The Relationship Between Barnacles and Green Sea Turtle Health

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Sea turtles harbor a variety of epibionts, including barnacles. Barnacle colonization may negatively affect the health of sea turtles, particularly by increasing the hydrodynamic drag and body weight of host turtles. Healthy turtles can typically overcome this type of burden, but sick or immunosuppressed turtles often experience a decrease in active behaviors (i.e. swimming and self-grooming), which could promote higher barnacle loads. To investigate the relationship between barnacle load and sea turtle condition, dorsal and ventral photographs and corresponding health information of green sea turtles (*Chelonia mydas*) captured from the St. Lucie Power Plant intake canal in Jensen Beach, Florida, were obtained. An analysis of these images provided the abundance, density, average size and clustering level of barnacles relative to turtle Body Condition Index (BCI). While no statistically significant relationships between the barnacle data and sea turtle body condition were found, the most emaciated turtles commonly hosted slightly smaller barnacles, and turtles in the middle of the BCI range hosted the highest density of barnacles. Barnacle distribution was highly variable between the BCI classes, suggesting that parameters other than those examined (e.g., sea turtle behaviors, migration patterns, and barnacle physiology) drive the observed epibiosis.

INTRODUCTION

Epibiosis

Epibiosis is a form of symbiosis involving two or more organisms whereby one organism (the basibiont or host) is externally colonized by another (typically smaller) species, the epibiont (Wahl, 1989). This type of association most often occurs in aquatic environments, and tens of thousands of marine species are documented to occur as epibionts (Wahl, 1989). Epibiosis allows organisms to colonize and occupy a wider range and diversity of habitats through the movements and migrations of the basibiont. It also provides alternative attachment opportunities for the larvae or spores of sessile animals and plants when substrate availability is limited in the surrounding marine environment (Wahl, 1989). Epibiotic relationships are potentially negative for the basibiont (e.g. increasing weight and decreasing flexibility), but positive attributes (e.g., increasing camouflage and protection/defense) have also been documented from this type of relationship (Wahl & Mark, 1999). While there are a number of well-known basiobionts (e.g., whales and horseshoe crabs), many epibiont studies focus on sea turtles.

A sea turtle's shell, or carapace, offers a motile stronghold for epibionts including amphipods, polychaete worms, barnacles, and a suite of other organisms (Frick et al., 2004). Loggerhead, *Carreta caretta*, and hawksbill, *Eretmochelys imbricata*, sea turtles are documented to host 150 to 200 species of epibionts, the largest number of symbiotic associates reported for any vertebrates examined to date (Frick & Pfaller, 2013). The settlement of epibiotic forms like barnacles depends on habitat characteristics such as surface texture (Wahl, 1999), space availability, chemical cues (Pawlik, 1992) and larval supply (Minchinton & Scheibling, 1991). For instance, barnacles attach to green sea turtles, *Chelonia mydas*, in a clumped distribution (Hayashi et al., 2008) and are more frequently found on the hard carapace surface of host turtles, as opposed to the soft tissue of the flippers or plastron (Gramentz 1988, Fuller et al. 2010, as cited in Frick & Pfaller 2013). Barnacle distribution on turtles is also influenced by host turtle behavior (Frick & McFall, 2007), size (Hayashi et al., 2008) and the intensity of water flow over the turtle's carapace (Pfaller et al., 2006).

Barnacle attachment creates hydrodynamic drag (Logan and Morreale 1994), and barnacle location likely affects the degree to which sea turtles are affected. In extreme cases, epibionts double the mass and volume of juvenile sea turtles (Bolten unpubl. data in Bjorndal 2003). Thus, increased drag could amplify the potentially negative effects of epibiosis on sea turtles in poor condition by further impeding the ability to forage. Interestingly, sea turtles exhibit self-grooming patterns, actively rubbing against sessile structures to remove epibiota (Heithaus et al., 2002; Schofield et al., 2006; Frick & McFall, 2007).

