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Residency and Sociality Analysis of Skin Lesions in Bottlenose Dolphins (Tursiops truncatus) of the St. Johns River, FL

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Residency and Sociality Analysis of Skin Lesions in Bottlenose Dolphins (*Tursiops truncatus*) of the St. Johns River, FL

by

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CERTIFICATE OF APPROVAL

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Abstract

Photo analysis of skin lesions is a non-invasive method to measure the health of cetacean populations. The St. Johns River (SJR) in NE Florida is an estuarine system inhabited by bottlenose dolphins that is characterized by high levels of anthropogenic activity, which can impact dolphin health. Social transmission of disease may influence lesion formation in dolphins; thus this study aimed to determine if skin lesion prevalence and body coverage differed based on 1) the amount of time spent in the SJR annually and 2) sociality measures. The dataset was restricted to three 12-month periods, based on the occurrence of an unusual mortality event (pre, during, and post-UME). High-quality photos were examined for lesions on the dorsal fin and total visible body. Body coverage was categorized as background (<5%), low (5-20%), medium (20-50%), or high (>50%). Lesion types were recorded based on a categorical system that included potentially pathogenic, rake mark associated, orange, hypopigmentation and hyperpigmentation categories. Individuals within the SJR vary in time spent within the river, and were categorized as residents, seasonals, or transients based on the monthly sighting rate in cold and warm seasons. Sociality analyses included resident and seasonal individuals and the mean half-weight index and degree centrality were compared to lesion prevalence and coverage. Residents exhibited the highest prevalence, followed by seasonals and transients. Coverage in lesioned individuals did not significantly differ among residency groups. Lesion prevalence decreased with each period of study. Overall lesion prevalence was higher than nearby sites, and potentially pathogenic lesions were observed at high levels. Half-weight indexes during the UME were correlated with lesion prevalence and coverage. All other associations between sociality and lesion measures were not significant. These results suggest that environmental factors primarily drive lesion occurrence in the SJR, but that sociality influences health during disease outbreaks.

Introduction

1.1 Background

Bottlenose dolphins are long-lived apex predators and may serve as a sentinel species within an ecosystem (Wells et al. 2004). By studying the health status of bottlenose dolphin populations, we can better understand the health of their ecosystems and mitigate harmful human impacts. Historically, health and environmental risk assessments have been conducted on bottlenose dolphin populations of interest (Wells et al. 2004; Bossart et al. 2017; Reif et al. 2017). However, these methods are expensive to conduct and include capture and release of animals involved, which is highly invasive. In addition, samples are taken opportunistically, and therefore provide limited interpretation of health status at the population level. However, alternative methods can be used to estimate overall population health, as skin lesions in cetaceans serve as an indicator of general health status (Wilson et al. 1999; Van Bressem et al. 2009; Hart et al. 2010). Photo analysis of skin lesions provides a non-invasive, relatively inexpensive method of measuring population health, while maximizing the proportion of individuals in a population that are sampled. Thus, photo analysis of skin lesions has been increasingly conducted in cetacean populations in recent years.

Cetacean skin lesions can be caused by different types of microbial infections such as bacterial, viral, and fungal infections (Mouton & Botha 2012), but have also been linked with certain environmental exposures, such as exposure to freshwater and some algal species (Nemoto et al. 1977; Barry et al. 2008; Mullin et al. 2015). Skin lesions from infectious diseases are prevented when healthy cetacean skin acts as an effective barrier against pathogens that may invade. The immune system of the individual also serves as a fail-safe for expelling any pathogens that enter the body to further prevent infection. However, if either the physical or immuno-barrier fails lesions may form (Mouton & Botha 2012). Exacerbation of skin lesions may occur due to advancing disease, secondary infections, or environmental conditions, and the epidermis may progress to an ulcerative state in some extreme cases (Mullin et al. 2015; Holyoake et al. 2009; Deming et al. 2020).

There are a variety of natural and anthropogenic properties of an environment that may make individuals more susceptible to lesion formation. Habitat characteristics such as low salinity and temperature have been linked with an increase in skin lesion prevalence (the proportion of individuals that exhibit lesions) and extent of coverage (the proportion of body area covered by lesions, Wilson et al., 1999; Mullin et al. 2015; Ewing et al. 2017; Fazioli & Mintzer 2020; Deming et al. 2020), as have high levels of human impact from noise pollution, chemical pollution, and habitat alteration (Van Bressem et al. 2007; Van Bressem et al. 2009; Rowe et al. 2010). These factors may act by degrading the quality of the epidermis so that microbes are able to directly cause lesions by penetrating the skin, such as in the case of temperature, salinity, and certain pollutants. In particular, salinity may have a substantial effect on epidermal quality in dolphins. Salinities of 15-35ppt have been identified as bottlenose dolphin habitat, but it has been shown that dolphins may tolerate habitats of >8ppt for short periods without visible signs of skin deterioration (Hornsby et al. 2017). Nevertheless, freshwater exposure events have been linked with an increase in skin lesion prevalence and coverage in dolphin populations (Mullin et al. 2015; Toms 2019; Fazioli & Mintzer 2020). With prolonged freshwater exposures visual cues such as skin pallor, wrinkling, roughening and sloughing of the skin, and coalescing of lesions that are 2-3 cm in diameter have been identified (Mase-Gutherie et al. 2005; Barry et al. 2008; Mullin et al. 2015; Ewing et al. 2017; Deming et al. 2020; McClain et al. 2020), though discriminating “freshwater skin lesions” from those associated with pathogenic disease has proven difficult. It has not been possible to link the etiology of skin lesions directly to freshwater exposure from stranding or biopsy data due to the difficultly of ruling out other causes that may occur as secondary infections (Holyoake et al. 2009; Deming et al. 2020). However, the visual cues mentioned are generally accepted as “freshwater skin lesions”. Additional factors such as anthropogenic noise and ingestion of pollutants may also contribute to a compromised immune system, indirectly leading to the formation of lesions (Mouton & Botha 2012).

Noises produced by human activities are a topic of concern regarding the health of cetacean populations. Chronic sounds in the environment may lead to increased stress in a population (National Research Council 2003). In the case of bottlenose dolphins, sounds caused by ship traffic and dredging

may lead to behavior changes (Pirotta et al. 2013), and acoustic masking can interfere with communication or echolocation sounds (Suedel et al. 2019). Physiological stress may lower the immune system, perhaps causing dolphins in habitats of increased anthropogenic noise to be more susceptible to infection (Wilson et al. 1999). In addition, human activities may interfere with prey availability in certain areas of the habitat (Clark et al. 2009) and may cause dolphins to shift to an unfavorable location with respect to prey abundance and type, salinity, or temperature (Pirotta et al. 2013; Pirotta et al. 2015; Marley et al. 2017), which may lead to further physiological stress. Many of the bacteria found in cetacean lesions generally occur secondarily as a result of a compromised immune system (Mouton & Botha 2012); therefore, recognizing factors that affect immune function are of importance in lesion analyses. Studies have suggested that a synergistic effect of environmental stressors and disease may contribute to degrading the health status of individuals and that dolphins within estuarine environments may be at particular risk for these reasons (Holyoake et al. 2009; Carmichael et al. 2012; Wilson et al. 1999).

