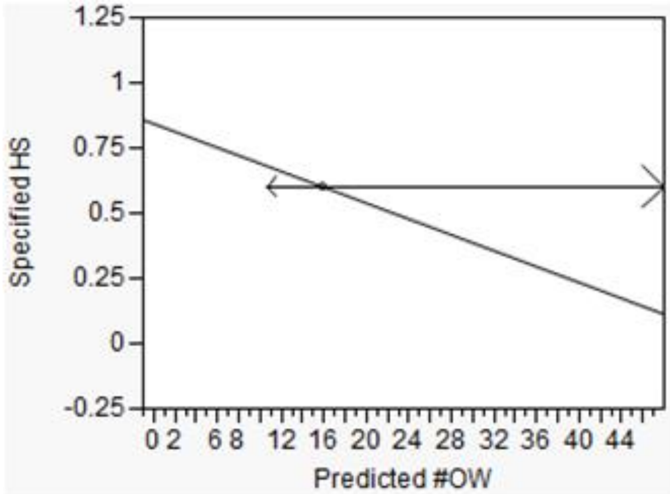
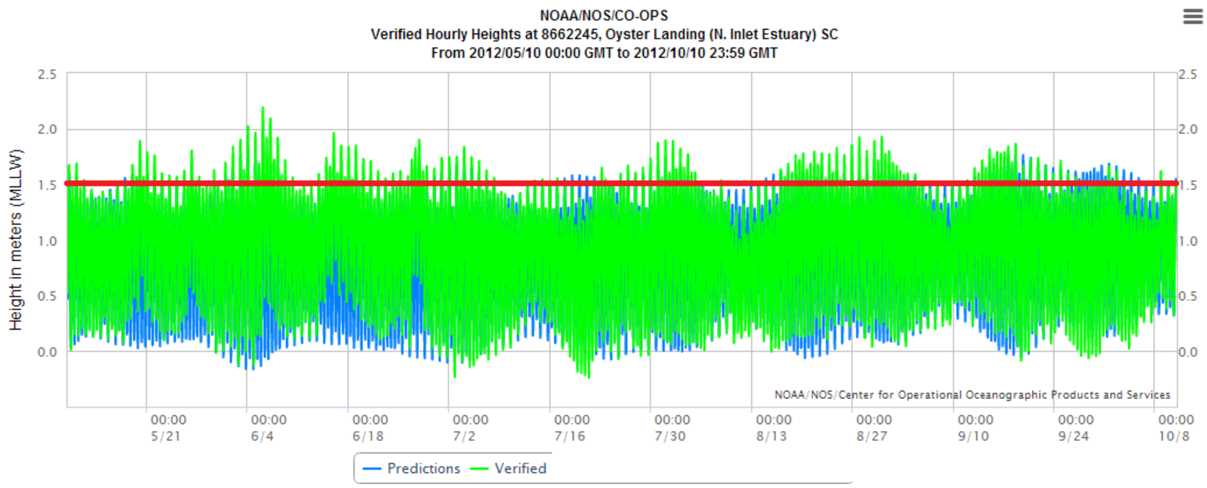


Figure 2.11: Predicted number of wash-overs (#OW) to decrease loggerhead HS below a specified 60% (considered a nest failure) during the 2012 nesting season on South Island at Tom Yawkey Wildlife Center, Georgetown County, South Carolina. It was predicted to take 16 (n = 70, 90% C.I. = [10.69, 47.98]) wash-over events to decrease HS below a predetermined value of 60% excluding nests that experienced storm-induced inundations and wash-away. The predicted number of wash-overs to decrease HS below the expected response could not be predicted for nests that experienced storm-induced inundation because all nests that experienced flooding or wash-away were below 60% HS.

a.



b.

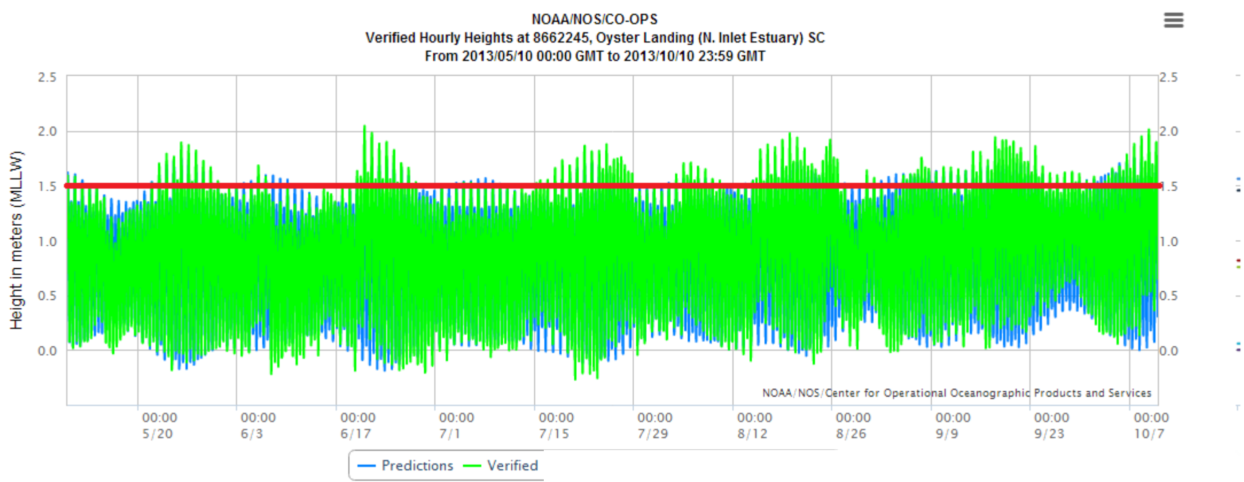


Figure 2.12: Plot of hourly tide data (height in meters) from Oyster Landing (North Inlet Estuary) tide station located at Hobcaw Barony, Georgetown County, South Carolina between a) May 10 - October 10, 2012 and b) May 10 - October 10, 2013. MLLW represents the tidal datum mean lower low water (NOAA/NOS/CO-OPS 2013). Nest wash-over and/or inundation often occurred when MLLW exceeded 1.5 meters (represented by the horizontal red line).

Table 2.1: Losses that occurred during the loggerhead sea turtle incubation period prior to hatching on South Island at Tom Yawkey Wildlife Center, Georgetown County, South Carolina during the nesting season (May - October) 2012 and 2013.

Year	2012	2013	TOTAL
Total nests	167	137	304
Eggs lost to probing	88	70	158
Eggs lost to coyotes	278	182	460
Eggs lost to ghost crabs	22	10	32
Eggs lost to others nesting efforts	0	25	25
Eggs lost - other	1	9	10
Nests lost to poaching	1	0	1
Nests depredated by ghost crabs	18	3	21
Nests depredated by coyotes	4	3	7
Nests washed away	9	0	9

Table 2.2: Nesting and wash-over data collected throughout the incubation period for all nests laid on South Island beach at the Tom Yawkey Wildlife Center, Georgetown County, SC during the 2012 nesting season. Red indicates nests with a hatch success < 60%. Nests that experienced partial depredation by coyotes throughout the incubation period are denoted by PD following the zone in which they were laid. Loss experienced during the incubation period includes eggs lost to probing and/or depredation by ghost crabs or coyotes.

Nest #	Zone	Inundated and/or washed-away by storm tides	# of wash-overs	Date laid	Hatch Success	Experienced loss during incubation
1	3-I	N	0	11-May-12	83.80%	N
2	3-I (PD)	N	0	16-May-12	12.70%	Y
3	1	Y	16***	16-May-12	0.00%	Y
4	1	N	11	16-May-12	63.50%	N
5	3-I	N	0	21-May-12	94.50%	N
6	1	N	24	21-May-12	0.00%	Y
7	1	N	5	22-May-12	91.50%	N
8	1	N	11	22-May-12	68.90%	N
9	1 (PD)	N	0	22-May-12	29.80%	Y

10	1 (PD)	N	0	23-May-12	7.00%	Y
11	1	N	13	24-May-12	85.20%	N
12	1	Y	9***	24-May-12	0.00%	Y
13	1	N	15**	25-May-12	17.20%	N
14	1	Y	8***	25-May-12	0.00%	Y
15	3-I	N	0	25-May-12	82.60%	Y
16	1	N	8**	26-May-12	20.70%	N
17	3-I	N	3*	28-May-12	0.00%	N
18	2	N	3	28-May-12	77.10%	N
19	1	Y	9***	28-May-12	0.00%	N
20	3-R	N	0	28-May-12	97.40%	Y
21	3-I	N	0	29-May-12	92.30%	N
22	1	N	11**	29-May-12	0.00%	N
23	1	Y	10***	29-May-12	0.00%	N
24	1	Y	8***	29-May-12	0.00%	N
25	2	N	2	29-May-12	37.40%	N
26	3-I	N	9*	30-May-12	0.00%	Y
27	2	N	0	31-May-12	86.10%	N
28	3-I	N	0	31-May-12	92.20%	N
29	1	Y	6***	31-May-12	0.00%	N
30	1	N	2	31-May-12	66.90%	Y
31	3-I	N	0	1-Jun-12	76.00%	Y
32	1	N	13**	2-Jun-12	0.00%	N
33	1	N	2**	2-Jun-12	43.30%	Y
34	3-I	N	0	3-Jun-12	67.30%	N
35	3-I	N	0	3-Jun-12	89.30%	N
36	3-I	N	0	6-Jun-12	95.50%	N
37	2	N	1	6-Jun-12	84.20%	N
38	2	N	0	7-Jun-12	0.00%	N
39	2	N	0	7-Jun-12	90.00%	N
40	3-I	N	0	8-Jun-12	61.40%	N
41	3-I	N	0	8-Jun-12	97.70%	N
42	2	N	1	8-Jun-12	76.50%	N
43	1	N	1	8-Jun-12	86.10%	N
44	2	N	0	8-Jun-12	92.80%	N
45	1	N	6	9-Jun-12	44.30%	N
46	1	Y	6***	9-Jun-12	0.00%	N
47	1	N	11	10-Jun-12	68.00%	N
48	1	N	13**	10-Jun-12	0.00%	Y

49	1	N	13	10-Jun-12	90.60%	N
50	3-I	N	0	10-Jun-12	47.70%	N
51	1	N	12**	11-Jun-12	14.30%	N
52	3-R	N	0	11-Jun-12	89.70%	N
53	1	N	10	13-Jun-12	69.80%	N
54	2	N	0	14-Jun-12	88.10%	N
55	3-I	N	0	16-Jun-12	85.50%	N
56	1	N	5	17-Jun-12	96.40%	Y
57	3-I	N	0	17-Jun-12	96.60%	N
58	1	N	1	17-Jun-12	84.40%	Y
59	1	N	9	18-Jun-12	60.50%	N
60	1	N	1	18-Jun-12	89.60%	N
61	2	N	0	18-Jun-12	73.60%	Y
62	2	N	1	18-Jun-12	96.10%	N
63	1	N	6	19-Jun-12	40.50%	N
64	1	N	11	19-Jun-12	29.50%	N
65	1	Y	3***	19-Jun-12	0.00%	N
66	1	N	3	20-Jun-12	41.00%	N
67	2	N	0	20-Jun-12	55.90%	N
68	1	N	3	20-Jun-12	80.90%	Y
69	3-R	N	0	21-Jun-12	51.20%	N
70	1	N	3	21-Jun-12	75.00%	N
71	1	N	7	22-Jun-12	72.60%	N
72	1	N	8	22-Jun-12	0.00%	N
73	3-R	N	0	22-Jun-12	87.30%	N
74	1	N	8	22-Jun-12	47.30%	N
75	1	N	1	23-Jun-12	96.90%	N
76	1	N	3	23-Jun-12	45.60%	N
77	1	N	11**	23-Jun-12	0.00%	N
78	2	N	3	24-Jun-12	79.40%	N
79	1	N	4	24-Jun-12	88.30%	N
80	3-R	N	0	25-Jun-12	92.00%	Y
81	3-R	N	0	25-Jun-12	44.90%	Y
82	3-I	N	0	26-Jun-12	22.80%	Y
83	1	N	3	26-Jun-12	61.40%	N
84	3-I	N	0	28-Jun-12	92.90%	N
85	3-I	N	1*	28-Jun-12	71.90%	N
86	3-I	N	0	28-Jun-12	79.20%	N
87	1	N	5	28-Jun-12	87.50%	N

88	3-R	N	0	29-Jun-12	78.90%	Y
89	3-R	N	0	29-Jun-12	91.90%	Y
90	1	N	8	29-Jun-12	75.40%	N
91	1	N	4	29-Jun-12	77.20%	N
92	1	Not monitored	Not monitored	30-Jun-12	33.30%	Y
93	1	N	6	30-Jun-12	95.40%	Y
94	1	N	4	30-Jun-12	86.50%	N
95	1	Wild nest	Wild nest	unknown	0.00%	Y
96	1	N	3	1-Jul-12	75.80%	N
97	3-R	N	0	1-Jul-12	82.40%	N
98	3-R	N	0	1-Jul-12	91.60%	N
99	1	N	2	2-Jul-12	93.20%	Y
100	3-R	N	0	3-Jul-12	86.70%	Y
101	2	N	1	3-Jul-12	93.10%	N
102	1	N	4	3-Jul-12	86.80%	N
103	1	N	4	3-Jul-12	73.20%	Y
104	3-I	N	0	4-Jul-12	84.40%	N
105	1	N	8	4-Jul-12	96.80%	N
106	2	N	2	4-Jul-12	95.60%	Y
107	3-I	N	0	4-Jul-12	83.50%	N
108	1	N	2	4-Jul-12	87.00%	Y
109	3-I	N	0	4-Jul-12	92.00%	N
110	1	N	4	4-Jul-12	95.60%	N
111	1	N	1	5-Jul-12	66.30%	N
112	1	N	7	5-Jul-12	75.40%	Y
113	3-R	N	0	6-Jul-12	89.30%	N
114	1	N	0	6-Jul-12	94.40%	N
115	1	N	2	6-Jul-12	97.60%	N
116	1	N	1	6-Jul-12	82.20%	N
117	1	N	1	7-Jul-12	80.70%	N
118	1	N	1	7-Jul-12	91.10%	N
119	2	N	0	7-Jul-12	64.70%	N
120	3-I	N	0	8-Jul-12	62.90%	Y
121	1	N	5**	8-Jul-12	31.00%	Y
122	3-I	N	0	8-Jul-12	87.60%	N
123	1	N	7	9-Jul-12	86.60%	N
124	1	N	2	11-Jul-12	72.20%	Y
125	2	N	0	11-Jul-12	92.70%	N
126	3-I	N	0	13-Jul-12	89.10%	N

127	1	N	2	13-Jul-12	0.00%	N
128	1	N	7	13-Jul-12	96.30%	N
129	1	N	3	14-Jul-12	71.60%	N
130	1	N	4**	14-Jul-12	45.60%	Y
131	3-R	N	0	14-Jul-12	84.60%	Y
132	1	N	1	15-Jul-12	63.70%	Y
133	1	N	7	16-Jul-12	96.90%	N
134	3-I	N	0	16-Jul-12	93.80%	N
135	1	N	0	17-Jul-12	93.20%	N
136	2	N	0	17-Jul-12	94.50%	N
137	1	N	1	17-Jul-12	56.50%	Y
138	1	N	10	18-Jul-12	65.50%	Y
139	1	N	2**	19-Jul-12	55.90%	N
140	2	N	0	19-Jul-12	91.40%	N
141	2	N	0	20-Jul-12	83.70%	Y
142	1	N	2	20-Jul-12	81.90%	N
143	1	N	4	21-Jul-12	78.30%	Y
144	1	N	3	22-Jul-12	79.30%	Y
145	1	N	6**	22-Jul-12	0.00%	N
146	2	N	0	22-Jul-12	59.40%	Y
147	3-R	N	0	22-Jul-12	65.30%	N
148	2	N	1	23-Jul-12	65.50%	Y
149	1	N	5	24-Jul-12	82.20%	N
150	1	N	1**	25-Jul-12	21.60%	N
151	1	N	0	26-Jul-12	75.40%	Y
152	2	N	1	27-Jul-12	98.80%	N
153	1	N	8	29-Jul-12	75.20%	N
154	2	N	1	30-Jul-12	64.60%	N
155	3-I	N	1*	31-Jul-12	85.20%	N
156	2	N	0	1-Aug-12	94.60%	Y
157	1	N	0	1-Aug-12	84.30%	N
158	1	N	1	1-Aug-12	75.70%	Y
159	1	N	2	3-Aug-12	69.50%	N
160	1	N	1	5-Aug-12	90.50%	Y
161	1	N	2	5-Aug-12	90.90%	Y
162	1	N	3	6-Aug-12	65.50%	Y
163	3-I	N	0	9-Aug-12	83.30%	N
164	3-I	N	0	9-Aug-12	83.70%	N
165	1	Not monitored	Not monitored	unknown	unknown	Not monitored

166	1	Not monitored	Not monitored	unknown	unknown	Not monitored
167	1	Wild nest	Wild nest	unknown	83.40%	Wild nest

* Nest was > 3m above SHTL and experienced wash-over during storm tide(s)
** Nest experienced storm-induced inundation while
*** Nest washed away during a storm tide

Table 2.3: Nesting and wash-over data collected throughout the incubation period for all nests laid on South Island beach at the Tom Yawkey Wildlife Center, Georgetown County, SC during the 2013 nesting season. Red indicates nests with a hatch success < 60%. Nests that experienced partial depredation by coyotes throughout the incubation period are denoted by PD following the zone in which they were laid. Loss experienced during the incubation period includes eggs lost to probing and/or depredation by ghost crabs or coyotes.