The expenditure of energy on self-grooming behaviors may differ between sick and healthy turtles, resulting in differences in barnacle patterns.

Previous work has shown no significant correlation between sea turtle size and epibiont load (Najera-Hillman, 2012). However, sea turtle epibiosis in the context of health is widely understudied and the vast majority of studies carried out to date examine only the diversity of epibionts (e.g., Frick et al., 2004; Pfaller et al., 2008) or the common locations of epibionts (e.g., Pfaller et al., 2006; Casale et al., 2012). For example, barnacles were most likely to settle on flippers and scutes in a study of loggerhead sea turtles in the Mediterranean (Casale et al., 2012). Although this data provides information on the attachment locations of certain species of barnacles, it does not relate spatial patterns of barnacle colonization to the health of sea turtles. Najera-Hillman (2012) reported the presence of barnacles in relationship to green sea turtle health but lacked data on the spatial characteristics of epibiotic barnacles such as clustering, size, and density.

Objective

This study investigates the relationship between barnacle abundance, size, density, clustering, and green sea turtle (*Chelonia mydas)* body condition. It is hypothesized that turtles with lower condition indices likely host a greater abundance of barnacles, due to changes in behavior (e.g., slower swimming, and decrease in self-grooming). This investigation indicates barnacles as a health factor for green sea turtles.

METHODS

For this study, 40 green sea turtles with varying body conditions were selected to assess barnacle loads. Barnacles visible on dorsal and ventral images from each turtle were noted, providing information on location, as well as the area and perimeter of each barnacle. These data were used to calculate clustering levels and density of barnacles for each turtle.

The St. Lucie Powerplant and Sea Turtle Dataset

The St. Lucie Power Plant is located in Jensen Beach, Florida and draws in water from the Atlantic Ocean at a rate of one million gallons per minute to cool the nuclear reactors that run it. Each year, hundreds of sea turtles are inadvertently drawn through the intake pipes into a secure water entrainment area of the power plant. Inwater Research Group captures, photographs, and collects morphometric data (weight, straight carapace length, plastron carapace length) according to Florida Fish and Wildlife Conservation Commission protocols (Marine Turtle Permit #125). Researchers oversee the safe return of turtles into the wild, or relocate sick or injured sea turtles to rehabilitation centers (e.g., Loggerhead Marinelife Center) until they are healthy enough to be released. As a result of these efforts, the power plant has maintained an extensive database on sea turtles in South Florida since 1976. This data set includes information on roughly 6500 green sea turtles, making it a highly informative and representative collection of green sea turtles in South Florida, the second largest nesting site of greens in the Western Hemisphere.

Photographic Data

Data for the years 2008-2013 was obtained, which included Excel spreadsheets with information on individual turtles' length, weight, condition, and tag ID as well as dorsal and ventral photos. To summarize each turtle's body condition, the formula was used:

$$BCI = \frac{Weight}{SCL^3} * 10^4$$

where BCI= body condition index, SCL= straight carapace length (cm), weight= total weight in kg (Bjorndal et al. 2000). the turtles were categorized as Poor <1.00, Average 1.00-1.10, Good 1.11-1.20, or Very good > 1.20 (Flint et al. 2009). A stratified sampling scheme was used to select individuals across all health classes. From each group, 10 individuals were randomly selected, resulting in a sample size of 40 turtles and 80 images (dorsal and ventral views).

Image Analysis

Photographs were analyzed using ImageJ, a java based analysis software that allowed us to identify the location, area, and perimeter of each barnacle as shown in Figure 1**.** The turtle's dorsal and ventral body surface areas including flippers and head were calculated (see Figure 2), and subsequently each visible barnacle was located (determined by x and y coordinates). The barnacle's area, perimeter, and location was then measured.

