BARNACLE GROWTH AS AN INDICATOR OF THE ONSET AND DURATION OF
THE CLINICAL SYMPTOMS OF DEBILITATED TURTLE SYNDROME AFFECTING
LOGGERHEAD (*Caretta caretta*) SEA TURTLES

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

MASTER OF SCIENCE

in

ENVIRONMENTAL STUDIES

by

KELLY SLOAN
MAY 2011

at

THE GRADUATE SCHOOL OF THE COLLEGE OF CHARLESTON

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ABSTRACT

BARNACLE GROWTH AS AN INDICATOR OF THE ONSET AND DURATION OF THE CLINICAL SYMPTOMS OF DEBILITATED TURTLE SYNDROME AFFECTING LOGGERHEAD (Caretta caretta) SEA TURTLES.

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Debilitated Turtle Syndrome (DTS) has become a growing concern for sea turtles in South Carolina, and in recent years (2000-2010) has accounted for an increasing percentage of loggerhead (Caretta caretta) strandings in the state. Although the causes of DTS are unknown, loggerheads stranding with DTS are characteristically emaciated, hypoglycemic, anemic, and heavily encrusted with epibiota. The illness is thought to ultimately weaken the turtle to the point that it floats at the water’s surface, restricting the animal to an environment that predisposes it to heavy recruitment of the barnacle Chelonibia testudinaria on the carapace and soft tissue. The time it takes for debilitated loggerheads to manifest this heavy barnacle load is unknown. Our study measured how barnacle growth rate correlates with several environmental factors and experimentally tested whether barnacle recruitment on loggerhead scute varied between debilitated and non-debilitated individuals. Floating arrays holding test panels consisting of four treatments (debilitated turtle scute, non-debilitated turtle scute, Plexiglas, and slate tile) were placed at four independent experimental sites near Charleston, South Carolina. Results from two seasons (2009 and 2010) indicate that the larvae of the turtle barnacle C. testudinaria recruit at significantly higher rates along the open shore but do not recruit differentially to the four substrates. Growth rates for this barnacle are also higher in open water but do not vary with substratum type. Overall, individual barnacles had a mean growth rate of roughly 6.3 mm²/day on sea turtle carapace substrates.
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INTRODUCTION

The loggerhead sea turtle (Caretta caretta) was listed as a threatened species in 1978 under the Endangered Species Act (ESA) and consequently all life stages of the loggerhead are federally protected. Loggerheads are also listed as Endangered on the International Union for the Conservation of Nature (ICUN) Red List. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) lists loggerhead turtles in CITES Appendix 1. This species is also protected by the South Carolina (SC) Nongame and Endangered Species Conservation Act of 1976 and was designated the official state reptile on July 28, 1988. The Northern Recovery Unit for loggerhead turtles consists of North Carolina, SC, Georgia, and Virginia, and is distinct geographically and genetically from the Peninsular Florida Recovery Unit (NMFS and USFWS, 2008). Sixty-eight percent of the total nesting effort for the Northern Recovery Unit is in SC. Therefore, conservation of females nesting in South Carolina is important for population management as well as genetic diversity.

It is important to document in-water mortality of sea turtles to identify threats to juvenile and adult sea turtles and subsequently implement appropriate management initiatives. The SC Department of Natural Resources (DNR) collects data on all sea turtle strandings in SC. Sources of mortality for sea turtles in SC include watercraft, entanglement, disease, dredge kills, cold-stunning, and pollution. Debilitated Turtle Syndrome (DTS) is a disease that affects primarily juvenile loggerheads. Debilitated turtles are characteristically emaciated, hypoglycemic, and encrusted in barnacles.
SCDNR data indicate that between 2 - 22% of loggerhead strandings in South Carolina from 2000-2010 exhibit symptoms of DTS (Figure 1). Historical stranding data since 1980 were examined and a stranded qualified as debilitated if it fit the criteria of being emaciated and having a heavy barnacle load (determined either by photos or comments on stranding form). Approximately 89% of debilitated turtles stranded in the northern part of the state (north of 32º32’06”N, 80º15’45”W; Figure 2), and 72% stranded during the months of April, May and June (Figure 3) from 1980 - 2010. Ninety-two percent of all debilitated strandings since 1980 were juveniles with a mean curved carapace length of 74.2 cm. It is also noteworthy that 37% of debilitated turtles strand alive.

Figure 1. Percent of loggerhead strandings in South Carolina exhibiting symptoms of Debilitated Turtle Syndrome from 1980 - 2010 (SCDNR unpublished data)
Figure 2. Total number of strandings exhibiting symptoms of Debilitated Turtle Syndrome by county from 2000 - 2010 (ordered from north to south)

Figure 3. Total number of strandings exhibiting symptoms of Debilitated Turtle Syndrome by month from 2000 - 2010
Debilitated turtles are emaciated, hypoglycemic, and anemic. Health assessment and necropsy data suggest that these turtles are being affected by a wide range of secondary bacterial infections and parasites while the primary causes of DTS are still unknown (Terry Norton, unpublished data). Data from previous research suggest that barnacles are not found on certain soft tissues in animals deemed healthy by veterinary standards. Presumably, the illness ultimately weakens the turtle to the point that it floats at the water’s surface, restricting the animal to an environment that predisposes it to heavy barnacle recruitment. Another probable cause of barnacle encrustation also relates to immobility. Healthy turtles often practice self-grooming and remove barnacles by wedging themselves into coral crevices or by actively scraping their carapaces against hard substrata (Frick and McFall, 2007). Sea turtles exhibiting DTS may be too ill to actively remove barnacles (Flint et al., 2010), allowing for especially heavy loads to accumulate on their carapaces and soft tissues.

Although debilitated turtles always present with heavy barnacle loads, a study investigating the relationship between epibiotic load (organisms living on the scute) and hematologic values produced highly variable results and found no statistically significant correlation between the two parameters (Stamper et. al, 2005). A more recent study also indicates that carapacial barnacle load on small immature green turtles (*Chelonia mydas*) was not determined to be a useful indicator of health status (Flint *et al.*, 2010). However, this study did find that greens with high levels of *Chelonibia testudinaria* on the plastron are more often clinically unhealthy than those with low counts.
Researchers and veterinarians suspect that disease rates in marine organisms have been increasing over the past few decades. Ward and Lafferty (2004) examined the potential for seemingly increasing rates of diseases to be correlated with increased documentation in recent years. Even after normalizing total disease reports to documentation rates, a higher rate of disease outbreaks in marine organisms since the 1970’s has been established (Ward and Lafferty, 2004; Harvell et al., 2002). These studies revealed increased disease reports specifically in sea turtles. Climate change has been implicated as a potential link to emerging marine diseases, as stressed hosts are more susceptible to infection (Ward and Lafferty, 2004). Disease could also increase with increased host density. The effects of these issues are unknown in sea turtles.

Some researchers have proposed that DTS begins with cold-stunning, when animals either deviate from their typical warm-water migratory pathways or when periods of severely cold weather cause water temperatures to rapidly drop in shallow water systems (Witherington and Ehrhart, 1989). Reptiles are ectotherms and therefore behavioral thermoregulation is an important aspect of the thermal biology of sea turtles (Jacobsen, 2007, p 4). All reptiles have a preferred optimum temperature zone that is regulated by behavioral and physiological mechanisms. This temperature may change based on the season of the year or time of day. If the temperature falls below the critical thermal minimum, the reptile will suffer from cold narcosis and will eventually lose the ability to actively swim (Jacobsen, 2007).