The St. Johns River (SJR) in northeast Florida is an approximately 500km long brackish river that empties into the Atlantic Ocean (Demort 1991). The lower basin of the SJR provides important habitat for a community of common bottlenose dolphins (*Tursiops truncatus*) that are part of the Jacksonville Estuarine System Stock (JES, Mazzoil et al. 2020; NOAA Fisheries 2015). The SJR environment is characterized by several potential lesion-causing factors. The lower basin of the SJR is an estuary comprised of brackish water. Salinity in this area highly variable (0-35 ppt) based on the distance from the river mouth, and changes in salinity at a given location may vary considerably in response tidal state, weather, and input from springs and groundwater connections (Pinto et al. 2018). Dolphins in the SJR routinely utilize areas with low salinity (<15ppt, Brown et al. 2018). The SJR is also known to endure significant runoff from industrial and urban sources and has been the site of recent algal blooms (Pinto et al. 2019) which have been linked to dolphin strandings (Brown et al. 2018). The lower basin of the SJR is an area of increased anthropogenic noise due to the presence of multiple shipping ports, military bases, recreational boat traffic and a port expansion project (King 2017). Estuarine dolphins have been shown to

be more at risk to epidemics when compared with coastal dolphins due to anthropogenic altering of habitats (Van Bressem et al. 2009), therefore it is likely that these factors have a cumulative effect on the health of the dolphins that inhabit the SJR.

Recent events suggest that dolphins that utilize the SJR may be vulnerable to declines in abundance. In May of 2010, the JES stock of bottlenose dolphins suffered an unusual mortality event (UME), meaning more deaths than would normally be expected for this population occurred. The cause of the 2010 UME could not be determined. Three years later a large-scale UME impacted dolphins from New York to Southern Florida and resulted in a more than three-fold increase in the number of bottlenose dolphin strandings in Florida from that of 2007-2012 (NOAA Fisheries 2019). NOAA Fisheries identified the cause of this UME, that spanned from July 1st, 2013-March 1st, 2015, as "infectious disease", particularly due to Cetacean Morbillivirus (CeMv). Though it was originally thought that only coastal stocks were affected by the 2013-2105 UME, NOAA identified the JES stock as impacted due to elevated mortalities and identification of CeMv+ individuals that coincided with the UME (NOAA Fisheries 2015) The SJR dolphin community is part of a strategic stock, in which "the level of direct human-caused mortality exceeds the potential biological removal level" (NOAA Fisheries 2015). The SJR community is also behaviorally and genetically distinct from other communities in the JES (Caldwell 2001), and likely incurs more cumulative threats to their health status than others in the JES. Therefore, information on the health of this community is needed, as concerns for the stock remain.

The cumulative effect of recent UMEs and the high levels of anthropogenic impact on the SJR environment emphasize the need to monitor the health of this community, and lesion analysis provides a non-invasive method to do so. In addition, this community serves as a particularly interesting study system because SJR dolphins do not use the habitat equally. Some dolphins exhibit site fidelity year-round, while others enter seasonally or on occasion (Caldwell 2001; Szott 2019). Therefore, the SJR dolphin community provides a natural experiment to study the relative health of individuals as a function of time spent within a specific habitat. Given the characteristics of the SJR environment, this is likely to be a key contributing factor to the health of individuals that use the area. Human activities and natural

environmental factors likely influence the dolphins that use the SJR; however, it is not known if increased time spent in the SJR has a significant decline on the health of individuals. By quantifying skin lesions within this community, we can compare the health of residents of the St. Johns River to non-residents and identify potential stressors. Therefore, the first aim of this study is to compare lesion prevalence and extent of coverage between defined residency groups. It is hypothesized that increased use of the SJR is associated with increased lesion prevalence and coverage.

While the amount of time spent in a compromised environment is likely to affect the health of individuals, other factors may also contribute to lesion formation in a population. Even in favorable conditions infectious diseases have the potential to spread rapidly through bottlenose dolphin populations due to behavioral characteristics of the species. Bottlenose dolphins live in societies with high fission-fusion dynamics (Connor et al. 2000) where individuals join and leave groups frequently. These fluid association patterns may allow for the rapid exchange of microbial organisms throughout the social network. Dolphins within a group often surface at the same time to breathe, allowing for the exchange of respiratory viruses and bacteria. They may also make tactile contact when socializing which can allow microbes to be spread directly to the skin. Due to these opportunities for transmission dolphin lesions can be linked to social network properties (Powell et al. 2020); however, very few studies have examined this connection in cetaceans. A recent study on *Tursiops aduncus* in Shark Bay, Australia found that one type of lesion presenting disease, tattoo skin disease (TSD), spread predictably through the population, with individuals contracting TSD that had been associated with other individuals with tattoo lesions (Powell et al. 2020). While lesion prevalence was not higher among more connected individuals, it was noted that the prevalence of TSD was relatively low, and that populations with a higher prevalence of disease may experience different results.

The SJR dolphin community differs in several ways from that of the Shark Bay, Australia population. Notable differences between these populations that are relevant to disease transmission include population density and levels of environmental degradation. The population density of both Shark Bay and SJR dolphins is thought to be relatively high compared to the average across bottlenose dolphin

populations (1.32 dolphins/km^2, Ermak et al. 2017). Shark Bay density estimates range from 0.92 dolphins/km^2 (Nicholson et al 2012) to 2.40 dolphins/km^2 (Watson-Capps 2005), with the SJR density significantly higher at 6.76 dolphins/km^2 (Ermak et al. 2017). A high population density may allow for more interactions between individuals, and therefore a faster transmission rate of diseases. These two habitats also differ in that Shark Bay is relatively pristine and free of human impact (Heithaus et al. 2007), especially when compared to the SJR. These differences would likely lead to different patterns of disease transmission. Poor environmental conditions may lead to immunosuppression, allowing for the spread of disease that would not be possible otherwise. Investigating lesion occurrence within the SJR social network may provide a better understanding of the cumulative effects of environment and sociality on the transmission of disease.

The multiple UMEs that SJR dolphins have faced emphasize the importance of understanding the spread of disease in this community. It has been shown that overlapping core areas and social mixing may provide a pathway for disease transmission between residency categories in this community (Szott 2019), but it is not known if the degree of social connectedness of an individual may play a role in the contraction of disease and expression of symptoms (lesions). With this information predicting the spread of disease in future outbreaks and identifying at-risk individuals in this population may be possible. Therefore, the second aim of this study was to determine if individuals with a higher degree centrality or mean half weight index (HWI) had an increased prevalence and coverage of lesions compared to individuals with lower measures. Mean half-weight index and degree centrality are both measures representing the connectedness of an individual in a social network. Degree centrality allows for a comparison of the number of contacts between individuals whereas HWI may represent the relative strength of an individual's social bonds. Both social parameters may provide explanations for increased transmission when elevated values are seen in lesioned individuals.

The social connectedness of an individual provides an alternative hypothesis of lesion occurrence in addition to that of environmental effects. Therefore, if sociality measures suggest that social factors are driving the occurrence of lesions in the population, then symptoms may not be attributed solely to the

amount of time spent in the SJR environment. However, these hypotheses are not mutually exclusive; both sociality and residency patterns may have a cumulative effect on the risk of an individual developing lesions.

Methods

2.1 Survey Methods

Ongoing weekly photo-identification surveys have been conducted in the St. Johns River since March of 2011. Surveys consist of traveling along a 40-km survey route from downtown Jacksonville (N30.1479, W-81.62987) to the river mouth (Mayport Inlet; N30.39904, W-81.39396), with the direction of travel alternated each week. The survey vessel travels at a speed of 10-12 km/h until dolphins are sighted. In addition to photo data collected using a professional grade digital camera, behavioral and environmental data are also collected. These include activity state, group size and composition, movement with respect to tidal state, surfacing patterns, water depth, water temperature, salinity and GPS coordinates of the sighting location. Group size and composition are based on a 10-m chain rule (Smolker et al. 1992). Once all dolphins in a group are photographed the sighting is ended and the vessel continues along the survey route.