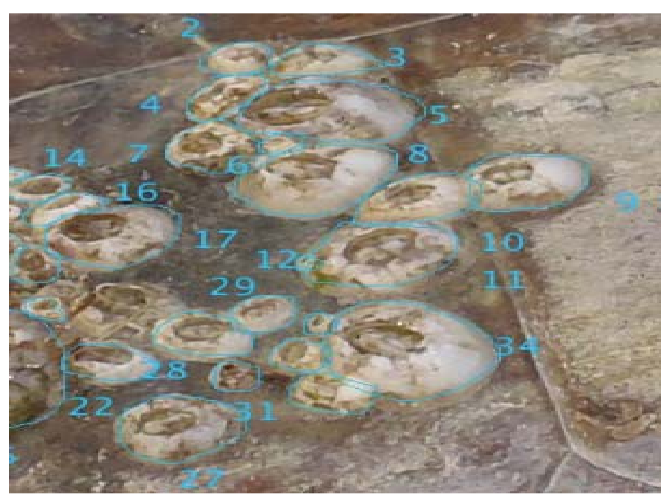

Figure 1. Use of ImageJ software to circle barnacles. Image courtesy of Inwater Research Group.

Figure 2. Ventral view. Image of turtle with BCI 1.08. Image courtesy of Inwater Research Group.

Statistical Analysis

Data points from ImageJ were used to calculate the density (number of individuals divided by the surface area of a given turtle), average area, and clustering levels of barnacles using the Clark Evans Nearest Neighbor method (CENNm). The CENNm is used to determine if a species is distributed in a clumped, uniform, or random manner (Blackith 1958). The following formula was used to calculate the clustering levels of barnacles:

$$R = \frac{mean\ distance}{{}^{1}/_{2}\sqrt{density}}$$

where mean distance is the average distances of an individual barnacle (center) to its nearest neighbor's center, and density is the density of barnacles on the sea turtle's carapace (n/cm^2). When R<1 barnacles are considered clustered, R=1 barnacles are randomly dispersed, and R>1 barnacles are evenly dispersed. Relationships between sea turtle BCI and barnacle clustering, mean barnacle area, density, and abundance were assessed with linear regressions.

RESULTS

Overall, the BCI of the sea turtle had little relationship to the data on corresponding barnacle populations. There was a slight yet insignificant decline in the density of barnacles as sea turtle BCI increased (Figure 3). There was no statistically significant relationship between BCI and CENNm R (clustering levels) (Figure 4). There was no relationship between BCI and total abundance (Figure 5). Lastly, there was a positive, but insignificant relationship between dorsal mean barnacle area and health (Figure 6). Interestingly, there was negative, significant relationship between ventral mean barnacle area and health (Figure 6). However, removal of a suspected outlier in the data (BCI=0.9) yielded a relationship that was not significant.

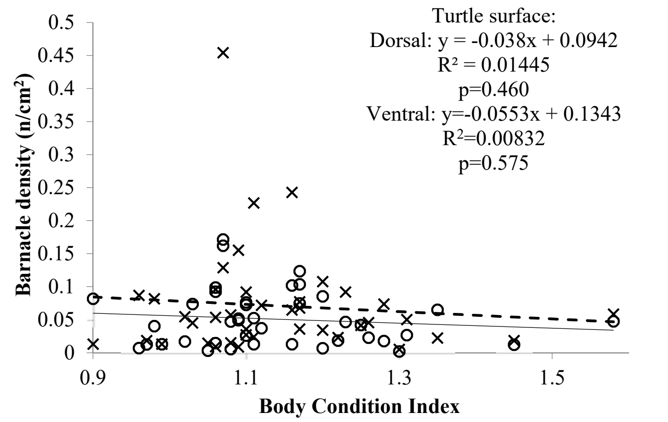

Figure 3. Body Condition Index (BCI) vs. Barnacle Density on the dorsal and ventral surfaces of green sea turtles. Symbols refer to the dorsal (O) and ventral (X) surfaces. Solid line and dashed lines indicate best fit for dorsal and ventral surfaces, respectively.