Nest #	Zone	Inundated and/or washed-away by storm tides	# of wash-overs	Date laid	Hatch Success	Experienced loss during incubation
1	3-I	N	0	26-May-13	39.9%	N
2	3-I	N	0	26-May-13	91.5%	N
3	3-I	N	0	26-May-13	88.8%	N
4	3-R	N	0	26-May-13	34.4%	Y
5	3-R	N	0	26-May-13	78.6%	Y
6	1	N	0	26-May-13	19.9%	N
7	3-I	N	0	26-May-13	75.9%	N
8	3-R	N	0	28-May-13	80.0%	N
9	2	N	1	29-May-13	72.3%	N
10	2	N	0	30-May-13	44.6%	N
11	3-R	N	0	30-May-13	88.9%	N
12	3-I	N	0	30-May-13	59.8%	N
13	2	N	2	31-May-13	86.1%	N
14	1	N	4	01-Jun-13	87.6%	N
15	3-R	N	0	02-Jun-13	7.0%	Y
16	2	N	2	02-Jun-13	87.3%	N
17	2	N	3	03-Jun-13	85.4%	N
18	3-R	N	1*	05-Jun-13	0%	N
19	3-I	N	1*	05-Jun-13	77.1%	Y

20	1	N	3	05-Jun-13	76.2%	Y
21	3-R	Not Monitored	Not Monitored	05-Jun-13	unknown	Y
22	3-R	Not Monitored	Not Monitored	05-Jun-13	unknown	Y
23	3-R	N	0	06-Jun-13	36.1%	N
24	2	N	0	07-Jun-13	50.0%	N
25	3-I	N	1*	07-Jun-13	48.8%	N
26	3-R	N	1*	08-Jun-13	0%	N
27	3-R	N	0	08-Jun-13	59.2%	N
28	1	N	2	08-Jun-13	23.5%	N
29	3-I	N	0	08-Jun-13	34.5%	N
30	3-I	N	1*	08-Jun-13	79.5%	N
31	2	N	0	08-Jun-13	70.3%	N
32	3-R	N	0	09-Jun-13	85.8%	N
33	2	N	3	09-Jun-13	81.4%	Y
34	3-R	N	0	10-Jun-13	28.9%	Y
35	1	N	4	10-Jun-13	74.6%	N
36	2	N	0	10-Jun-13	39.1%	N
37	2	N	2	10-Jun-13	88.3%	N
38	3-R	N	0	11-Jun-13	78.2%	Y
39	3-R	N	0	13-Jun-13	85.6%	N
40	1	N	4	13-Jun-13	96.1%	N
41	2	N	1	13-Jun-13	77.0%	Y
42	2	N	0	13-Jun-13	85.7%	N
43	3-R	N	3*	14-Jun-13	64.1%	Y
44	3-R	N	0	14-Jun-13	86.5%	N
45	1	N	9	14-Jun-13	61.2%	N
46	2	N	0	15-Jun-13	52.8%	Y
47	3-R	N	0	15-Jun-13	92.4%	N
48	3-R	N	0	15-Jun-13	85.8%	N
49	1	N	0	15-Jun-13	64.0%	Y
50	2	N	0	16-Jun-13	63.2%	Y
51	3-R	N	0	17-Jun-13	95.1%	N
52	Not Monitored	Not Monitored	Not Monitored	17-Jun-13	unknown	unknown
53	3-R	N	0	18-Jun-13	87.8%	N
54	3-I	N	0	18-Jun-13	0%	N
55	2	N	0	19-Jun-13	93.3%	Y
56	3-I	N	0	19-Jun-13	55.2%	Y

57	3-R	N	0	20-Jun-13	81.1%	Y
58	3-R	N	0	20-Jun-13	88.0%	Y
59	1	N	1	20-Jun-13	95.8%	N
60	3-I	N	0	21-Jun-13	52.8%	N
61	3-I	N	0	21-Jun-13	60.3%	Y
62	2	N	11	21-Jun-13	67.2%	Y
63	3-R	N	0	21-Jun-13	85.1%	Y
64	3-I	N	0	22-Jun-13	34.4%	N
65	2	N	0	23-Jun-13	90.2%	N
66	3-I	N	0	22-Jun-13	88.5%	N
67	3-R	N	0	24-Jun-13	92.3%	N
68	2	N	0	24-Jun-13	96.0%	N
69	3-R	N	0	25-Jun-13	84.9%	N
70	2	N	0	26-Jun-13	89.1%	N
71	3-I	N	0	26-Jun-13	94.6%	N
72	1	N	11**	26-Jun-13	14.6%	N
73	3-R	N	0	26-Jun-13	0%	Y
74	3-I	N	0	26-Jun-13	70.7%	Y
75	3-I	N	0	27-Jun-13	86.5%	N
76	3-R	N	0	27-Jun-13	91.6%	N
77	2	N	0	28-Jun-13	96.3%	N
78	3-I	N	0	28-Jun-13	93.9%	N
79	3-R	N	0	30-Jun-13	84.7%	N
80	2	N	8	30-Jun-13	99.0%	N
81	3-R	N	0	30-Jun-13	22.8%	N
82	1	N	0	02-Jul-13	95.9%	N
83	2	N	0	03-Jul-13	12.5%	Y
84	3-R	N	0	03-Jul-13	95.6%	Y
85	3-I	N	0	03-Jul-13	94.3%	Y
86	3-I	N	0	03-Jul-13	73.9%	N
87	3-R	N	0	04-Jul-13	97.2%	N
88	3-I	N	0	04-Jul-13	82.0%	N
89	3-I	N	0	04-Jul-13	82.7%	N
90	2	N	0	04-Jul-13	95.0%	N
91	3-I	N	0	04-Jul-13	93.6%	N
92	1	N	0	07-Jul-13	91.0%	N
93	2	N	0	07-Jul-13	65.7%	N
94	3-I	N	0	07-Jul-13	81.4%	N
95	3-R	N	0	09-Jul-13	72.1%	Y

96	3-R	N	0	09-Jul-13	79.4%	N
97	3-I	N	0	09-Jul-13	75.0%	N
98	1	N	14**	10-Jul-13	37.8%	N
99	3-I	N	0	10-Jul-13	75.5%	N
100	3-R	N	0	10-Jul-13	91.9%	N
101	2	N	0	12-Jul-13	74.8%	N
102	3-R	N	0	12-Jul-13	85.2%	N
103	1	N	0	12-Jl-13	94.6%	N
104	3-R	N	0	13-Jul-13	87.4%	N
105	3-R	N	0	13-Jul-13	90.3%	N
106	2	N	0	13-Jul-13	88.0%	N
107	3-R	N	0	14-Jul-13	27.8%	N
108	3-R	N	0	15-Jul-23	91.0%	Y
109	3-R	N	0	15-Jul-13	91.7%	N
110	2	Y	1	16-Jul-13	76.9%	N
111	2	N	0	16-Jul-13	64.5%	N
112	1	Y	1	17-Jul-13	5.0%	Y
113	3-R	N	0	17-Jul-13	94.7%	N
114	3-R	N	0	18-Jul-13	86.4%	N
115	1	N	2	18-Jul-13	84.1%	N
116	3-R	N	0	20-Jul-13	86.3%	N
117	3-R	N	0	22-Jul-13	86.8%	N
118	3-I	N	0	24-Jul-13	89.0%	N
119	3-R	N	0	25-Jul-13	80.4%	N
120	1	N	0	27-Jul-13	88.1%	N
121	3-I	N	0	27-Jul-13	90.6%	N
122	3-R	N	0	27-Jul-13	83.7%	N
123	3-R	N	0	28-Jul-13	94.5%	Y
124	2	N	0	28-Jul-13	93.2%	N
125	3-R	N	0	01-Aug-13	88.2%	N
126	3-I	N	0	02-Aug-13	89.4%	N
127	3-I	N	0	03-Aug-13	76.7%	N
128	3-R	N	0	03-Aug-13	86.2%	Y
129	1	N	2	06-Aug-13	75.8%	N
130	3-R	N	0	07-Aug-13	92.8%	N
131	2	N	0	08-Aug-13	87.3%	N
132	2	N	0	10-Aug-13	66.7%	N
133	3-R (PD)	N	0	11-Aug-13	76.3%	Y
134	3-I	Wild Nest	Wild Nest	unknown	unknown	unknown

135	1	Wild Nest	Wild Nest	unknown	unknown	unknown
136	2	Wild Nest	Wild Nest	unknown	unknown	unknown
137	2	Wild Nest	Wild Nest	unknown	unknown	unknown

* Nest was > 3m above SHTL and experienced wash-over during storm tide(s) and/or extreme spring tides
** Nest experienced inundation caused by extreme spring tides

Table 2.4: Staging criteria (revised from Whitmore and Dutton 1985) for unhatched eggs in nests that experienced wash-over during the 2013 loggerhead nesting season on South Island at Tom Yawkey Wildlife Center, Georgetown County, South Carolina. Days of the incubation period assume an average incubation period of 60 days reported for loggerheads nesting in South Carolina. Incubation duration (in days) of each staged nest was divided into thirds to determine stage each wash-over occurred.

Developmental Stage	Staging Criteria	Days of Incubation Period (approximate)
Undetermined	-no visible embryo or blood formation -no white circle on shell (an indication of membrane attachment and fertility) - egg with decaying yolk - fungal and/or root invasion	
Early	- blood formation - white circle on shell (indicates fertility) - eyes usually present - embryo lacks pigmentation - no carapace - embryo < 10 mm in length	Day 1- 19 (1st third)
Mid	- carapace present - white embryo without dark scutes - pigmented eyes - unpigmented body 10 – 30 mm in length	Day 20- 39 (2nd third)
Late	- fully formed scutes - pigmented embryo usually >30 mm in length	Day 40 – 60 (final third)

Table 2.5: Comparison of mean HS between different beach zones on South Island at Tom Yawkey Wildlife Center, Georgetown County, South Carolina during the 2012 and 2013 loggerhead nesting seasons using linear contrasts.

	2012		**2013**	
Zone	**mean HS**	**n**	**mean HS**	**n**
1	58.48% **b***	90	65.88% **a**	15
2	77.43% **a**	25	75.46% **a**	24
3-I	75.82% **a**	30	71.79% **a**	25
3-R	80.94% **a**	14	73.54% **a**	50

* Dissimilar letters indicate a significant difference in mean HS among zones (α = 0.10).

Table 2.6: Mean HS by beach zone during the 2012 and 2013 loggerhead nesting seasons on South Island at Tom Yawkey Wildlife Center, Georgetown County, South Carolina.

contrast	**coefficients**	**mean comparison (of zones)**	**p-value (α = .10)**	**F**
2012 season				
L1	(1,-1,0,0)	mean 1 to mean 2	< 0.01*	7.8
L2	(1,0,0,-1)	mean 1 to mean 3-R	0.01*	6.8
L3	(0,1,-1,0)	mean 2 to mean 3-I	0.80	0.04
2013 season				
L1	(1,-1,0,0)	mean 1 to mean 2	0.20	1.59
L2	(1,0,0,-1)	mean 1 to mean 3-R	0.28	1.18
L3	(0,1,-1,0)	mean 2 to mean 3-I	0.58	0.31

* Dissimilar letters indicate a significant difference in mean HS among zones (α = 0.10).

Table 2.7: Mean HS by whether nests experienced loss (due to probing and/or depredation) for each loggerhead nesting season on South Island at Tom Yawkey Wildlife Center, Georgetown County, South Carolina.

Loss	**2012**	**2013**
	Mean HS	**Mean HS**
Yes	69.2% $_{\mathbf{a}}$	64.5% $_{\mathbf{b}*}$
No	71.2% $_{\mathbf{a}}$	74.9% $_{\mathbf{a}}$

*Dissimilar letters indicate a significant difference in mean HS based on loss occurrence ($\alpha = 0.10$).

Table 2.8: Mean HS by whether nests experienced a wash-over event(s) for each year and across all years on South Island at Tom Yawkey Wildlife Center in Georgetown County, South Carolina.

Wash-over	**2012**	**2013**	**2012 + 2013**
	Mean HS	**Mean HS**	**Mean HS**
Yes	65.7% $_{\mathbf{a}*}$	65.5% $_{\mathbf{a}}$	64.5% $_{\mathbf{a}}$
No	80.4% $_{\mathbf{b}}$	77.5% $_{\mathbf{b}}$	78.5% $_{\mathbf{b}}$

*Dissimilar letters indicate a significant difference in mean HS for wash-over nests within each year and across all years.

Table 2.9: Data collected for nests that experienced inundation by storm tides or extreme spring tides during the 2012 and 2013 loggerhead nesting seasons on South Island at Tom Yawkey Wildlife Center, Georgetown County, South Carolina.

Nest #	Year	Date(s) of tidal event(s)	Wash away	# Wash -overs	# of times inundated	Tropical Storm, Hurricane or Spring Tide	HS
3	2012	7 June	Y	16*	0*	H Chris	0%
13	2012	25 May; 7 and 27 June	N	15	3	TS Beryl, H Chris, TS Debby	17.2%
16	2012	7 and 27 June	N	8	2	H Chris, TS Debby	20.7%
19	2012	7 and 27 June	Y	9*	1*	H Chris, TS Debby	0%
22	2012	7 and 27 June	N	11	2	H Chris, TS Debby	0%
23	2012	27 June	Y	10*	0*	TS Debby	0%
24	2012	27 June	Y	8*	0*	TS Debby	0%
29	2012	7 June	Y	6*	0*	H Chris	0%
32	2012	7 June, 27 June	N	13	2	H Chris, TS Debby	0%
46	2012	27 June	Y	6*	0*	TS Debby	0%
51	2012	27 June	N	12	1	TS Debby	14.3%
65	2012	27 June	Y	3*	0*	TS Debby	0%
77	2012	27 June	N	11	1	TS Debby	0%
139	2012	25 August	N	2	1	H Isaac	55.9%
145	2012	27 August	N	6	1	H Isaac	0%
150	2012	25 August	N	1	1	H Isaac	21.6%
72	2013	22, 23, 24 July; 20, 21, 22 August	N	11	6	Extreme Spring Tides	14.6%
98	2013	23 July; 21 & 22 Aug	N	14	3	Extreme Spring Tides	37.8%

* Number of wash-over and/or inundation events experienced by a nest prior to wash-away.

LITERATURE CITED

Ackerman, R.A. 1997. The nest environment and the embryonic development of sea turtles. In The Biology of Sea Turtles (eds P.L. Lutz and J.A. Musick), pp.83-106. CRC, Boca Raton, Florida.

Bimbi, M.K. 2009. Effects of relocation and environmental factors on loggerhead sea turtle (*Caretta caretta*) nests on Cape Island. Thesis, College of Charleston, Charleston, South Carolina, USA.

Bishop, G.A. and B.K. Meyer. 2011. Sea Turtle Habitat Deterioration on St. Catherines Island: Defining the Modern Transgression. Anthropological Papers American Museum of Natural History 94: 271-295.

Box, G.E.P. 1953. Non-normality and tests on variances. Biometrika 40: 318-335.

Bustard R.H. and P. Greenham P. 1968. Physical and chemical factors affecting hatching in the green sea turtle, *Chelonia mydas* (L.). Ecology 49: 269-276.

Carthy, R.R. 1996. The role of the eggshell and nest chamber in loggerhead turtle (*Caretta caretta*) egg incubation, Ph. D. Dissertation. University of Florida, Gainesville, 121 pp.

Carthy, R.R., A.M. Foley and Y. Matsuzawa. 2003. Incubation environment of loggerhead turtle nests: effects on hatching success and hatchling characteristics. In loggerhead sea turtles (eds A.B. Bolten and B.E. Witherington), pp. 144-153. Washington, DC: Smithsonian Press.

Caut, S., E. Guirlet, and M. Girondot. 2010. Effect of tidal overwash on the embryonic development of leatherback turtles in French Guiana. Marine Environmental Research 69: 254-261.

Coll, G.E. 2010. Sea Turtle Nest Management: Examining the Use of Relocation as a Management Tool on Three South Carolina Beaches. Thesis, College of Charleston, Charleston, South Carolina, USA.

Collins, S.A. 2012. Reproductive Ecology of American Oystercatchers in the Cape Romain Region of South Carolina: Implications for Conservation. Thesis, Clemson University, Clemson, South Carolina, USA.

Conant, T.A., P.H. Dutton, T. Eguchi, S.P. Epperly, C.C. Fahy, M.H. Godfrey, S.L. MacPherson, E.E. Possardt,B.A. Schroeder, J.A. Seminoff, M.L. Snover, C.M. Upite, and B.E. Witherington. 2009. Loggerhead Sea Turtle (*Caretta caretta*)

2009 status review under the U.S. Endangered Species Act. Report to the National Marine Fisheries Service, Silver Spring, Maryland, USA. 219 p.

Davenport, J. 1989. Sea turtles and the greenhouse effect. British Herpetological Society Bulletin 29: 11-15.

Dodd, C.K. and U.S. Fish and Wildlife Service. 1988. Synopsis of the biological data on the loggerhead sea turtle: *Caretta caretta* (Linnaeus, 1758).Washington, DC: Springfield, VA: Fish and Wildlife Service, U.S. Dept. of the Interior.

Dodd, M.G. and A. H. Mackinnon. 2008.Loggerhead Turtle Nesting in Georgia. Annual Report submitted to U.S. Fish and Wildlife Service for grant E-5- Amendment 8 "Coordination of loggerhead sea turtle nest protection in Georgia". 43 pp.

Eckert, K.L. and S.A. Eckert. 1985. Tagging and nesting research of leatherback sea turtles (*Dermochelys coriacea*) on Sandy Point, St. Croix, USVI, 1985. Annual Report. U.S. Fish and Wildlife Service, USFWS Ref. MIN 54-8680431. 58 pp.

Eckert, K.L. and S.A. Eckert. 1990. Embryo Mortality and Hatch Success in *In Situ* and Translocated Leatherback Sea Turtle *Dermochelys coriacea* Eggs. Biological Conservation. 53: 37-46.