Although unhealthy turtles are known to carry heavy barnacle loads, healthy animals also host a diversity of epibionts. At least 100 invertebrates have been documented on loggerheads (Frick et al., 1998). Fourteen documented species of barnacles have been
found on loggerhead sea turtles, all belonging to a single Balanomorph family, the Coronulidae (Epibiont Research Cooperative, 2007). Nine species have been reported on Atlantic loggerheads. Of particular interest is the barnacle *Chelonibia testudinaria*, which accumulates in large numbers on the carapace, plastron (ventral surface of turtle), and soft tissues of debilitated turtles. *Chelonibia testudinaria* is the most conspicuous and comprehensively studied of the turtle barnacles (Zardus and Hadfield, 2004). Like all barnacles of the genus *Chelonibia*, *C. testudinaria* is distinct because it has eight wall plates. This species has been found on the head, carapace, plastron, skin, and one nail of sea turtles (Epibiont Research Cooperative, 2007).

These barnacles are obligate commensals of turtles and they are commonly referred to as “turtle barnacles.” Turtle barnacles exhibit high host specificity, and it is generally believed that they rarely occur on anything other than sea turtles (Zardus and Hadfield, 2004). Barnacle-turtle associations have probably been evolving for millions of years and the commensal relationship has likely arisen many times throughout barnacle evolution (Zardus and Hadfield, 2004). The effects of barnacles on sea turtles are not clear but they may have a negative effect due to increased drag, or they may be advantageous for reasons such as protection from predators. In general, epizoic barnacles, or those that live on the surface of an animal, are considered to have a commensal relationship (where one organism benefits while the other is unaffected) and have no harmful effect on the host (Zardus and Hadfield, 2004).

Barnacles are hermaphroditic and copulatory (Anderson, 1994). They are not self fertile because they are either sequential hermaphrodites or they alternate between male and female during breeding. Breeding occurs via cross-fertilization with receptive
neighbors. In some circumstances, a small individual attaches directly to another full size hermaphrodite and instead of growing large remains as a small male, acting as a sperm donor (Zardus and Hadfield, 2004). Darwin (1854) termed these tiny, male-only individuals “complemental males.”

Barnacle larvae go through six distinct naupilar stages, the first two of which may be retained in the mantle cavity (Zardus and Hadfield, 2004). The seventh instar is the cyprid stage, which occurs approximately nine days after hatching. The role of the cyprid is to successfully locate and attach to surfaces conducive to adult growth and survival. For turtle barnacles, locating a specific host animal is akin to finding a spatially limited target, which means that the larvae will likely need to actively seek out the organism. The main mechanism of transport for all larvae is the current (Railkin, 2004), and barnacle cyprids also use the current to orient themselves and swim. Nauplii and cyprids have chitinous shells that allow them to swim more efficiently by compensating for their negative buoyancy (Railkin, 2004). *Heterosaccus dollfusi* cyprids were shown to be capable of modifying their swimming pattern, direction, velocity, and turning rate to navigate in changing environmental conditions (Pasternak, 2004).

Host-specific barnacles, such as *C. testudinaria*, colonize with more precision than generalized settlers (Anderson, 1994). The swimming cyprid encounters a substratum, at which point the exploratory behavior begins. The cyprid attaches itself to the substratum by antennules and then explores it by walking on the antennules. First, the cyprid walks uniformly, and if the conditions seem suitable it will continue for a specific settling location by frequently changing walking direction. If the substratum is unfavorable, the cyprid swims off. Once it finds a suitable location the cyprid begins to attach itself by
means of an attachment disc. The glands on the disc secrete a proteinaceous material that secures the developing juvenile to the substratum.

Permanent attachment is accomplished by secretion of cement (Anderson, 1994). Cyprids have two cement glands that form during the stage VI nauplius. Secretion accumulates in the ducts and is released when the cyprid is ready to permanently attach. The liquid cement hardens within one to three hours into a quinone-tanned protein.

Locating a suitable habitat is critical for the survival of barnacles and has a strong impact on dispersal and recruitment (Pasternak et al., 2002; Railkin, 2004). Reproduction is dependent on colonial settlement due to the need for cross-fertilization. When looking for settlement sites on hosts and conspecific adults, the most reliable environmental cues are soluble chemical metabolites that are released from the target organisms. The role of protein cues from conspecifics for inducing settlement has been shown in intertidal barnacles (Crisp and Meadows, 1962). Light and gravity are also important cues directing settlement. Cyprids have chemoreceptors and mechanoreceptors located on the antennulæ, carapace, and caudal appendages, and in especially high density on the attachment disc (located underneath the third segment of the antennulæ). Other sensory organs that have been mentioned in substrate selection are the nauplius eye, compound eyes, the setae of caudal appendages, and sensory organs positioned on the surface of the carapace (Railkin, 2004).

The survival and growth of epizootic barnacles is also strongly dependent on the location and orientation of the animal (Pasternak et al., 2002). Orientation is determined at the time of cyprid settlement. It is particularly simple on an animal such as a turtle because in these circumstances the barnacles are exposed to uni-directional currents due
to the nature of turtle locomotion (Pasternak et al., 2002). Turtles offer patchy environments for epibionts to select from (Hayashi and Tsuji, 2008). *Chelonibia testudinaria* on loggerhead carapaces tend to orient themselves so that their rostrum faces the oncoming current (Pasternack, 2002).

Once a turtle barnacle has settled on a sea turtle, there may be differential persistence among regions of the carapace due to probabilities of desiccation, food availability, and abrasion from contact with flippers or other objects that the turtle may scratch against (Pfaller et al., 2006). Studies investigating loggerhead carapace epibionts have found that the highest densities of barnacles were found on the posterial and vertebral zones (Pfaller et al., 2006; Matsuura and Nakamura, 1993). Hayashi and Tsuji (2008) also confirmed aggregated distributions of barnacles on green turtles captured in the wild by fishermen. These patterns could be due to differential recruitment, survival, or both. Water flow, turtle behavior, interactions among epibionts, and varying tolerance to desiccation and physical trauma are very likely to play a role in the observed settling patterns (Frick et al., 2004).

Similar to recruitment, barnacle growth is also intimately linked to environmental conditions. In balanomorphs, the primary determinant of wall plate growth rate is immersion time and body growth is primarily dependent on the feeding regime (Anderson, 1994). Feeding is generally passive except in very slow water flow and is accomplished by spreading thoracic appendages to form a fan that faces towards the incoming flow. After the food has been captured, the cirri withdraw into the mantle cavity and transfer the particles to the mouth (Pasternak et al., 2002). Cirral activity only
occurs when the animal is immersed (Anderson, 1994), so body growth is maximized when immersion time is greatest.

Recent studies have investigated the relationship between barnacle growth and upwelling events, as these events normally carry a large supply of food. A study in Oregon demonstrated that growth rates were low during upwelling events. Since upwelling events carry high levels of phytoplankton, the author concluded that factors other than phytoplankton contribute to variation in barnacle growth (Sanford and Menge, 2001). However, most literature suggests that food concentration and water velocity has an influence on growth and reproduction of barnacle populations (Bertness et al., 1991 and Moore, 1936).

Other factors affecting barnacle growth rates include temperature, current flow, tidal amplitude, food supply, population density, parasitic infections, seasonal balance between reproductive and vegetative activity, and simultaneous presence of other plant or animal species (Anderson, 1994). External growth is also manipulated by erosion, which can produce significant variation between species and individuals of similar age. Complicating the matter further is the interaction of these discrete factors when acting simultaneously through the life of the barnacle. Previous studies have noted that even linear measurements of growth rates can yield variations within species by up to a factor of seven (Anderson, 1994).

The time it takes for heavy barnacle loads to manifest on the carapace and soft tissue of loggerheads exhibiting symptoms of DTS is unknown. Although the sea turtle literature is replete with scattered studies cataloging sea-turtle epibionts, research characterizing barnacle recruitment and growth rates on sea turtle shells is largely absent.
This study assessed barnacle recruitment and growth rates in an effort to develop a protocol that allows scientists and veterinarians to use barnacles as a biomarker for estimating the length of time that a debilitated loggerhead has been passively floating.