2.2 Study Periods and Data Set

Photo data from three 12-month periods ranging from 2012-2016 were evaluated. Each period was chosen from a 20-month range before (Nov. 2011-June 2013) during (July 2013-Feb. 2015) or after (Mar. 2015-Oct. 2016) the 2013-2015 UME, so that results could be interpreted while taking this health event into account. Periods were defined as pre-UME (May 2012-Apr. 2013), during-UME (Nov. 2013-Oct.2014), and post-UME (May 2015-Apr. 2016). The months for each of the periods were selected by defining the seasonal classification for each month based on the average temperature in the SJR (warm ≥22.55°C, cold <22.5°C, Karczmarki et al. 2000). Periods comprised of one complete warm season (>1 month directly before and after the season = cold) and one complete cold season (>1 month directly before and after the season = warm) and included 12 consecutive months within the specified range. Therefore, gaps between periods are consistent with overlapping seasons between pre-UME, during-

UME, and post-UME periods. Individual dolphins not sighted in a particular period and calves that were not yet weaned were excluded from that period of analysis. Calf identification was based on body length (75% of cow's length) and observations of infant position (Mann 1999; Mazzoil et al. 2020).

2.3 Photo Data Set and Lesion Scoring

Photo data from surveys were initially processed following standard dolphin photo-identification protocols; the best photo of each individual from each sighting was compared to a catalog to confirm the presence and identity of individuals in each sighting, based on unique dorsal fin qualities including shape, scars, and nicks (Würsig & Würsig 1977; Würsig and Jefferson 1990).

Unedited photos of identified non-calf individuals were rated for quality and ability to detect lesion presence/absence using the the Fazioli and Mintzer (2020) rating system. Qualifying photos included those with good to excellent focus, and that were properly lit so that skin details could be observed (Fazioli & Mintzer 2020; Urian and Wells 1996). Best photos for each individual were selected by month, and multiple photos from the same sighting of an individual were used in some cases to provide greater confidence in lesion presence if qualifications were met in each photo (Toms et al 2020; Fazioli and Mintzer 2020), especially if photos of both left and right sides of the body were available. Additional photos assisted with discerning between watermarks and/or glare and lesions.

Dorsal fin only (D) and total visible body (D+B) data sets were created based on guidelines in Toms et al. (2020). Photos for the D analysis exhibited, at minimum, the entire dorsal fin. Only photos exhibiting >10% of the body (including the dorsal fin) were included in the D+B data set. Visual guides from Toms (2019) were used in selection of photos for the D+B data set. Visual guides provided multiple examples of photos in which the entire body of an animal was isolated and 10% of pixels were counted, to quantitatively illustrate 10% body portions surrounding the dorsal fin. Examples of photos with qualifying body portions for each analysis are provided in Fig. 2. Photos used for the D+B data set were eligible for use in the D data set but in some cases the best photo for D analysis and D+B analysis differed.

Dorsal fin only analysis offers a standardized measure for lesion analysis, as the dorsal fin is the standard target of photographs in cetacean surveys and provides a distinct boundary for the detection of lesions where the dorsal fin meets the body. However, defining this boundary as the shortest distance between the anterior and posterior fin meeting point or the natural curvature of the fin has been identified as an issue (Toms et al. 2020). Furthermore, Toms et al. (2020) quantified differences in estimating lesion presence and coverage for D vs. D+B measures and suggested that that D analysis underestimates prevalence and coverage of lesions compared to D+B. However D+B is less standardized, as boundaries on the total visible body area are not defined above the 10% of visible body estimate. While D+B estimates are less standardized they may be more accurate, as lesions may concentrate in areas of the body other than the dorsal fin (Bearzi et al. 2009). Toms et al. (2020) noted that when the two measures agree, estimates can be interpreted with greater confidence. Hence, D and D+B measures are used in this study, providing an enhanced proxy for lesion prevalence and coverage in SJR dolphins that increases accuracy while remaining comparable to previous studies. In either case, the dorsal surface is used as a proxy to the total body, therefore results of this study should be considered minimum estimates of the true prevalence and coverage, as false negatives may occur in the detection of lesions.

For the D+B data set lesions that appeared on the body surface above the water were considered. If lesions were detected in any photos on the visible body portion the photo was scored as “present” for D+B. If lesions were detected above the natural meeting point of the dorsal fin and the body for any of the photos the photo was scored as “present” for D.

To analyze the severity of lesions in each photograph an extent of coverage analysis was performed using methods modified from Fazioli and Mintzer (2020) and Toms et al. (2020). Photos that met the lesion presence criteria were categorized by two raters based on extent of body coverage using visual guides provided from Fazioli and Mintzer (2020). Guide photos included raw photos that qualified for D+B analysis that were duplicated, with isolated body portions exhibiting lesions that were traced and outlined. Exact calculations of lesion coverage were included for each guide photo. Visual guides used to estimate coverage included photos with 6%, 8%, 27%, 41%, 51%, and 93% coverage. Categories of

lesion coverage included background levels (<5% coverage of visible epidermis), low (5-20% coverage), medium (20-50% coverage), or high (>50% coverage, Fig. 3). If high quality photos of both sides of the individual were available for a sighting, the extent of coverage was averaged between the two photographs. If a discrepancy occurred between the two raters, a third rater served as a tie breaker. First and third raters were experienced in lesion scoring (completed >12 months of data as a second rater or had significant background knowledge of cetacean lesions), while second raters began as inexperienced in lesion detection (outside of initial training).

Lesions were categorized by type using a modified condensed category system suggested in Toms et al. (2020). Previously used categorical systems have low inter-rater reliability and include distinctions that may actually be representative of stages of disease (Geraci et al. 1979; Wilson et al. 1999; Wilson et al. 1990; Toms et al. 2020), and the objectives of this study are not dependent on such specified data. Examples of photos for each lesion type in this study are presented in Fig. 4. The potentially pathogenic (PP) category includes defined lesion types from several studies (Hart et al. 2012; Geraci et al. 1979; Van Bressem and Van Waerebeek 1996; Wilson et al. 1997) that have been associated with pathogenic microbes. These include previously described tattoo, lunar, dark fringe, white fringe, cloudy white spots, spotted, vesicular, and dark spots categories. Rake mark associated (RMA) lesions occur only on or directly adjacent to rake marks. Inter-rater reliability for scoring RMA-PP and RMA-other has shown to be poor (Toms et al. 2020), therefore these categories have been condensed into RMA for this study. PP lesions were recorded only if at least one lesion of this type did not occur on a rake mark. The orange (O) category combined previously described orange hue and orange patches categories from Wilson et al. (1997). Hypopigmentation includes any light, non-potentially pathogenic patches including previously defined white freckles, white amorphous, and mottled (if primarily light in pigmentation). Hyperpigmentation includes, dark non-potentially pathogenic patches and mottled (if primarily dark in pigmentation). Operational definitions and photos from Toms et al. (2020) for each of the previously defined categories were provided to raters for lesion type analysis and raters categorized

lesions into the condensed category system based on the organization of the previously defined categories in the new system.

The proportion of individuals in each presence and extent of coverage category was calculated for each study period and averaged across all periods. Individuals were coded as "lesions present" in a period if any photos of the individual displayed lesions in the period. Only individuals coded as "lesions present" were scored for lesion coverage. Individuals were coded for extent of coverage in a period based on the maximum extent of coverage score displayed in any photo of the individual for the period. Therefore, estimates of prevalence and coverage reflect the maximum display of lesions from the data in each time period. The prevalence of each lesion type was calculated for each period, and results were averaged between periods. Prevalence for each lesion type was defined as the proportion of lesioned individuals in the data set that displayed the lesion type within a period.

