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## Resource Allocation and Phenotypic Plasticity of Simultaneous Hermaphroditic Turtle Barnacles (*Chelonibia Testudinaria*)

Kevin C. Cash

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# Thesis of Kevin C. Cash

Submitted in Partial Fulfillment of the Requirements for the Degree of

## Master of Science Marine Science

Nova Southeastern University  
Halmos College of Arts and Sciences

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Approved:  
Thesis Committee

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HALMOS COLLEGE OF ARTS AND SCIENCES

Resource Allocation and Phenotypic Plasticity of Simultaneous  
Hermaphroditic Turtle Barnacles (*Chelonibia testudinaria*)

By

Kevin Cash

Submitted to the Faculty of  
Halmos College of Arts and Sciences  
in partial fulfillment of the requirements for  
the degree of Master of Science with a specialty in:

Marine Biology

Nova Southeastern University

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## ABSTRACT

This research addresses the knowledge gap of phenotypic plasticity in a commonly found and important species of epizoic barnacle, *Chelonibia testudinaria*. Limited research has been published regarding how phenotypic expression is mediated the spatial distribution of barnacles on a mobile host. To investigate this potential relationship, barnacles were collected from the backs of turtles along the beaches of Fort Lauderdale Florida. These barnacles were assessed for various phenotypic traits as well as their corresponding spatial distribution on the turtle carapace. Barnacles were safely removed from the carapace using a chisel before their preservation in ethanol. Barnacles were then numbered according to position on the carapace, then dissected, measured, and photographed under a microscope. Barnacles of clustered communities were fertilized at 68% greater rates than solitary individuals. To determine spatial distribution, each turtle's carapace was separated into four different positions. Overall, barnacles were more abundant in the posterior position, followed by central, central costal and anterior scutes. Diameter, total length, and annulation/segmentation was measured for each barnacle's penis, as well as their first cirri and sixth cirri. ANOVAS, factorial ANCOVAS, and logistical regressions were used to determine how functional morphology of the penis and feeding cirri change dependent upon different environmental and physical conditions. Internal body weight and position on the carapace were associated with penis morphology such that larger barnacles bear larger penises. Central costal barnacles possess a penis 27% larger compared to those settled on the anterior portion of the carapace. Feeding structures were also significantly different between individuals of different internal body weight, position and whether the barnacle was considered solitary. Larger barnacles have larger feeding cirri. Anterior barnacle's cirrus 1 segmentations were lesser when compared to all other positions. Crowded barnacles were observed to have thicker cirrus 1, compared to solitary individuals. Posterior barnacles have longer cirrus 6 compared to central costal individuals. While similarities between *C. testudinaria* and other species of acorn barnacles were expected, there are some notable differences. *C. testudinaria* seemingly did not adjust their morphology to combat the harsher environmental impacts of the high flow conditions experienced at the anterior portion of the turtle carapace. Barnacles residing within their preferred location of the posterior portion of the turtle carapace demonstrated morphological adjustments of their sixth cirri. Crowded individuals had thicker first cirri, likely accommodating for eddying caused by surrounding barnacles. Lastly, rather than adjusting their structural morphology in response to local condition, it appears that *C. testudinaria* rely on increased recruitment and simply move to a more desired location.

Keywords: Invertebrates, Barnacles, *Caretta caretta*, *Chelonia mydas*, Sea Turtle, Fort Lauderdale, Florida, Ecology, Evolution, Spatial Distribution, Fertility Probability

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## INTRODUCTION

Barnacles are ancient organisms that date as far back as the middle Cambrian period of the Paleozoic era (Walters 2007). Barnacles have been, and continue to be, a successful lineage as there are about 1,400 living species documented and widely distributed around the world (Newman 1980). Barnacles were originally classified as mollusks (Linnaeus 1758) due to their outward appearance. They secrete a calcareous shell referred to as a carapace, resembling the outer shell and general lack of segmentation observed in the molluscs. This outer shell completely encompasses the innards of the organism, providing both defense and structural stability. Despite these similarities, barnacles were different enough for Lamarck in 1809 that he suggested placing them in a class of their own known as Cirripedia (Lamarck 1809). In 1830, barnacles were again reclassified by John Vaughn Thompson (Thompson J. V. 1830) as crustaceans where they remain today. The defining characteristic that ultimately differentiated barnacles from mollusks was their larval state (Bosc 1802). Closer observation showed these cirripeds do in fact share characteristic traits found within crustaceans. They molt, have jointed limbs and even similar jaws to those of crustaceans (Winsor 1969). It was then that barnacles were classified as Cirripedia under the subphylum Crustacea. Research on barnacles began around 200 years ago. One influential researcher was Charles Darwin. Even though Cirripedia research was one of Darwin's lesser known works, Darwin's primary focus was on classification between species. More modern research has shifted to evolutionary patterns and fitness consequences (Crisp, D. J. 1983, Fusco & Minelli 2010, Kelly & Sanford 2010, Hoch et al. 2016). Many of these studies focus on key sexual characteristics displayed by these unique hermaphroditic animals.

### *Unique sexual production for non-epibiotic hermaphroditic barnacles*

Barnacles are simultaneous hermaphrodites that reproduce by means of pseudo-copulation with neighboring barnacles (Barnes et al. 1977). Most barnacles are sessile organisms, making it difficult for individuals of this nature to fertilize egg masses not found nearby (Hoch 2010). If there are no potential mates within the area, barnacles typically cannot move to a new location in search of a partner. A unique feature used to combat this issue is the barnacle's extremely long flexible penis (Darwin 1854a). Barnacles have the largest penis proportional to their body size of the entire animal kingdom (Darwin 1854a). When their penis is



flaccid, it is still longer than its largest feeding cirri (Klepal et al. 1972). During the mating season, an individual can act as a functional male or a functional female, but not at the same time. The penis grows rapidly before their mating season (Klepal 1990) and is shed during the first post-mating molt (Crisps and Patel 1958). In search of a mate, the individual extends its penis out several times its flaccid size (Klepal 1990). The penis has a series of small “segmentations”, that are actually folds in the cuticle of the penis that allows for it to extend in search of a mate (Klepal et al. 1972, Hoch et al. 2016). These segmentations are referred to as annulations which can be counted to better measure the penis length of the individual barnacle, rather than solely relying on total flaccid length (Hoch 2008, Neufeld & Rankine 2012). The annulations provide a better idea of a barnacle’s penis full extension length (Neufeld & Palmer 2008). Once a functional male has found a functional female, sperm is released from the penis and deposited inside the mantle of the receptive female. It has been documented that functional females can accept up to six penises during one mating session, though the amount of success is unclear (Barnes et al. 1977).

Barnacles exhibit a diverse number of sexual systems (Darwin 1851). A few of these systems include simultaneous hermaphroditism, androdioecy and dioecy. Simultaneous hermaphroditism is when an individual has the ability to possess both male and female reproductive systems. Androdioecy refers to a population where you can find both hermaphrodites and strict males. Dioecy is when a community is comprised of strict males and strict females (Yusa et al. 2013). Whenever strict males make up a community of barnacles, they are always smaller than the rest of the population they reside in and are referred to as dwarf males (Høeg 1995).

#### *Dwarf male barnacles*

*C. testudinaria* exhibit androdioecy which is the coexistence of males and hermaphrodites (Darwin 1851). Small hermaphroditic barnacles that act as males during the mating process are referred to as complementary males, whereas non-hermaphroditic males are called dwarf males. Dwarf male barnacles are smaller barnacles growing upon the outer shell of larger hermaphroditic barnacles (Zardus & Hadfield 2004, Lin et al. 2015). Dwarf males act as a potential sperm donor for the larger individual it grows upon. These specialized males tend to remain extremely small in size and typically live for a short amount of time (Zardus & Hadfield

2004). In general dwarf male barnacles tend to do well in smaller mating groups commonly found in deep sea environments or when barnacles are attached symbiotically (Ghiselin 1974, Zardus & Hadfield 2004). This is due to the fact that sperm competition is intense between hermaphrodites and smaller individual males. Dwarf males have few advantages over complementary males (Crisp 1983), including earlier maturation, allowing for reduced generation time (Foster 1984, Zardus & Hadfield 2004) and closer proximity of mates to one another. Growing on top of larger females/ hermaphrodites increases fertilization success per sperm (Ghiselin 1974; Gotelli and Spivey 1983; Urano *et al.* 2009) and could allow for dwarf males to utilize resources in aspects other than male morphology. In some cases, if there is not enough successful fertilization between hermaphrodites, their male function can be lost permanently. This causes the community to rely solely on whatever dwarf males are within close proximity (Newman 1980; Charnov 1987; Urano *et al.* 2009).