There were two primary objectives of this study. The first goal was to develop a trajectory for barnacle growth rates on loggerhead sea turtles (*Caretta caretta*), focusing on the turtle barnacle, *Chelonibia testudinaria*. A growth curve for this species of barnacle on sea turtle carapaces currently does not exist. The second objective was to quantify recruitment rates of barnacles on loggerhead sea turtle shell. Several additional questions were also of interest. Secondary objectives were to determine variability in growth and recruitment rates among four experimental sites, determine variability in growth and recruitment rates between debilitated vs. non-debilitated loggerhead scutes, and to identify where barnacle settlement occurs (inshore vs. offshore).
MATERIALS AND METHODS

Field Seasons

The 2009 and 2010 field seasons extended from May through October and April through October, respectively. Significant modifications to the experimental design, made between the 2009 and 2010 season for improved sampling methodology, are detailed below.

Experimental Arrays

A series of 0.9 m x 0.6 m floating arrays holding barnacle settlement panels were constructed from either ¾” polyvinyl chloride (PVC) pipe or aluminum (Figure 4A and 4B). Each array carried a total of 12 test panels. The test panels consisted of three panels each of Plexiglas, slate, debilitated loggerhead turtle carapace, and non-debilitated loggerhead turtle carapace. The Plexiglas and slate panels provided two control conditions to identify and compare recruitment and growth rates on alternate substrata. Panels were randomly assigned positions on the arrays.

PVC arrays were used for inshore locations. Styrofoam pipe insulation was attached to the top of the PVC array, allowing the test panels to float at a uniform depth of 30.5 cm.

Figure 4. Experimental arrays: A) PVC array used for three inshore sites B) Aluminum array placed offshore
below the water’s surface. Marine grade rope was tied to two corners of each array and used to attach arrays at sites.

An array designed for offshore conditions was similar in design to the PVC arrays but was constructed of welded aluminum (Figure 4B) and was attached to a U.S. Coast Guard buoy using a steel cable and galvanized shackles. Crab pot buoys made of PVC were attached to the top of the array instead of Styrofoam pipe insulation.

Due to the high percentage of panels that were physically removed by wave action in 2009 (particularly offshore), two zip ties were used as reinforcement on the edges of the panels at all sites in 2010.

Array Deployment

Arrays were deployed twice in 2009 from May through July and September through October. In 2010 the panels on the array were changed approximately once per month between the months of April and October. They arrays were deployed 6 times with roughly one week intervals between deployments.

Obtaining and Preparing Carapace Samples

The Sea Turtle Stranding and Salvage Network (STSSN) was established in 1980 to document sources of mortality and injury to sea turtles. Network members report every sea turtle that strands in South Carolina to the South Carolina Department of Natural Resources (SCDNR). Stranding forms detailing the stranding date, location, species, morphometrics, and relevant facts regarding the stranding event are submitted for every observed sea turtle. Network members characterize the condition of the animal (alive, fresh dead, moderately decomposed, severely decomposed, dried carcass, or skeleton). SCDNR employees transport live turtles to the SC Aquarium Sea Turtle Rescue Program
for rehabilitation. Post-mortem necropsies are performed on freshly dead carcasses to determine possible causes of mortality. For the purposes of this study, carapace samples were taken from dead hosts with undamaged, intact scutes, regardless of condition code. Not all animals used in this study were necropsied. Each sample was stored individually in a Ziploc© bag labeled with the STSSN identification number.

In 2009, 10.5 cm (total surface area = 86.5 cm²) circular scute samples were extracted from the lateral scutes of host turtles using a circular drill bit. Although the bone, scute, and connective tissue were all removed together with the drill bit, the scute sample peeled off of the bone during storage and only the scute layer was used. Once the scute layer detached from the bone they were attached to circular Plexiglas pieces with Z spar marine epoxy.

A circular saw was used instead of the hole saw in the 2010 season for increased efficiency in sampling and square, 10.2 cm x 10.2 cm (total surface area = 104.04 cm²) samples of scute, connective tissue, and bone were taken from lateral scutes. Scutes did not readily peel away from the majority of samples in 2010, making it necessary to use the entire bone and scute as a sample. One 0.6 cm hole was drilled through each sample and the sample was bolted directly to the array.

All sites had a total of 3 panels of each substrate, so the total area for each treatment was 259.8 cm² in 2009 and 313.2 cm² in 2010.

Experimental Sites

Arrays were placed at independent sites around Charleston, South Carolina. In 2009, the inshore sites were the South Carolina Aquarium (Aquarium), Folly Beach, and Isle of Palms (IOP). There was also one nearshore site referred to in this study as “offshore.”
“Offshore” indicates that the array was in an exposed environment that was not as sheltered as the inshore sites (Figure 5).

The Aquarium site is located in the Charleston Harbor, 2.8 km southwest of where the Cooper and Ashley rivers meet (32°47’28”N, 79°55’30”W; Figure 6A). The Aquarium location was once a superfund site. The array was tied to cleats on a cement pier located underneath the Aquarium. The area beneath the pier is shallow, and even at the deepest point there are only approximately 1.5 m of water at low tide. The array was initially hung in the water at the center of the pier, but after learning that the array was exposed to air at low tide the array was moved to the area with the deepest water, where it was continuously submerged. This site also provided habitat to extensive oyster reefs and other marine life. Watercraft such as container ships, yachts, cruise ships, recreational boats frequently operate in the proximity of the Aquarium.

Figure 5. Inshore and offshore array deployment sites in 2009 near Charleston, South Carolina
The Folly array was anchored to a private dock located 4 km from the north mouth of the Folly River (32°40′10″N, 79°54′58″W; Figure 6B). A long walkway/catwalk (0.2 km) extended over the marsh and ended in a ramp leading to a deep-water floating dock. The intertidal habitat around the array supported extensive oyster beds that were exposed at low tide. The dock is a popular area for fishing, as the oyster bed provides habitat for many other marine species, particularly black drum (Pogonias cromis). The location of the dock at a bend in the river made it vulnerable to wave action from boat wakes. The array was positioned behind the deck of the floating dock, sheltering it from heavy turbulence. The deck was a simple rectangle, allowing ample water flow around the array.

Figure 6. Experimental sites: A) South Carolina Aquarium B) Folly C) Isle of Palms and D) Offshore
The Isle of Palms site was located in Hamlin Creek approximately 0.45 km north of Breach Inlet (32°46′45″N, 79°48′27″W; Figure 6C). Similar to the Folly site, this floating dock belongs to a private owner and was over an oyster reef. The dock owner and his neighbors harvest oysters from this area for commercial purposes. This area is heavily used by recreational and commercial watercraft. Unlike the Folly site, the base of this drive-on dock was shaped like a “U,” which restricted water flow around the array. Additionally, the pilings were installed close to the dock, leaving no space to place the array that was away from boat usage without it touching a piling and placing it in a corner.

In 2009, the offshore array was positioned approximately 4.1 km southeast of the northern tip of Morris Island (32° 43′ 0″ N, 79° 49′ 60″W; Figure 6D). The water depth at the site was 10 feet at high tide. The Charleston Harbor jetties were north of the offshore buoy and to access the buoy it was necessary to drive through Dynamite Hole and along the coast of Morris Island to avoid a sandbar, and then turn east and backtrack toward the jetties. Even on calm days this site was rough, frequently experiencing white caps (Beaufort scale 4).

The Aquarium and IOP sites were eliminated in the 2010 season and a site in Dewees Inlet was added (Figure 7). The Dewees array was anchored to a private floating dock on Dewees Island located approximately 0.8 km northwest of the mouth of Dewees Inlet (32°49′41″N, 79°43′35″W; Figure 8). The walkway of the dock was short due to the immediate deep-water access. This dock was shaped like an “F,” and the array was positioned on the deck arm closest to shore to avoid boat interference. Currents were
Figure 7. Inshore and offshore array deployment sites in 2010 near Charleston, South Carolina.

Figure 8. Dewees Island deployment site in 2010.
extremely strong as this site. Fishermen were frequently seen around the dock and reported good fishing.

The Folly site was used again during the 2010 season. The offshore buoy was moved to a more sheltered location inside the jetties and southeast of Sullivan’s Island for increased protection and accessibility (32°44’10”N, 79°49’42”W).