Eskew, T.S. 2012. Best Management Practices for Reducing Coyote Depredation on Loggerhead Sea Turtles in South Carolina. Thesis, Clemson University, Clemson, South Carolina, USA.

Fish M.R., I.M. Cote, J.A. Gill, A.P. Jones, S. Renshoff, and A.R. Watkinson. 2005. Predicting the impact of sea-level rise on Caribbean sea turtle nesting habitat. Conservation Biology 19: 482-491.

Foley, A.M. 1998. The nesting ecology of the loggerhead turtle (*Caretta caretta*) in the Ten Thousand Islands, Florida (incubation environment, hatching success). Ph.D. Dissertation. University of South Florida, Tampa. 164 pp.

Foley A.M., S.A. Peck, and G.R. Harman GR. 2006. Effects of sand characteristics and inundation on the hatching success of loggerhead sea turtle (*Caretta caretta*) clutches on low-relief mangrove islands in southwest Florida. Chelonian Conservation Biology 5: 32-41.

Freidlin, B. and J.L. Gastwirth. 2000. Should the median test be retired from general use? American Statistician 54: 161-164.

Garmestani, A.S., H.F. Percival, K.M. Portier and K.G. Rice. 2000. Nest-site selection by the loggerhead sea turtle in Florida's Ten Thousand Islands. Journal of Herpetology 34: 504-510.

Godley, B.J., A.C. Broderick and N. Mrosovsky. 2001. Estimating hatchling sex ratios of loggerhead turtles in Cyprus from incubation durations. Marine Ecology Progress Series 210: 195-201.

Goldenberg, S. B., C.W. Landsea , A.M. Mestas-Nu˜nez and W.M. Gray. 2001. The Recent Increase in Atlantic Hurricane Activity: Causes and Implications. Science 293: 474-479.

Hamann, M., M.H. Godfrey, J.A. Seminoff, K. Arthur, P.C.R. Barata, K.A. Bjorndal, A.B. Bolten, A.C. Broderick, L.M. Campbell, C. Carreras, P. Casale, M. Chaloupka, S.K.F. Chan, M.S. Coyne, L.B. Crowder, C.E. Diez, P.H. Dutton, S.P. Epperly, N.N. FitzSimmons, A. Formia, M. Girondot, G.C. Hays, I.S. Cheng, Y. Kaska, R. Lewison, J.A. Mortimer, W.J. Nichols, R.D. Reina, K. Shanker, J.R. Spotila, J. Tomas, B.P. Wallace, T.M. Work, J. Zbinden, and B.J. Godley. 2010. Global research priorities for sea turtles: informing management and conservation in the 21st century. Endangered Species Research 11: 245-269.

Hanson, J., T. Wibbels and R.E. Martin. 1998. Predicted female bias in sex ratios of hatchling loggerhead sea turtles from a Florida nesting beach. Canadian Journal of Zoology 76: 1850-1861.

Hawkes, L.A., A.C. Broderick, M.H. Godfrey and B.J. Godley. 2007. Investigating the potential impacts of climate change on a marine turtle population. Global Change Biology 13: 923-932.

Hawkes, L.A., A.C. Broderick, M.H. Godfrey and B.J. Godley. 2009. Climate Change and Marine Turtles. Endangered Species Research 7: 137-154.

Herrera, A.E. 2006. The effects of nest management methods on sex ratio and hatching success of leatherback turtles (*Dermochelys coriacea*). Biological Conservation.

Hilterman, M.L. 2001. The sea turtles of Suriname, 2000. Biotopic technical report. Commissioned by the WWF-Guianas, Paramaribo, Suriname. 61 pp.

Hilterman M.L. and E. Goverse. 2003. Aspects of nesting and success of the leatherback turtles *(Dermochelys coriacea)* in Suriname, 2002. World Wildlife Fund Guianas/ Bio Foundation, Amsterdam.

Hopkins, S.R. and T.M. Murphy. 1983. Management of loggerhead turtle nesting beaches in South Carolina. Study Completion Report to U.S. Fish and Wildlife Service. South Carolina Wildlife and Marine Resources Department. Charleston, South Carolina.

IPCC, 2007. Climate change: the physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M.Tignor, H.L. Miller, editors. Cambridge, United Kingdom and New York, NY, USA: Cambridge University Press.

Janzen, F.J. and G.L. Paukstis. 1991. Environmental sex determination in reptiles-ecology, evolution, and experimental design. Quarterly Review of Biology 66: 149-179.

Janzen, F.J. 1994. Climate change and temperature-dependent sex determination in reptiles. Proceedings of the National Academy of Sciences USA 91:7487–7490.

Johnston, K., E. Koepfler, M. James, S. Dawsey, E. Freeman, P. Schneider, M. Schneider and B. Brabson. 2007. Spatial and Temporal Influence of Meteorological and Substrate Factors upon Loggerhead Sea Turtle Nests in South Carolina. Proceedings of the Twenty-Eighth Annual Symposium on Sea Turtle Biology and Conservation.

Klein, R.J.T. and R.J. Nicholls. 1999. Assessment of coastal vulnerability to climate change. AMBIO 28: 182–187.

Landsea, C.W. 1993. A climatology of intense (or major) Atlantic hurricanes. Monthly Weather Review 121: 1703–1713.

Leblanc, A.M., K.K. Drake, K.L. Williams, M.G. Frick, T. Wibbels and D.C. Rostal. 2012. Nest temperatures and hatchling sex ratios from loggerhead turtle nests incubated under natural field conditions in Georgia, United States. Chelonian Conservation and Biology 11: 108-116.

Limpus, C.J., V. Baker, and J.D. Miller. 1979. Movement induced mortality of loggerhead eggs. Herpetologica 35: 335-338.

Limpus, C.J. 1985. A study of the loggerhead turtle, *Caretta caretta*, in eastern Australia. PhD thesis, University of Queensland, Brisbane, Australia.

Magrin, G., C. G. García, D. C. Choque, A. R. M. J.C. Giménez, G. J. Nagy, C. Nobre and A. Villamizar. 2007. Latin America, in Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, edited by M. L. Parry and O. F. Canziani.

Marcovaldi, M.A., M.H. Godfrey and N. Mrosovsky. 1997. Estimating sex ratios of loggerhead turtles in Brazil from pivotal incubation durations. Canadian Journal of Zoology 75: 755-770.

Margaritoulis, D. and A.F. Rees, A.F. 2003. Nest inundation by seawater: a threat to mitigate or a natural "masculinising" factor? In: Proceedings of the twenty third symposium on sea turtle biology and conservation. 17-21 March 2003. Kuala Lumpur, Malaysia.

Matsuzawa, Y., K. Sato, W. Sakamoto and K.A. Bjorndal. 2002. Seasonal fluctuations in sand temperature: effects on the incubation period and mortality of loggerhead sea turtle (*Caretta caretta*) pre-emergent hatchlings in Minabe, Japan. Marine Biology 140: 639-646.

McElroy, M. 2009. The effect of screening and relocation on hatching and emergence success of loggerhead sea turtle nests at Sapelo Island, Georgia. Thesis, University of Georgia, Athens, Georgia, USA.

McGehee, M.A. 1979. Factors affecting the hatching success of loggerhead sea turtle eggs (*Caretta caretta*). MS thesis, University of Central Florida, Orlando.

Milton, S.L., S. Leone-Kabler, A.A. Schulman and P.L. Lutz. 1994. Effects of Hurricane Andrew on the sea turtle nesting beaches of south Florida. Bulletin of Marine Science 54: 974-981.

Mitchell, N. J., M. R. Kearney, N. J. Nelson, and W. P. Porter. 2008. Predicting the fate of a living fossil: How will global warming affect sex determination and hatching phenology in tuatara? Proceedings of the Royal Society Bulletin 275: 2185-2193.

Mortimer, J. 1982. Factors influencing beach selection by nesting sea turtles. In Biology and Conservation of Sea Turtles. Bjordnal, K. Ed. Smithsonian Institution Press. Washington DC.

Mrosovsky, N. and C.L. Yntema. 1980. Temperature dependence of sexual differentiation in sea turtles: Implications for conservation practices. Biological Conservation 18: 271-280.

Mrosovsky, N., P.H. Dutton and C.P. Whitmore. 1983. Sex ratios of two species of sea turtle nesting in Suriname. Canadian Journal of Zoology 62: 2227–2239.

Mrosovsky, N. 1988. Pivotal temperatures for loggerhead turtles (*Caretta caretta*) from northern and Southern nesting beaches. Canadian Journal of Zoology 66: 661-669.

Mrosovsky, N. and C. Pieau. 1991. Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex determination in reptiles. Amphibia-Reptilia 12: 169-179.

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

Mrosovsky, N. 1994. Sex ratios of sea turtles. The Journal of Experimental Zoology 270: 16-27.

Mrosovsky, N., S. Kamel, A.F. Rees, and D. Margaritoulis. 2002. Pivotal temperature for loggerhead turtles (*Caretta caretta*) from Kyparissia Bay, Greece. Canadian Journal of Zoology 80: 2118–2124.

Mrosovsky, N. 2006. Distorting gene pools by conservation: assessing the case of doomed turtle eggs. Environmental Management 38: 523–531.

Mrosovsky, N. 2008. Against Oversimplifying the Issues on Relocating Turtle Eggs. Environmental Management 41:465-467.

National Marine Fisheries Service (NMFS) and U.S. Fish and Wildlife Service (USFWS). 1991. Recovery Plan for U.S. Population of the Loggerhead Turtle. National Marine Fisheries Service, Washingon D.C.

National Marine Fisheries Service (NMFS) and U.S. Fish and Wildlife Service (USFWS). 2008. Recovery Plan for the Northwest Atlantic Population of the Loggerhead Sea Turtle (*Caretta caretta*), Second Revision. National Marine Fisheries Service, Silver Spring, Maryland.

National Oceanic and Atmospheric Administration (NOAA). 2012. National Weather Service Forecast Office. Preliminary Monthly Climate Data (CF6). Available from: http://www.nws.noaa.gov/climate/index.php?wfo=chs

Patino-Martinez, J., A. Marco, L. Quinones and L. Hawkes. 2012. A potential tool to mitigate the impacts of climate change to the Caribbean leatherback sea turtle. Global Change Biology 18: 401-411.

Pike, D.A. and J.C. Stiner. 2007. Sea turtle species vary in their susceptibility to tropical cyclones. Oecologia 153: 471-478.

Pike D.A. 2008. Natural beaches confer fitness benefits to nesting marine turtles. Biology Letters 4: 704–706.

Provancha, J.A. and L.M. Erhart. 1987. Sea turtle nesting trends at Kennedy Space

Center and Cape Canaveral Air Force Station, Florida, and relationships with factors influencing nest-site selection. In: Witzell, W.N. (eds), Ecology of East Florida Sea Turtles: Proceedings of the Cape Canaveral, Florida Sea Turtle Workshop Miami, Florida 26-27 February 1985, pp 33-44. NOAA Technical Report NMFS 53.

Rahmstorf S. 2007. A semiempirical approach to projecting future sea-level rise. Science 315:368-370.

Rees, A.F. and D. Margaritoulis. 2004. Beach temperatures, incubation durations and estimated hatchling sex ratio for loggerhead nests in Kyparissia Bay, Greece. B.C.G. Testudo 6: 23-36.

Schmid J.L., D.A. Addison, M.A. Donnelly, M.A. Shirley and T. Wibbels. 2008. The effect of Australian Pine (*Casuarina equisetifolia*) removal on loggerhead sea turtle (*Caretta caretta*) incubation temperatures on Keewaydin island, Florida. Journal of Coastal Research 55: 214–220.

Schulz, J.P. 1975. Sea turtles nesting in Surinam. Zoologische Verhandelingen 143: 1-143.

Shamblin, B.M., M.G. Dodd, K.L. Williams, M.G. Frick, R. Bells and C.J. Nairn. 2011. Loggerhead turtle eggshells as a source of maternal nuclear genomic DNA for population genetic studies. Molecular Ecology Resources 11: 110-115.

Shaw, K.R. 2013. Effects of Inundation on Hatch Success of Loggerhead Sea Turtle (*Caretta caretta)* Nests. Thesis, University of Miami, Miami, Florida, USA.

South Carolina Department of Natural Resources (SCDNR). 2010. SCDNR Managed Lands.Columbia, South Carolina. Available from: https://www.dnr.sc.gov/mlands/managedland?p_id=64 (accessed November 2012).

South Carolina Department of Natural Resources (SCDNR). 2012. Guidelines for Marine Turtle Permit Holders. Nest Protection Management. Charleston, South Carolina. Available from: www.dnr.sc.gov/seaturtle/nt/nestguide.pdf (accessed June 2012).

South Carolina Department of Natural Resources (SCDNR). 2014. Guidelines for Marine Turtle Permit Holders. Nest Protection Management. Charleston, South Carolina. Available from: www.dnr.sc.gov/seaturtle/nt/nestguide.pdf (accessed February 2014).

Stancyk, S.E., O.R. Talbert and J.M. Dean. 1980. Nesting Activity of the Loggerhead

Turtle *Caretta caretta* in South Carolina, II. Protection of Nests from Raccoon Predation by Transplantation. Biological Conservation 18: 289-298.

Standora, E. A. and J. R. Spotila. 1985. Temperature dependent sex determination in sea. turtles. Copeia 1985:779-782

Tuttle, J.A. 2007. Loggerhead Sea Turtle (*Caretta caretta*) Nesting on a Georgia Barrier Island: Effects of Nest Relocation. Thesis, Georgia Southern University, Statesboro, Georgia, USA.

Trullas S.C. and F.V. Paladino. 2007. Micro-environment of olive ridley turtle nests deposited during an aggregated nest event. Journal of Zoology 272: 367–376.

Webster, P. J., G.J. Holland, J.A. Curry and H.R. Chang. 2005. Changes in Tropical Cyclone Number, Duration, and Intensity in a Warming Environment. Science 309: 1844-1846.

Wibbels, T., R.E. Martin, D.W. Owens and M.S. Amoss Jr. 1991. Female-biased sex ratio of immature loggerhead sea turtles inhabiting the Atlantic coastal waters of Florida. Canadian Journal of Zoology 69: 2973-2977.

Whitmore, C. P. and P.H. Dutton. 1985. Infertility, embryonic mortality and nest-site selection in leatherback and green sea turtles in Suriname. Biological Conservation. 34: 251-272.

Williams, T., A.T. Chow and B. Song. 2012. Historical Visualization Evidence on Forest-Salt Marsh Transition in Winyah Bay, South Carolina: A Retrospective Study in Sea Level Rise. Baruch Institute of Coastal Ecology and Forest Science, Clemson University. Wetland Science and Practice 29: 5-17.

Wood, D.W. and K.A. Bjorndal. 2000. Relation of temperature, moisture, salinity, and slope to nest site selection in loggerhead sea turtles. Copeia 2000: 119-128.

Wyneken, J., T.J. Burke, M. Salmon and D.K. Pedersen. 1988. Egg failure in natural and relocated sea turtle nests. Journal of Herpetology 22: 88-96.

Yntema, C.L. and N. Mrosovsky. 1980. Sexual differentiation in hatchling loggerheads (*Caretta caretta*) incubated at different controlled temperatures. Herpetologica 36: 33-36.

CHAPTER III

ASSESSMENT OF THE PROBE STICK TO LOCATE NEST CAVITIES: EFFECTS OF PROBING ON EGG BREAKAGE THROUGHOUT THE CLUTCH

INTRODUCTION

While there are several methods utilized by surveyors across the Southeast to locate the nest cavity of loggerhead sea turtle nests, beaches in South Carolina use a probe stick (SCDNR 2014). This tool makes finding nests more time efficient and less labor intensive for statewide nesting beach survey (SNBS) participants who are often volunteers. However, methods used to locate the clutch of sea turtle eggs differs by state. In Florida, nest location protocol states all participants are to locate the clutch by digging gently and systematically by hand into the nest site. Nest survey participants are only authorized to probe the sand with their hands, without the assistance of shovels or other tools (FWC 2002). In Georgia the probe is used as a nest location tool at the discretion of the program and often as a last resort (Bishop 2001; Dodd and Mackinnon 2008). In South Carolina, authorized personnel are also allowed to locate the clutch by carefully digging shallow holes and probing with their fingers into the nest feeling for the softer sand that is evident over the clutch (SCDNR 2014). Unless specifically stated on the project permit, this is the only method participants should use to locate the clutch. Other personnel are able to obtain permits that allow for location of the clutch using a probe stick. Probing a nest by a project participant is allowed only if the appropriate training has been conducted by SCDNR Marine Turtle Conservation Program personnel or another experienced participant who possesses a current SCDNR Letter of Authorization under their primary permit holder (SCDNR 2014).

The probe used to locate the clutch is a tapered, T-handled dowel constructed of either wood or metal. The probe can be gently inserted into the body pit to locate the clutch more efficiently. A depression is felt in the sand when the probe is inserted into the egg chamber. While locating the clutch of loggerheads and other species of sea turtles may be less time and labor intensive when using the probe, the use of this tool is controversial (Bishop 2001). This method can lead to the incidental breakage (puncture) of one or more eggs. It is well known that the insertion of the probe stick often causes direct egg breakage at the top and/or center of the clutch (D.B. Griffin and C.P. Hope, personal communication; Bishop 2001; FWC 2002). Total egg loss attributed to probing throughout South Carolina was reported as 506 eggs for the 2012 season and 513 eggs for the 2013 season (C.P. Hope, personal communication).