### *Phenotypic plasticity*

Barnacles exhibit phenotypic plasticity, the ability for an organism to change its morphology in response to inputs from the environment (West-Eberhard 2003, Price 2003, Ewers-Saucedo *et al.* 2015). This change may involve its morphology, physiological state or even behavior (Fusco & Minelli, 2010). Studies have found that many factors can influence barnacle growth rate. For example (Doell *et al.* 2017) determined that some influential factors determining barnacle growth rate included host origin, substrate type, laboratory of field conditions as well as seasonality. Barnacles typically change their sexual function, as described above, or even their body structure, dependent upon environmental fluctuations (Hoch 2010). When barnacles are exposed to higher wave energy, penis diameter is increased by thickening both the cuticle and the muscles (Hoch 2010, Hoch *et al.* 2016). When barnacles are exposed to areas of lower wave energy, barnacles were observed to have longer (25%) and thinner penises (Neufield & Palmer 2008). It is also interesting that even though penis diameter increased, the overall mass of the penis did not increase (Hoch 2010). When distance between mates increased, barnacles developed longer penises allowing them to reach farther distances by adding more ringed annulations (Hoch *et al.* 2016). It is common for barnacles to adjust their morphology dependent upon environmental influences allowing them to increase their mating success (Hoch *et al.* 2016). Phenotypic plasticity is not only used to increase sexual success, but it can also

increase success in other aspects of an individual's life (Arsenault et al. 2001). Barnacle cirri are also receptive to phenotypic plasticity. Individuals have adapted to have long, thin feeding cirri in areas with little to no wave action, and short, thick cirri in areas where they are often exposed to heavy wave action (Lopez et al. 2007, Marchinko 2007, Neufeld & Palmer 2008). These morphological adaptations help the individual to be better suited to withstand harsher physical environmental conditions caused by water-flow as well as social conditions of living within crowded or secluded areas (Lopez et al. 2007). Barnacles that are feeding within areas of heavy wave action have short, stocky appendages allowing them to withstand drag forces put onto them (Marchinko 2007).

### *Turtle barnacles*

Epizoic barnacles are those that live on the surface of another living animal, the relationship between the host and surface dweller is considered commensal. These species have been found to date to at least 5-15 million years ago. Examples include Porifera, Cnidaria, Mollusca and Arthropoda (Frick et al. 1998, Fuller 2010). A few marine organisms that host epizoic barnacles include whales, turtles and crustaceans (Darwin 1851, 1854a). Barnacles are so common upon sea turtles that more than 94 % of nesting logger head sea turtles host at least one species at any one time (Frick et al. 1998, Rawson 2003). Host movement creates water flow allowing for increased access to potential nutrients (Fuller et al. 2010). One of the most common genera of acorn barnacles residing on mobile hosts is *Chelonibia*. Within the genus of *Chelonibia* there were formerly three main recognized species, *C. testudinaria*, *Chelonibia patula* and *Chelonibia manati*. However, it was found that these three species are genetically indistinct from one another (Zardus et al. 2014, Doell et al. 2017). The distinction is the host they reside on. *C. patula* is mainly found attached to crabs, *C. manati* mainly attached to sirenians, manatees and dugongs, and *C. testudinaria* predominately is associated with sea turtles. (Zardus et al. 2014). With this discovery, all three species are recognized as *C. testudinaria* (Doell et al. 2017). *C. testudinaria* is one of the most common and largest acorn barnacles residing on sea turtles. *C. testudinaria* are nearly host specific and rarely reside on anything other than sea turtles (Zardus & Hadfield 2004). Turtles have been observed to have shown the presence of *C. testudinaria* year-round off the coast of Georgia and Florida (Frick & Slay 2000).

*C. testudinaria* is not only the most common epizoic organism found upon sea turtles, but also typically the largest epizoic organism residing on sea turtles. These barnacles can reach a rostro carinal length (The distance from the most anterior plate of the shell to the most posterior; the total length of the barnacle) of 120 mm (Zardus & Hadfield 2004). *C. testudinaria* grow linearly, growing at  $4.28 \text{ mm}^2 \text{ day}^{-1}$  in basal area and  $0.07 \text{ mm day}^{-1}$  in rostro-carinal length (Sloan et al. 2014, Ewers-Saucedo et al. 2015, Doell et al. 2017). *C. testudinaria* grows until it is removed from the host due to molting or the host rubbing against a hard substrate. It is typical for epizoic barnacles to be removed because of molting (Cheang et al. 2013). Sea turtle's individual scutes molt in bits rather than full sheets, meaning that molting can be a relatively long process, compared to crustaceans who molt more often and at a quicker rate (Monroe 1981, Zardus & Hadfield 2004, Cheang et al. 2013). Sea turtles molt approximately every year (Monroe 1981, Zardus & Hadfield 2004, Kelly & Sanford 2010). Because *C. testudinaria* longevity is at the mercy of molting frequency of its host, life span is not exactly known (Zardus & Hadfield 2004). However, life span can be roughly estimated for individuals residing on the same hosts. This is to say, the larger the barnacle is typically means the older the barnacle (Cheang et al. 2013).

### *Life history*

There is a paucity of information on the overall life history of the turtle barnacle *C. testudinaria*, including their breeding cycle and frequency of reproduction (Zardus & Hadfield 2004, Kelly & Sanford 2010). This is likely due to the difficulties of monitoring individuals residing on mobile hosts. The turtles these barnacles reside on migrate great distances, and rarely come onto dry land other than for mating purposes, making it difficult for longitudinal measures of individual barnacles in a natural setting. Typically, larval development of intertidal acorn barnacles includes seven instars (molting periods; Zardus & Hadfield 2004). The first six instars are nauplii, unsegmented larvae; the first few may reside within the mantle of the adult barnacle before being released into the water column with the rest of the plankton (Barnes et al. 1977). The seventh is referred to as a cyprid, the last larval stage before adulthood, prioritizing settlement over feeding (Zardus & Hadfield 2004). There are many factors that can impact the larval period in barnacles such as climate, proper site attachment as well as individual species (Pineda & Lopez 2002, Sloan et al. 2014, Zardus 2021).

*C. testudinaria* follows a typical barnacle larval development, similar to that of the intertidal acorn barnacle. The first stage nauplii hatch and reside within the mantle of the adult throughout the first instar of its life cycle. The larval *C. testudinaria* is then released into the water column to feed amongst the rest of the plankton until they develop into the non-feeding cyprid searching for a suitable turtle host to attach to (Zardus & Hadfield 2004, Zardus 2021). It is a difficult task for these newly settled individuals to succeed in their lives as they need time to settle onto their host and find proper nutrients before the host migrates to areas that may be less productive (Zardus & Hadfield 2004, Nájera-Hillman et al. 2012). For example, loggerhead sea turtles migrate large distances throughout its life. Juvenile loggerhead turtles spend upwards of 6 years in productive oceanic nursery grounds, whereas older individuals who have left the nursery phase, travel on large seasonal migrations (Rawson et al. 2003, Bowen et al. 2004). It is likely that turtle barnacles tend to reproduce and attach to their new hosts near shore where turtles are commonly feeding or mating (Zardus & Hadfield 2004).

### *Spatial distribution*

Even though *C. testudinaria*'s life history is not well documented, there have been many studies determining the optimal position individuals prefer for attachment on their mobile turtle hosts. Their most frequent attachment position is on the carapace of turtles followed by the plastron, then fewer common locations found on more flexible body parts with smaller scales like neck, flippers, or the tail (Frick & Ross 2001, Zardus & Hadfield 2004, Nájera-Hillman et al. 2012). Turtles provide a patchy environment for barnacles to settle within an otherwise inhabitable open ocean (Hayashi & Tsuji 2008). This patchy environment means that *C. testudinaria* tends to live within clusters, called hummocks, at areas of more optimal distributions (Hayashi and Tsuji 2008). Optimal spatial distribution is determined based on water flow as well as inter- and intraspecies competition (Pfaller et al. 2006, Habibi et al. 2020). Water flow affects all stages of an organism's life from dispersal to recruitment as well as overall survival (Larsson & Jonsson 2006). The overall survival of the organism is apparent in why individual barnacles tend to migrate to better locations amongst their hosts' carapace (Moriarty et al. 2004). *C. testudinaria* has been observed to actively seek out preferred areas of water flow, by means of post-settlement locomotion (Moriarty et al. 2004). Some adult barnacles are able to rotate or move short distances by lateral pressure of a neighboring barnacle (Moriarty et al.

2004). Pedunculate barnacles can even relocate by extending their peduncles down the substrate they are attached to (Hoffman 1989, Kugele & Yule 2000, Moriarty et al. 2004). It is likely that cyprids find a suitable location to settle on the carapace but end up moving as an adult barnacle to find an optimal position for feeding caused by waterflow across the shape of the turtle's carapace (Moriarty et al. 2004). This optimal spatial distribution appears to be on the posterior portion of the carapace (Frick et al. 1998, Frick et al. 2003, Fuller et al. 2010). This is because the posterior scutes have a lower water flow rate as compared to anterior scutes (Nájera-Hillman et al. 2012). Posterior scutes could favor larvae colonization due to the lower amount of drag than anterior regions (Schärer 2001). Other studies suggest that these barnacles were more abundant in the central scutes (Nájera-Hillman et al. 2012). However (Fuller et al. 2010) discovered that loggerheads tend to have larger barnacles aggregating towards the anterior half of the carapace. Whereas for green sea turtles, most of the barnacle coverage was located on the marginal scutes. Central and marginal scutes could be favored due the ability to keep sediments and food particles more effectively due to the flow patterns eddying around unique carapace features in those locations (Schärer 2001, Nájera-Hillman et al. 2012). Knowing how these epizoic barnacles spatially distribute amongst the host carapace provides important indicators into movement patterns, migration routes, geographical distribution as well as health of the sea turtles they are attached to (Habibi et al. 2020).