**Experimental Protocol**

The arrays were inspected weekly for signs of recruitment. In some cases, particularly offshore, logistical complications limited the visits to every other week. The panels were photographed and each barnacle was individually identified and measured. Measurements included maximum length and width.

**Barnacle Recruitment**

A simplistic method to derive recruitment rates for each site and substrate was calculated by dividing total barnacle count by the days elapsed between deployment and removal of array. A recruitment rate including an offset was generated to incorporate the variable length of time that each array was deployed. The data were then log-transformed to reduce skew and increase homoscedasticity of the data. Differences in recruitment among sites and substrates were calculated using an Analysis of Variance (ANOVA). An ANOVA was also used to examine interaction between sites and substrates.

**Barnacle Survival**

Survival rates were calculated for each site and substrate by dividing the total number of barnacles that survived longer than one visit by the total barnacle count.
Barnacle Growth

An adapted von Bertalanffy growth interval equation developed especially for situations where age of organism is unknown was attempted (Eckert and Eckert, 1987). The von Bertalanffy growth equation predicts that growth slows to an undetectable rate at some point in time, but the maximum area values for *C. testudinaria* did not converge in this study. The time intervals used in our study was not long enough to observe a decrease in the barnacle growth rates and this kept the non-linear algorithm from converging. Instead, linear regression procedures were used to provide estimates of intrinsic growth rates because they more accurately described our data due to the lack of convergence of maximum barnacle size to an asymptote.

Applying a linear regression to the full dataset would have been inappropriate because the initial size did not represent the size at first attachment in most cases. Not knowing the length of time that a barnacle had been growing prior to the first visit complicates the construction of the growth curve. A three-step method was used to overcome this issue and calculate a linear model for barnacle growth. *Chelonibia testudinaria* has been documented to metamorphose at length of 0.8 mm (Zardus and Hadfield 2004). Therefore, all barnacles first observed at 1 mm were assumed to be captured within one day of settlement. First, a linear regression was fit using only those barnacles with an initial size of 1 mm. Then, using the slopes calculated from barnacles with known initial dates, all data were adjusted to determine the amount of time each barnacle had actually been growing when first encountered based on the size at which it was first observed. For example, if an offshore barnacle was first seen at 7 mm, this observed value would be divided by 5.02 mm$^2$/day, shifting the initial size along the x axis to the estimated day
that it was actually first seen. In order to anchor all three slopes through the origin of the graph, each initial and final size value was reduced by 1 mm². Thus, the pairs of data were shifted by one unit but the numbers of days elapsed between initial and final observation was not altered. Finally, a linear model was used to determine the growth curves produced using the entire adjusted dataset.

Additionally, phytoplankton biomass was measured in 2010 to determine its effect on growth rate. One liter of seawater was collected, filtered and stored in a lightproof container in a -80º freezer. Samples were analyzed for fluorescence on a fluorometer to indicate phytoplankton biomass. A t-test was used to detect differences in phytoplankton abundance between the Folly and offshore sites. To measure the effect of phytoplankton and seasonality on barnacle growth, a weekly growth rate was calculated by subtracting the area of the barnacle on week 1 from the area of the barnacle on week 2, and dividing the difference by the exact number of days between visits.
RESULTS

In 2009, all sites experienced barnacle recruitment. In 2010, only the offshore site supported barnacle recruitment. Neither the Dewees nor the Folly site supported any recruitment of *Chelonibia testudinaria* in 2010. Therefore, these sites have no results to report. No barnacles survived from one visit to the next at the IOP site, so no growth data could be generated at that site. Over the course of both seasons, samples of sea turtle carapace (both DTS and non-DTS) were taken from a total of 14 different animals.

**Barnacle Recruitment**

The magnitude of overall recruitment across the season was much greater in 2009 than in 2010 (Figure 9). In 2009 and 2010, 343 and 27 *Chelonibia testudinaria* barnacles recruited across all sites and panels, respectively.

![Figure 9. Total number of *Chelonibia testudinaria* barnacles that recruited across all sites and panels in 2009 and 2010](image-url)
Overall, mean barnacle recruitment rate across both seasons was highest at the offshore site (Table 1). Among the inshore sites, the Folly site had the highest recruitment rate, but the differences among inshore sites were small. Mean recruitment rates varied approximately less than 0.066 barnacles per day (translating to less than one barnacle per week) among inshore sites.

A recruitment rate using data from both seasons including an offset was generated to account for the variable length of time that each array was deployed (Figure 10). Recruitment data were log-transformed to produce a more symmetric (less skewed) dataset (Figure 11). The resulting log-transformed data also exhibit improved homogeneity of variance. There was significant spatial variation in recruitment among sites using transformed data (F= 8.1032, p = 0.0003). The offshore site also had the highest recruitment using the transformed data with adjustment for days exposed.

<table>
<thead>
<tr>
<th>DEPLOYMENT SITE</th>
<th>MEAN RECRUITMENT RATE (Barnacles/Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQUARIUM</td>
<td>0.180</td>
</tr>
<tr>
<td>FOLLY</td>
<td>0.114</td>
</tr>
<tr>
<td>ISLE OF PALMS</td>
<td>0.128</td>
</tr>
<tr>
<td>OFFSHORE</td>
<td>0.313</td>
</tr>
</tbody>
</table>

Table 1. Mean recruitment rates (2009 and 2010 data combined) for each site calculated by dividing total barnacle count for each site by the number of days elapsed between deployment and removal of array
Figure 10. Total recruitment at each site adjusted for days exposed. Median barnacle count at each site is represented by the dark line in the center of the box. The sample minimum, lower quartile (bottom line of box), upper quartile (top line of box), and sample maximum are displayed. Outliers are represented by circles.
The magnitude of barnacle recruitment and the effect of experimental substrates did not vary among sites. No significant differences in recruitment were seen among substrate ($F = 0.5164, p = 0.67$). Although not significant, non-DTS panels had the highest average recruitment rate (Table 2). Confidence intervals around mean recruitment rate means were too large to draw conclusions regarding differences in recruitment among substrates.

Figure 11. Log-transformed recruitment data at each site adjusted for days exposed
Table 2. Mean recruitment rate for each substrate. Rates calculated by dividing total barnacle count for each substrate by the number of days elapsed between deployment and removal of array.

<table>
<thead>
<tr>
<th>PANEL SUBSTRATE</th>
<th>MEAN RECRUITMENT RATE (Barnacles/Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEBILITATED TURTLE SCUTE</td>
<td>0.151</td>
</tr>
<tr>
<td>NON-DEBILITATED TURTLE SCUTE</td>
<td>0.248</td>
</tr>
<tr>
<td>PLEXIGLAS</td>
<td>0.128</td>
</tr>
<tr>
<td>SLATE</td>
<td>0.194</td>
</tr>
</tbody>
</table>

The transformation of the substrate recruitment data considerably corrected the skew and differences in variability, demonstrated by the similarly sized boxes around each median barnacle count for all substrates (Figure 13). However, the large “whiskers” on the transformed data plot illustrate the high variability in recruitment rates within each type of substrate.
Figure 12. Recruitment data for each substrate adjusted for days exposed. Median barnacle count at each substrate is represented by the dark line in the center of the box. The sample minimum, lower quartile (bottom line of box), upper quartile (top line of box), and sample maximum are displayed. Outliers are displayed by circles. DTS=Debilitated turtle scute and non-DTS=Non-Debilitated turtle scute.
The highest recruitment rate was seen on non-DTS panels at the offshore site (Table 3). Slate exhibited high recruitment rates offshore and at Folly. DTS panels at the Aquarium also displayed high rates of recruitment, while DTS panels at Folly had recruitment rates uncharacteristically low for that particular site.
There was suggestive evidence of interaction between site and substrate on barnacle recruitment ($F = 2.0904, p = 0.058$). When an interaction plot of log-transformed barnacle count is plotted against site and substrate, it is apparent that the high level of recruitment on slate and non-DTS caused some interaction offshore. There is also some indication of interaction with DTS panels at Folly having lower recruitment counts.