During nest relocations, participants sometimes report eggs are found broken at the center or bottom of the clutch, but with no sign of direct puncture caused by the probe (i.e. yolk and/or albumen on the probe tip). These eggs are recorded as ‘broken in nest’ as opposed to the loss being attributed to probing. While the direct breakage of eggs by the probe is a common and well-known cause of egg loss near the top of the clutch, it has been suggested breakage of eggs that are found broken (but not punctured directly by the probe) in the bottom or center of a clutch may be caused indirectly by pressure created in the nest when the probe is inserted into the substrate. Conversely, others attribute this loss to the females nesting efforts during oviposition (D.B. Griffin and C.P. Hope, personal communication). Egg loss quantified as ‘broken in nest’ was higher than loss reported by probing during the past two consecutive loggerhead nesting seasons in South Carolina. A total of 989 and 1,399 eggs were reported as ‘broken in nest’ during the 2012 and 2013 nesting seasons in South Carolina, respectively (C.P. Hope, personal communication). Further investigation is necessary in order to determine whether the use of this tool, used to more efficiently locate loggerhead clutches in South Carolina, is causing significant egg loss throughout the clutch and/or decreased hatch success (HS) when compared to alternative nest location methods. If eggs found ‘broken in nest’ are being indirectly ruptured by pressure created by insertion of the probe during nest locations, it is essential to accurately quantify this as additional loss caused by the probing method.

RESEARCH GOALS AND HYPOTHESES

The purpose of this study was to assess the use of the probe stick to locate the clutch of loggerhead sea turtle nests in South Carolina. The goal of this study was to quantify egg loss associated with two nest location methods 1) probing and 2) hand digging the body pit to determine whether use of this tool is correlated with significantly higher loss and/ or decreased HS when compared to the alternative method (i.e. hand digging the body pit). Specifically, it was determined 1) whether the number of eggs found broken inside the nest cavity was significantly greater when using the probe to locate the clutch compared to an alternative method (hand digging) used in the Southeast 2) whether nests found with the probe have significantly lower HS than nests found digging by hand, and 3) whether nests that experience egg breakage during the clutch location procedure exhibit significantly lower HS than nests that do not experience egg loss. By comparing the number of broken eggs per clutch with sign of direct puncture, the number of broken eggs per clutch with no sign of direct puncture, and their location in the nest cavity based on the nest location method, the influence of the probe on egg breakage throughout the clutch was tested, specifically to determine whether eggs found broken at the center or bottom of the clutch (with no sign of direct puncture) during nest relocations is correlated with probe use (suggesting pressure created by insertion of the probe into the substrate may cause indirect egg breakage) or if no correlation exists between nest location method and eggs found broken throughout the clutch (suggesting nesting efforts of female loggerheads may be the cause of eggs found broken with no sign of direct puncture by the probe). By comparing the HS of nests found with the probe, nests found by hand digging, and nests that experienced egg loss during the location procedure, it was determined whether use of the probe significantly decreases HS of loggerhead nests.

Hypotheses:

H_O 1: The mean number of broken eggs found in nests does not significantly differ between the two nest location methods (probed vs. dug).

H_A 1: Nests located with the probe will have a significantly greater mean number of broken eggs throughout the clutch than nests found by hand digging.

H_O 2: Mean HS does not significantly vary based on method used to locate the nest cavity.

H_A 2: Mean HS is different between method used (probed vs. dug).

H_O 3: Mean HS does not significantly vary based on whether the nest experienced egg breakage when located.

H_A 3: Nests that have experienced egg loss when located have significantly lower mean HS than nests that did not experience egg loss.

MATERIALS AND METHODS

Study Site: Loggerhead sea turtle nesting data were collected 11 May - 14 October 2012 and 11 May - 11 October 2013 at the Tom Yawkey Wildlife Center (TYWC), a publically managed wildlife center located near Georgetown, South Carolina (33.2°N, -79.2°W). The South Carolina Department of Natural Resources (SCDNR) manages the TYWC. It is separated from the mainland by the Intracoastal Waterway and consists of Cat Island, North Island, Sand Island, and South Island (Figure 2.1). The property is managed as a wildlife center with severely limited public access and is composed of approximately 9,700 hectares of managed wetlands surrounded by tidal marsh, longleaf pine (*Pinus palustris*) forest, ocean beach and maritime forest (SCDNR 2010).

Loggerhead nesting surveys used in this study were conducted on South Island and Sand Island beaches. Sea turtles had access to the full length and width of beach since no structures such as seawalls exist. South Island consists of 6.08 km of undisturbed, beach managed for sea turtle and shorebird nesting. Nesting beach surveys have been annually conducted on South Island since 1977. This site has averaged 175 nests per season since annual surveys began and is considered a high density nesting beach for loggerheads in the state of South Carolina (SCDNR 2010). The dominant flora include sea oats (*Uniola paniculata*), seacoast marsh elder (*Iva imbricata*), and seaside panicum (*Panicum amarum*) which contribute to the establishment and maintenance of coastal dunes that provide suitable loggerhead nesting habitat. The maritime forest behind the dunes is characterized by a variety of salt-tolerant evergreens such as wax myrtle (*Myrica cerifera*), yaupon (*Ilex vomitoria*), live oak (*Quercus virginiana*), red bay (*Persea borbonia*), southern magnolia (*Magnolia grandiflora*), cabbage palmetto (*Sabal palmetto*), saw palmetto (*Serenoa repens*) and loblolly pine (*Pinus taeda*). Erosional forces that occurred between the 2011 and 2012 nesting seasons created foredunes that are steeply scarped beginning slightly south of the beach entrance (33.149°N, -79.224°W) and extending north of the entrance to approximately (33.168°N, -79.199°W) leaving the beach with what appears to be less suitable nesting habitat than in prior years (i.e. a narrower beach with steeper dunes). These scarped dunes prevent most sea turtles from crawling to higher dune elevations or into vegetated areas of the dunes to lay eggs (personal observation). The south end of the beach consists of a flat wash-out section that experiences flooding during spring tides and is also less suitable for nesting. While the north end of the beach past the scarped dunes does consist of well-established dunes exceeding 2 m tall, the path to reach dunes at the far north end extends a great distance beyond the tide line and is covered with dense wrack (defined as vegetation, largely *Spartina*, cast on the shore), debris and often trash. Dunes along the entirety of South Island began to re-establish prior to the end of the 2013 season.

Sand Island is separated from South Island by a tidal creek that is approximately 50 - 100 m wide. Width and direction of this tidal creek alters after storm activity. The construction of the south jetty during the late 1890's led to the formation of Sand Island following the accumulation of sand around the jetty. Nesting surveys began in 2008 with an estimated nest count of 100 nests annually (SCDNR 2014). Sand Island consists of 5.15 km of undisturbed, limited access beach with the island's south end beginning at the tidal creek and extending northward to Winyah Bay. By the 1950's, salt tolerant plant communities became established on the island, with wax myrtles being the tallest vegetation present (SCDNR 2014). Beach morning glory (*Ipomoea pes-caprae*) degrades nesting habitat at the north end of Sand Island during the latter half of the nesting season due to potential root invasion into incubating nests. Flat wash-out areas unsuitable for nesting characterize the habitat south of the jetty. In August 2011, Hurricane Irene flattened this section almost entirely with the exception of three well-established dunes. Small dunes began to re-establish prior to the end of the 2012 nesting season, but storm surge from Hurricane Sandy in October 2012 flattened this area once more and steeply scarped dunes that partially remained. Erosional forces also created scarped dunes north of the jetty, some exceeding 3 m tall, that turtles were unable to climb. The beach is also narrow on the north end and is prone to inundation during high tide events (personal observations). Prior to the 2013 nesting season, several tall dunes re-established to provide suitable loggerhead nesting habitat both south and north of the jetty but high tide events during August and September 2013 flattened this area once more and steeply scarped dunes that partially remained.

Nest location and identification: Nest surveys on South Island were conducted by project participants at sunrise seven days a week throughout the nesting season which ranged from 11 May - 18 August 2012 and 11 May - 16 August 2013. Nest surveys on Sand Island rotated around the low tide schedule each day. Kayaks were used to cross the inlet no sooner than two hours before low tide and returning no later than two hours after low tide as a safety precaution due to the presence of swift currents and rough water that begin to form closer to high tide. Nesting beaches were patrolled by use of truck (South Island only), ATV or by foot. Nests were located by following crawls to the body pit constructed by the female during the previous night's nesting attempt. A line was drawn through all tracks so data was not collected more than once per attempt. To determine whether a clutch was deposited or if the crawl was false, meaning a non-nesting emergence where no eggs were deposited, a probe was carefully inserted into the sea turtle nest body pit for a sample of nests. Clutches were found when a depression was felt when probing the sand. An alternate method that consisted of digging the body pit by hand was used to locate the nest cavity for a sample of nests that were determined to need relocation. All emergences were recorded as a nest or false crawl. If eggs were located, one was excavated and stored in a 50 mL vial containing 95 % ethanol for use in the NRU loggerhead DNA genetic fingerprinting study (Shamblin et al. 2011). Any eggs broken by the probe were removed so as not to attract predators or cause microbial contamination that could spread to the rest of the incubating clutch (Wyneken et al. 1988). If an egg was broken by the probe, that egg was used as the genetic sample. In the absence of broken eggs, one egg was collected from the nest for genetic testing.

All nests were protected with approximately 1.2 m X 1.2 m plastic or metal screens and staked at the four corners to deter predators such as raccoons (*Procyon lotor*) and coyotes (*Canis latrans*). Beginning 31 August 2013, screens on Sand Island were replaced with self-releasing metal cages due to an increase in coyote presence and nest depredation at this site. Several markers were used: brightly colored flagging tape was tied to two stakes and a flag was inserted into the center of the nest. All markers were labeled with the date the nest was laid and the nest number. Coordinates were taken for each nest using a Garmin GPSMAP 60CSx.Throughout the incubation period, nests were monitored approximately 5 days/week for signs of depredation and/or disturbance.

Nest relocation: Only relocated nests were used in this investigation. Nests that were partially depredated by coyotes on the night of oviposition were relocated but excluded from this study. Relocation criteria differed among sites and between years:

- Sand Island 2012 and 2013 – all nests laid below spring high tide line (SHTL)
- South Island 2012 – all nests laid > 3 m below SHTL
- South Island 2013 – all nests laid > 3 m below SHTL; 2/3 nests laid ≤ 3 m below SHTL

Nests relocated on Sand Island were vulnerable to erosion or tidal wash-over including those laid below the SHTL and in flat wash-out areas prone to flooding. Nests relocated on South Island consisted of only a sample of nests that were vulnerable to erosion or wash-over since a sample of vulnerable nests were left *in situ* to examine the effects of wash-over on HS (see Chapter II). The criteria used for selection of artificial nest locations were based on the SHTL, dune height and vegetation. If a well-established dune without dense vegetation was located directly inland of the original nest site, the nest was relocated to this dune. If this type of dune was not located directly inland of the original nest site, the closest suitable site to the original location was chosen as the relocation site. This was done in order to recreate the conditions of the original site and to minimize disturbance. Once an appropriate site was determined, an egg chamber approximately 20 - 25 cm in diameter and the same depth as the initial nest was constructed using a shovel, hands, or shells. Clutches were excavated from their *in situ* location and transferred using a plastic bucket to the new site where the eggs were carefully placed into the newly constructed chamber in the same layer as they were laid in the original chamber. Nests were covered with damp, cool sand from the original chamber, protected with screening and marked as previously described.

Hatching: Nest inventories were conducted 21 July - 9 October 2012 and 25 July - 11 October 2013. All relocated nests laid on South Island and Sand Island were inventoried with the exception of two nests laid on a separate beach on Winyah Bay. Nests were checked daily for signs of emergence beginning on day 45 of the incubation period (SCDNR 2014). Field signs used to determine emergence activity included a crater in the center of the nest or the presence of hatchling tracks. Nests were excavated 3 days after the first sign of emergence. Nests where emergence signs were not evident were inventoried 75 days after the date they were laid, with an exception being nests laid in

May 2012. These nests were inventoried 80 days after being laid if emergence signs were not observed due to a potentially extended incubation period caused by unseasonably cool and rainy conditions (SCDNR 2012). Contents of the egg chamber were counted and clutch size was determined. Eggs were recounted during inventories even though they had been previously counted during relocation. The clutch count determined during excavation was used in analyses. The number of unhatched eggs, hatched eggs (defined as an intact shell greater than or equal to 50%), pipped eggs (defined as an egg broken by a hatchling that dies before it is able to fully emerge from the egg), live hatchlings and dead hatchlings were also counted. All contents which included unhatched eggs, shells from hatched eggs, and dead hatchlings were discarded into the ocean so they did not attract predators.

Experimental Design: Only relocated nests were included in this study. The number of eggs broken in each clutch upon location of the nest cavity, the location of each broken egg in the clutch and if sign of direct puncture was evident based on method used to locate the nest cavity (probing vs. hand digging) was examined. The HS of a sample of nests relocated to new nest sites from their *in situ* locations was examined based on 1) method used to locate the nest cavity and 2) whether nests experienced egg breakage (i.e. loss) upon location was also examined.

Nests relocated on Sand Island were vulnerable to erosion or tidal wash-over including those laid below the SHTL and in flat wash-out areas prone to flooding. Nests relocated on South Island consisted of only a sample of nests that were vulnerable to erosion or wash-over since a sample of vulnerable nests were left *in situ* to examine the effects of wash-over on HS. During the 2012 season, the probe was used to locate the nest cavity for approximately 50% of these relocated nests (probing was performed by the project manager only to reduce error) while the nest cavities of the other 50% of relocated nests were located by hand digging (conducted by all project participants). All probed nests were located with aluminum probes. In order to increase sample size for this investigation during the 2013 season, sampling methods were revised (see relocation criteria above).

A 30 minute time limit was placed on nest location via hand digging. After failure to locate the eggs after 30 minutes, body pits were checked with the probe to determine if the emergence was a successful nesting attempt or a non-nesting emergence (i.e. false crawl). The method used to locate the nest cavity, the number of broken eggs found per clutch, the location of each egg in the clutch, and whether the egg appeared to be directly broken by the probe was recorded for all relocated nests (Table 3.1). An indication of direct egg breakage by the probe was the sign of yolk and/or albumen on the probe tip.

Statistical Analyses: All nests that were relocated on South Island, with the exception of those relocated due to partial depredation, were included in the investigation of possible egg breakage cause. A sample of relocated nests on Sand Island that were not inventoried (n = 8, 2012; n = 10, 2013) were excluded from analyses of HS. Nests that experienced depredation and/or tidal wash-over were excluded from analyses of HS. Only probed nests located by the project leader were included in analyses to control for operator error. The level of significance was $\alpha = 0.05$ for all comparisons since there was concern of making a Type II error, but also did not want to inflate the likelihood of making a Type I error.

ANOVA and t-tests were used to test hypotheses. These methods require certain assumptions for the hypothesis test results to be valid. These assumptions were evaluated in each of the following hypothesis test analyses. The assumption of normality was tested using the Shapiro Wilk W statistic and graphically using normal quantile plots and histograms. In some cases the normality assumption was found to be violated. The assumption of equal variance was tested with Levene's Test. In some cases the equal variance assumption was found to be violated. Fortunately, hypothesis test results using methods that allow for violation of assumptions (i.e. transformations, nonparametrics) yielded results similar to the original ANOVA and t-test results. This was most likely due to the violations not being too severe and the large sample sizes resulting in somewhat robust ANOVA and t-tests (Box 1953). Therefore we chose to use the standard (i.e. parametric) ANOVA and t-test results (t-tests were adjusted for unequal variance where appropriate) because these tests are more statistically powerful and mean was the measure of central tendency of interest for this study. Also, non-parametric tests can be less efficient, less powerful and do not always control the probability of Type II error (Freidlin and Gastwirth 2000). All statistical calculations were performed with JMP software (V.9, SAS). Hatch success was calculated for each nest as ([# hatched eggs / clutch size] * 100) for all investigations.

Nest Location Method and Egg Breakage Throughout the Clutch

The analysis for hypothesis 1 (H_O 1: The mean number of broken eggs found in nests does not significantly differ between the two nest location methods (probed vs. dug)) was based on a one factor completely randomized design (CRD). The model for this investigation was $y = \mu + \tau + \varepsilon$, where y = egg breakage (loss), μ = overall mean, τ = treatment (method) and ε = error.

Pearson's chi-squared was used to determine whether a correlation existed between method used to locate the nest cavity and whether eggs were found broken. A one-tailed t-test assuming unequal variance was used to determine if the mean number of eggs found broken in a clutch significantly differed based on method use to locate the nest cavity. A t-test assuming unequal variance was used to examine the relationship between location method and eggs found broken throughout the clutch since Levene's homogeneity of variance test indicated unequal variance between the mean number of eggs found broken in dug nests and probed nests ($F = 181.04$, $p < 0.01$).When examining the relationship between location method and the number of eggs found broken, data from the 2012 and 2013 seasons were combined for analysis since the relationship was consistent within each year and for overall years (Table 3.2).

HS vs. Clutch Location Method

The analysis for hypothesis 2 (H_O 2: Mean HS does not significantly vary based on method used to locate the nest cavity (probed vs. dug)) was based on a one factor CRD. The model for this investigation was $y = \mu + \tau + \varepsilon$, where y = HS, μ = overall mean, τ = treatment (nest location method) and ε = error. Only probed nests located by the project leader were included in analyses to control for operator error.

Mean (± SD) HS was calculated for relocated nests based on the method used to locate the nest cavity. The impact of nest location method on HS was determined using a pooled t-test (two-tailed). A pooled t-test assuming equal variance was used to examine the relationship between location method and HS since Levene's homogeneity of variance test indicated equal variance between HS of dug nests and probed nests ($F = 0.26$, $p = 0.61$).When examining the relationship between location method and HS, data from the 2012 and 2013 seasons were combined for analysis since the relationship was consistent within each year and for overall years (Table 3.3).

HS vs. Clutch Location Method and Loss

The analysis for hypothesis 3 (H_O 3: Mean HS does not significantly vary based on whether the nest experienced egg breakage when located) was based on a 2 X 2 factorial CRD, where loss and nest location method were the treatments. The model for this investigation was: $y = \mu + \tau + \varepsilon$ (or, $y = \mu + M + L + \varepsilon$), where y = HS, μ = overall mean, τ = treatments (method and loss) and ε = error.