### *Importance*

Epizoic barnacles can act as valuable indicator of overall health of the host they reside upon (Flint et al. 2009). Analysis of the epizoic barnacles can determine habitat (Pfaller et al. 2014) and even movement (Killingley and Lutcavage 1983) of the host animal. Barnacle abundance is not determined by turtle size, instead healthier turtles tend to have a lesser number of barnacles. Without conducting more research on these epibionts, the level of difficulty utilizing these indicators increases drastically (Doell et al. 2017). Although turtle barnacles are widely known, research on them is scarce due to difficulties in their assessment. As *C. testudinaria* reside upon mobile sea turtles, collection and observation is problematic (Cheang et al. 2013). This study presents information vital to the understanding of how *C. testudinaria* adapt to a multitude of variables in a natural setting. Specifically, answers to these several questions will bridge the gap of the current knowledge of *C. testudinaria*. First, does the barnacle's

anatomy of penis and cirri vary with body size? Second, how does barnacle position on the turtle affect the morphology of the penis and feeding cirri? Third, how do barnacles more distant from each other differ in the morphology of the penis and feeding cirri compared to barnacles growing within a cluster? Fourth, how does social condition affect fertility probability? Lastly, what is the difference in spatial distribution of barnacles on the carapace of *Caretta caretta* compared to *Chelonia mydas*?

Overall, this research serves to bridge the knowledge gap existing on a commonly found and important species of epizoic barnacle. Here, I investigated these questions by describing the distribution and abundance of *C. testudinaria* distribution on turtles found on beaches in Broward County, Florida collected in the years of 2019 and 2020.

## **MATERIALS AND METHODS**

### *Study organism*

*C. testudinaria* is a species of epibiont acorn barnacle that utilizes sea turtles, most commonly loggerhead sea turtles (*Caretta caretta*), as their permanent residence. Loggerhead sea turtles are the most common species of sea turtle that nests in South Florida (Species of Sea Turtles Found in Florida, 2020, Slagle et al. 2020). These barnacles are filter feeders, meaning they extract food particles from the water using their cirri, grabbing onto whatever floats into their reach. Competitive interactions are common as they tend to be found aggregated in tight clusters (Cheang et al. 2003). *C. testudinaria* is unique from other barnacles as they are sessile, but mobile at the same time. Residing on a moving object (sea turtle) affects their ability to filter feed, so they are dependent on host trajectory and locations.

### *Study area*

Samples were collected from nesting sea turtles on Fort Lauderdale Beach, Fort Lauderdale, Florida. Approximately ranging from the Southernmost point, the Fort Lauderdale Jetties (26°05'42.4"N, 80°06'17.5"W), and the Northernmost located approximately at the Royal Ambassador Hotel (26°10'26.3"N, 80°05'50.0"W).

### *Sampling protocol*

The removal of epibiont barnacles was already a sampling protocol for tag placement that is integrated into the turtle tagging program run through the Nova Southeastern University's Conservation, Movement, and Ecosystem Dynamics Laboratory. The protocol was modified to preserve barnacles removed from the carapace of the nesting turtles rather than immediate disposal. All activities were performed by trained and authorized personnel on Florida Fish and Wildlife Conservation Commission Marine Turtle Permit #255.

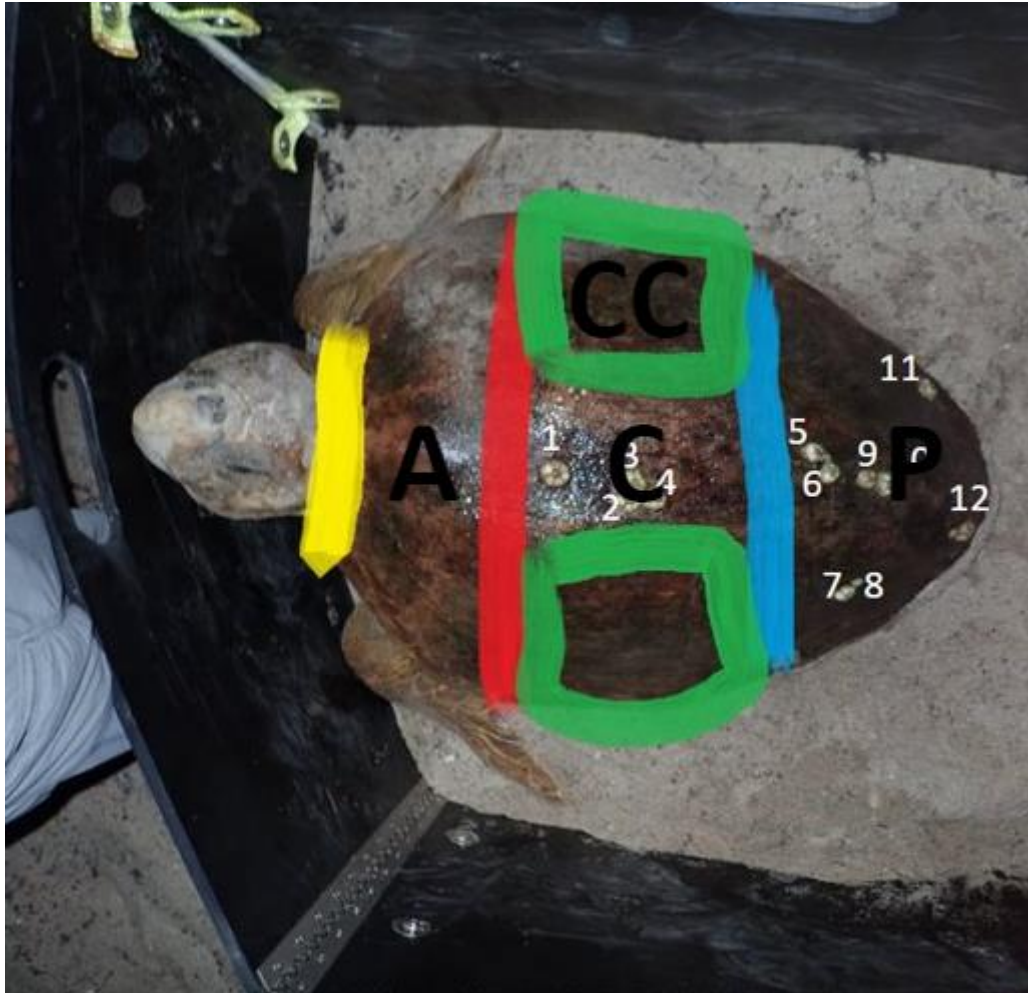
Nightly patrols spanned from 21:00 – 05:00 hours on most nights, weather permitting. The loggerhead sea turtle nesting period ranges from April to September in Florida, with peaks in June and July (Brost et al. 2015, Slagle et al. 2020). Patrols involved locating nesting and false crawling female loggerhead and green sea turtles. Spotted individuals were categorized by determining whether she was heading towards a nest or returning to the ocean. For individuals heading towards the ocean, a corral barrier was placed around the turtle to facilitate sampling. Turtles were cataloged by name and number. Carapaces were then cleaned with seawater to produce clearer photos of barnacle positioning from above. Close-up images of barnacle clusters (hummocks) were also taken to determine individuality of the barnacles before removal and separation from clusters. Barnacles were then carefully removed using a chisel or paint chipper dependent on the size, location, and structural soundness. Removed barnacles were placed inside of a containment bottle, rinsed with saltwater, and then preserved with ethanol for transportation to NSU's Environmental Science Laboratory located in the Parker Building at Nova's main campus in Davie, FL.

### *Laboratory protocol*

Barnacles were compared to the top-down photos taken prior to removal. These photographic images determined individuality and position for each barnacle. Barnacles were numbered based on position and separated into individual containment bags filled with ethanol. Photos of the turtle's carapace were separated into 4 different positions: anterior, posterior, central and central costal scutes (Figure 1). All of the barnacles photographed were considered when determining how many individuals were located within each position, rather than only using the dissected individuals. These data were used to determine the total spatial distribution on the host. Barnacles were weighed using a Mettler Toledo EI204 analytical balance (220g x .01



mg) to determine total weight. Soft tissue weight was determined after dissection. Measurements of shell height, rostral-carinal length, base length, and aperture length were recorded using a digital caliper. These measurements were used to determine size and volume of the shell. Volume was calculated based on the formula for a truncated cone. Shell volume =  $(1/3) * \pi * \text{height} * (r^2 + r * R + R^2)$ , where R is the radius of the basal opening, and r is the radius of the aperture.



*Figure 1.* This figure shows how each turtle was positionally categorized. Those positions are anterior (A), posterior (P), central (C) and central Costal (CC) scutes. Additionally figure 1 shows how individuals were numbered during the positioning process.

Following proper identification and measurement, barnacles were then dissected. Many acorn barnacles have a baseplate, separating the internal organs from the substrate they reside on, however *C. testudinaria* does not possess this baseplate. Instead *Chelonibia* have a slightly

opaque film on the dorsal sector of the organism. This film was first removed using forceps to access the internal structure. The internal structure was carefully removed from the protective shell and placed under a dissecting microscope for examination of sexual and feeding structures. Individuals were also observed to possess eggs or not. Cirri 1 and Cirri 6 (right) as well as the penis were removed from the body and placed onto slides in respected order from left to right across one slide per individual. The slides were then covered for microscopic photography. A Hirox- KH- 7700 3D digital video microscope was used to photograph the small internal structures removed from each barnacle. Most structures could be photographed at low range, 35X magnification, however for some smaller individuals the use of stronger magnification was necessary up to 100X magnification. Cirri 1 and 6 were measured for total length, basal thickness as well as number of segmentations along the cirri branch. The penis was measured for total length, basal thickness as well as number of annulations.

Once all individuals were photographed, pictures were uploaded to a computer and measured for length, thickness, as well as counting total number of segmentations/ annulations using the imaging software Image J (v. 1.53e). Image J uses known distances, accounted for by the calibrated scale on the Hirox digital video microscope, and converts the unknown pixel length into accurate measurable distances. Using Image J's plugin feature, I used an adaptation of a downloadable plugin designed to assess the age of trees by automatically counting tree rings. This plugin allowed for a quicker, and still accurate means of counting hundreds of annulations. However, the plugin needed confirmation by eye due to instances of miscounting closely associated annulations.