<table>
<thead>
<tr>
<th></th>
<th>Aquarium</th>
<th>Folly</th>
<th>Isle of Palms</th>
<th>Offshore</th>
</tr>
</thead>
<tbody>
<tr>
<td>Debilitated Turtle Scute</td>
<td>0.270</td>
<td>0.074</td>
<td>0.176</td>
<td>0.179</td>
</tr>
<tr>
<td>Non-Debilitated Turtle Scute</td>
<td>0.162</td>
<td>0.081</td>
<td>0.088</td>
<td>0.470</td>
</tr>
<tr>
<td>Plexiglas</td>
<td>0.126</td>
<td>0.116</td>
<td>0.123</td>
<td>0.151</td>
</tr>
<tr>
<td>Slate</td>
<td>0.108</td>
<td>0.219</td>
<td>0.088</td>
<td>0.286</td>
</tr>
</tbody>
</table>

Table 3. Mean recruitment rates for each site and substrate. Rates calculated by dividing total barnacle count for each substrate at each site individually by the number of days elapsed between deployment and removal of array.
Barnacle Survival

Percentage of barnacles that survived from one visit to the next was lower at the three inshore sites than the one offshore site (Table 4). Survival was not systematically different among substrates. Survival was highest offshore on the slate substrate. However, the slate substrate did not consistently encourage high survival levels; survival on slate was low at the Folly and Aquarium sites relative to other substrates. Non-DTS panels did consistently support high survival, but relative survival compared to other
substrates varied at each site. Barnacles at the Isle of Palms site never survived from one visit to the next. Survival was also generally lower at the Aquarium. It is important to note that the time interval between array deployment and the first visit varied by site. Due to inaccessibility, the offshore site was checked at two-week intervals (versus one week for inshore sites). Therefore, the offshore barnacles that survived beyond the first visit actually survived twice as long as those from the other sites.

**Barnacle Growth**

Paired size measurements from 98 individual barnacles (on a total of 25 panels) were fit to a linear regression. Using the subset of data that only included barnacles first observed on day one of growth, the offshore slope was 5.02 mm²/day, the Folly slope was 4.79 mm²/day, and the Aquarium slope was 1.98 mm²/day (Figure 15). The growth lines resulting from the adjusted dataset that includes all barnacles, regardless of day of initial observation, are plotted in Figure 16.

<table>
<thead>
<tr>
<th>SITE/SUBSTRATE</th>
<th>DTS</th>
<th>NON-DTS</th>
<th>PLEXIGLAS</th>
<th>SLATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>OFFSHORE (2009)</td>
<td>0.33</td>
<td>0.46</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>OFFSHORE (2010)</td>
<td>NA</td>
<td>0.46</td>
<td>NA</td>
<td>0.67</td>
</tr>
<tr>
<td>FOLLY (2009)</td>
<td>0.25</td>
<td>0.36</td>
<td>0.33</td>
<td>0.02</td>
</tr>
<tr>
<td>ISLE OF PALMS (2009)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AQUARIUM (2009)</td>
<td>0.27</td>
<td>0</td>
<td>0.07</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4. Percentage survival at each site and substrate. DTS=debilitated turtle scute and non-DTS=non-debilitated turtle scute. NA indicates no settlement on the substrate at that site.
Considerable variability in growth rates existed within and among sites when all data are used. Growth rates offshore were substantially higher than at the two inshore sites (7.20 mm²/day ± 0.2641 SE). Folly barnacles grew at an average rate of 4.73 mm²/day ± 0.5045 SE. Aquarium barnacles were the slowest growing at an average rate of 2.34 mm²/day ± 0.9681 SE. No growth rate was established for IOP because no barnacle survived from one week to the next. Constructing confidence intervals with a desired coverage percentage was not possible due to the approximations of initial date used in the analysis. However, approximating two standard errors provides strong evidence that the

Figure 15. Barnacle growth established using paired size data from barnacles first seen at 1 mm² (day 1). Green points represent offshore barnacles, red points represent Folly, and black points represent the Aquarium barnacles.
offshore site supported higher growth rates than the Folly and Aquarium sites. Growth rates for each substrate were also calculated using the same three-step method (Figure 17). There were no substantial differences in growth rates seen among substrates. The highest rates were seen on non-DTS panels 6.3 mm²/day ± 0.28 SE. Plexiglas and DTS panels supported very similar growth rates (5.4 mm²/day ± 0.61 SE and 4.8

Figure 16. Growth lines established using paired size data from barnacles. Data adjusted using calculations to determine the actual first day of observation. Green points represent offshore barnacles, red points represent Folly, and black points represent the Aquarium barnacles.
Barnacle growth rates were then regressed against days regardless of site or substrate (Figure 18). This model predicts that individual barnacles grew at an average rate of 5.95 mm$^2$/day (±0.234 SE).
No seasonal patterns emerged when 2009 growth data were plotted against time (Figures 19 and 20). There are also no evident differences among sites or substrates. It is important to note that the data on these charts include all growth rates, regardless of barnacle age. If data deviate from a linear pattern, a growth curve could confound the seasonal curve. Because our growth rates are linear, plotting all data together does not obscure any seasonal pattern. It is also important to note that many barnacles have more than one growth rate over a period of time, so the dataset is not independent.
Figure 19. Growth rates for all barnacles (at all ages) across the season (2009)

Figure 20. Growth rates for all barnacles (all ages) across the season (2009)
Phytoplankton Biomass

Quantitative phytoplankton samples were taken in 2010 only (Table 5). No significant differences were seen in plankton abundance between the offshore and Folly sites ($t = 2.06, p = 0.34$). Phytoplankton data for Dewees and IOP were not included in the t-test because of the lack of barnacle recruitment and/or growth at these sites. A slight increase in plankton levels can be seen in late July/early August (Figure 21).

<table>
<thead>
<tr>
<th>SITE</th>
<th>µL/L</th>
<th>SITE</th>
<th>µL/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEWEES</td>
<td>3.02</td>
<td>ISLE OF PALMS</td>
<td>3.08</td>
</tr>
<tr>
<td>DEWEES</td>
<td>9.12</td>
<td>ISLE OF PALMS</td>
<td>3.29</td>
</tr>
<tr>
<td>DEWEES</td>
<td>12.62</td>
<td>ISLE OF PALMS</td>
<td>5.34</td>
</tr>
<tr>
<td>FOLLY</td>
<td>2.81</td>
<td>OFFSHORE</td>
<td>9.50</td>
</tr>
<tr>
<td>FOLLY</td>
<td>3.83</td>
<td>OFFSHORE</td>
<td>1.23</td>
</tr>
<tr>
<td>FOLLY</td>
<td>4.11</td>
<td>OFFSHORE</td>
<td>2.05</td>
</tr>
<tr>
<td>FOLLY</td>
<td>5.06</td>
<td>OFFSHORE</td>
<td>2.81</td>
</tr>
<tr>
<td>FOLLY</td>
<td>5.26</td>
<td>OFFSHORE</td>
<td>4.14</td>
</tr>
<tr>
<td>FOLLY</td>
<td>5.90</td>
<td>OFFSHORE</td>
<td>4.63</td>
</tr>
<tr>
<td>FOLLY</td>
<td>7.52</td>
<td>OFFSHORE</td>
<td>4.75</td>
</tr>
<tr>
<td>FOLLY</td>
<td>9.28</td>
<td>OFFSHORE</td>
<td>4.89</td>
</tr>
<tr>
<td>FOLLY</td>
<td>14.82</td>
<td>OFFSHORE</td>
<td>8.62</td>
</tr>
<tr>
<td>FOLLY</td>
<td>4.14</td>
<td>OFFSHORE</td>
<td>8.59</td>
</tr>
<tr>
<td>FOLLY</td>
<td>8.96</td>
<td>OFFSHORE</td>
<td>4.46</td>
</tr>
<tr>
<td>FOLLY</td>
<td>8.15</td>
<td>OFFSHORE</td>
<td>5.76</td>
</tr>
<tr>
<td>FOLLY</td>
<td>5.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FOLLY</td>
<td>3.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FOLLY</td>
<td>4.15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Phytoplankton results from four experimental sites
This change is more pronounced at Folly than offshore. When the limited growth data from 2010 are plotted against phytoplankton levels, no trend emerges (Figure 21).