2.4 Residency Status Analysis

The sighting history of individuals was used to determine residency status for each year of study using methods from Szott (2019). Seasons were defined as Pre-UME warm (6 mo., May-Oct.), and cold (6 mo., Nov.-Apr.), During-UME cold (6 mo., Nov.-Apr.), and warm (6 mo., May-Oct.), and Post-UME warm (7 mo., May-Nov.) and cold (5 mo., Dec.-Apr.). A monthly sighting rate (MSR) for each individual was calculated by dividing the number of months an individual was sighted in a season by the total number of months in the season. Individuals were divided into residency categories based on the MSR value. To decrease bias, the MSR category thresholds were relative values specific to each season in the study, with high MSRs in the 3rd tertile (top 33.33%), and medium and low MSRs in the 2nd and 1st tertiles, respectively. Residents (R) were defined as those with high or medium MSRs in both warm and cold seasons, seasonal residents (S) were individuals with a high or medium MSR in one season and a low MSR in the other, and transients (T) were defined as those with a low MSR in both seasons (Szott 2019).

For both the D and D+B data sets, the proportion of lesioned individuals in each presence and extent of coverage category was averaged across periods. Lesion prevalence and extent of coverage

measures were compared between residency groups using SPSS (V24). A Fisher's exact test was used to determine if the proportion of individuals with lesions differed significantly between residency categories for each time period and measure (D vs. D+B). Variances between residency groups were not homogeneous, therefore a Kruskal-Wallis tests were performed for each period and measure (D vs. D+B) to test for significant differences in the extent of coverage categories between residency groups.

2.5 Sociality Analysis

The different sighting patterns among residency groups may cause bias when estimating sociality. Resident individuals by definition are sighted more frequently than seasonals and transients. For this reason, transient individuals were excluded from the sociality analyses, and the analyses were restricted to warm season months, when seasonal and resident individuals are sighted at a similar rate.

Sighting histories were used to calculate the mean half weight index (HWI) for each resident and seasonal individual sighted ≥10 times in the period using SOCPROG (Version 2.9, Whitehead 2009). The half weight index accounts for possible missed observations of individuals, therefore attempting to minimize bias in the estimate of association of individuals (Cairns and Schwager 1987). The Mean HWI is defined as the average of all HWI's for an individual, therefore providing a proxy to general sociability based on the strength of an individual's social bonds (Whitehead 2009). UCINET 6 software (Borgatti et al. 2002) was used to calculate degree centrality (DC), or the number of unique associates.

The mean degree centrality and HWI was calculated for each period of study. Tests of linearity for lesion prevalence and extent of coverage data with respect to HWI and DC failed. Therefore, binary logistic regressions were completed for each sociality measure (HWI and DC) to determine if there was a significant association with lesion presence in each period; each measure of lesion presence (D and D+B) was analyzed separately. Spearman's rank tests were used to determine if there was a significant association between lesion coverage and each sociality measure for each period and each measure of coverage (D and D+B).

Results

3.1 Sample Size and Lesion Prevalence between Residency Groups

The D data set consisted of 1528 high-quality photos that were used for lesion scoring. The D+B data set included 1378 high-quality photos. Photos were selected from 63 survey days in warm season months (D: 1118 photos, D+B: 1032 photos) and 54 survey days in cold season months (D: 410 photos, D+B: 346 photos). The high-quality photo sample contained a total of 405 individuals (D and D+B) and included more residents (range: 104-133individuals/year) than seasonals (94-106 individuals/year) and transients (58-66 individuals/year). The Pre-UME period had the greatest sample size (305 individuals) followed by the During-UME period (289 individuals) and the Post-UME period (256 individuals). Inter-rater reliability for all measures of prevalence, extent, and type was good. First and second rater disagreed in 3.66% of cases for D prevalence, 0.93% of cases for D+B prevalence, 4.67% of cases for D extent of coverage, 2.92% of cases for D+B extent of coverage, and 3.95% of cases for lesion type (D+B).

The mean prevalence of lesions in the SJR community across the three years of study was 0.64±0.11 (mean±SE) for D and 0.64±0.10 for D+B. Prevalence was highest in the year preceding the UME and declined in each subsequent study period (Table 1). When analyzing residency categories separately, prevalence was consistently highest in the Pre-UME period, followed by the During UME and Post-UME periods for all residency groups (Fig. 5). A significant association was found between lesion prevalence and residency status for all periods of study, for both D and D+B data sets ($p<.001$, Fisher's exact test, Table A1). For each year, the proportion of lesioned individuals was highest in residents (D: mean ± SE=0.83± .08, D+B: 0.82± 0.09), followed by seasonals (D: 0.58±0.10 and D+B: 0.60± 0.11), and transients (0.34± 0.12 for D, 0.37± 0.13 for D+B, Fig.5). The D and D+B data sets produced similar results without an obvious trend of underestimation or overestimation of lesion prevalence by either method.

3.2 Lesion Coverage among Residency Groups

There was not a significant difference in lesion coverage between residency groups for any period of study in either D or D+B data sets (Table 2). For each residency group the majority of lesioned individuals in each year exhibited background level coverage (Fig. 6). Mean results for the three periods combined revealed low coverage as the second most common level of coverage among all residency

groups (Fig. 6d). No consistent pattern of coverage proportion between residency groups across time periods was observed for any level of coverage (Fig. 6). Mean results for the three periods combined also revealed no significant difference in any level of coverage between residency groups (Fig. 6d).

Despite the high prevalence of lesions in SJR dolphins, high and medium coverage cases were rare in all residency groups (Fig. 6). For all individuals sampled the proportion with background (mean±SE; D: 0.53±0.05, D+B: 0.47±0.05) and low coverage (D: 0.08±0.04, D+B: 0.11±0.05) were much higher than those with medium (D: 0.02±0.01, D+B: 0.04±0.01) and high coverage (D: 0.01±0.00, D+B: 0.02±0.01, Fig. 7).

3.3 Lesion Prevalence and Coverage Compared with Sociality

Sample sizes for the sociality analyses were highest in the Pre-UME period (D: 206, D+B:207) followed by During-UME (D: 141, D+B: 144) and Post-UME (D: 60 D+B: 52, Table 4).

Mean HWI was highest Post-UME (mean±SE; 0.05±0.00) followed by Pre-UME (0.04±0.00) and During UME (0.03±0.00). Mean degree centrality was highest Post-UME (29.27±1.30), followed by Pre-UME (28.62±1.23) and During UME (20.01±0.95).

The logistic regression models predicting lesion prevalence based on mean HWI was statistically significant ($p<0.05$) for the During-UME (D and D+B) period (Table 3). However, Nagelkerke's R^2 value was low (D and D+B: $R^2=0.01$), so the relationship between variables in the model may not be interpreted with great confidence. Models for all other time periods and measures were not significant. None of the logistic regression models predicting lesion prevalence based on degree centrality were significant for any period or measure (Table 3). Spearman's rank tests revealed a significant negative monotonic association between mean HWI and lesion coverage in the During-UME (D and D+B), but not in any other periods (Table 4). No significant association between degree centrality and lesion coverage was observed for any time period or measure.

3.4 Lesion Types

The most prominent lesion type in all periods of study was potentially pathogenic (PP), followed by rake mark associated (RMA, Fig. 8). There was a consistent pattern across all years of study in which

the orange lesion type (O) was the next most common, followed by hypopigmentation (HYPO) and hyperpigmentation (HYPER), though O and HYPO were at equal proportions in the Post-UME period. The proportion of lesioned individuals with PP and RMA types was highest Post-UME. Proportions of HYPO, HYPER, and O were slightly higher Pre-UME compared to other periods.

Discussion

4.1 Residency and Lesion Occurrence

To our knowledge, this study was the first to compare cetacean skin lesion prevalence and extent of coverage with residency status. The significant association between residency status and lesion prevalence (both D and D+B data sets) in all time periods suggests that dolphin health is impacted by the amount of time spent in the SJR annually. Prevalence results indicate that residents of the SJR are in relatively poor health compared to seasonal and transient dolphins, though coverage results suggest that severity in lesioned individuals between residency groups does not differ. These results suggest that the SJR environment acts to promote the initial occurrence of lesions, most likely due to the combination of several environmental characteristics.