### *Statistical analysis*

The hypotheses were statistically analyzed by means of a factorial analysis of covariance (factorial ANCOVA) using the statistical analysis software R version 1.4.1717. This analysis tested for interactions between position, crowding, and measured data with body size as the covariate. Three different variables determined body size: total weight, internal weight, and volume. Internal weight represented body size in accordance with previous studies (Li & Denny 2004). Correlation coefficients determined which body size variable best suited as a covariate.

Within this factorial ANCOVA, dependent variables were tested against two categorical variables, using body size as the covariate. Dependent variables consisted of penis diameter, measured penis length, penis length determined by counted annulations, cirrus diameter,

measured cirrus length, as well as cirrus length determined by counted segmentations for both cirrus 1 and cirrus 6. The two categorical variables being tested included position the barnacle resided on the turtle's carapace, and whether the barnacle grew within a cluster of other barnacle/barnacles or if the individual barnacle was considered solitary. We used both variables to ensure that significant interactions between the categorical variables and the dependent variables to account for multicollinearity. Tukey post-hoc tests determined differences between specific variable groupings.

A logistic regression served to predict the likelihood of a variable changing based on one or more independent variables. For this analysis, the dependent variable was whether the individual barnacle was observed to have eggs within its inner mass, represented as the 1 value, or the individual barnacle was not observed to have eggs within its inner mass, represented as the 0 value. The independent variables were crowding as well as internal volume of the barnacle. The internal volume was used rather than either measurement of mass as individuals containing eggs were heavier than similarly sized eggless counterparts. The crowding variable was used as a predictor as it was expected that individuals within a crowd were more likely to contain eggs than solitary individuals.

## RESULTS

### *Variable correlation*

While no dwarf males were obtained in this investigation, understanding their potential impacts on other barnacles was still a vital aspect of this project. Three different body weight measurements were collected to be used as body size within this research: total weight of the individual, internal weight, and internal volume of the shell. Total weight was calculated as the combined weight of the shell and the soft body tissue. Internal weight was measured as just the soft body tissue. Internal volume was measured using the equation for a truncated cone. All three variables were used to represent body size as all three are highly correlated to one another. Total weight and internal weight had a correlation coefficient of 0.8 ( $p < 2.2e-16$ ,  $df = 720$ ), total weight and internal volume had a correlation coefficient of 0.8 ( $p < 2.2e-16$ ,  $df = 720$ ) and internal weight and internal volume had a correlation coefficient of 0.9 ( $p < 2.2e-16$ ,  $df = 720$ ). Each appendage collected was measured for three different variables: diameter at the base of the structure, total length, and annulations (penis) and segmentations (cirrus). penis length was

highly correlated to penis annulation, having a correlation coefficient of 0.8 ( $p < 2.2e-16$ ,  $df = 720$ ). However, penis length was only slightly correlated to penis diameter with a correlation coefficient of 0.5 ( $p < 2.2e-16$ ,  $df = 720$ ), and penis annulations were not strongly correlated to penis diameter with a correlation coefficient of 0.4 ( $p < 2.2e-16$ ,  $df = 720$ ). cirri length and segmentations had a correlation coefficient of 0.8 ( $p < 2.2e-16$ ,  $df = 720$ ) (cirrus 1) and 0.8 ( $p < 2.2e-16$ ,  $df = 720$ ) (cirrus 6), cirri length and cirri diameter had a correlation coefficient of 0.8 ( $p < 2.2e-16$ ,  $df = 720$ ) (cirrus 1) and 0.8 ( $p < 2.2e-16$ ,  $df = 720$ ) (cirrus 6) and cirri diameter and segmentation had a correlation coefficient of 0.7 ( $p < 2.2e-16$ ,  $df = 720$ ) (cirrus 1) and 0.8 ( $p < 2.2e-16$ ,  $df = 720$ ) (cirrus 6).

### *Barnacle morphology*

Due to collinearity of body size measurements, internal weight was used as a covariate within this analysis. Penis diameter was strongly positively associated with both body size ( $p < 2.2e-16$ ,  $F$  value = 487.97) and if the individual was found within a cluster or considered solitary ( $p < 0.0005$ ,  $F$  value = 12.79) (see figure 2). Position on the turtle's carapace was not a significant predictor of penis diameter.

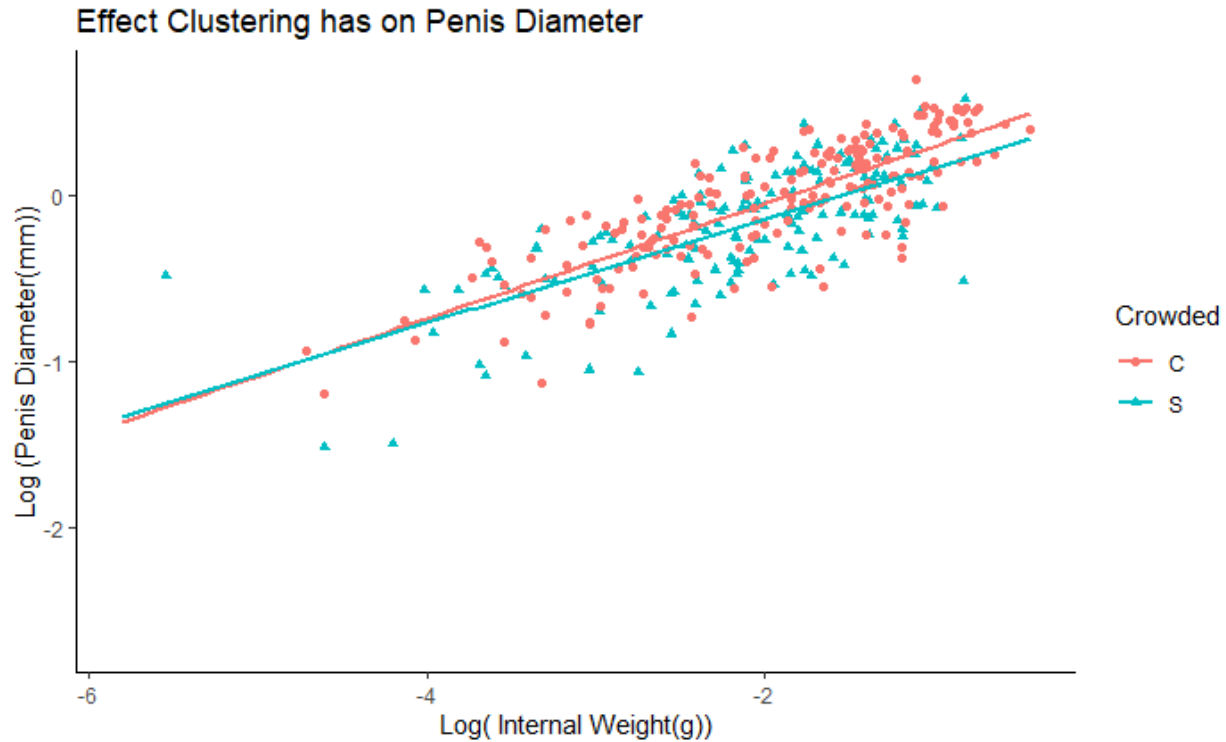
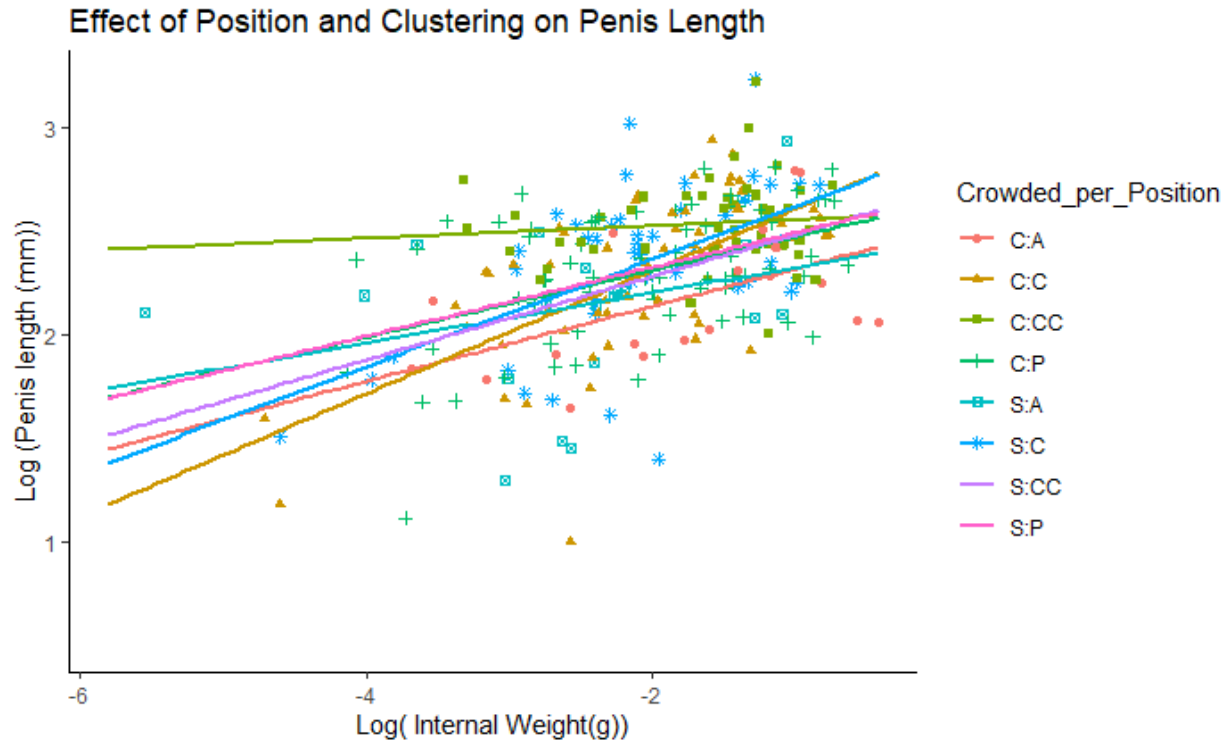