Figure 21. Phytoplankton levels plotted with growth rates for 2010 at Folly and offshore
DISCUSSION

Barnacle Recruitment

Seasonal Variation in Recruitment

Annual variability in recruitment was seen in *Chelonibia testudinaria*. Recruitment was substantially higher in 2009. It is important to note that two of the four sites were changed between the 2009 and 2010 seasons, and the differences in recruitment could be site specific.

However, temporal variability in recruitment is commonly seen in many barnacle species on all spatial scales at any given time (Caffey, 1985; Navarrete and Wieters, 2000). For example, substantial annual variation in the recruitment of *Semibalanus balanoides* was seen consistently in a long-term study from 1961-1981 (Kendall *et al.*, 1985). Differences in densities of settlers are thought to be related to the annual variation in larval densities (Caffey, 1985). Erratic fluctuations in abundance of marine populations are not limited to barnacles. Other marine invertebrates show similar inconsistencies in annual larval densities. In two long term studies of *Asterias forbesi* and *Crassostrea virginica*, large fluctuations in annual concentrations of larvae were noted for both species, and there was no relationship between the degree of fluctuations and environmental factors (Loosanoff 1964, 1966). Increased recruitment rates observed at the Folly and offshore sites in 2009 were likely at least partially due to higher larval densities during this season.

Events in the offshore waters affecting quantity of larvae contribute to oscillations in many marine organisms including sardines, lobsters, squid, and crabs (Roughgarden *et al.*, 1988). Factors affecting larval arrival and availability, particularly at inshore sites,
include reproductive output of adults, winds, currents, tides, and tidally generated waves (Raimondi, 1990). Shoreline topography and changes in larval mortality have also been attributed to large scale spatial variability in settlement due to altered concentrations of larvae in the water column (Gaines and Roughgarden, 1985). The mechanisms of temporal variability in larval recruitment are complex and it is likely that many factors contributed to the decreased barnacle recruitment in the 2010 season.

Evidence suggests that competition by other barnacles could also explain the low recruitment rates in 2010. Other species of barnacles, specifically *Balanus eburneus*, settled at high densities on experimental panels in 2010, which was not the case in 2009. Secondary barnacle species may act as recruitment inducers (Morse and Morse, 1984), or in some cases, inhibitors (Rittschof *et al.*, 1985). Additionally, differences in tolerances of heat, desiccation, or other environmental characteristics can cause interspecific competition among adult barnacles of different species (Connell, 1961). It could be argued that the presence of other species reduced recruitment either during settlement or by competition after recruitment. Perhaps some unmeasured environmental factor changed between 2009 and 2010, causing *B. eburneus* to thrive at the expense of *C. testudinaria*.

Intraspecific interactions may also play a role in barnacle settlement. The presence of previously settled conspecifics may cause an increase in additional settlement for these colonial settlers. Fewer initial recruits to encourage colonization of other larvae may help explain the low recruitment seen in 2010. If reduced larval availability led to decreased initial settlement, overall settlement may have been exponentially reduced by colonization behaviors. However, conspecifics may also reduce the rate of settlement by
simply occupying potential settling space or by consuming potential settlers while filter feeding (Raimondi, 1990).

Barnacle larvae are known to select habitats with specific chemical complexes and textural qualities (Anderson, 1994). The change in protocol from 2009 to 2010 involving a shift from attaching scutes with marine epoxy to Plexiglas to simply drilling through the center of the bone and scute complex could have had an impact on the chemical properties of the panel. For example, if some chemical of the epoxy is naturally appealing to *C. testudinaria*, the change in protocol between the seasons may have affected recruitment rates. It is also possible that *B. emburneus* are more sensitive to the marine epoxy used in 2009 and the discontinued use of the chemicals increased recruitment of this species, thus increasing interspecific competition for the turtle barnacles.

Recruitment can occur in bursts instead of occurring steadily (Caffey, 1985), and it is possible that the pulses in 2010 were missed due to the changed protocol of removing the array from the water once per month to change the panels. However, the arrays were removed in 2010 on only five occasions for a maximum out-of-water duration of seven days. So, the likelihood of consistently missing pulses was small for the remaining several months that the array was in the water.

Although barnacles are known to have predators (Connell, 1961), the role of predation was not assessed in this study. Future studies should characterize the community on the panels to fully understand the interaction of different organisms with the species of interest. It is possible that more predation existed in 2010 and although recruitment did occur, the small nauplii were depredated in the intervals between site visits.
Several accounts of increased recruitment following El Niño events have been documented on geographically broad scales (Connolly and Roughgarden, 1999; Navarrete and Wieters, 2000; Roughgarden et al., 1998). Stationary or onshore moving waters produced during El Niño events contribute to the distribution of barnacle larvae moving closer to shore. Increased onshore transport can strongly affect benthic communities and increase the availability of nearshore barnacle larvae. Conversely, ocean cooling caused by La Niña may have the opposite effect on larval supply. Weather conditions in 2010 were characteristically those of a La Niña event and may have contributed to reduced recruitment.

It is likely that the reduced recruitment observed in 2010 was caused by multiple confounding factors. The consequences of seasonal variability in recruitment have implications for sea turtle epibiont communities. Temporal patchiness in larval distribution may reduce the potential for sea turtles to encounter larvae in seasons with lower larval supply. It is plausible that debilitated turtles stranding in these seasons may appear less encrusted that those that strand in years with abundant larvae. However, although temporal patchiness occurs, sea turtles are highly. It is unlikely that they would fail to encounter an area with at least a moderate abundance of *C. testudinaria*.

**Spatial variation in recruitment**

*Chelonibia testudinaria* preferentially settled on the offshore panels. Identical experimental conditions were presented at each site, so the probability or ability to settle did not vary among sites. The spatial variability seen among sites is likely caused by spatial variation in larval concentrations. Local barnacle populations fluctuate according to the relative availability of larvae (Caffey, 1985). Physical and biological processes
disperse larvae and patchy arrival to different habitats results. The offshore site may have more *C. testudinaria* larvae due to its proximity to foraging sea turtles.

Among the inshore sites, recruitment was highest at Folly. Large differences in recruitment can be expected, even within small distances. In a study with sites separated by only a few meters, barnacle settlement rates regularly differed among them (Gaines and Roughgarden, 1985). The Folly River, Dewees Inlet, and Hamlin Creek all have high water velocities, and therefore similar recruitment could be expected at the Folly, Dewees, and IOP sites. However, the random delivery of larvae can contribute to the variance observed. Patchiness in settlement has been seen within very small scales (less than 3 m²), so these results are not unusual (Caffey, 1985).

Raimondi (1990) saw spatial variability in settlement only when overall recruitment rates were high. The scale of our recruitment may have been too low to observe discrete differences among inshore sites. Additionally, a larger sample size may have reduced variability in recruitment rates and increased consistency among individual panel recruitment rates.

In most species of barnacles, the presence of surviving barnacles encourages further recruitment. However, this density dependent recruitment should not be an issue in our study since established *C. testudinaria* colonies were not found at any concentration at any of the sites used.

Settlement is directly proportional to the amount of available space on a substrate (Gaines and Roughgarden, 1985). Studies have shown that the large degree of variability in recruitment, which was also seen in this study, could be attributed to the available space on the panels at any given time. In 2009, when the majority of the data were
collected, the panels remained in the water for extended periods of time. As barnacles recruited to the panels, the amount of available habitat decreased, which may have been reflected in reduced recruitment rates later in the season. An initially large recruitment rate on an individual panel followed by gradually decreasing recruitment rates would produce variable overall rates.