Differences in lesion prevalence among residency groups may be due in part to the portion of the SJR used by each group. Within the SJR study area, salinity continuously decreases with distance from the mouth of the river and reaches levels markedly lower than that of the adjacent Atlantic Ocean where transients likely reside when not in the SJR (Mazzoil et al. 2020). Previous spatial analyses of the SJR community have found that the home ranges of resident and seasonal dolphins overlap, but residents use the upriver portion more often than seasonals and transients do. Additionally, transient core areas are located closer to the mouth of the river than resident and seasonal core areas (Szott 2019). These habitat use differences, especially increased use of lower salinity areas, may contribute to greater lesion formation among residents.

While this study did not aim to identify the individual habitat factors that contribute to lesion occurrence, our prevalence results follow expected patterns based on what is currently known about the natural and anthropogenic environmental factors that promote skin lesions in wild dolphin populations.

Resident SJR dolphins, who spend more time in low salinity areas than seasonals or transients, had the highest lesion prevalence; this finding is consistent with previous studies on the effect of salinity on lesion formation (Hart et al. 2012; Rowe et al. 2010; Taylor et al. 2020; Wilson et al. 1999; Fazioli & Mintzer 2020; Fury et al. 2012; Mullin et al. 2015; Toms 2019). In a study of bottlenose dolphins in the Southern US, Ewing et al. (2017) found that pathophysiological effects from abrupt freshwater exposure were more related to the degree of salinity as opposed to the amount of time spent in the low salinity area. However, their results were based on short-term freshwater exposure as opposed to continuous exposure over several years, as in the SJR, and did not account for lesion prevalence. Another study on dolphins exposed to salinities ranging from 0-30 ppt identified both the salinity level and the amount of time in low salinity environments as significant factors in the prevalence and severity of skin lesions (McClain et al. 2020). Therefore, it is possible that prolonged exposure to slightly lower salinity areas may increase lesion presence based on the duration of exposure, but other environmental factors may also help explain the differences lesion presence among residency groups.

It has been hypothesized that dolphins in estuarine habitats may have adapted to fluctuations in salinity, which could prevent them from exhibiting freshwater induced lesions (Hornsby et al. 2017; Toms 2019; McClain et al. 2020). Therefore, other factors may be responsible for the higher levels of prevalence and extent of coverage in residents of the SJR. In addition to salinity, temperature, pollutants, and other anthropogenic influences have been associated with lesion presence (Geraci et al. 1979; Hart 2011; Hart et al. 2012; Kiszka et al. 2009; Murdoch et al. 2010; Van Bressem et al. 2009a; Van Bressem et al. 2009b; Wilson et al. 1999). While temperature is relatively uniform between the SJR and the nearby Atlantic, and both areas experience harmful algal blooms (Brown et al. 2018), the SJR has more condensed habitat disturbance by dredging, commercial shipping, military operations, and recreational boats (King 2017). These anthropogenic disturbances may contribute to the difference in lesion presence among residency groups, as residents would likely be exposed to a greater combination of lesion promoting factors for a longer period than seasonal and transient dolphins.

Comparing prevalence among other estuarine populations may aid in interpreting specific factors that lead to elevated lesion prevalence in a population. Mean prevalence results for SJR dolphins suggest an elevated proportion of individuals with lesions (D: 64%) compared to other nearby estuarine sites (Hart et al. 2012; Taylor et al. 2020). Two prior studies (Hart et al. 2012; Taylor et al. 2020) used dorsal fin only data to compare sites on the Southern Atlantic coast of the US. Lesion prevalence ranged from 53% in Brunswick, GA to 32% in Sarasota Bay, FL, with Charleston, SC and Roanoke Sound, NC falling in between. The Brunswick, GA site, located approximately 100 km NNE of the SJR, is characterized by freshwater inflow from the Altamaha River with salinity ranging from 0.1-33.0 ppt (avg=25.1ppt, Hart et al. 2012). Hart et al. (2012) attributed the increased prevalence of lesions in this population to colder temperatures, lower average salinity with greater fluctuations, and increased exposure to PCB's when compared to other sites. The Brunswick site is comparable to the area of the SJR routinely used by dolphins in temperature and salinity (0-35.0ppt). However, these two sites likely differ with respect to pollutants; Brunswick is on the Environmental Protection Agency's National Priorities List due to contamination of groundwater, soil, and sediment (USEPA 2020). While contaminants such as PCBs, organochlorine pesticides, and mercury are at unsatisfactory levels in the lower SJR basin (Pinto et al. 2018), the EPA designation is not shared by both sites. Therefore, the synergistic effects of multiple stressors may have caused the prevalence of lesions in the SJR to exceed that of Brunswick.

Disease outbreaks represent one circumstance that may lead to an increase in lesion prevalence. The selected periods for this study are based around an unusual mortality event (2013-2015) caused by an infectious disease (Cetacean morbillivirus), allowing for the interpretation of lesion presence with respect to the event. Taylor et al. (2020) conducted lesion analysis on estuarine bottlenose dolphins from 2012-2014 in the Roanoke Sound, which was likely also affected by the 2013-2015 Cetacean Morbillivirus UME. Unlike the results reported in Taylor et al. (2020) the SJR dolphin community exhibited a trend of decreasing lesion prevalence with each subsequent time period. The highest prevalence of lesions in the SJR occurred in the year leading up to the UME, suggesting that the SJR community may have been immunocompromised when the UME initiated.

Lesion prevalence provides a measure of the proportion of the population experiencing health effects, while extent of coverage can be used to assess the severity of epidemiological symptoms in lesioned individuals. This study was also the first to assess lesion coverage with respect to residency status, but the results indicate that the duration of exposure to multiple lesion causing factors does not affect the severity of lesions in individuals. This may be attributed to individuals acclimating to factors in their environment, thereby preventing the exacerbation of lesions. Transients had similar levels of coverage to residents and seasonals, but transients are not found in areas of low salinity, therefore it is unlikely that freshwater exposure is not driving lesion coverage. Significant differences in prevalence, but not coverage between residency groups demonstrate a pattern that is likely to occur in other estuarine populations, though this pattern has not been assessed outside of the SJR.

Previous studies have assessed extent of lesion coverage by specific lesion types (Bearzi et al. 2009) and interpreted findings in the context of freshwater exposure events (Fazioli and Mintzer 2020; Rowe et al. 2010; Toms 2019). The methodology used to assess extent of lesion coverage in this study was similar to that of recent studies in estuarine environments (Fazioli and Mintzer 2020; Toms 2019), thereby enabling comparison of results across studies and populations. Mean lesion coverage across the SJR community was similar to that of Galveston Bay, TX (Fazioli & Mintzer 2020) and Pensacola Bay, FL (Toms 2019) in the baseline time periods before low salinity exposure. In all populations that have been studied, the majority of lesioned individuals exhibit low/background level coverage under normal conditions (Fazioli & Mintzer 2020; Toms 2019; Bearzi et al. 2009). It has been suggested that a low level of coverage may be standard to estuarine populations, as these habitats likely contain features that influence epidermal integrity (Toms 2019). A low proportion of individuals with medium and high levels of coverage is also shared among the SJR, Galveston Bay, and Pensacola Bay sites (during periods without flooding disturbance), suggesting that coverage of medium and high levels occurs in estuarine populations, but is rare without significant drops in salinity. These comparisons suggest that lesion coverage in the SJR is within a normal range for estuarine populations when not experiencing a flooding event. However, Fazioli and Mintzer (2020) and Toms (2019) demonstrated that flooding events

significantly increase lesion coverage in a population. Therefore large scale environmental disturbances such as floods would likely have differential effects on lesion coverage between residency groups in the SJR; residency groups may experience differing levels of disturbance and may have acclimated physiologically or behaviorally to low salinity environments in ways that allow them to mitigate coverage. For instance, residents may be more acclimated to changes in salinity due to past exposure to low salinity areas, or past experiences with low salinity may have behavioral influences on residents, making them more likely to change their spatial use based on the salinity level in the environment.