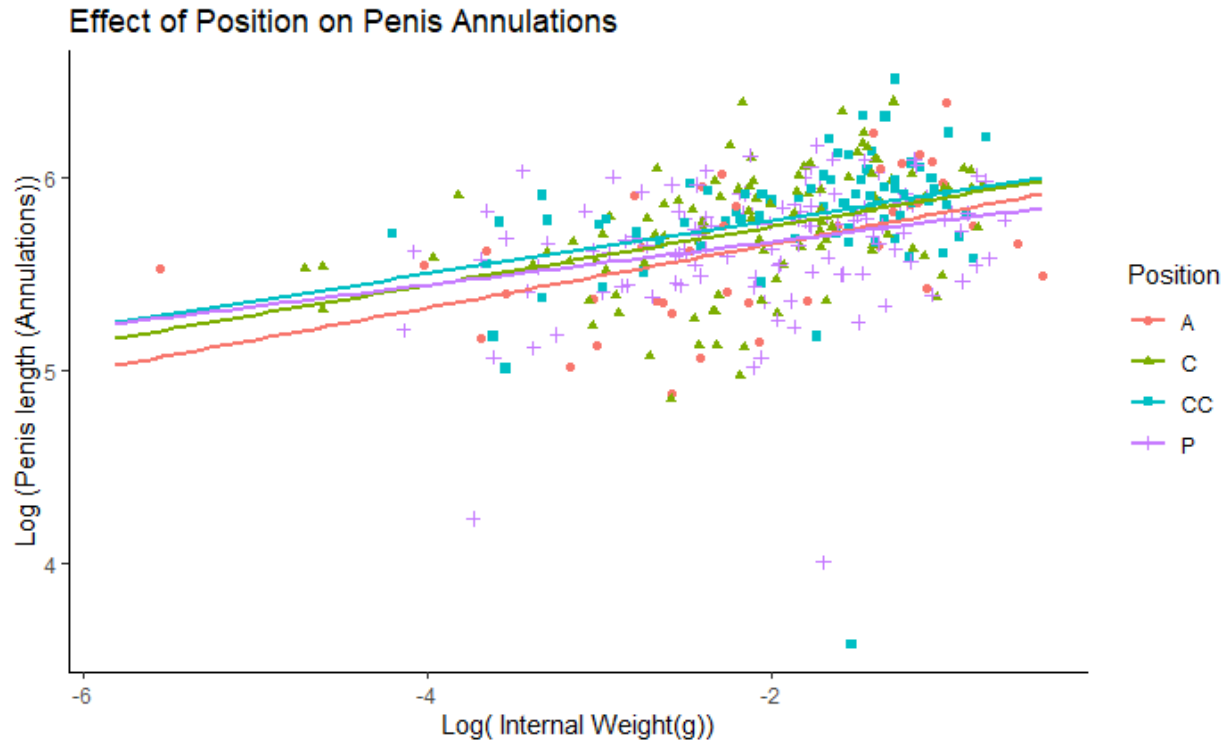
Figure 2. (*Effect clustering has on penis diameter*): This figure shows the significance clustering has on penis diameter ( $p < 0.0005$ ). Within the legend the social condition is represented by crowded (C) and solitary (S).

Penis length was positively associated with body size ( $p < 2.2e-16$ , F value = 487.97), position on the turtle's carapace ( $p < 0.005$ , F value = 0.7) (see figure 3), the interaction between position and body size ( $p < 0.05$ , F value = 1.16) as well as the interaction between position and clustering ( $p < 0.01$ , F value = 1.33). Individuals within central costal scutes have a longer penis compared to anterior barnacles (Tukey:  $p < 0.005$ ). Similarly, within that same grouping there was a significance interaction between position and clustering. Individuals that were within a cluster on central costal scutes have a significantly longer penis than individuals within a cluster on anterior scutes (Tukey:  $p < 0.005$ ), as well as individuals within a cluster on central costal scutes have a significantly longer penis than individuals living solitary on anterior scutes (Tukey:  $p < 0.05$ ). There was no relationship with other groupings. Penis length was not significantly associated with whether the individual was within a cluster or solitary nor any interaction between clustering and body size.



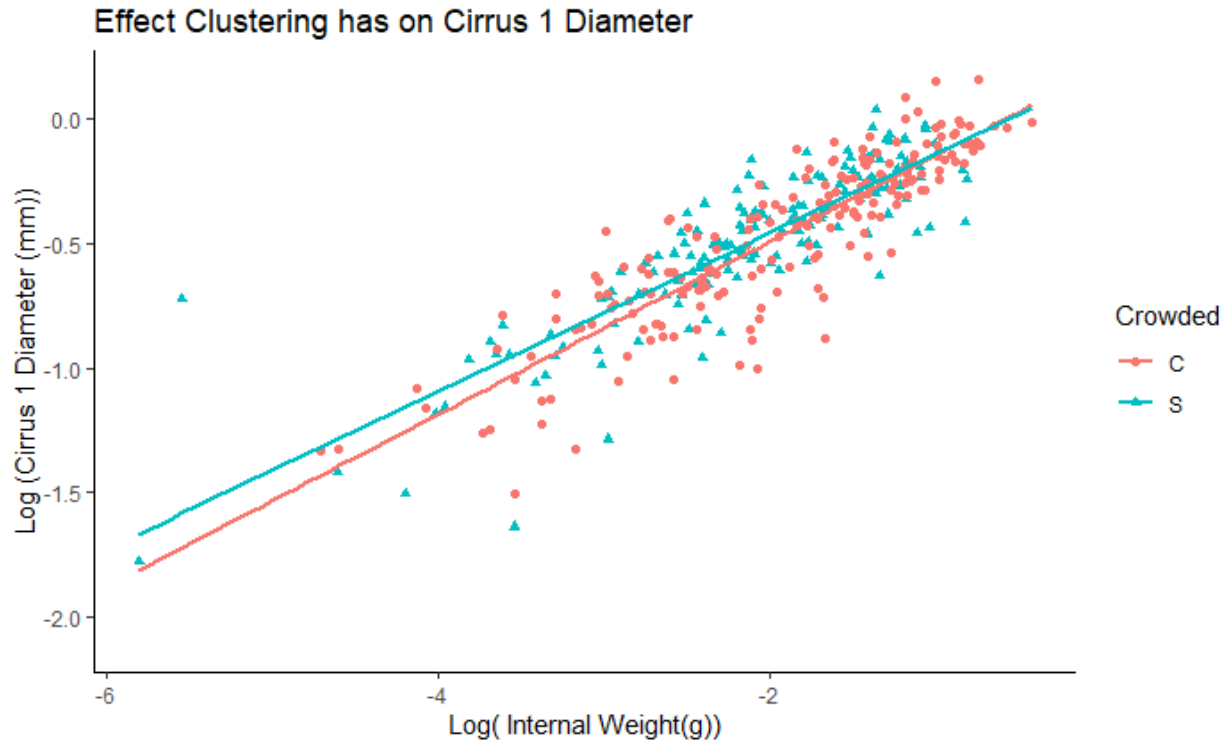
*Figure 3.* This figure shows the positive relationship between position and penis length ( $p < 0.005$ ) as well as the significance between central costal individuals and anterior individuals. Also depicted is the interaction between barnacles within a cluster on central costal scutes and barnacles within a cluster on anterior scutes (Tukey:  $p < 0.005$ ), as well as individuals within a cluster on central costal scutes and individuals living solitary on anterior scutes (Tukey:  $p < 0.05$ ). The first column within the legend represents crowded (C) and solitary (S). The second column represents the position; anterior (A), central (C), central costal (CC) and posterior (P).

Penis annulation was positively associated with both body size ( $p < 3e-12$ , F value = 52.4) and position ( $p < 0.05$ , F value = 2.89) (see figure 4). However, there was no difference between any of the positions against another. The post hoc Tukey tests showed no significant difference between any positions in particular. Penis annulation was not significantly associated by individuals residing within a cluster or solitary.



*Figure 4.* This figure shows the significance position has on penis annulation ( $p < 0.05$ ). Clustering was not included within this figure as it was not a significant interaction. Within the legend the positions are anterior (A), central (C), central costal (CC) and posterior (P).

Cirrus 1 diameter was positively associated with body size ( $p < 2.2e-16$ , F value = 1117.36) as well as whether the individual was crowded or solitary ( $p < 0.05$ , F value = 4.4892) (see figure 5). Position was not associated with cirrus 1 diameter, and there was no interaction between crowded and position as well as between crowding and body size.