Mean (± SD) HS was calculated for relocated nests based on the method used to locate the nest cavity and whether eggs were found broken when the nest was located. When examining the relationship between location method and loss on HS, data from the 2012 and 2013 seasons was combined for analysis since the relationship did not vary between seasons (Table 3.4). ANOVA was used to test for differences in HS between nests found hand digging with no loss, nests found hand digging with loss, nests located with the probe that experienced no loss, and nests found by the probe that contained broken eggs. Interaction effects between method and los were not examined with two-way ANOVA due to missing data since no nests located by hand digging contained broken eggs. Follow-up tests were not performed since ANOVA detected no significant differences between groups ($F = 1.78$, $p = 0.17$).

RESULTS

Clutch Location Method and Egg Breakage Throughout the Clutch

Pearson's chi squared detected a correlation between method used to locate the nest cavity and whether eggs were found broken ($\chi^2 = 34.56$, $p < 0.01$). A one tailed t-test assuming unequal variance indicated mean number of eggs found broken in a clutch significantly differed based on method use to locate the nest cavity, probed (n = 63, mean eggs found broken = 1.24 ± 1.75, 95% C.I. = [0.80, 1.68]) vs. dug (n = 67, mean eggs found broken = 0 ± 0, 95% C.I. = [0, 0]) ($t = 5.62$, $p < 0.01$) (Table 3.5).

HS vs. Clutch Location Method and Loss

A pooled t-test (two-tailed) detected no significant difference between mean HS and method used to locate the nest cavity, probed (n = 50, mean HS = 79.7% ± 17.3%, 95 % C.I. = [74.8%, 84.6%]) vs. dug (n = 51, mean HS = 78.0% ± 18.2%, 95% C.I. = [72.9%, 83.1%]) ($t = 0.47$, $p = 0.64$). ANOVA detected no significant difference between mean HS of nests found hand digging with no loss (n = 51, mean HS = 78.0% ± 18.2%, 95% C.I. = [73.2%, 82.9%]) , nests located with the probe that experienced no loss (n =

30, mean HS = 83.4% ± 11.6%, 95% C.I. = [77.0%, 89.7%]), and nests found by the probe that contained broken eggs (n = 20, mean HS = 74.1% ± 22.6%, 95% C.I. = [66.3%, 81.9%]) ($F = 1.78$, $p = 0.17$). No nests located by hand digging contained broken eggs, so this group was not included in the above ANOVA (Figure 3.2).

DISCUSSION

The probe stick can be used as a tool to more efficiently locate the nest cavity of loggerhead sea turtles nesting in the southeastern U.S. While this nest location tool has the ability to decrease the time and labor involved in locating the nest cavity during nesting beach surveys, it can lead to substantial egg breakage throughout the clutch if the procedure is not properly conducted by trained personnel (SCDNR 2014). While probing has been known as a cause of direct egg breakage (i.e. puncture) mainly at the top and center of loggerhead clutches, this study was the first to assess whether this tool may be responsible for eggs found broken in the nest cavity upon location but with no sign of direct puncture (possibly due to indirect egg breakage at the bottom and center of clutches caused by pressure created by insertion of the probe into the substrate). Also, this study was the first to assess whether use of this tool significantly impacts HS of loggerhead nests in South Carolina.

Nest Location Method and Egg Breakage Throughout the Clutch

No eggs were found broken in nests located by hand digging the body pit during the 2012 or 2013 nesting seasons at TYWC. Out of the 130 nests included in this study, all nests that contained broken eggs upon location were found using the probe. The strong correlation between probing and presence of broken eggs throughout the clutch, and the lack of eggs broken in nests found hand digging, suggest that eggs reported as broken by project participants during nest relocations are not damaged during nesting efforts of female loggerheads. However, while the results of this study suggest a correlation exists between the use of the probe as a nest location method and whether or not broken eggs are found in a nest upon location, this study does not provide evidence for causation. In order to determine if the probe is indeed the cause of indirect egg breakage, future research should be conducted. For example, in order to determine if pressure created by the probe during insertion into the body pit is an indirect cause of egg breakage, an artificial nest experiment could be designed in the field or laboratory. If an artificial nest contained pressure censors throughout the chamber, researchers may be able to determine if the probe is exerting pressure at the center and/or bottom of a clutch and if this pressure is enough to cause egg breakage. Also, in order to definitively exclude female oviposition as a cause of eggs found broken at the bottom of the clutch, participants could perform night surveys and monitor the fate of each egg as it is deposited by the female. However, the latter is time consuming, costly, and likely to cause increased disturbance that could be detrimental to nesting females and is not recommended.

A potential source of error in this study was the difficulty in quantifying total eggs broken directly vs. indirectly by the probe. During the 2012 and 2013 nesting seasons combined, only 5 nests (approximately 8% of nests found with broken eggs) contained eggs broken at the center and/or bottom of the clutch with no sign of direct breakage caused by the probe (i.e. no yolk and/or albumen was present on the probe tip). All other nests in this study that contained broken eggs (n = 58) were also found with the probe, but in these nests the probe tip was covered with contents from the egg(s), evidence of direct puncture.

It was not possible to definitively quantify the number of eggs broken indirectly (i.e. not punctured). It is possible that no eggs were broken indirectly due to pressure and that all broken eggs with no sign of direct puncture were actually punctured. Yolk and/or albumen from the directly punctured egg(s) may have been cleaned from the probe tip during removal of the tool from the sand. However, this is unlikely since sand has a tendency to stick to ruptured egg contents on the metal probe instead of removing them (personal observation) and because the probe tip was thoroughly examined for sign after every insertion.

HS vs. Clutch Location Method and Loss

The use of the probe as a tool to locate the nest cavity of loggerheads at TYWC did not significantly impact HS of the relocated nests included in this study. While nests located by hand digging contained no broken eggs upon location, these nests did not exhibit significantly different mean HS than nests located with the probing method. In fact, mean HS of nests located with the probe was slightly higher within seasons and overall seasons (Table 3.3). This analysis did not account for the occurrence of broken eggs.

When incorporating whether broken eggs were present upon nest location, the results of this study suggest that egg breakage (i.e. loss) did not negatively impact HS of loggerhead nests at TYWC. While mean HS of nests found with the probe that experienced loss was slightly lower (mean HS = 74.1%) than mean HS of nests found probing (mean HS = 83.4%) and digging (mean HS = 78.0%) that did not experience loss, this difference in mean HS did not significantly differ from nests with no egg breakage located by either method. It is important to note that loss in this study is defined only as eggs found broken in the nest upon location. Nests that experienced loss by any other means (depredation, storm tides, exposure, etc.) were not included in this investigation.

Nests probed by participants other than the project leader were excluded from analyses to reduce error. While all probed nests included in the investigations explored in this chapter were located using the same model aluminum probe stick (as opposed to an alternative wooden dowel model), two separate probes were used. The difference in probe stick was not accounted for and is a potential source of error in the aforementioned investigations.

CONCLUSIONS

Using the probe as a tool to locate loggerhead clutches is more time efficient and less labor intensive than the alternative method of hand digging the body pit. Although a significantly higher number of eggs were found broken in nests located with the probe, HS did not significantly vary based on nest location method and whether or not nests experienced loss upon location. While a strong correlation exists between probing and egg loss throughout the clutch, future research should be conducted to definitively prove causation of eggs found broken during relocations with no sign of direct puncture. These findings suggest that the probe is an appropriate tool to aid in the location of nest cavities and its use is not detrimental to loggerhead HS in South Carolina. The management implications of this study are discussed in Chapter IV.

Figure 3.1: Study area within the Tom Yawkey Wildlife Center, Georgetown County, South Carolina. Loggerhead sea turtle nests in this study occurred along the Atlantic coast of South Island (outlined in yellow) and Sand Island (outlined in orange) beaches (image Eskew 2012).

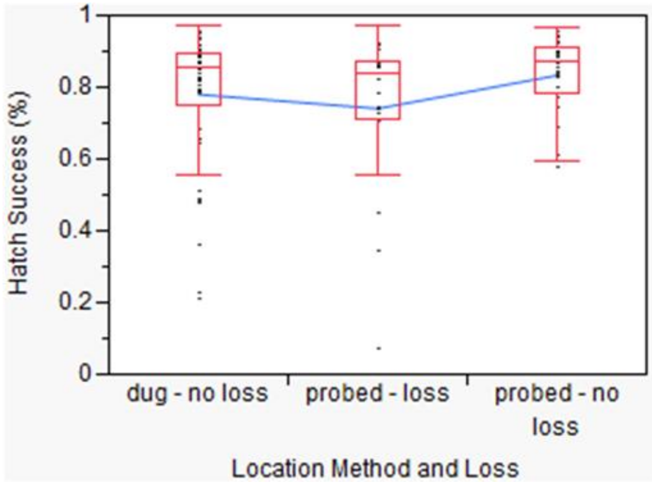


Figure 3.2: Comparison of mean HS between the 3 combinations of method used to locate the clutch and whether eggs were found broken inside (loss) on South Island and Sand Island beaches at Tom Yawkey Wildlife Center, Georgetown County, South Carolina. ANOVA indicated there was no significant difference in mean HS between nests found hand digging with no loss (n = 51), nests located with the probe that experienced no loss (n = 30), and nests found by the probe that contained broken eggs (n = 20) (F = 1.78, p = 0.17).

Table 3.1: Nesting data collected for relocated nests laid on South Island and Sand Island beaches at the Tom Yawkey Wildlife Center, Georgetown County, South Carolina during the 2012 and 2013 loggerhead nesting seasons. Data includes method used to locate the nest cavity, whether eggs were found broken upon location and their position in the clutch. Red indicates nests with a hatch success < 60% (considered a nest failure).

Nest #	Site	Year	Method	# eggs broken top clutch	# eggs broken mid clutch	# eggs broken bottom clutch	Hatch Success
11	Sand	2012	dug	0	0	0	unknown
12	Sand	2012	probed	2	0	0	85.9%
36	Sand	2012	probed	1	0	4	74.3%
40	Sand	2012	dug	0	0	0	75.2%
44	Sand	2012	probed	2	0	0	87.5%
47	Sand	2012	probed	2	1	0	unknown
50	Sand	2012	probed	2	1	0	70.6%

52	Sand	2012	probed	2	3	0	72.8%
55	Sand	2012	dug	0	0	0	unknown
64	Sand	2012	dug	0	0	0	48.0%
77	Sand	2012	dug	0	0	0	unknown
81	Sand	2012	probed	2	1	0	unknown
84	Sand	2012	dug	0	0	0	91.9%
94	Sand	2012	probed	0	0	0	unknown
96	Sand	2012	probed	0	0	0	95.8%
97	Sand	2012	dug	0	0	0	unknown
98	Sand	2012	dug	0	0	0	83.7%
104	Sand	2012	probed	0	0	0	58.0%
114	Sand	2012	dug	0	0	0	93.8%
117	Sand	2012	probed	2	1	0	unknown
20	South	2012	probed	1	2	0	97.4%
52	South	2012	probed	0	0	0	89.7%
69	South	2012	dug	0	0	0	51.2%
73	South	2012	probed	0	0	0	87.3%
80	South	2012	probed	1	0	0	92.0%
81	South	2012	probed	2	0	0	44.9%
88	South	2012	dug	0	0	0	78.9%
89	South	2012	probed	3	0	0	91.9%
97	South	2012	dug	0	0	0	82.4%
98	South	2012	dug	0	0	0	91.6%
100	South	2012	probed	3	2	0	86.7%
113	South	2012	dug	0	0	0	89.3%
131	South	2012	dug	0	0	0	84.6%
147	South	2012	dug	0	0	0	65.3%
4	South	2013	probed	2	2	0	34.4%
5	South	2013	dug	0	0	0	78.6%
8	South	2013	probed	0	0	0	80.0%
11	South	2013	dug	0	0	0	88.9%
15	South	2013	probed	3	0	0	7.1%
18	South	2013	dug	0	0	0	0%
23	South	2013	dug	0	0	0	36.1%
26	South	2013	probed	0	0	0	0%
27	South	2013	probed	0	0	0	59.2%
32	South	2013	probed	0	0	0	85.8%
34	South	2013	dug	0	0	0	28.9%
38	South	2013	probed	0	0	3	78.2%
39	South	2013	dug	0	0	0	85.6%
44	South	2013	dug	0	0	0	86.5%
48	South	2013	dug	0	0	0	85.8%

51	South	2013	dug	0	0	0	95.1%
57	South	2013	dug	0	0	0	81.1%
69	South	2013	dug	0	0	0	84.9%
76	South	2013	dug	0	0	0	91.6%
81	South	2013	dug	0	0	0	22.8%
87	South	2013	dug	0	0	0	97.2%
96	South	2013	dug	0	0	0	79.4%
102	South	2013	dug	0	0	0	85.2%
105	South	2013	dug	0	0	0	90.3%
114	South	2013	dug	0	0	0	86.4%
116	South	2013	dug	0	0	0	86.3%
119	South	2013	dug	0	0	0	80.4%
125	South	2013	dug	0	0	0	88.2%
1	Sand	2013	probed	0	0	0	unknown
2	Sand	2013	dug	0	0	0	48.4%
3	Sand	2013	probed	0	0	0	25.9%
8	Sand	2013	dug	0	0	0	59.5%
9	Sand	2013	probed	2	1	0	41.7%
12	Sand	2013	dug	0	0	0	87.2%
20	Sand	2013	probed	1	0	0	59.4%
24	Sand	2013	dug	0	0	0	48.7%
25	Sand	2013	probed	0	0	4	73.9%
27	Sand	2013	dug	0	0	0	29.0%
28	Sand	2013	probed	0	0	0	61.0%
30	Sand	2013	dug	0	0	0	82.5%
31	Sand	2013	probed	0	0	0	92.1%
32	Sand	2013	dug	0	0	0	85.8%
35	Sand	2013	probed	0	0	0	unknown
38	Sand	2013	dug	0	0	0	71.9%
42	Sand	2013	probed	0	0	0	74.2%
43	Sand	2013	probed	1	0	1	82.5%
44	Sand	2013	dug	0	0	0	88.3%
47	Sand	2013	probed	0	0	0	86.8%
55	Sand	2013	probed	0	0	0	68.9%
56	Sand	2013	probed	0	0	0	unknown
57	Sand	2013	probed	2	1	0	90.4%
59	Sand	2013	probed	2	2	0	85.6%
60	Sand	2013	dug	0	0	0	78.9%
61	Sand	2013	probed	0	0	0	78.6%
65	Sand	2013	probed	1	2	4	55.6%
66	Sand	2013	dug	0	0	0	65.5%
70	Sand	2013	probed	0	0	0	90.0%

72	Sand	2013	dug	0	0	0	68.3%
73	Sand	2013	probed	0	0	0	83.9%
76	Sand	2013	dug	0	0	0	85.5%
77	Sand	2013	probed	0	0	0	93.8%
78	Sand	2013	dug	0	0	0	89.2%
79	Sand	2013	probed	0	0	0	88.1%
82	Sand	2013	dug	0	0	0	21.0%
83	Sand	2013	probed	0	0	0	91.0%
84	Sand	2013	dug	0	0	0	64.7%
85	Sand	2013	probed	2	0	0	71.5%
88	Sand	2013	dug	0	0	0	55.6%
106	Sand	2013	probed	0	0	0	90.1%
107	Sand	2013	dug	0	0	0	81.6%
131	Sand	2013	dug	0	0	0	79.3%
132	Sand	2013	probed	0	0	0	82.7%
136	Sand	2013	probed	0	0	0	89.0%
142	Sand	2013	dug	0	0	0	90.4%
144	Sand	2013	probed	0	0	0	96.9%
146	Sand	2013	dug	0	0	0	72.5%
150	Sand	2013	probed	0	0	0	84.7%
155	Sand	2013	dug	0	0	0	89.9%
156	Sand	2013	probed	0	0	0	93.0%
160	Sand	2013	probed	0	0	0	77.0%
161	Sand	2013	probed	1	0	0	85.3%
162	Sand	2013	dug	0	0	0	86.6%
163	Sand	2013	probed	0	0	0	90.1%
169	Sand	2013	dug	0	0	0	89.0%
172	Sand	2013	probed	0	0	0	96.4%
173	Sand	2013	dug	0	0	0	82.8%
174	Sand	2013	probed	0	0	0	83.2%
177	Sand	2013	dug	0	0	0	93.1%
180	Sand	2013	probed	1	0	0	85.3%
184	Sand	2013	dug	0	0	0	95.6%
188	Sand	2013	probed	0	0	0	94.2%
195	Sand	2013	dug	0	0	0	unknown
198	Sand	2013	dug	0	0	0	unknown
202	Sand	2013	probed	0	0	0	unknown
207	Sand	2013	dug	0	0	0	unknown
213	Sand	2013	probed	0	0	0	unknown

Table 3.2: Mean number of eggs found broken in the clutch upon location by method used to locate the nest cavity (probed vs. dug) for each year and across all years during the 2012 and 2013 loggerhead nesting seasons on South Island and Sand Island beaches at Tom Yawkey Wildlife Center, Georgetown County, South Carolina.

Location Method	2012	2013	2012 + 2013
	Mean # eggs found broken (loss)	Mean # eggs found broken (loss)	Mean # eggs found broken (loss)
Probed	2.22 $_{b*}$	0.84 $_{b}$	1.24 $_{b}$
Dug	0 $_{a}$	0 $_{a}$	0 $_{a}$

*Dissimilar letters indicate a significant difference in mean number or broken eggs between nest location methods within each year and across years (α = 0.05).

Table 3.3: Mean HS (%) by method used to locate the nest cavity (probed vs. dug) for each year and across all years on South Island and Sand Island beaches during the 2012 and 2013 loggerhead nesting seasons at Tom Yawkey Wildlife Center, Georgetown County, South Carolina.