In addition to studying lesion prevalence and extent of coverage, the analysis of lesion types may provide a better understanding of lesion drivers. The proportion of PP and RMA lesions was highest Post-UME. This suggests that when lesion occurrence is at low levels, as in the Post-UME period, lesioned individuals are more likely to have lesions caused by pathogens. However when lesion prevalence is higher, such as in Pre-UME and During-UME periods, HYPO, HYPER, and O make up a greater proportion of lesions seen on individuals. This suggests that lesion prevalence levels in the SJR are generally associated with environmental variables, as the lesion types that are not associated with pathogenic disease follow proportional trends of overall lesion occurrence.

Potentially pathogenic lesions were observed at a higher level than any other lesion type in SJR dolphins, suggesting that pathogenic disease prevalence is high in the SJR community. Categorizing lesions into broader categories allowed for a comparison of pathogenic disease between the SJR and Pensacola Bay, FL. The elevated proportion of PP lesions compared to other lesion types in the SJR community is consistent with the Pensacola Bay population (Toms 2019). In considering disease prevalence estimates for this community it is important to note that the proportion of PP in the population is likely higher than estimated, as PP is defined by pathogenic etiology that does not occur on a rake mark, and RMA encompasses both pathogenic and non-pathogenic etiology on rake marks. However, Toms et al. (2020) suggested that the PP category may encompass some lesions with causes other than pathogens (i.e. freshwater exposure), since distinctions between the appearances of PP lesions and freshwater lesions have not been recorded. It is also important to note that some lesion types may resolve

more quickly than others (Gonzalvo et al. 2015). Therefore, lesions that have not resolved between periods of study may be counted more than once, inflating prevalence and coverage results in later periods. However, the decreasing proportion of lesion prevalence with each time period in this study suggests that gaps between study periods may allow sufficient time for most lesions to resolve.

The goal of this study was to evaluate skin lesion prevalence and extent of coverage in an environment containing several factors that may contribute to lesion formation. Other studies have shown that natural and anthropogenic factors may contribute to an increased prevalence and/or severity of lesions. To our knowledge, this is the first study to demonstrate that the amount of time spent in an area with multiple potential lesion-causing factors is associated with symptomatic presentation of lesions. The results of this study contribute to understanding how several environmental factors may combine to affect skin diseases, and how lesion presentation may differ within a population. The identification of this site as a lesion promoting environment may impact the management of the SJR, as the health of the resident dolphin population is clearly compromised due to environmental factors. Lesions are widely used to estimate the health of cetacean populations, and trends across populations of this sentinel species reinforce the importance of habitat management in estuarine systems in general.

4.2 Sociality and Lesion Occurrence

In addition to determining if the SJR is a lesion promoting environment, another goal of this study was to identify if social differences among individuals influence lesion occurrence. It was predicted that a positive correlation may exist between HWI or degree centrality and lesion occurrence; however, the During-UME period produced significant negative correlations between the mean HWI and both prevalence and coverage levels (though logistic regression models did not explain much of the variance between HWI and lesion presence). Neither social measure (mean half-weight index and degree centrality) was consistently associated with lesion prevalence or differences in extent of coverage across all periods. These results suggest that during infectious disease events an individual's mean bond strength, but not the number of associates, significantly contributes to the severity of lesions. This is likely because individuals with weaker bonds may spend less time with associates and therefore do not

have as much opportunity to acquire immunity to infection as those with stronger bonds. When infection does occur in individuals with low HWI's via a highly contagious disease (such as morbillivirus), the immune system may be less equipped to fight pathogens due to a low exposure to past infections. This could lead to the exacerbation of disease symptoms, resulting in a higher coverage of lesions.

A variety of measures can be used to assess the sociality of individuals, with each representing slightly different social phenomena. Depending on the measure used, results in disease studies can differ, and may provide different information about how disease is spread. Powell et al. (2020) found that the proportion of symptomatic contacts an individual had was significantly associated with the occurrence of tattoo skin disease (TSD) in Shark Bay, Australia, but that degree centrality was not. Powell et al. (2020) also used focal follow data to compare the proportion of time spent socializing and time spent in groups between TSD lesioned and non-TSD individuals (*Tursiops aduncus*), and found that TSD was not associated with time spent socializing or time spent in groups. Focal follows are difficult to complete in the SJR due to the turbidity of the water and high levels of vessel traffic, and the focus of this study was not the presentation of a particular disease. In addition, the level of TSD in Shark Bay is low, so the results of Powell et al. (2020) do not provide a direct comparison to this study. However, the results of Powell et al. (2020) in conjunction with this study suggest that the number of associates an individual has does not influence lesion occurrence. Furthermore, the results of our study provide stronger evidence that increased sociality in general does not lead to an increase in overall lesion occurrence; but the disease status of associates does matter.

Disease transmission is influenced by sociality in populations, thus, lesion studies that take sociality into account contribute to a more complete understanding of lesion occurrence. Differences in sociality among periods may explain patterns in lesion occurrence. In this study, individuals had fewer associates and bonds between associates were weaker During the UME than before and after the UME, based on degree centrality and HWI results. Although the size and composition of the social network differed among time periods and direct comparisons are difficult, sociality in the SJR likely decreased during the UME. This reduction in social measures during the UME may explain the decrease in lesion

prevalence from Pre-UME to During-UME periods, as a network that is less social and less connected is less likely to spread disease in general. However, Post-UME lesion prevalence was lower than During-UME prevalence, even though sociality measures were highest Post-UME of any time period. This may be due to an increase in acquired immunity to morbillivirus in the population Post-UME. Therefore, when immunity to infectious disease is low in the population sociality may have a greater influence on lesion presence than when immunity is high.

Skin lesions are not specific to Cetacean Morbillivirus, so the transmission pattern of the virus through live animals cannot be concluded based on the results of this study. However, our results show that decreased sociality in the SJR was associated with decreased lesion prevalence During-UME compared to Pre-UME, which may have occurred due to lower levels of transmission during the UME. During the UME HWI was associated with lesion occurrence, but this did not coincide with an increased prevalence of lesions in the population, and the relationship between HWI and lesion occurrence was negative, providing further indication that transmission of disease was not the primary driver of lesion development during the study periods.

This study was the first to describe the occurrence of dolphin skin lesions surrounding a UME using sociality measures. This comparison of lesion prevalence to sociality patterns provides a better understanding of how sociality may influence health in other populations, particularly in the context of infectious disease events. Our results suggest that the connectedness of individuals is not positively associated with the presentation of a non-specialized disease symptom; therefore, a rapid transmission of disease between individuals due to high levels of sociality is not likely the cause of elevated lesion presence in this population compared to other estuarine populations. These results provide support for other possible factors outside of disease transmission (e.g., environmental characteristics) that may affect overall lesion occurrence in a population.

Conclusions

This study was the first to compare lesion prevalence and coverage among residency groups within a population. The occurrence of lesions is likely due to environmental features of the SJR, as

prevalence of lesions was associated with the amount of time spent annually in the SJR. An association between individual social factors, such as the number of associates or the mean bond strength and lesion occurrence, would help explain lesion occurrence via elevated disease transmission; however, the lack of significant findings in the sociality analysis of lesion occurrence allows for the rejection of this alternative hypothesis. An elevated prevalence of lesions in SJR dolphins compared to nearby estuarine sites was confirmed by this study, and lesion prevalence was significantly higher among residents of the SJR. These results further emphasize the need for changes in management of the SJR environment and future research on the presentation of disease in this population.