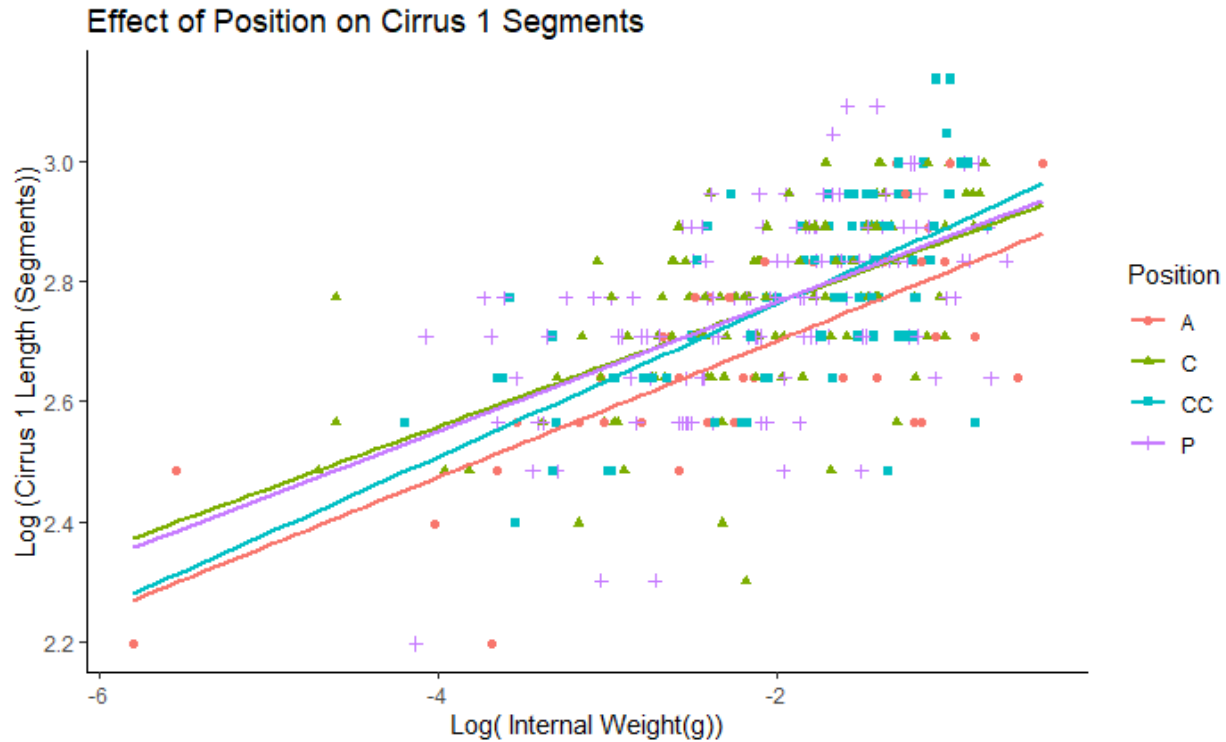


*Figure 5.* This figure shows the significance crowding has on cirrus 1 diameter ( $p < 0.05$ ). Position was not included as there was no significant effect. Within the legend the social condition is represented by crowded (C) and solitary (S).

Cirrus 1 length was positively associated with body size ( $p < 2.2e-16$ , F value = 1130.73). Position and crowding were not significantly associated with cirrus 1 length. There were no significant interactions between all other variables.

Cirrus 1 segmentations was positively associated with body size ( $p < 2.2e-16$ , F value = 204.15) as well as position on the turtle's carapace ( $p < 0.05$ , F value = 3.21) (see figure 6). The difference between individuals residing within the anterior portion of the shell and central (Tukey:  $p < 0.05$ ), central costal (Tukey:  $p < 0.05$ ) and posterior (Tukey:  $p < 0.05$ ) positions were all determined to be significantly greater. There was no difference between the rest of the positions. Crowding was also not associated with cirrus 1 segmentations. There were no interactions between all remaining variables.

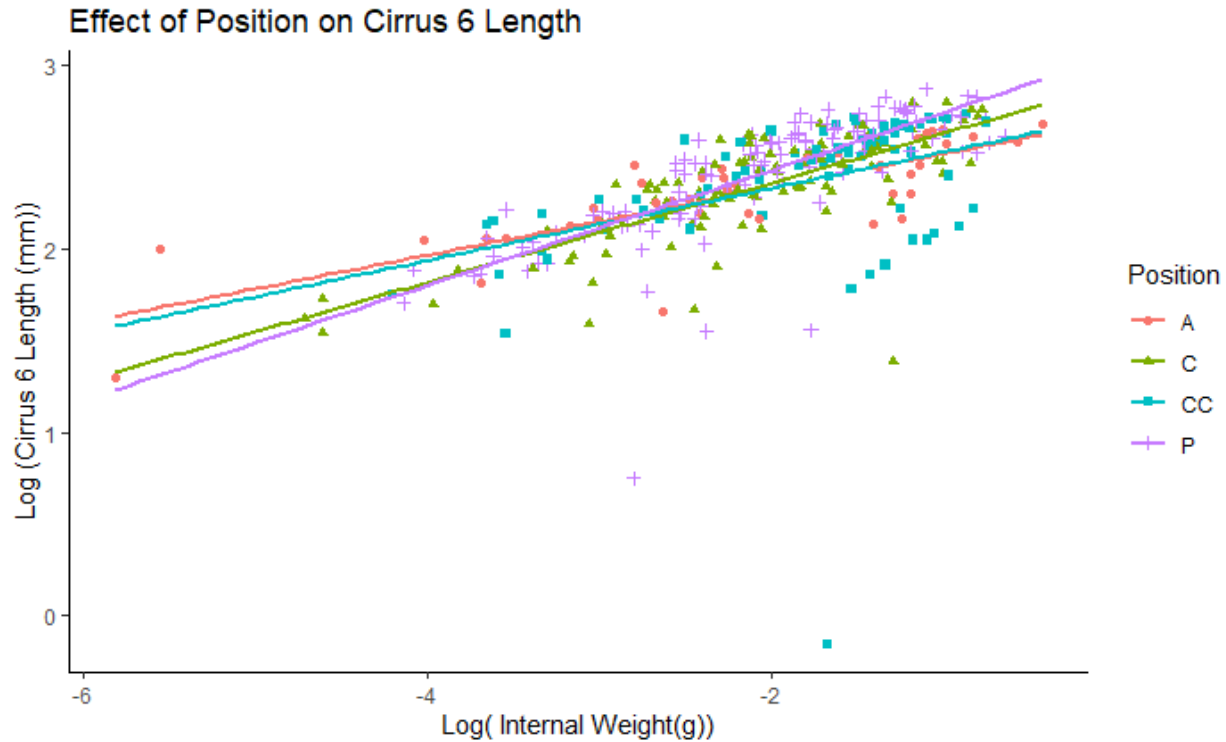




*Figure 6.* This figure shows the significance position has on cirrus 1 segmentations ( $p < 0.05$ ), specifically the difference between the anterior portion of the shell and central (Tukey:  $p < 0.05$ ), central costal (Tukey:  $p < 0.05$ ) and posterior (Tukey:  $p < 0.05$ ) scutes. Crowding was excluded as crowding had no significant effect on cirrus 1 segmentations. Within the legend the positions are anterior (A), central (C), central costal (CC) and posterior (P).

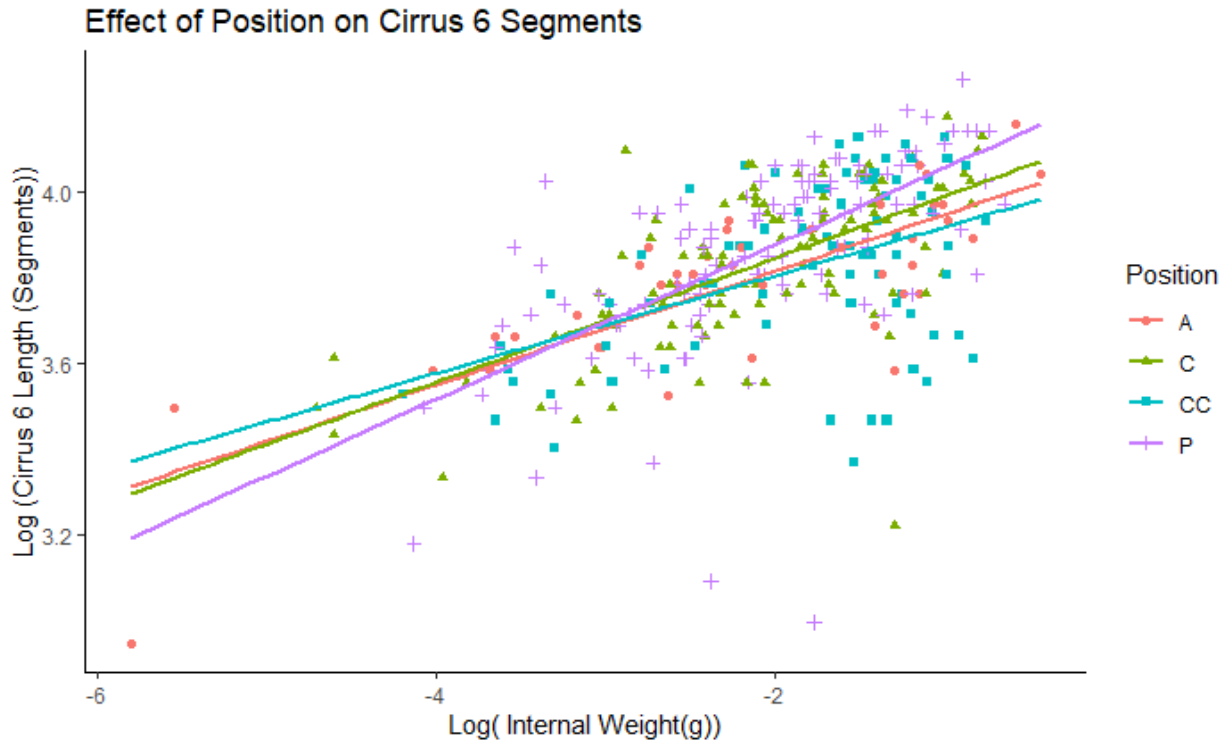
Cirrus 6 diameter was positively associated with body size ( $p < 2.2e-16$ , F value = 676.98). Position and crowding did not significantly affect cirrus 6 diameter. However, there was a significance between the interaction body size has on both position ( $p < 0.0003$ , F value = 6.46) and crowding ( $p < 0.03$ , F value = 4.57). The interaction between crowding and position was not significant.