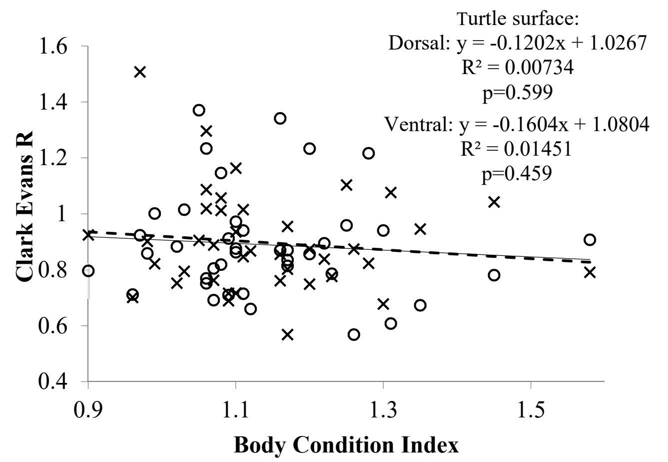

Figure 4. Body Condition Index (BCI) vs. Clustering of barnacles on the dorsal and ventral surfaces of green sea turtles. R<1: clustered; R=1: randomly dispersed; R>1: evenly dispersed. Symbols refer to the dorsal (O) and ventral (X) surfaces. Solid line and dashed lines indicate best fit for dorsal and ventral surfaces, respectively.

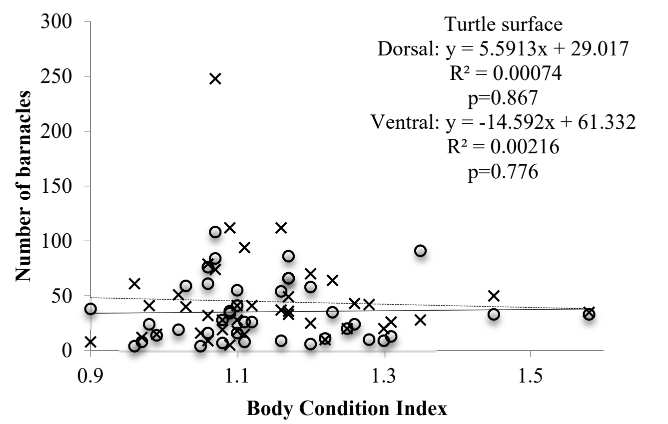

Figure 5. Body Condition Index (BCI) vs Barnacle Abundance on the dorsal and ventral surfaces of green sea turtles. Symbols refer to the dorsal (O) and ventral (X) surfaces. Solid line and dashed lines indicate best fit for dorsal and ventral surfaces, respectively.

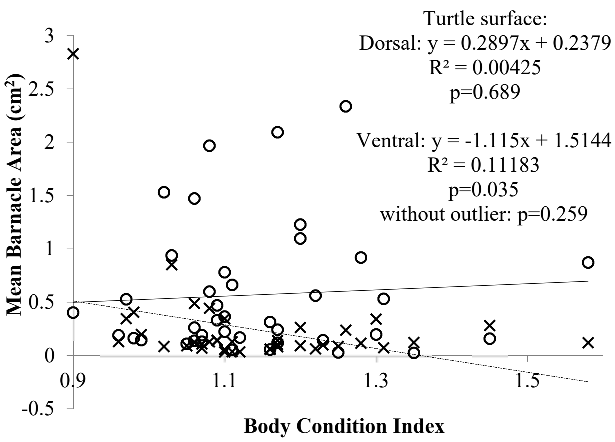

Figure 6. Body Condition Index (BCI) vs. Mean Barnacle Area on the dorsal and ventral surfaces of green sea turtles. Symbols refer to the dorsal (O) and ventral (X) surfaces. Solid line and dashed lines indicate best fit for dorsal and ventral surfaces, respectively.