Location Method	2012	2013	2012 + 2013
	Mean HS	Mean HS	Mean HS
Probed	81.1% $_{a*}$	79.1% $_{a}$	79.7% $_{a}$
Dug	77.4% $_{a}$	78.2% $_{a}$	78.0% $_{a}$

*Dissimilar letters indicate a significant difference in mean HS between nest location methods within each year and across years (α = 0.05).

Table 3.4: Mean HS (%) by nest location method and whether eggs were found broken in the clutch upon location for each year and across all years on South Island and Sand Island beaches during the 2012 and 2013 loggerhead nesting seasons at Tom Yawkey Wildlife Center, Georgetown County, South Carolina. No nests that were located by hand digging contained broken eggs.

Location Method and Loss	2012 Mean HS	2013 Mean HS	2012 + 2013 Mean HS
Probed – no loss	82.7% $_{a*}$	83.4% $_a$	83.4% $_a$
Probed - loss	80.4% $_a$	67.8% $_a$	74.1% $_a$
Dug – no loss	77.4% $_a$	78.2% $_a$	78.0% $_a$

*Dissimilar letters indicate a significant difference in mean HS between nest location methods and whether loss was evident upon location ($\alpha = 0.05$).

Table 3.5: Nesting survey data by method used to locate the nest cavity collected during the 2012 and 2013 loggerhead nesting seasons at Tom Yawkey Wildlife Center, Georgetown County, South Carolina.

	2012	**2013**	**TOTAL**
PROBED			
# of nests (n)	18	45	63
# eggs broken	40	38	78
% of nests w/ broken eggs	72%	29%	41%
DUG			
# of nests (n)	16	51	67
# eggs broken	0	0	**0**
% of nests w/ broken eggs	0%	0%	**0%**

LITERATURE CITED

Bishop, G.A. 2001. St. Catherine's Island Sea Turtle Conservation Program. Available from http://www.scistp.org/conservation/index.php (accessed October 2013).

Box, G.E.P. 1953. Non-normality and tests on variances. Biometrika 40: 318-335.

Dodd, M.G. and A.H. Mackinnon. 2008. Loggerhead Turtle Nesting in Georgia. Annual Report submitted to U.S. Fish and Wildlife Service for grant E-5-Amendment 8 "Coordination of loggerhead sea turtle nest protection in Georgia".43 pp.

Florida Fish and Wildlife Conservation Commission (FWC). 2002. Sea Turtle Conservation Guidelines. Available from http://el.erdc.usace.army.mil/flshore/pdfs/Guidelines.pdf. (accessed March 2013).

Shamblin, B.M., M.G. Dodd, K.L. Williams, M.G. Frick, R. Bells and C.J. Nairn. 2011. Loggerhead turtle eggshells as a source of maternal nuclear genomic DNA for population genetic studies. Molecular Ecology Resources 11: 110-115.

South Carolina Department of Natural Resources (SCDNR). 2010. SCDNR Managed Lands. Columbia, South Carolina. Available from: https://www.dnr.sc.gov/mlands/managedland?p_id=64 (accessed November 2012).

South Carolina Department of Natural Resources (SCDNR). 2014. Guidelines for Marine Turtle Permit Holders. Nest Protection Management. Charleston, South Carolina. Available from: www.dnr.sc.gov/seaturtle/nt/nestguide.pdf (accessed February 2014).

South Carolina Department of Natural Resources (SCDNR). 2012. Marine Turtle Conservation Guidelines. Section 2 – Nesting Beach Survey Activities. Charleston, South Carolina. Available from: http://www.dnr.sc.gov/seaturtle/volres/MT%20Guidelines%20Section%202.pdf (accessed February 2013).

Wyneken, J., T.J. Burke, M. Salmon and D.K. Pedersen. 1988. Egg failure in natural and relocated sea turtle nests. Journal of Herpetology 22: 88-96.

CHAPTER IV

CONSERVATION & MANAGEMENT IMPLICATIONS

After the 1978 listing of the loggerhead sea turtle as threatened under the Endangered Species Act, a recovery plan became established (NMFS and USFWS 1991). The main objectives of this plan include an increase in the number of nests as a corresponding result of an increase in nesting females; the annual nest count for the NRU reach 14,000 + with the loggerhead sea turtle populations of North Carolina, South Carolina, and Georgia to return to pre-listing levels (approximate nest distributions: NC = 2,000 nests/season; SC = 9,000 nests/season; GA = 3,000 nests/season); and that all priority goals be successfully implemented. Recovery efforts are implemented to protect both marine and nesting habitat and are achieved through conservation law and policy, fisheries management, public outreach, and nesting/habitat management (NMFS and USFWS 1991, 2008). The Recovery Plan for the U.S. population of loggerheads (1991, 2008) states that the central actions on nesting beaches needed to attain recovery are 1) to provide long-term protection through the development and implementation of legislation at the local, state, federal, and international levels, 2) ensure a minimum 60% HS through the application of scientifically based nest management plans including the management of sufficient nesting habitat, 3) ensure nest counts for each recovery unit increase as a result of an increase of nesting females and 4) minimize unsustainable harvest.

The application of appropriate management techniques is essential to the conservation and recovery of the species. In 1977, the South Carolina Department of

Natural Resources (SCDNR) Marine Turtle Conservation Program began conducting beach management research throughout the state. Research and conservation management activities have been cautiously evaluated in order to determine which have proven successful and if the benefits of these actions outweigh potential risks. The periodic reassessment of management practices based on recent findings is essential to develop the most operative strategies. This study reassessed the use of two management tools currently utilized as part of the protection effort for loggerhead sea turtles nesting in the southeastern United States, with a primary focus on South Carolina barrier island nesting beaches. The nest management tools reassessed in this study included 1) the relocation of all nests laid seaward of the SHTL, and 2) use of the probe stick to locate the nest cavity.

Reassessing Nest Relocation as a Management Tool: Examining the Effects of Nest Location, Tidal Wash-over and Inundation on Hatch Success of Loggerhead Sea Turtles Nesting within the Tom Yawkey Wildlife Center, Georgetown County, South Carolina

One goal of the U.S. Loggerhead Recovery Plan is to assess the impact of nest management activities such as nest relocations on sex ratios, hatchling fitness, and productivity (NMFS and USFWS 1991, 2008). Currently, the recovery plan states nests vulnerable to erosion and with high probabilities of tidal inundation should be relocated from their original site to a more suitable site on higher grounds. The use of relocation as a management tool should occur only as a last resort if the nest is presumably doomed (NMFS and USFWS 1991, 2008; SCDNR 2014).

Researchers suggest nest relocation as a conservation strategy is beneficial because it has shown to greatly increase productivity (Stancyk et al. 1980; Hopkins and Murphy 1983; Wyneken et al. 1988; Eckert and Eckert 1990; Tuttle 2007; Bishop and Meyer 2011). However, it has been reported in the southeastern U.S. that no significant differences were detected between the hatch and emergence success of *in situ* and relocated loggerhead clutches (Bimbi 2009; McElroy 2009). Other studies suggest relocated nests had significantly lower hatch and emergence success than *in situ* nests (Schulz 1975; Eckert and Eckert 1985; Herrera 2006). While nest relocations are increasing due to a loss of suitable nesting habitat as beaches throughout the state face increased erosion, many of these relocations are unnecessary and are often conducted due to a misconception of concerned volunteers and project participants that the occurrence of any tidal wash-over will negatively influence HS, even of nests marginally landward of the SHTL (Coll 2010; D. B. Griffin and C. P. Hope, personal communication). Recovery plans suggest further research evaluating the tolerance of eggs to tidal threats should be conducted to develop operative nest management guidelines relative to such threats. An evaluation regarding the appropriateness of manipulative nest management tools such as relocation has also been recommended (NMFS and USFWS 1991, 2008).

While nest relocations have the ability to increase productivity, studies have revealed several concerns regarding their use including movement-induced mortality, artificial selection, alteration of the incubating environment, female-biased sex ratios and an increase in time and labor for volunteer workers (Limpus et al. 1979; Carthy 1996; Mrosovsky 2006, 2008; Tuttle 2007; Pfaller et al. 2008). There has been discussion that relocating vulnerable clutches may exert selective pressures and cause gene pool distortion since a nonrandom sample of the population is targeted for this management strategy (Mrosovsky 2006, 2008). It has been suggested that if individual females within a population show consistency in nest placement, for example above or below the SHTL, and if nest-site selection is heritable, then the relocation of vulnerable clutches could cause artificial selection and the maintenance of traits favoring unsuccessful nest sites (Mrosovsky 2006; Pfaller et al. 2008). Mrosovsky (2005) reported high consistency in the nest placement of individual hawksbills (*Eretmochelys imbricata*) in the French West Indies, some laying consistently close to the water. However, nest placement studies report varying degrees of temporal and spatial scattering in leatherbacks (Mrosovsky 2005), greens (Bjorndal and Bolten 1992) and loggerheads (Hays and Speakman 1993; Pfaller et al. 2008) as an evolved strategy to cope with unpredictable threats that may vary seasonally such as the increased risk of storm-generated erosion and inundation of nests incubating closer to the water, and threats such as heat-related mortality and predation that increase with nesting at higher dune elevations and/or closer to the vegetation line. Nest scattering ensures reproductive effort is not completely diminished if certain incubation environments become unsuitable for survival within a season and also increases variation of hatchling characteristics (Foley 1998). Conservation actions that decrease the variability of incubating environments, such as relocations, may lead to reduced hatchling variability and survival rates than leaving nests *in situ* over a broader array of incubating environments (Carthy et al. 2003). Nest relocation is being reevaluated as a conservation tool by the International Union for Conservation of Nature

Species Survival Commission (IUCN/SSC) Marine Turtle Specialist Group (MTSG) due to this recent debate on the possibility of relocation altering the gene pool (Pfaller et al. 2008).

Nest-site selection by individual females is a key component that influences survival of their offspring since physical parameters of the incubating environment greatly influence embryonic development, HS, and ultimately fitness (Garmestani et al. 2000; Wood and Bjorndal 2000). It has been determined incubation environment influences hatchling sex (Yntema and Mrosovsky 1982), size and growth (Foley 1998), locomotor abilities, and survivorship (Fisher 2012). Relocating nests seaward of the SHTL regarded as doomed could cause them to incubate at higher temperatures than if left *in situ* since temperature varies between different zones of the beach (Standora and Spotila 1985). Incubating at higher temperatures can have a feminizing effect (Yntema and Mrosovsky 1980; Janzen and Paukstis 1991; Mrosovsky 1994) and has been shown to decrease emergence success due to high rates of heat-related mortality (Matsuzawa et al. 2002). Climate change also has the potential to greatly increase sand temperatures, influencing cohort sex ratios and ultimately population dynamics (Janzen 1994; Mitchell et al. 2008). Predominantly female hatchlings are produced at most loggerhead nesting sites (Wibbels et al. 1991; Mrosovsky and Provancha 1992; Mrosovsky 1994; Marcovaldi et al. 1997; Hanson et al. 1998; Godley et al. 2001; Rees and Margaritoulis 2004; Hawkes et al. 2007) including those in Florida (Mrosovsky and Provancha 1989; Mrosovsky 1994; Hanson et al. 1998), Georgia (LeBlanc et al. 2012) and South Carolina (Johnston et al. 2007; Tuttle 2007) and may become more female-skewed as global temperatures continue to rise. Johnston et al. (2007) reported average nest temperatures exceeded a pivotal temperature of 29.2°C, resulting in female-biased sex ratios at several sites in South Carolina, with percent females averaging approximately 83%. This percentage was greater than the average reported by Mrosovsky (1984) possibly due to recent climate change along coastal South Carolina.

Variations in the incubation temperature of nests can be attributed to moisture due to rainfall and/or storm tides (Schmid et al. 2008). If wash-over and/or inundation events cause cooling below the pivotal range of 29-30°C male hatchlings will develop (Mrosovsky and Provancha 1991). While incubating closer to the SHTL increases the chance of storm-induced inundation and/or wash-away, nests laid in this zone will incubate at cooler temperatures and have greater potential to produce male hatchlings. The long-term survival of loggerheads is dependent on a sufficient range of incubation temperatures to ensure that an adequate ratio of male to female hatchlings is produced (Davenport 1989, 1997).

Predicted rise in global mean temperatures and sea level may lead to an increase in tidal wash-over, nest inundations and vulnerability of nesting beaches to erosion (Fish et al. 2005). Inundation, erosion and accretion are the major abiotic factors impacting incubating eggs (NMFS and USFWS 1991, 2008). Strong winds and storm events may cause sand to accrete over incubating nests. Increased nest depth caused by accretion of sand over the nest can modify temperature, moisture content, and gas exchange. If late-term nests are impacted by sand compaction, hatchlings may suffocate, become trapped beneath protective screening, or face exhaustion during emergence which can increase terrestrial and marine predation risk (Erhart and Witherington 1987; Horrocks and Scott 1991; Milton et al. 1994; Eckert et al. 1999).

A disadvantage of leaving low beach nests *in situ* during the present study, was an increase in time and labor costs to project participants due to the high occurrence of accretion over nests incubating in this zone. While the labor involved in relocating a nest was initially greater than leaving a nest *in situ*, participants often unburied low nests daily beginning at day 45 of the incubation period to ensure hatchlings would not be adversely impacted by sand compaction. Dune collapse occurred frequently during the 2012 season covering nests at the base of steep escarpments on the interior of South Island often burying the screen and stakes. Locating nests with plastic screens required tremendous time and labor costs due to the 3 – 5 m accuracy of the GPS unit. Nests incubating at higher beach elevations further from the SHTL (*in situ* and relocated) did not experience this level of accretion and required lower maintenance. Based on the results of this study, the use of metal screens at low beach areas is recommended so these nests can be more easily located with a metal detector. The use of multiple nest markers upon location such as flagging vegetation behind the nest site and inserting additional stakes in the dunes is also recommended in case of severe accretion.

This study supports the claim that nest relocations have the ability to increase loggerhead HS when compared to nests left to incubate in vulnerable areas below the SHTL, and can also decrease accretion related labor costs to project participants throughout the nesting season. However, while the results of the present study and previous investigations support the conclusion that the occurrence of frequent wash-over and inundation can significantly decrease HS in loggerheads (Foley et al. 2006; Pike and Stiner 2007; Coll 2010; Shaw 2013) and other species of sea turtles (Whitmore and Dutton 1985; Caut et al. 2010), it is essential to remember that some hatchlings are still produced in these nests (Whitmore and Dutton 1985; Hilterman 2001; Foley et al. 2006; Mrosovsky 2006; Pike and Stiner 2007; Caut et al. 2010; Coll 2010; Shaw 2013). Throughout the course of this study, many nests survived tidal events remarkably well. Results from the 2012 season suggest that the occurrence of inundation caused by storm surge may have more adverse effects on loggerhead HS than numerous wash-overs experienced during normal high tide events.

While HS of nests left seaward of the SHTL may have been significantly lower than HS reported in other zones during the 2012 season, it was only slightly lower than the recommended 60% (58.5%). Also, in 2013 the HS of *in situ* nests below the SHTL did not differ from that of other locations and treatments (i.e. relocation). Due to the concerns regarding the use of nest relocations, the results of this study indicate that while relocation is still an important nest management tool for sites that face severe erosion and tidal inundation, it should be utilized conservatively. Relocation of low nests on a particular beach should be considered only when it is certain that all eggs will be destroyed if the clutch is not moved.

Incorporating slope and elevation in combination with distance above the SHTL could enable participants to choose the most successful relocation sites. Due to the high tolerance of loggerhead clutches to withstand varying levels of tidal influences and the dynamic nature of the nesting beach, areas that have been rendered unsuitable for incubation should be continually assessed. The use of relocation may be more effectively managed by nesting projects based on site-specific recommendations derived from personal observations identifying high risk areas vulnerable to inundation, wash-away, depredation and decreased HS.

Site Specific Recommendations – South Island

One objective of the present study was to identify areas at high risk of tidal inundation and nest wash-away on South Island. Since climate change will likely increase the occurrence of inundation due to rapidly rising sea level along coastal South Carolina, it is imperative to identify high risk areas at a local scale and determine areas where nests will likely be destroyed without the use of nest relocations. ArcGIS v. 10.0 (ESRI) was used to map nest locations and occurrences of inundation, wash-away and/or nest failure during both seasons.

This investigation identified one high risk area located on the interior of the beach north of the entrance. The interior of South Island beach appears to be less suitable for nesting based on a grouping of nest failures caused by inundation and wash-away evident on this stretch of beach (Figure 4.1). The majority of nests that experienced wash-away or inundation caused by either storm surge or other extreme spring tide events during the 2012 and 2013 seasons were located in this area (83%, n = 15). Erosional forces create foredunes that are steeply scarped beginning slightly south of the beach entrance (33.149°N, -79.224°W) and extending north of the entrance to approximately (33.168°N, -79.199°W) leaving the beach with what appears to be less suitable nesting habitat than in prior years (i.e. a narrower beach with steeper dunes). During the 2012 season, many nests were deposited at the base of escarpments. These scarped dunes most likely prevented sea turtles from reaching higher elevations, thus causing them to deposit clutches in low beach areas closer to the SHTL with increased vulnerability to tidal influences (personal observations). While dunes along the entirety of South Island began to re-establish prior to the end of the 2013 season, spring tides continue to create escarpments at the interior of this site. However, mean HS of nests incubating below the SHTL by < 3 m (often at the base of these escarpments) did not significantly differ from the HS of relocated and *in situ* nests in zones above the SHTL in 2013, and was just slightly below the 60% recommended by the U.S. Loggerhead Recovery Plan (1991, 2008) in 2012.