Cirrus 6 length was positively associated with body size ( $p < 2.2e-16$ , F value = 244.4) and Position ( $p < 0.05$ , F value = 3.04) (see figure 7). Cirrus 6 length was significantly longer for individuals within the posterior position compared to those within the central costal scutes (Tukey:  $p < 0.05$ ). The interaction between body size and position was also significant ( $p < 0.05$ ). Crowding did not significantly affect cirrus 6 length.



*Figure 7.* This figure shows the positive association between position and cirrus 6 length ( $p < 0.05$ ). Specifically, the difference between the posterior and central costal positions ( $p < 0.05$ ). Crowding was excluded as it was no associated with cirrus 6 length. Within the legend the positions are anterior (A), central (C), central costal (CC) and posterior (P).

Cirrus 6 segmentations was positively associated with body size ( $p < 2.2e-16$ , F value = 201.57) and position on the turtle's carapace ( $p < 0.05$ , F value = 3.72) (see figure 8). Cirrus 6 segmentations were significantly different for individuals within the posterior position compared to those within the central costal scutes (Tukey:  $p < 0.05$ ). The difference between the rest of the positions were determined not to be significant. Crowding did not significantly affect cirrus 6 segmentations. The interactions between all variables were determined not to be significant.



*Figure 8.* This figure shows the positive association position has on cirrus 6 segmentations ( $p < 0.05$ ), specifically the difference between posterior position compared to those within the central costal scutes (Tukey:  $p < 0.05$ ). Crowding was excluded as it does not have a significant effect on cirrus 6 segmentations. Within the legend the positions are anterior (A), central (C), central costal (CC) and posterior (P).

#### *Fertility probability*

Internal volume did not have a significant effect on predicting whether barnacles contained eggs or not (see figure 9). However, proximity to potential mates was significant in predicting fertility. Keeping all variables constant, when crowding increases by one unit, the barnacle is 0.679 times more likely to have eggs. In other words, individuals within a crowded community were 68% more likely to be fertilized than their solitary counterparts.

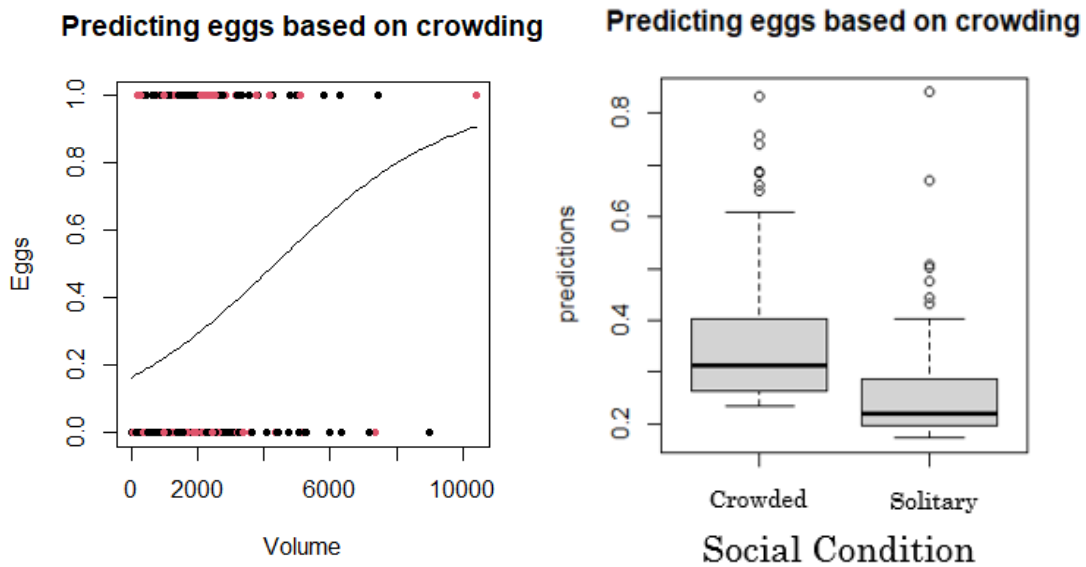


Figure 9. This figure shows the logistic regression predicting the likelihood barnacles have eggs (1) or not (0) compared to internal volume and proximity to other barnacles. Solitary barnacles are represented by red dots, and crowded barnacles by black.

### *Spatial distribution*

Spatial distribution for all barnacles observed was determined based on top-down photographs taken on the night of collection (see figure 10). There were two main species of sea turtle observed, Loggerhead (*Caretta caretta*) and Green (*Chelonia mydas*). Each turtle carapace was separated into 4 generalized scute positions, anterior, posterior, central and central costal (see figure 10). The largest amount of barnacle distribution was on posterior scutes (N = 357) for loggerhead sea turtles but was central costal scutes (N = 39) for the greens used within this study. The next highest distribution number was central scutes for both loggerhead (N = 204) and green (N = 15) sea turtles. The second lowest distribution number was central costal scutes (N = 159) for loggerhead and anterior scutes (N = 6) for green sea turtles. The lowest distribution determined within this study was the anterior scutes (N = 99) for loggerhead and the posterior scutes (N = 1) for green sea turtles.

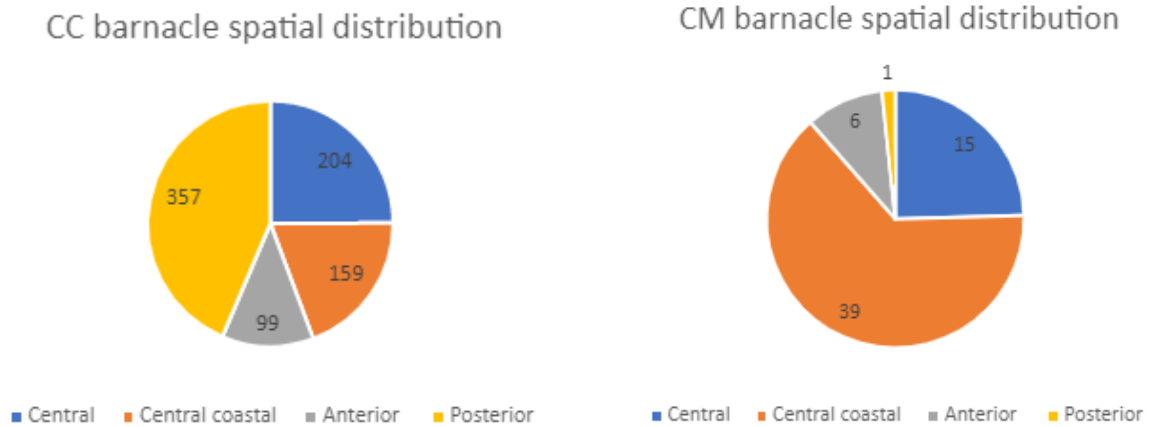


Figure 10. This figure shows the distribution of total barnacles residing on the carapace of both species of sea turtle *Chelonia mydas*, green sea turtle, and *Caretta caretta*, loggerhead sea turtle.

## DISCUSSION

### *Correlation statistics*

Many variables were interrelated. For instance, penis length as well as penis annulations were strongly correlated, as they are both measurements of the total length of the penis. However, it is still vital to measure both data since barnacles have the ability to extend and retract their penis (Hoch 2008, Yuen & Hoch 2010). It is understood that penis annulation tends to provide a more accurate length measurement (Hoch 2008, Neufeld & Rankine 2012). This is measured to determine the fully extended length of the penis. Due to the nature of the collection process, it was not possible to relax the barnacles prior to preservation to avoid retraction of their soft tissues. When collecting these barnacles, limiting the stress of host turtles was the main priority so we could not spend the required time to put the barnacles into a relaxed state before euthanasia. Therefore, despite their collinearity, both length variables were considered. Taken together, these provide a better understanding of the penis morphology and the variables associated with it. When it comes to the feeding cirri, all the measurements were strongly related. This is likely due to; 1. the cirri do not have annulations, but segmentations instead, meaning that they do not extend and retract to a longer length, 2. the elasticity of the structure is lacking, meaning that the structures overall measurements are likely predetermined, rather than having more phenotypic elasticity.

### *Penis morphology*

Barnacle penis morphology was significantly affected by the individuals body size. Typically, a larger individual possessed a larger penis in all facets, diameter, length, and total annulations, than those of a smaller body size. A bigger body allows for larger appendages to be housed. One can only grow as large as its internal volume. *C. testudinaria*'s penis length was also influenced by position on the turtle carapace. Measured length and penis annulations were significantly associated with position on the host. The only significant difference between positions was for measured penis length between anterior barnacles and central costal scutes. Barnacles residing on central costal scutes had a longer penis length on average 27.27% longer, than those within the anterior position of the turtle's carapace. Barnacles residing on anterior scutes could require a shorter penis, owing to a few different factors. First, these individuals within the anterior portion could be smaller/ younger than those residing on the central costal scutes. The anterior portion may be a better recruitment site for newer barnacles to settle, meaning the younger barnacles would have a smaller penis than older more established individuals. It is suggested that barnacle larvae could favor certain positions due to environmental conditions such as water flow (Schärer 2001). This is supported by barnacles being 14.74% larger, on average, within costal central scutes compared to those on anterior scutes.