A cumulative effect of natural factors and high levels of anthropogenic impact in the SJR likely contributes to the elevated lesion prevalence in residents. Therefore, management changes for the SJR should focus on reducing habitat disturbance by boating, shipping, and dredging as well as reducing contaminants in the SJR system. Further lesion studies in this population should include etiology, environmental disturbance, and spatial research to better understand the cause of differing lesion occurrence in the SJR. While the general occurrence of lesions in the SJR was not consistently associated with the two social measures assessed, it is still likely that sociality plays a role in the transmission of specific diseases, particularly during epidemics. Since SJR dolphins experienced an infectious disease UME in the past, future studies comparing lesions and sociality may still be important in understanding the health of this population. Further epidemiological research in this population should include an assessment of pathogenic lesion location in the network to provide a more complete understanding of how diseases spread through the social network in the SJR.

In addition to influencing the management of the SJR, this study provides support to substantiate that lesion occurrence is more influenced by cumulative environmental effects than social transmission. This is especially important to consider in populations that inhabit environments with multiple stressors, as baseline health may be low, and cutaneous symptoms of disease may be exacerbated by increased human impact or natural disturbances.

Acknowledgements

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Tables

Table 1: Dorsal fin only (D) and total visible body (D+B) prevalence of lesions and 95% confidence intervals for each period of study.

	D	95% CI	D+B	95% CI
Pre-UME	0.83	0.80-0.85	0.84	0.82-0.86
During UME	0.57	0.54-0.60	0.59	0.56-0.62
Post-UME	0.51	0.47-0.55	0.49	0.46-0.52

Table 2: Results of Kruskal-Wallis tests comparing lesion coverage between residency groups. Mean rank coverage scores were calculated based on categorical coverage values (1=background, 2=low, 3=medium, 4=high).

		Pre-UME		During UME		Post-UME	
		D	D+B	D	D+B	D	D+B
	χ^2	0.72	2.36	0.79	0.81	1.87	0.65
	df	2	2	2	2	2	2
	N	252	257	165	170	132	127
	p	0.70	0.31	0.67	0.67	0.39	0.72
Mean Rank	R	128.25	131.88	81.64	87.71	66.62	65.51
	S	126.71	131.12	84.05	82.94	68.42	61.85
	T	119.8	115.4	87.5	81.24	59	62.67

Table 3: Binary logistic regression results for lesion prevalence and sociality measures (mean half-weight index and degree centrality). R^2 represents Nagelkerke's R^2.

		Pre-UME		During UME		Post-UME	
		D	D+B	D	D+B	D	D+B
HWI	χ^2	2.48	5.34	10.43	8.75	3.99	8.31
	df	5	5	3	3	6	6
	p	0.78	0.38	0.02*	0.03*	0.68	0.22
	R^2	0.12	0.08	0.01	0.01	0.01	0.02
	% of cases	95.8	96.3	67.8	69.2	65.1	69.8
	B (slope)	54.30	47.48	-11.04	-8.61	8.30	11.13
DC	χ^2	5.58	8.16	9.37	9.24	5.51	5.71
	df	7	7	8	8	8	8
	p	0.59	0.32	0.31	0.32	0.70	0.68
	R^2	0.14	0.12	0.00	0.00	0.03	0.05
	% of cases	95.8	96.3	67.8	69.2	66.3	70.9
	B (slope)	0.08	0.08	0.00	0.00	0.02	0.02

Table 4: Spearman's Rank correlation results for lesion coverage and sociality measures (mean half-weight index and degree centrality).

		Pre-UME		During UME		Post-UME	
		D	D+B	D	D+B	D	D+B
HWI	r_s	-0.10	-0.05	-0.17	-0.22	0.02	0.05
	N	206	207	141	144	60	52
	p	0.152	0.465	0.049*	0.010*	0.909	0.709
DC	r_s	-0.11	-0.06	-0.16	-0.13	0.00	0.04
	N	206	207	141	144	60	52
	p	0.133	0.358	0.062	0.131	0.998	0.797

Figures

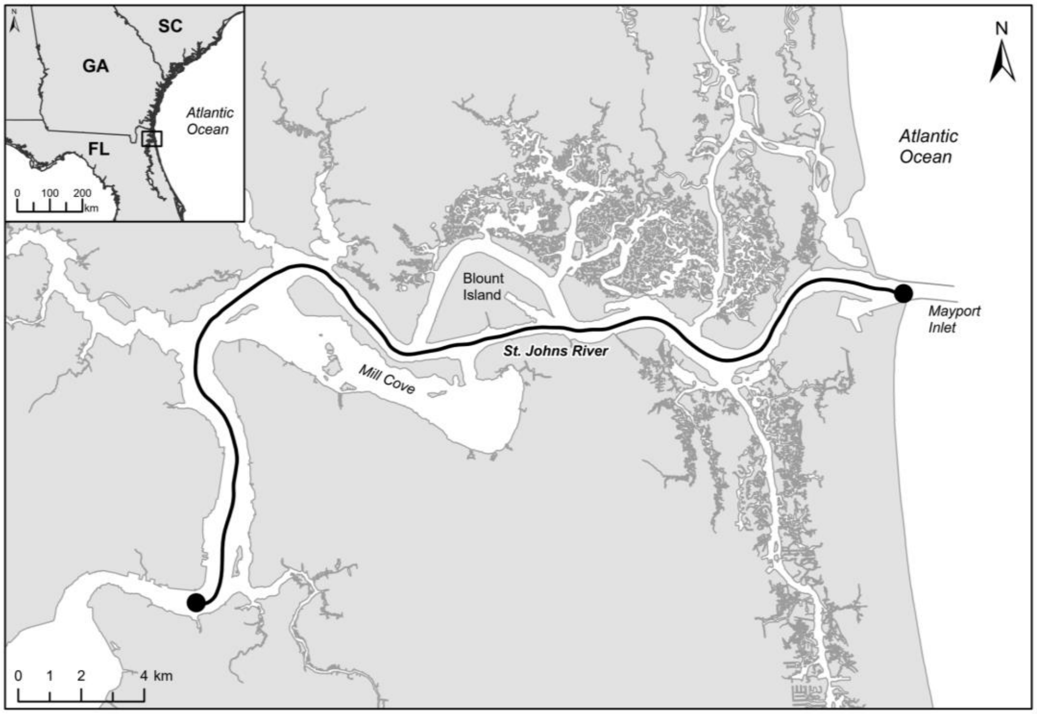


Figure 1: Map of the study area and survey route (black line) in the St. Johns River, FL.

Figure 2: Examples of photos eligible for D (left) and D+B (right) analysis based on the estimated percentage of visible body.