The results of this study suggest that sea turtles are more likely to recruit barnacles in offshore environments rather than inshore (i.e., inlets, harbors, estuaries). Future studies should focus on characterizing larval abundance in different locations, as this is likely directly related to recruitment.

**Substrate variation in recruitment**

There were no significant, consistent differences in recruitment among substrates. Previous studies indicate that turtle barnacles preferentially settle on sea turtles (Zardus, unpublished data). The insignificant differences in substrate preferences by *C. testudinaria* suggest that free roaming larvae may settle on suitable substratum with less discrimination than previously thought. If a cyprid is floating at the mercy of the current, it may opportunistically take advantage of any hard surface it encounters so long as it meets standard minimum requirements.

*Chelonibia testudinaria* may also have adaptive responses only to substrates they would naturally encounter. Hard surfaces that a turtle barnacle finds in a natural environment would not typically include slate or Plexiglas. If another natural substrate had been substituted, such as a whale or rock, a significant difference in recruitment rates may have been observed between turtle scute and the natural substrate.
There are several methodology issues that may explain why carapace samples were not preferred by *C. testudinaria* in this study. First, the structural integrity of the DTS panels was very poor in many cases. Although efforts were taken to use high quality samples, there were often open patches or areas with very thin layers of keratin. The poor health of the animal at the time of stranding and the carapace encrustation by barnacles were likely the cause of the compromised scute condition. There is little doubt that the chemical properties of the DTS panels differed from the non-DTS panels due to the heavy load of barnacles previously occupying the DTS scutes. Given these circumstances, high barnacle settlement was expected on DTS panels, but did not occur.

An early experiment by Crisp and Barnes (1954) provided evidence that barnacles settle in grooves and concavities both larger and smaller than their own body size, a response they termed *rugophilic*. Their results are reinforced by recent literature stating that larvae respond to surface texture during site selection (Hills and Thomason, 1998). The purchased slate panels had textural inconsistencies, and despite efforts taken to minimize the presence of grooves in panels, they were often present. If turtle barnacles first settled on a slate panel, they may have permanently settled despite the presence of sea turtle carapace samples nearby.

Finally, there is evidence that larvae avoid substratum previously occupied by predators (Johnson and Strathmann, 1989). Turtle barnacles uniquely settle on motile hosts, reducing the relevance of predators. However, it was not feasible to eliminate the possibility that predators (such as small crabs living on host carapaces) left cues on carapace samples while the turtle was still alive (both DTS and non-DTS) or when the array was placed in the water, thus reducing the appeal of these panels to barnacles.
Although this could have reduced barnacle larvae’s attraction to the turtle shells, it is not unreasonable for a turtle barnacle to encounter a situation when their sea turtle host contains crabs or other predators among their epibiotic communities. For this reason, turtle barnacles may be not be adapted to respond the same way as other barnacles with known aversion to predator cues. It would be interesting to research the relationships of barnacle predators on sea turtles to document their effects on barnacle recruitment.

**Barnacle Survival**

The survival values offered by this study provide only general approximations of relative survival. Standardization of time intervals between visits is necessary to calculate survivorship that is accurately comparable among sites.

Barnacle survival was highest offshore, with almost 50% of recruits surviving beyond the first visit. Folly had intermediate survival rates, and the Aquarium and Isle of Palms had very low survival rates. Many factors contribute to barnacle survival, and barnacle survival varies greatly over space and time (Caffey, 1985). Benthic marine invertebrates typically exhibit an age-specific mortality pattern, with mortality decreasing with increasing age (Foster, 1971; Connell, 1972). First day mortality has been shown to be extremely high in the barnacle *Balanus glandula*, followed by a sharp increase in survivorship (Gosselin and Qian, 1996). Our study only observed panels once per week, so it is possible, and highly likely, that first day settlement and mortality was missed for many barnacles.

Gosselin and Qian (1996) concluded that first day mortality did not coincide with elevated densities of grazers (predators), stress from desiccation, wave exposure, or vulnerability due to size. However, several factors were reported as important. Selective
pressures such as time of settlement relative to tidal cycle, specific location of settlement, and energy reserves at settlement (i.e., energy needed for extensive changes associated with metamorphosis immediately after settlement) may determine the success of the recruits on the first day (Gosselin and Qian, 1996). Future studies specifically considering survival should note these variables.

Natural communities interact in complex ways and post-recruitment survival can be influenced by the surrounding community. For example, physical interference from nearby plant and animal colonizers may interfere with food supply to the barnacle or increase sedimentation by reducing water velocity (Leonard, 1999). Alternatively, vegetative growth may increase survival by reducing desiccation when exposed at low tides. Leonard (1999) found that mechanical effects from algae (algal whiplash) reduced recruitment, but there was actually a positive influence of algal canopies on post recruitment survival. The organismal community was not recorded in our study but should be documented and studied in future research.

Another example of the complex interactions seen in nature is intra- and interspecific competition. The relationship between different species of mussels and barnacles was researched with acorn barnacles (*Semibalanus balanoides*; Stephens and Bertness, 1991). While mussels can out-compete barnacles for space, mussels may increase barnacle survivorship by buffering from temperature extremes during thermally stressful conditions. Although the experimental designs at each of the study sites were consistent, the overall habitats were highly variable. The offshore array was exposed to high-energy wave action and accumulated less algal/plant species than the other arrays. The Folly array was located at a bend in the river and may have experienced stronger currents than
the Isle of Palms or Aquarium sites. Although the water velocity could have inhibited growth on the array, the Folly array collected a diverse community of invertebrates and plants in addition to turtle barnacles in 2009. The effects of the other organisms remain unknown. Both the Folly and Isle of Palms sites supported extensive oyster reefs, which may act similarly to mussels in increasing competition and/or buffering from harsh environmental conditions.

Raimondi (1990) reported that early recruits more often survived to maturity than did those that recruited later in the reproductive season, likely because they exploited the majority of suitable settlement sites. Our results support this finding, as survival rates were high on slate, likely because of the crevices uniquely present on this substrate. There may be an advantage conferred on initial settlers, but this detail is beyond the scope of this study.

**Barnacle Growth**

Little is known about the growth of *C. testudinaria*, but if it can be assumed to follow growth patterns similar to other barnacle species, the predicted growth would reach asymptotic size at some determined time. Our data appear to have a slight exponential shape, suggesting that the observation window was not long enough to capture the convergence seen when asymptotic growth occurs. The rapid barnacle growth seen in small *C. testudinaria* barnacles may continue until a size larger than we observed. This assumption is supported by observations of turtle barnacles on carapaces of loggerhead strandings that are much larger than those seen on the experimental panels (even up to approximately 60 cm² on a sea turtle carapace), providing evidence that these barnacles do indeed reach sizes substantially larger than were seen in this study.
Many barnacle species face constant danger of predation, supporting the need to grow and reach maturity as quickly as possible to decrease risks from predators. Escaping large predators is less urgent on motile hosts such as sea turtles, which may result in slower growth rates due to the less adventurous feeding tactics (reduced cirral movement) outlined in Pasternak et al. (2002).

On the other hand, turtle barnacles may be more short-lived than other barnacle species due to the transient nature of their host substrate. Scutes are sloughed regularly on loggerheads, which may have implications on survival techniques. Other than leatherbacks, the integument of all sea turtles shells is covered by β-keratin (Jacobsen 2007). The surface of the shell is unique in that it is mostly ossified. The basal surface of the β-keratin cells have long processes that interdigitate with the connective tissue beneath. Epidermal growth occurs by replacing the older layer of epidermis with a new inner epidermis, and is continuous in turtles. When growth occurs, new keratin is produced at the seams, which are where the scutes meet. The length of time between shedding scutes in sea turtles is currently unknown, but it is possible that it occurs in approximately one-year intervals based on other turtle species. Therefore, it may have been presumed that turtle barnacles grow faster and reach maturity more quickly than very long-lived barnacles because they have a shorter relative lifespan on their transient host substrate (scute).