Visual inspection of the maps created for this investigation suggests that the north and south ends of South Island beach were suitable nesting areas with many successful nests (Figure 4.2; Figure 4.3). The only nest to wash-away on the south end was mistakenly left to incubate below the SHTL by > 3 m and experienced repeated inundation and partial wash-away. The south end consists of a flat wash-out section that often experiences flooding during spring tides. While low nests are vulnerable to tidal influences on the south end, elevated dunes located south of the beach entrance provide suitable nesting habitat (Figure 4.3). The north end of the beach past the scarped dunes consists of well-established dunes exceeding 2 m tall, but the path to reach dunes at the far north end extends a great distance beyond the tide line and is covered with dense wrack. While dunes on the ends of South Island are far from the ocean and may be difficult for sea turtles to access for nesting and for post-emergence hatchlings to orient to the water, these areas had low probability of inundation and wash-away during the 2012 and 2013 seasons.

Risk maps can be used to help managers and project leaders determine where high risk nesting areas might be located on beaches and to determine if high risk areas are consistently those below the SHTL, and low risk areas above the SHTL. These maps could also enable managers to select the most ideal relocation sites based on a variety of factors shown to influence HS at a given local site such as slope, elevation, previous areas prone to depredation, and distance to features such as dune vegetation, maritime forest, salt marsh and ocean.

Risk classes on South Island beach were created in ArcGIS v. 10.0 (ESRI) by incorporating distance of nests above or below the SHTL, elevation, and slope. These variables were used to create risk maps since they are known to influence HS of loggerhead sea turtle nests. Risk was classified according to the likelihood of inundation at a given nest site based on the above variables. Inundation risk classes ranged from lowest probability of inundation (one) to highest probability of inundation (seven). The risk classification map was examined to determine its potential to accurately predict suitable and unsuitable nesting habitat on South Island beach during the 2012 loggerhead nesting season.

Field Collected Data: ArcGIS v. 10.0 (ESRI) was used to create a risk map for South Island's nesting habitat using remotely sensed and field collected data. Field collected data used in this investigation included coordinates for each nest using a hand held GPS (Garmin GPSMAP 60CSx), distance above or below the SHTL, number of wash-away and inundation events that occurred throughout the incubation period, and hatch success (HS). Nest surveys were conducted between 11 May and 20 August 2012. Nest inventories were conducted beginning 21 July and ending 9 October 2012 in order to determine HS.

GIS Data*:* The data used to create risk maps included elevation, slope, and distance of nests above or below the SHTL. Digital Elevation Models (DEM) and digital orthophotos were acquired from USGS data services. Elevation, slope and distance from the SHTL influence whether or not nests experience tidal wash-over, inundation, or wash-away during storm surge.

Methods: The nesting data collected during the 2012 season was imported into ArcGIS v. 10.0 (ESRI) for storage, visualization, and analysis. A spatial data layer representing the locations of individual nests as points was created from the recorded GPS coordinates. The 2012 SHTL (characterized by scarped dunes and the wrack line resulting from the equinoctial spring tide that occurred in April) was marked with a Garmin GPS unit and added using a line shapefile. To get distance from the SHTL, multiple buffers were placed on either side of the SHTL (3, 6, 12 m). Tide buffer layers were converted to raster and reclassified to highlight different areas of the beach above and below the SHTL. The slope layer was derived from DEM with ‘slope’ tool and converted to a raster. Elevation and slope were reclassified using the ‘reclassify’ tool to denote ranges of values in each for later use in the weighted overlay. Nesting risk classes were delineated by assigning subjective weights to the layer classes that included elevation, slope, and distance from the SHTL. Weights were assigned according to the potential effect of the variable on HS based on probability of inundation from wash-over events or wash-away by storm surge. Each variable was classified according to levels of risk within each variable. Slope classes were created based on the findings of Wood and Bjorndal (2000) that a positive relationship exists between slope and HS due to the reduced chance of inundation at increased slopes, while nests in flat and poorly drained areas are more susceptible to mortality induced by inundation and/or heavy rainfall than those incubating in elevated dunes (Kraemer and Bell 1980; Foley et al. 2006). Elevation classes were assigned in the same manner as slope classes. Risk category increased closer to the SHTL due to an inverse relationship between distance from the SHTL and probability of inundation and/or wash-away.

Nesting habitat was classified by seven categories of risk ranging from very high to very low. The highest risk class was assigned a class of seven and the lowest risk class was assigned a class of one (Table 4.1). Modelbuilder was used to connect reclassified elevation, slope, and distance from SHTL to the 'weighted overlay' tool. The rasters were weighted by influence and field values that were assigned a risk level. After the model was run, the resulting risk layer was added to the map.

Maps were examined to determine which risk class each nest was deposited to determine if nests that experienced inundation, wash-away and/or nest failure were located in higher risk areas. This would suggest that inundation risk maps created using elevation, slope, and distance of nests above or below the SHTL as variables may be accurate predictors of unsuitable nesting habitat. Inundation risk maps were able to accurately predict areas of unsuitable nesting habitat based on the visual inspection of nests that experienced inundation and wash-away in each risk zone. While the dynamic nature of the nesting beach makes it extremely difficult to classify areas where nests will likely fail based on elevation, slope and distance of the nest above or below the SHTL, risk maps generated with these variables placed all inundation and wash-away events in risk classes of three or higher (Figure 4.4). No nests that incubated in risk class one or two experienced inundation or wash-away, however, some of these nests experienced wash-over. Wash-over events occurred at varying locations across the study site and throughout areas of all risk classes, indicating the potential uncertainty of predicting areas more suitable for nesting in terms of inundation risk.

The negative consequences of nesting close to the ocean include an increased vulnerability to tidal influences including inundation and complete or partial wash-away. However, nests incubating further from the ocean face a different set of threats. Hatchlings are more likely to experience disorientation/misorientation and increased post-emergence exposure time to terrestrial predators (Godfrey and Barreto 1995; Blamires and Guinea 1998). Also, eggs in clutches incubating closer to vegetation have an elevated chance of root infiltration (Whitmore and Dutton 1985). Addition of a predation risk layer to nesting risk assessment maps is suggested to determine if there is an increased predation risk associated with nesting higher on South Island beach and if so, does this threat outweigh the inundation risk of nesting lower on the beach. The predation risk layer should include elevation, slope, distance of nests to back marsh, forest edge and/or other dune vegetation, distance of nests to the SHTL, and areas prone to predation in the past based on previous years of collected data. Due to trade-offs between nesting close to the ocean vs. nesting high on the beach, a predation risk layer should be incorporated into the nesting risk assessment of South Island to more accurately evaluate the suitability of nesting habitat.

Sources of error for this investigation include 1) the land cover and DEM being obtained in 2010 and 2) slope and elevation values being derived from the DEM and not measured in the field throughout the season. It is possible that the generated risk maps did not better predict high risk areas due to the inaccuracy of the slope and elevation values used in spatial analyses. To more accurately depict conditions of this site, slope and elevation at each nest location could be measured in the field. Also, variables such as whether a nest was relocated or whether a nest experienced any loss throughout the incubation period (due to predation or methods used by project participants to locate the clutch) should be included in risk maps when considering influences on HS. Additional variables in the risk classification of South Island beach could lead to a more accurate prediction of unsuitable nesting habitat to aid in management decisions. Spatial statistical analyses should be conducted using the grouping analysis tool in ArcGIS to identify any significant areas of high and/or low clustering of nest inundations and wash-away.

Due to the dynamic nature of nesting beaches including South Island it is essential to continually assess sites for high risk areas within and between seasons as well as after storm events that have the ability to displace sand and drastically change beach topography. Sound protocols applied at a regional or local scale, regarding nest management and relocation decisions, may be inappropriate due to the dynamic nature of beaches and the changing topographic features and processes (i.e. accretion and erosion) that have the ability to influence nest success within and between seasons. Risk maps should be created for future nesting seasons to determine which factors are best at predicting unsuitable nest sites before these maps are used as tools in management decisions such as if and where nest relocations should be conducted. If similar areas continually experience severe erosion (such as the interior of the beach where steep escarpments form during spring tides and storm tides), flooding (such as low areas on the south end), and/or depredation, relocations should be performed at these locations. However, if prolonged assessment of the site determines certain areas are not prone to nest failures, leaving nests deposited in these areas to incubate *in situ* is recommended due to the potentially negative impacts of relocations outweighing the unpredictability and infrequency of extreme tidal events. South Island should also be assessed annually for not only inundation risks, but predation risks as well.

The current management strategy on South Island beach incorporates activities intended to maximize the reproductive success of loggerheads nesting at this site, including the relocation of nests deposited in unsuitable areas. Judgment varies regarding what sites are suitable for nests to remain *in situ*. Nest protection guidelines provided by the SCDNR Marine Turtle Conservation Program state no action should be taken to relocate nests that will not be destroyed *in situ* (SCDNR 2014). While nests deposited slightly above the SHTL may still wash-over during storm surge, inundation and wash-away did not occur at these nest sites. Furthermore, no discernible effects of wash-over were evident on the HS of *in situ* nests marginally landward of the SHTL. Results of this study suggest these nests are in suitable areas for incubation. Due to the potential negative impacts associated with relocation, it is imperative to adhere to current protocols regarding their use and minimize human intervention unless certain nests will be destroyed at their *in situ* locations.

Continued implementation of nest relocation is highly recommended for 1) nests laid > 3 m below the SHTL due to the high probability of inundation/wash-away and vehicular use for management actions and 2) high-risk areas that have been documented to regularly experience erosion and inundation, such as flat wash-out areas, and the base of eroding dunes and steep escarpments commonly located on the interior of the South Island beach north of the entrance. It is suggested relocation continue to be utilized as a management tool across South Island adhering to current protocol as described in the U.S. Loggerhead Recovery Plan (2008) and the SCDNR Marine Turtle Conservation Program nesting guidelines (2014) until additional years of data are collected to more accurately determine if areas considered suitable now remain so. Additionally, monitoring should continue in order to assess the impacts of management efforts and the suitability of nesting habitat across South Island beach to aid in the protection effort for loggerhead sea turtles at a local scale.

Assessment of the Probe Stick to Locate Nest Cavities: Effects of Probing on Egg Breakage throughout the Clutch

A probe stick is a tool used by statewide nesting beach survey participants that makes locating loggerhead nests more time efficient and less labor intensive than the alternative clutch location method involving hand digging the body pit. Since the majority of nest protection participants are volunteers (1,100 + statewide), it is important to consider the time and labor cost of each method. While nests located with the probe did not contain broken eggs during the 2012 and 2013 loggerhead nesting seasons at TYWC, the time and labor invested into the location of a nest by hand digging was substantially higher than the time and labor expended when utilizing the probe to find the nest cavity. Not only did the hand digging method often exceed 20 minutes before the clutch was found by participants, false crawls containing body pits and/or areas of disturbance were often dug to ensure eggs were not deposited during emergence. Bearing in mind the intense heat and humidity that coincides with the nesting season in South Carolina, the physical exertion and loss of efficiency brought on by this method may supersede its benefits. A survey that would inquire if volunteers would continue to participate in the program without a probe as a tool to assist in nest location may provide insight into the willingness of participants to utilize a more laborious method to minimize egg loss during daily nesting beach surveys.

Although a significantly higher number of eggs were found broken in nests located with the probe, this study suggests HS does not significantly vary based on nest location method or whether or not nests experienced loss upon location. However, egg breakage caused directly or indirectly by the probe could potentially decrease HS of *in situ* nests if the participant is unaware of the loss. When relocating nests, all broken eggs are evident, removed, and the contents gently cleaned from the shells of the intact eggs that remain in the clutch. This protocol (SCDNR 2014) is followed so odor from broken eggs does not attract predators and/or lead to fungal invasion throughout the clutch

(Wyneken et al. 1988). Egg breakage caused by probing in *in situ* nests may go unnoticed and ultimately lead to a decrease in HS.

The results of this study suggest that eggs reported as broken by project participants during nest relocations are not damaged during nesting efforts of female loggerheads due to 1) the lack of eggs broken in nests found hand digging and 2) a strong correlation evident between probing and presence of broken eggs throughout the clutch. Loss attributed to probing will be more accurately quantified in the SCNDR database on seaturtle.org. Eggs found broken (with no sign of direct puncture) at the bottom or center of a clutch will be recorded as loss due to probing as opposed to ‘broken in nest’. Loss recorded as ‘broken in nest’ statewide nearly doubled that of loss attributed to probing in 2012 (n = 989 eggs, ‘broken in nest’; n = 506 eggs, probing loss) and more than doubled the recorded probing loss in 2013 (n = 1,399 eggs, ‘broken in nest’; n = 513 eggs, probing loss). A more accurate quantification of eggs being lost statewide due to the probing method will help focus management efforts on nesting beaches. Participants having difficulty minimizing loss while using the probe will be more easily recognized and will receive additional training provided by the Marine Turtle Conservation Program team where necessary. Removal of participants from their projects probing permit may result if additional training does not improve their use of the probing method to locate the clutch (C. P. Hope, personal communication).

While locating the clutch of loggerheads and other species of sea turtles may be less time and labor intensive when using the probe, the use of this tool remains controversial (Bishop 2001). This method can lead to the incidental breakage (puncture) of one or more eggs (D.B. Griffin and C.P. Hope, personal communication; Bishop 2001; FWC 2002). However, the continued use of this tool on nesting beaches by properly trained participants is recommended based on its efficiency and results from this investigation that indicate HS is not significantly altered by nest location method and loss upon detection. These findings suggest that the probe is an appropriate tool to aid in the location of nest cavities and its use is not detrimental to loggerhead HS in South Carolina.

Figure 4.1: Map displaying area of high inundation risk on South Island beach at Tom Yawkey Wildlife Center, Georgetown County, South Carolina during the 2012 and 2013 loggerhead nesting seasons. High risk areas are located in the interior of the beach north of the entrance. Nests that experienced wash-away are denoted with a black X. Nests that experienced inundation are denoted with a red square. All nests that experienced inundation were nest failures (defined as HS < 60%) although viable offspring were often produced in these nests.

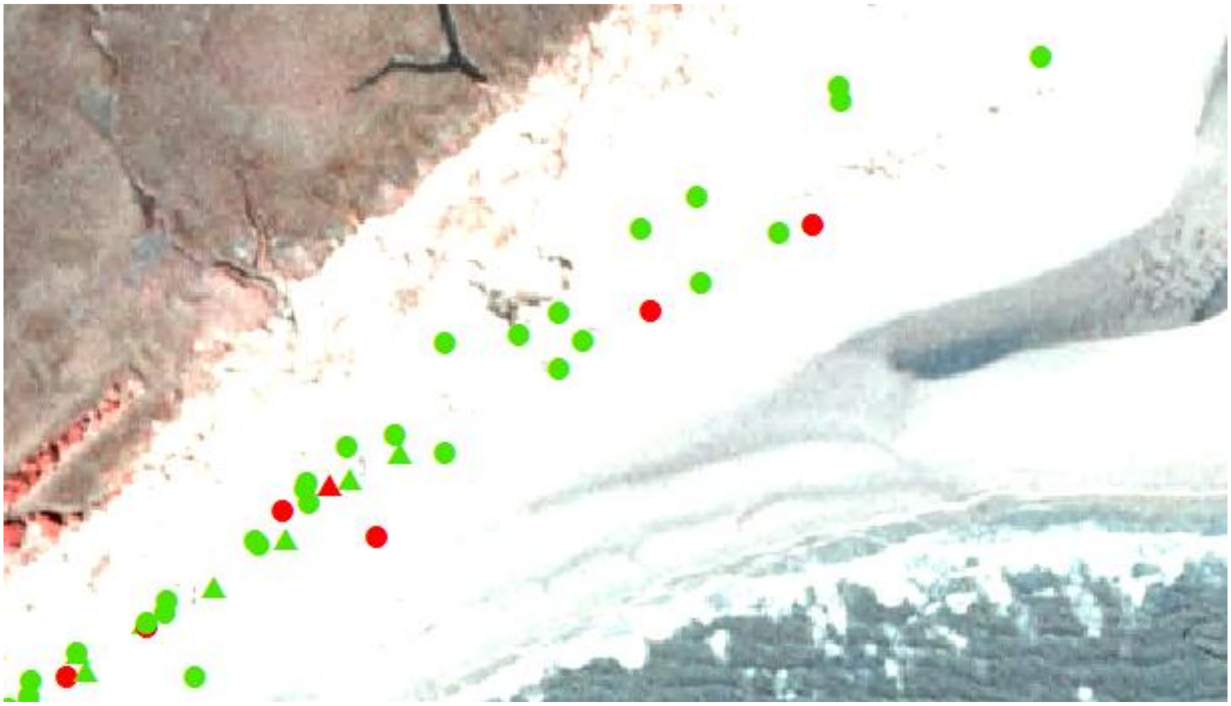


Figure 4.2: Map displaying area of suitable nesting habitat with a low inundation risk on South Island beach at Tom Yawkey Wildlife Center, Georgetown County, South Carolina during the 2012 and 2013 loggerhead nesting seasons. Low risk areas are located at the north end of the site. Nests that experienced wash-over are represented by triangles. Nests that did not experience any tidal influence are denoted by circles. Red symbols represent nest failures (defined as HS < 60%). Green symbols represent successful nest sites (defined as HS ≥ 60%).

Figure 4.3: Map displaying area of suitable nesting habitat with a low inundation risk on South Island beach at Tom Yawkey Wildlife Center, Georgetown County, South Carolina during the 2012 and 2013 loggerhead nesting seasons. This low risk area is located at the south end of the site. Nests that experienced wash-over are represented by triangles. Nests that inundated are denoted by squares. Nests that did not experience any tidal influence are denoted by circles. The black X at the southern tip symbolizes a nest left > 3 m below the SHTL that washed away. Red symbols represent nest failures (defined as HS < 60%). Green symbols represent successful nest sites (defined as HS ≥ 60%).