It's also interesting to note that penis diameter was not influenced by position. It is common to see intertidal barnacles have penises with a larger diameter in areas of higher wave action (Hoch 2010, Hoch et al. 2016). Assuming water flow patterns vary with position on the carapace, it was predicted that some positions would require a larger penis diameter to combat the difference in water flow. Given that position on the turtle's carapace was not significant with penis diameter, it is likely that flow patterns for each position on the turtle's carapace is not harsh enough to induce phenotypic plasticity. Another possible explanation is that perhaps it is more energy efficient to move to the preferred location (Moriarty et al. 2004) on the turtle rather than adjusting its penis morphology. The barnacle might not remain in the recruitment site long enough to warrant costly adjustments to morphology.

Surprisingly, clustering was not associated with penis morphology as it was expected that individuals at a greater distance to their closest mate would require a longer penis to fertilize

their eggs (Hoch 2008, Yuen & Hoch 2010). Similarly, it was expected that individuals of close proximity would need a much shorter length of penis to properly fertilize potential mates. The only relationship between clustering and penis morphology was the interaction between position and penis length. The two significant groupings were clustered anterior compared to clustered central costal (Tukey:  $p < 0.005$ ), and solitary anterior compared to clustered central costal (Tukey:  $p < 0.05$ ). It is likely that the position is a stronger moderator of morphology than clustering.

### *Cirrus morphology*

Barnacles feeding cirri was significantly associated with body size. Larger barnacles can house larger feeding appendages. Overall, cirrus morphology was related to both position and clustering. However, when comparing differences within cirrus 1 morphology, only position was associated with segmentations ( $p < 0.05$ ), and clustering was only associated with diameter ( $p < 0.05$ ). Neither variable was related to the overall length for cirrus 1. Cirrus 1 segmentations were significantly different between anterior barnacles compared to central (Tukey:  $p = 0.05$ ), central costal (Tukey:  $p = 0.05$ ) and posterior (Tukey:  $p = 0.05$ ). On average, cirrus 1 segmentations for individuals residing within the anterior portion of the turtle's carapace were less than the other three positions. Although overall length was not significantly affected by position, it is interesting that number of segmentations were significantly different for anterior barnacles compared to the rest of the positions on the turtle's carapace. A possible explanation for this would be similar to that of the penis morphology explained above when talking about the difference between anterior barnacles and other positions. Perhaps the anterior positioned barnacles are younger, meaning that they have not had enough time to properly develop the number of segments compared to barnacles on the other positions. Since cirrus 1 is the shortest cirri, as it is the first one, this could explain why total length was not significantly affected. Crowding significantly affected cirrus 1 diameter. On average crowded individuals had a thicker cirrus 1 diameter. This could be due to individuals within a close proximity to one another attempting to obtain any advantage they can when it comes to feeding against close competitors. Crowding tends to make filter feeding more difficult (Cheang et al. 2003). Cirrus 1's purpose is to hold the food for consumption and deny undesired particles (Anderson 1981, Chan et al. 2008). Solitary individuals may not require a thicker cirrus 1 as feeding may be more readily

available. Another possible reason for this significance could be within hummocks the water flow patterns are higher requiring sturdier appendages.

Cirrus 6 was affected by weight as well as position, but not significantly affected by crowding. Position significantly affected both length variables for cirrus 6, total length ( $p < 0.05$ ) and segmentations ( $p < 0.05$ ). Posterior barnacles had a significantly different total length (Tukey:  $p < 0.05$ ) and segmentations (Tukey:  $p < 0.01$ ) compared to those residing in central costal areas. There were no other significant differences between positions. On average, length was longer for barnacles residing on the posterior position compared to those on costal central scutes. This significant difference is likely due to flow patterns across the turtle's carapace. Barnacles residing on the posterior portion of the turtle's carapace experience less water flow, allowing for longer cirri to be supported (Schärer 2001). Longer cirri would also suggest that the individual must extend further to obtain their nutrients. Perhaps, individuals residing on the posterior portion of the turtle's carapace do not receive the proper amount of nutrients that other positions would receive. This would suggest that posterior barnacles exhibit some form of phenotypic plasticity when it comes to feeding.

#### *Fertility probability*

Barnacles are more likely to be fertilized within a clustered community. Clustered barnacles are 68% more likely to have been fertilized than not. This statistic makes sense because it is a lot less likely to be fertilized within a solitary community. Internal volume does not have a significance when predicting fertility. It would make sense for internal volume to help predict barnacles being fertilized, as the larger the volume the more space available for egg production. However, this is just an assumption when in fact there are other variables to consider when determining fertility probability like proximity to potential mates and penis size.

#### *Spatial distribution*

Barnacles collected from logger head sea turtles (*Caretta caretta*) had a higher distribution on the posterior portion of the host's carapace. Followed by central, central costal and lastly being the anterior scutes. It has been suggested that the optimal position for barnacles would be the posterior portion of the carapace (Frick et al. 1998, Frick et al. 2003, Moriarty et al. 2004, Fuller et al. 2010). However, when it came to green sea turtles (*Chelonia mydas*) the



greatest numbers were found on the central costal position of the carapace. Followed by central, anterior, and lastly being posterior scutes. Barnacles on green sea turtles have been documented to favor marginal or central scutes (Schärer 2001, Nájera-Hillman et al. 2012). Spatial distribution of barnacles appears to vary between species of turtle. A few explanations for this include the sample size could be way off. Thirty loggerhead sea turtles were sampled and only three green sea turtles. Typically, the green sea turtles that were observed to have been nesting did not have many or any barnacles on their carapace. Another possible explanation could be where the barnacles settle based on the water column each species of turtle typically resides in. Perhaps there could be a significant difference in flow across the different species of turtle's carapace, meaning that some locations are better for the barnacles. Flow patterns eddy around the unique carapace features for each different species of sea turtle (Schärer 2001, Nájera-Hillman et al. 2012). It is also possible that the green sea turtle, who were observed to have fewer barnacles, ha a better way of removing epizoic barnacles. Lastly these barnacles could have been younger and have not had enough time to move to the posterior position yet.

## CONCLUSION

In conclusion this research yielded some very interesting results. Some similarities between *C. testudinaria* and other acorn barnacles were expected, however there are some key differences between the species. These barnacles appear to not be significantly affected by many variables we would expect when living on a moving host. Intertidal acorn barnacles exhibit phenotypic plasticity based on many environmental factors. Wave action and crowding significantly affects penis and cirrus morphology amongst intertidal species (Li & Denny 2004, Hoch et al. 2016, Neufeld & Rankine, 2012). However similar variables did not have such a drastic change on *C. testudinaria*. Intertidal barnacles had thicker structures in areas of greater wave action (Hoch et al. 2016), but when it came to *C. testudinaria*, these barnacles were not as affected by more dynamic environmental conditions. Barnacles residing within the anterior portion of the turtle's carapace experience the highest level of flow (Schärer 2001) similar to barnacles residing on or around the tide line. These individuals collected from the anterior portion of the turtle's carapace did not have thicker sexual or feeding structures compared to areas of lesser drag. Knowing that *C. testudinaria* is not completely sessile and can move to

preferred areas with less water flow (Moriarty et al. 2004), it is safe to conclude that modifying the structural soundness of the barnacles' appendages is not worth the energy. The biggest impact is indeed the host they live on. Host movement creates water flow, allowing for the potential to obtain a greater amount of nutrients. No matter where these barnacles reside on their host, theoretically they should have access to plenty of food particles without needing to adjust their structural morphology. However, whenever the individual reaches its preferred location, *C. testudinaria* adjusts its morphology. Barnacles residing on the posterior portion of the turtle's carapace, an area of lower water flow (Schärer 2001), exhibited phenotypic plasticity when it came to their cirri. Barnacles had significantly longer cirrus 6, the active feeding cirri. Similar to intertidal species, the expectation for longer appendages in areas of less flow is indeed met. In areas of clustering, more difficult areas to filter feed (Cheang et al. 2003), the only significant adjustment barnacles made was a stockier cirrus 1. A thicker first cirrus allows for barnacles to better hold onto food particles in areas of eddying and greater feeding competition. Combining all of the information obtained from this thesis, we can conclude that the anterior portion of a turtle's carapace is a more readily available recruitment site for larval *C. testudinaria*. Barnacles found within the anterior portion of the turtle's carapace tend to be on average smaller than other positions and were determined to have a penis 27% smaller than those on marginal scutes. Ultimately this project aided in closing a significant gap in scientific knowledge pertaining to these tough to observe barnacles. We now have a better understanding of how barnacles congregate on the backs of a mobile host and how different locations affect barnacle morphology.

Barnacles that reside on the backs of moving hosts face environmental factors unique to these epizoic organisms. Water flow patterns change based on the water column the host resides in as well as these barnacles are at the mercy of their host when it comes to food particle collection. These epizoic barnacles combat difficult environmental factors by means of phenotypic plasticity as well as moving to a more beneficial location on the host. This research offers an important understanding on how organisms may adapt to distinct environmental factors differently from what may have been expected. Many organisms adapt phenotypic plasticity when impacted by different environmental factors, and this specific species of barnacle provides an unprecedented understanding of how a sedentary species adapted to move locations rather than adjust their structural morphology. Perhaps other sessile invertebrates have adapted

different means of conserving energy when it pertains to adjusting to environmental constraints. Plans for future research include attempting to determine which individual barnacles may be the potential parent for fertilized eggs, and how position potentially affects successful mating.

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