The linear model presented by our data indicates a growth rate of 6.3 mm$^2$/day on non-DTS sea turtle carapaces. This predicted rate has a large standard error that reflects the high variability seen in growth rates. As previously mentioned, studies have noted that linear measurements of growth rates can yield variations within species by up to a
factor of seven (Anderson, 1994). Regional environmental conditions, including temperature, salinity, and nutrient levels have major impacts on the growth and reproduction of barnacles (Crisp, 1960). Local conditions, specifically water flow, orientation of the barnacle relative to water flow, and presence of other individuals, also influences growth rates.

The offshore array was exposed to ideal conditions for barnacle growth. Salinity was high and heavy water flow theoretically provided abundant nutrient levels. Phytoplankton levels were not different between the Folly and offshore sites, indicating that both sites had adequate conditions for growth. However, although the plankton levels were relatively similar, the high rate of water flow over the barnacles offshore may have actually supplied more food to the barnacles. Additionally, some research indicates that the greatest growth takes place at the lowest tides (Moore, 1934). This evidence suggests that high concentrations of food due to tidal fluctuations would have actually contributed to faster growth at the Folly site rather than offshore.

Omitting intermediate size values taken over a series of weeks simplifies growth and in many cases may not capture a true representation of overall growth patterns. For example, panel crowding was an issue offshore, which may have caused growth to slow later in the season due to lack of space or competition for food. Although problems are associated with quantifying growth this way, examining individual growth rates may more accurately characterize the true patterns in growth behaviors. Substantial divergence among individual behavior contributes to the high overall variability in the growth rate model. This observation may suggest that growth rates can differ even among individual scutes. Further research is necessary to clarify this concept.
When growth concepts are applied to a debilitated turtle, several assumptions regarding environmental parameters must be considered. The animal is presumably floating at the water’s surface, which implies that the top of the carapace is not consistently submerged. Under these circumstances, barnacles located laterally/marginally may have greater growth rates due to longer submergence times. Additionally, growth rates increase when the barnacles are kept free from other organisms (Barnes, 1955). Debilitated turtles are characteristically encrusted in epibiota, potentially providing competition for food supplies and possibly reduced growth rates.

Debilitated Turtle Syndrome

Turtles suffering from DTS are lethargic, emaciated, and covered in small barnacles. When selecting barnacles to use as biomarkers for barnacle age, any large barnacles that were likely present before debilitated (especially those normally found on the vertebral scutes of healthy turtles) are not of interest. The cohorts of similarly sized barnacles can be assumed as those that recruited after the turtle began floating. These smaller barnacles tend to measure between 1 cm$^2$ and 4 cm$^2$ on debilitated turtles. Using the overall growth rate of 6 mm$^2$/day, barnacle ages can be estimated at approximately 16 days and 67 days, respectively. In some cases it may be more appropriate to use the offshore growth rate of 7.2 mm$^2$/day, as sea turtles are often found in nearshore environments similar to that of our offshore buoy. A 4 cm$^2$ barnacle would take approximately 56 days to reach this size using the offshore growth rate.

Three possible explanations for the initial causes of debilitation deserve mention. First, during the cold winter months, juveniles have been observed to dig head first into the mud and enter a period of dormancy, a phenomenon known as brumation (Lutz and
Musick, 1997). Brumation has been reported for juvenile loggerheads in the Canaveral Ship Channel (Carr et al., 1980; Ogren and McVea, 1982). Although it has not been documented, it is possible that juvenile loggerheads also overwinter in South Carolina. These animals are likely less physiologically and nutritionally fit than those juveniles who spend the winter foraging. These symptoms may severely weaken the turtle causing slowed movement, subsequently allowing for heavy recruitment of barnacles.

Second, sea turtles have been reported to suffer from cold stunning at temperatures under 8°C in the wild (Morreale et al., 1992). Therefore, a cold-stunning event occurs when the water falls below 10°C for a period of several days or more. One documented cold stunning event in the Indian River Lagoon, FL, affected hundreds of green turtles during an atypically cold winter in 1984-5 (Wilcox, 1896). More recently, in 2009 there were two events with cold-water anomalies affecting large numbers of loggerhead and green sea turtles. Another cold-stunning event in January, 2010, affected 4,612 sea turtles (4,365 greens, 111 loggerheads, 73 Kemp’s ridleys (Lepidochelys kempii), and 63 hawksbills (Eretmochelys imbricata); Brian Stacy, pers. comm.). Cold-stunned reptiles are unable to swim due to cold narcosis (Jacobsen, 2007). Similar to brumation, the lack of movement by these animals may results in heavy barnacle loads.

Finally, it is also possible that the recent increase in DTS is simply due to natural disease. This perceived rise in affected animals may not be due to an increased rate of infection, but instead may reflect increases proportional to the increased number of juveniles in the population. Nest protection efforts by SCDNR since 1980 (and in FL, GA and NC) have increased hatchling productivity. A study comparing hatch success before and after nest protection reported an increase from approximately 3.0% to 84.4% on Cape
Island, the highest density nesting beach in SC (Hopkins-Murphy and Seithel, 2005). The increased numbers of hatchlings produced over the past 30 years should have resulted in an increase in the number of juvenile loggerheads. Significant increases in catch rates of subadult loggerheads (65.1 to 75.0 cm straight carapace length) have been reported off the Atlantic coast between 2000-2003 and 2008 (Arendt et al., 2009). Therefore, overall proportions of debilitated turtles may not be increasing, but the disease may be becoming more noticeable due to the higher numbers of juveniles in the environment.
CONCLUSIONS AND RECOMMENDATIONS

Identifying the length of time a turtle has been debilitated using barnacle size as a metric will be difficult due to the inconsistencies seen in growth rates. However, the growth rates provided by this study can estimate acceptable predictions of barnacle age. The estimated size of barnacles seen on debilitated turtles is between 1 cm$^2$ to 4 cm$^2$. Using the overall growth rate established for this study (6 mm$^2$/day), these barnacles having been growing for approximately 16 to 67 days. By providing this information to wildlife managers and veterinarians, these diagnostics can play a constructive role in conservation.

Future studies investigating barnacle growth and recruitment should develop standardized procedures to attempt to characterize the sources of variability observed in this study. Describing organismal communities on experimental substrates may allow calculations of predation and competition. Alternatively, laboratory experiments can play a crucial role in eliminating confounding environmental factors and standardizing variables such as nutrient levels and water flow, both of which contribute significantly to barnacle growth.

Recovery Action 47.3 of the Loggerhead Recovery Plan recommends that managers “develop a manual for the assessment and treatment of loggerhead diseases and injuries,” emphasizing the importance of identifying causes, symptoms and treatment of DTS (NMFS and USFWS, 2008). Natural disease affecting increased populations of juvenile loggerheads is a possible cause of debilitation. Brumation and cold-stunning are two equally plausible explanations for DTS. Although this study cannot rule out cold-stunning as a possible cause of debilitation, further research would be necessary to
empirically confirm cold-stunning as the original cause of debilitation. Stable isotope analysis is widely used to investigate animal diets and habitat use, and has recently been used to establish a dichotomy in migration strategies among juvenile Atlantic loggerheads (Mansfield et al., 2009). Similar studies using stable isotope analysis may reveal differences in foraging habits and migratory patterns between healthy and debilitated juvenile loggerheads.

The establishment of sustainable environmental strategies and reduction of mortality rates of this threatened species will strengthen conservation efforts. For long lived species such as sea turtles, the loss of even a few individuals in some circumstances may have long term implications on the population (Congden et al., 2003 and Crouse et al., 1987). Future research and funding is needed to determine the cause of initial debilitation. Identifying the cause of morbidity and mortality in debilitated turtles will ultimately support conservation of these juveniles until reproductive age, and thus help preserve the species.
LITERATURE CITED


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