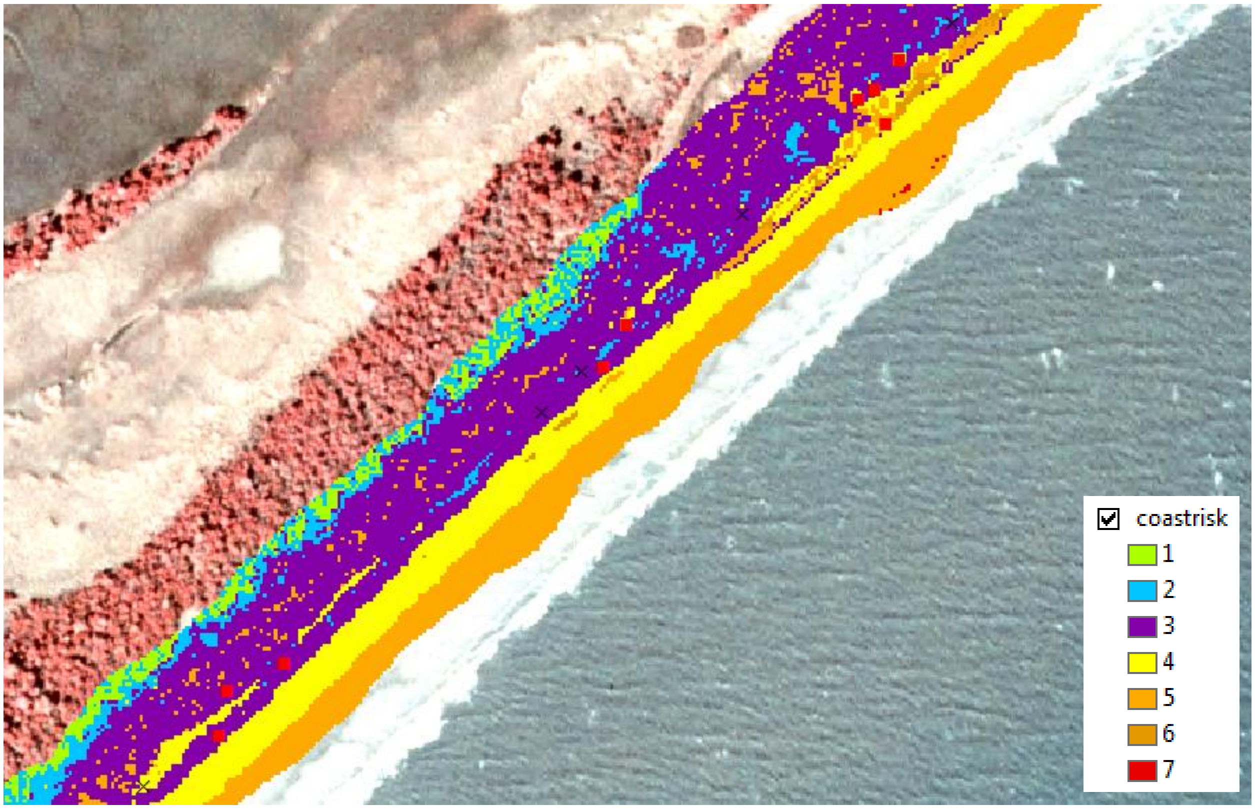


Figure 4.4: Inundation risk map of nesting habitat on South Island beach at Tom Yawkey Wildlife Center, Georgetown County, South Carolina during the 2012 loggerhead nesting season. Risk classes are color coded ranging from lowest inundation risk (1 = green) to highest inundation risk (7 = red). Nests that experienced wash-away are denoted with a black X. Nests that experienced inundation are denoted with a red square. All nests that experienced inundation were nest failures (defined as HS < 60%) although viable offspring were often still produced in these nests.

Table 4.1: Variables and assigned weights used to determine inundation risk classes on South Island beach at Tom Yawkey Wildlife Center, Georgetown County, South Carolina.

Variables	**Weights**	**Risk Classes**
Elevation	35	very high risk (lowest elevation) high risk medium risk low risk very low risk (highest elevation)
Slope	35	high risk (slope of 0) medium high risk medium low risk low risk
Distance above or below SHTL	30	very high risk (furthest below) high risk medium high risk medium low risk low risk very low risk (furthest above)

LITERATURE CITED

Bimbi, M.K. 2009. Effects of relocation and environmental factors on loggerhead sea turtle (*Caretta caretta*) nests on Cape Island. Thesis, College of Charleston, Charleston, South Carolina, USA.

Bishop, G.A. and B.K. Meyer. 2011. Sea Turtle Habitat Deterioration on St. Catherines Island:Defining the Modern Transgression. Anthropological Papers American Museum of Natural History 94: 271-295.

Blamires, S.J. and M.L. Guinea. 1998. Implications of nest site selection on egg predation at the sea turtle rookery at Fog Bay. *In* R. Kennett, A. Webb, G. Duff, M. Guinea, and G. Hill (eds.). Marine turtle conservation and management in Northern Australia, 20-24. Proceedings of a workshop held at the Centre for Indigenous Natural and Cultural Resource Management and centre for Tropical Wetlands Management, Northern Territory University, Darwin, Australia.

Carthy, R. 1996. The role of the eggshell and nest chamber in loggerhead turtle (*Caretta caretta*) egg incubation. Ph.D. Dissertation, University of Florida, Gainesville.

Caut, S., E. Guirlet, and M. Girondot. 2010. Effect of tidal overwash on the embryonic development of leatherback turtles in French Guiana. Marine Environmental Research 69: 254-261.

Coll, G.E. 2010. Sea Turtle Nest Management: Examining the Use of Relocation as a Management Tool on Three South Carolina Beaches. Thesis, College of Charleston, Charleston, South Carolina, USA.

Davenport, J. 1989. Sea turtles and the greenhouse effect. British Herpetological Society Bulletin 29: 11-15.

Davenport, J. 1997. Temperature and the life-history strategies of sea turtles. Journal of Thermal Biology 22: 479-488.

Eckert, K.L. and S.A. Eckert. 1985. Tagging and nesting research of leatherback sea turtles (*Dermochelys coriacea*) on Sandy Point, St. Croix, USVI, 1985. Annual Report. U.S. Fish and Wildlife Service, USFWS Ref. MIN 54-8680431. 58 pp.

Eckert, K.L. and S.A. Eckert. 1990. Embryo Mortality and Hatch Success in *In Situ* and Translocated Leatherback Sea Turtle *Dermochelys coriacea* Eggs. Biological Conservation. 53: 37-46.

Eckert, K. L., K.A. Bjorndal, F.A.Abreu-Grobois and M. Donnelly (Editors). 1999. Research and Management Techniques for the Conservation of Sea Turtles. IUCN/SSC MarineTurtle Specialist Group Publication No. 4.

Erhart, L.M. and B.E. Witherington. 1987. Human and natural causes of marine turtle nest and hatchling mortality and their relationship to hatchling production on an important Florida nesting beach. Florida Game and Fresh Water Fish Commission, Nongame Wildlife Program, Technical Report No.1:i-x, 1-141.

Ernest, R.G. and R.E. Martin. 1993. Sea turtle protection program performed in support of velocity cap repairs, Florida Power and Light Company, St. Lucie Plant, 1991. Report prepared for Florida Power and Light Company. Applied Biology, Inc., Jensen Beach, Florida. 51 pp.

Eskew, T.S. 2012. Best Management Practices for Reducing Coyote Depredation on Loggerhead Sea Turtles in South Carolina. Thesis, Clemson University, Clemson, South Carolina, USA.

Fish M.R., I.M. Cote, J.A. Gill, A.P. Jones, S. Renshoff and A.R. Watkinson. 2005. Predicting the impact of sea-level rise on Caribbean sea turtle nesting habitat. Conservation Biology 19: 482-491.

Florida Fish and Wildlife Conservation Commission (FWC). 2002. Sea Turtle Conservation Guidelines. Available from http://el.erdc.usace.army.mil/flshore/pdfs/Guidelines.pdf. (accessed March 2013).

Foley, A.M. 1998. Effects of egg position on characteristics of hatchlings: phenotypic plasticity, nesting strategies, and a possible advantage of temperature-dependent sex determination. Ph.D. Dissertation. University of South Florida.

Foley A.M., S.A. Peck and G.R. Harman GR. 2006. Effects of sand characteristics and inundation on the hatching success of loggerhead sea turtle (*Caretta caretta*) clutches on low-relief mangrove islands in southwest Florida. Chelonian Conservation Biology 5: 32-41.

Garmestani, A.S., H.F. Percival, K.M. Portier and K.G. Rice. 2000. Nest-site selection by the loggerhead sea turtle in Florida's Ten Thousand Islands. Journal of Herpetology 34: 504-510.

Godfrey, M.H. and R. Barreto. 1995. Beach vegetation and sea-finding orientation of turtle hatchlings. Biological Conservation 74: 29 -32.

Godley, B.J., A.C. Broderick and N. Mrosovsky. 2001. Estimating hatchling sex ratios of loggerhead turtles in Cyprus from incubation durations. Marine Ecology Progress Series 210: 195-201.

Hanson, J., T. Wibbels and R.E. Martin. 1998. Predicted female bias in sex ratios of hatchling loggerhead sea turtles from a Florida nesting beach. Canadian Journal of Zoology 76: 1850-1861.

Hawkes, L.A., A.C. Broderick, M.H. Godfrey and B.J. Godley. 2007. Investigating the potential impacts of climate change on a marine turtle population. Global Change Biology 13: 923-932.

Herrera, A.E. 2006. The effects of nest management methods on sex ratio and hatching success of leatherback turtles (*Dermochelys coriacea*). Biological Conservation.

Hilterman, M.L. 2001. The sea turtles of Suriname, 2000. Biotopic technical report. Commissioned by the WWF-Guianas, Paramaribo, Suriname. 61 pp.

Hopkins, S.R. and T.M. Murphy. 1983. Management of loggerhead turtle nesting beaches in South Carolina. Study Completion Report to U.S. Fish and Wildlife Service. South Carolina Wildlife and Marine Resources Department. Charleston, South Carolina.

Horrocks, J.A. and N. Scott. 1991. Nest site location and nest success in the hawksbill turtle, *Eretmochelys imbricata,* in Barbados, West Indies. Marine Ecology Progress Series 69: 1–8.

Janzen, F.J. and G.L. Paukstis. 1991. Environmental sex determination in reptiles-ecology,evolution, and experimental design. Quarterly Review of Biology 66: 149-179.

Johnston, K., E. Koepfler, M. James, S. Dawsey, E. Freeman, P. Schneider, M. Schneider and B. Brabson. 2007. Spatial and Temporal Influence of Meteorological and Substrate Factors upon Loggerhead Sea Turtle Nests in South Carolina. Proceedings of the Twenty-Eighth Annual Symposium on Sea Turtle Biology and Conservation.

Kraemer, J. E. and R. Bell. 1980. Rain-induced mortality of eggs and hatchlings of ` loggerhead sea turtles (*Caretta caretta*) on the Georgia coast. Herpetologica 36:72-77.

Leblanc, A.M., K.K. Drake, K.L. Williams, M.G. Frick, T. Wibbels and D.C. Rostal. 2012. Nest temperatures and hatchling sex ratios from loggerhead turtle nests incubated under natural field conditions in Georgia, United States. Chelonian Conservation and Biology 11: 108-116.

Marcovaldi, M.A., M.H. Godfrey and N. Mrosovsky. 1997. Estimating sex ratios of loggerhead turtles in Brazil from pivotal incubation durations. Canadian Journal of Zoology 75: 755-770.

Matsuzawa, Y., K. Sato, W. Sakamoto and K.A. Bjorndal. 2002. Seasonal fluctuations in sand temperature: effects on the incubation period and mortality of loggerhead sea turtle (*Caretta caretta*) pre-emergent hatchlings in Minabe, Japan. Marine Biology 140: 639-646.

McElroy, M. 2009. The effect of screening and relocation on hatching and emergence success of loggerhead sea turtle nests at Sapelo Island, Georgia. Thesis, University of Georgia, Athens, Georgia, USA.

Milton, S.L., S. Leone-Kabler, A.A. Schulman and P.L. Lutz. 1994. Effects of Hurricane Andrew on the sea turtle nesting beaches of south Florida. Bulletin of Marine Science 54: 974-981.

Mitchell, N. J., M. R. Kearney, N. J. Nelson, and W. P. Porter. 2008. Predicting the fate of a living fossil: How will global warming affect sex determination and hatching phenology in tuatara? Proceedings of the Royal Society Bulletin 275: 2185-2193.

Mrosovsky, N. and C.L. Yntema. 1980. Temperature dependence of sexual differentiation in sea turtles: Implications for conservation practices. Biological Conservation 18: 271-280.

Mrosovsky, N., P.H. Dutton and C.P. Whitmore. 1983. Sex ratios of two species of sea turtle nesting in Suriname. Canadian Journal of Zoology 62: 2227–2239.

Mrosovsky, N. 1988. Pivotal temperatures for loggerhead turtles (*Caretta caretta*) from northern and Southern nesting beaches. Canadian Journal of Zoology 66: 661-669.

Mrosovsky, N. and C. Pieau. 1991. Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex determination in reptiles. Amphibia-Reptilia 12: 169-179.

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

Mrosovsky, N. 1994. Sex ratios of sea turtles. The Journal of Experimental Zoology 270: 16-27.

Mrosovsky, N., S. Kamel, A.F. Rees and D. Margaritoulis. 2002. Pivotal temperature for loggerhead turtles (*Caretta caretta*) from Kyparissia Bay, Greece. Canadian Journal of Zoology 80: 2118–2124.

Mrosovsky, N. 2006. Distorting gene pools by conservation: assessing the case of doomed turtle eggs. Environmental Management 38: 523–531.

Mrosovsky, N. 2008. Against Oversimplifying the Issues on Relocating Turtle Eggs. Environmental Management 41:465-467.

National Marine Fisheries Service (NMFS) and U.S. Fish and Wildlife Service (USFWS). 1991. Recovery Plan for U.S. Population of the Loggerhead Turtle. National Marine Fisheries Service, Washingon D.C.

National Marine Fisheries Service (NMFS) and U.S. Fish and Wildlife Service (USFWS). 2008. Recovery Plan for the Northwest Atlantic Population of the Loggerhead Sea Turtle (*Caretta caretta*), Second Revision.National Marine Fisheries Service, Silver Spring, Maryland.

Pfaller, J.B., C.J. Limpus, and K.A. Bjorndal. 2008. Nest-site selection in individual loggerhead turtles and consequences for doomed-egg relocation. Conservation Biology 23:72–80.

Pike, D.A. and J.C. Stiner. 2007. Sea turtle species vary in their susceptibility to tropical cyclones. Oecologia 153: 471-478.

Pike D.A. 2008. Natural beaches confer fitness benefits to nesting marine turtles. Biology Letters 4: 704–706.

Rees, A.F. and D. Margaritoulis. 2004. Beach temperatures, incubation durations and estimated hatchling sex ratio for loggerhead nests in Kyparissia Bay, Greece. B.C.G. Testudo 6: 23-36.

Schmid J.L., D.A. Addison, M.A. Donnelly, M.A. Shirley and T. Wibbels. 2008. The effect of Australian Pine (*Casuarina equisetifolia*) removal on loggerhead sea turtle (*Caretta caretta*) incubation temperatures on Keewaydin island, Florida. Journal of Coastal Research 55: 214–220.

Schulz, J.P. 1975. Sea turtles nesting in Surinam. Zoologische Verhandelingen 143: 1-143.

Shaw, K.R. 2013. Effects of Inundation on Hatch Success of Loggerhead Sea Turtle (*Caretta caretta)* Nests. Thesis, University of Miami, Miami, Florida, USA.

South Carolina Department of Natural Resources (SCDNR). 2014. Guidelines for Marine

Turtle Permit Holders. Nest Protection Management. Charleston, South Carolina. Available from: www.dnr.sc.gov/seaturtle/nt/nestguide.pdf (accessed February 2014).

South Carolina Department of Natural Resources (SCDNR). 2014. Sea Turtle Nest Monitoring System: Sand Island Beach Description. Available from: http://seaturtle.org/nestdb/index.shtml?year=2014&view_beach=39 (accessed January 2014)

Stancyk, S.E., O.R. Talbert and J.M. Dean. 1980. Nesting Activity of the Loggerhead Turtle *Caretta Caretta* in South Carolina, II. Protection of Nests from Raccoon Predation by Transplantation. Biological Conservation 18: 289-298.

Tuttle, J.A. 2007. Loggerhead Sea Turtle (*Caretta caretta*) Nesting on a Georgia Barrier Island: Effects of Nest Relocation. Thesis, Georgia Southern University, Statesboro, Georgia, USA.

Wibbels, T., R.E. Martin, D.W. Owens and M.S. Amoss Jr. 1991. Female-biased sex ratio of immature loggerhead sea turtles inhabiting the Atlantic coastal waters of Florida. Canadian Journal of Zoology 69: 2973-2977.

Whitmore, C. P. and P.H. Dutton. 1985. Infertility, embryonic mortality and nest-site selection in leatherback and green sea turtles in Suriname. Biological Conservation. 34: 251-272.

Wood, D.W. and K.A. Bjorndal. 2000. Relation of temperature, moisture, salinity, and slope to nest site selection in loggerhead sea turtles. Copeia 2000: 119-128.

Wyneken, J., T.J. Burke, M. Salmon and D.K. Pedersen. 1988. Egg failure in natural and relocated sea turtle nests. Journal of Herpetology 22: 88-96.

Yntema, C.L. and N. Mrosovsky. 1980. Sexual differentiation in hatchling loggerheads (*Caretta caretta*) incubated at different controlled temperatures. Herpetologica 36: 33-36.

Yntema, C.L. and N. Mrosovsky. 1982. Critical periods and pivotal temperatures for sexual differentiation in loggerhead sea turtles. Canadian Journal of Zoology 60: 1012-1016.