DISCUSSION

No relationship between barnacle characteristics (density, size, and spatial distribution) and sea turtle condition was found. Many factors (environmental, physiological, and behavioral) contribute to the recruitment and survival of barnacles on sea turtles. While one study on green sea turtles in Mexico (Najera-Hillman, 2012) found a significant negative relationship between sea turtle health (using BCI) and barnacle abundance, this relationship may not be entirely representative of all green sea turtle populations. Understanding the role of these factors is likely key to understanding the relationship between barnacles and sea turtle health.

Many of the factors that affect barnacle settlement are environmental in nature. A major factor that contributes to the variability of barnacle settlement on sea turtles is water flow rate. It has been noted that carapace hydrodynamics create zones of differential drag and flow patterns (Logan & Morreale, 1994). As a result, a higher water flow rate near the anterior region of sea turtles may result in greater posterior settlement of barnacles (Pfaller et al., 2006). The differences in shell and body shape among turtle samples might influence these flow rates and affect barnacle settlement patterns. Movement by sea turtles is also likely to influence the complexion and intensity of epibiont communities as host turtles travel vast distances. For instance, turtles migrate from oceanic to coastal to terrestrial environments (e.g., nesting females) and less tolerant barnacles may die off when exposed to these different areas (Frick & Pfaller, 2013), which could effectively clear the carapace of barnacles.

Physical trauma can also affect barnacle abundance and distribution. Removal may sometimes be intentional (i.e. through self-grooming) (Heithaus et al., 2002; Schofield et al., 2006; Frick & McFall, 2007), but unintentional removal of barnacles could also occur through accidental scraping against hard surfaces or during mating behaviors (Pfaller et al., 2006). This removal would affect the abundance of barnacles on various regions of the body. Another factor that may affect barnacle death rates is sea turtle swimming behaviors. At times, marine turtles float at the surface with parts of their carapace sticking up above the water (Pfaller et al., 2006). Epibionts in these regions could experience desiccation, possibly altering the barnacle load. Barnacle recruitment could also be restricted by the host turtle's limb movements as well as the natural shedding of skin (Frick & Pfaller, 2013).

Finally, interactions among the epibionts themselves could affect the distribution of barnacles. For example, competition among barnacle species has been shown to play a role in their distribution (Najera-Hillman, 2012). At least one study has indicated the presence of niche partitioning between barnacle species for optimal locations on the turtles' body (Casale et al., 2012). This competition could lower epibiont variability among turtles. Additionally, barnacle species on sea turtles would be determined by the supply of larvae within the geographic location of the host turtle (Frick & Pfaller, 2013). The green sea turtles sampled at St. Lucie Power Plant could differ greatly from one another with respect to their realized geographic range. Because of the variety of factors that affect epibiont communities, the use of barnacle epibiota for the assessment of sea turtle health has not been widely explored and remains controversial.

Limitations

One limitation of this study includes the small sample size (N=40). This sample size was limited by the categorization of turtles using BCI (Flint et al., 2009). The "Poor" category (BCI< 1.00) only contained 6 turtles. Further limitations included blurry or unusable photos for analysis. Last, it is possible that the sample of turtles taken from the St. Lucie Power Plant might not be entirely representative of green sea turtles in South Florida because these turtles all had the ability to swim into the intake pipe. More debilitated turtles might not have the strength to do so, which could relate to the small number of turtles in the "poor" category. Also noted was that the Clark Evans Nearest Neighbor test utilizes the center coordinate of the barnacle to test for nearest neighbor. This neglects to take into account the individual barnacle's area when calculating the nearest neighbor distance. As a result, this could artificially inflate random and even distributions.

CONLCUSION

In conclusion, we did not find a relationship between barnacle distribution and size and sea turtle condition. This is likely due to the fact that many factors play a role in epibiont distribution including water flow rate, sea turtle behavior, and recruitment dynamics (Frick & Pfaller, 2013). Epibiont and sea turtle relationships are generally understudied. Of the few studies available, some have concluded that barnacles could be used as a health indicator for sea turtles (Najera-Hillman, 2012), while others have concluded that they cannot (Stamper, 2005). Further studies in this area may help us better understand the complex relationship between sea turtles and epibionts.

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