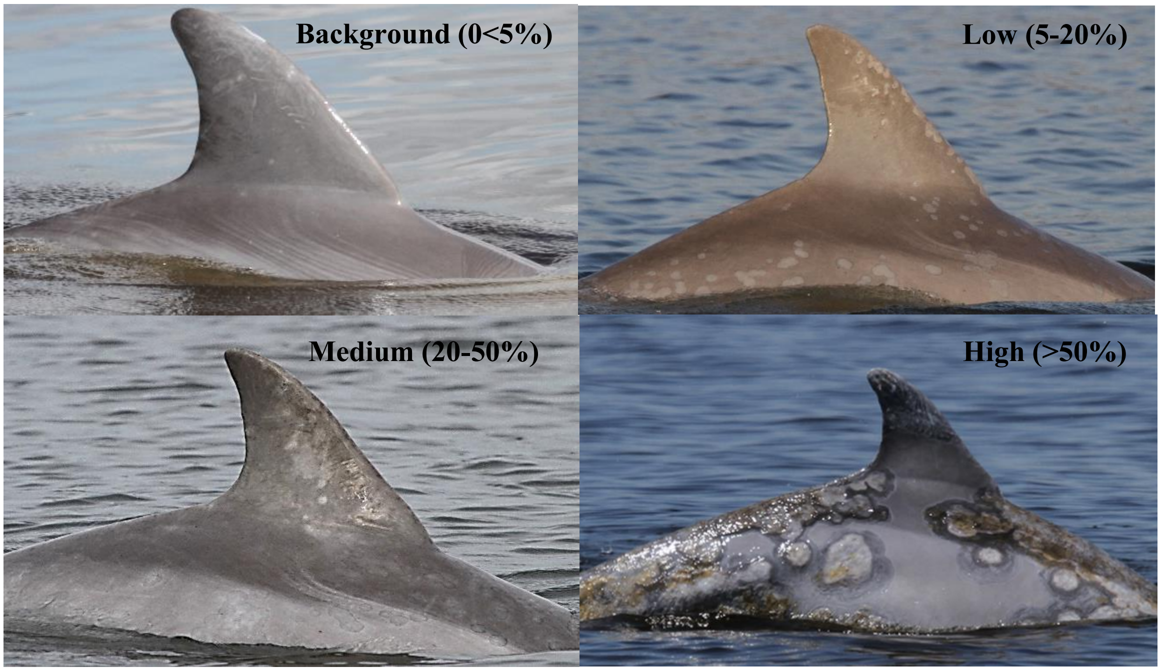


Figure 3: Examples of photos in each extent of coverage category (D+B).

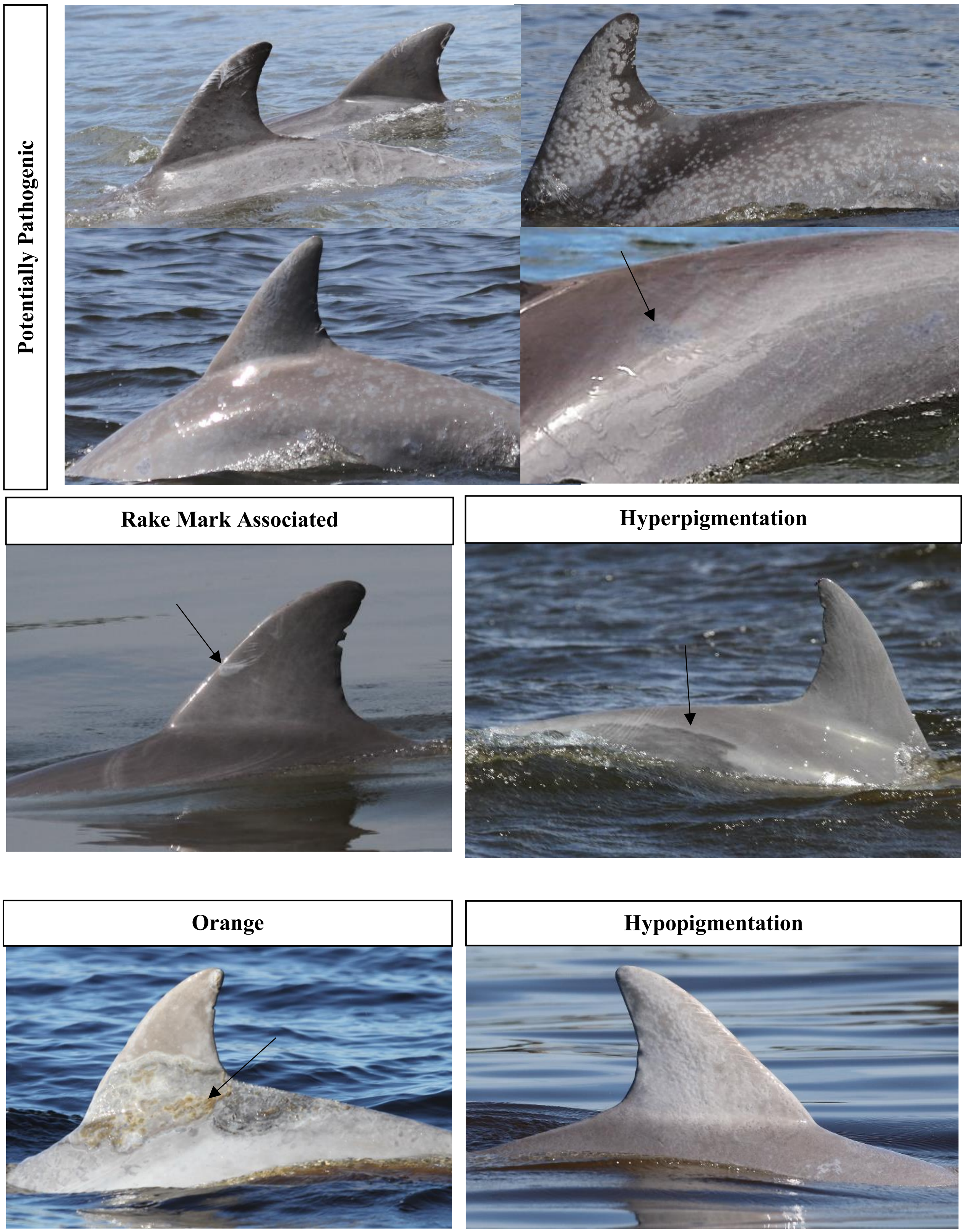
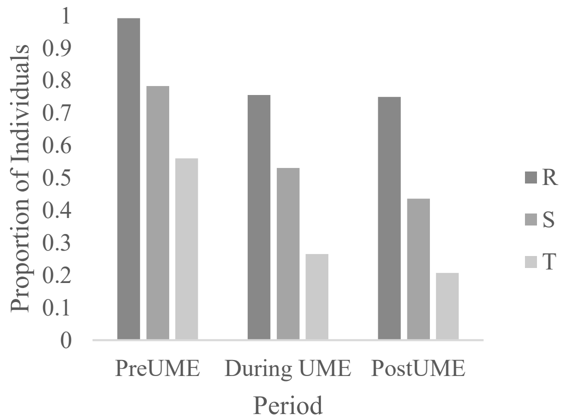
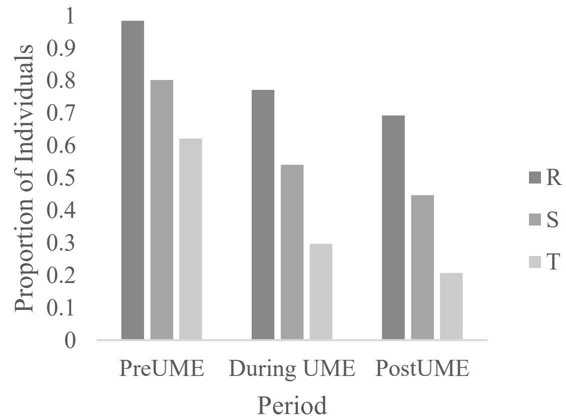


Figure 4: Examples of photos with lesions in each lesion type category.

(a)



(b)



(c)

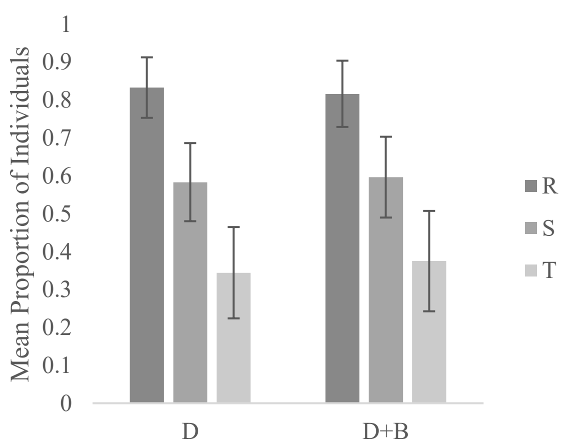
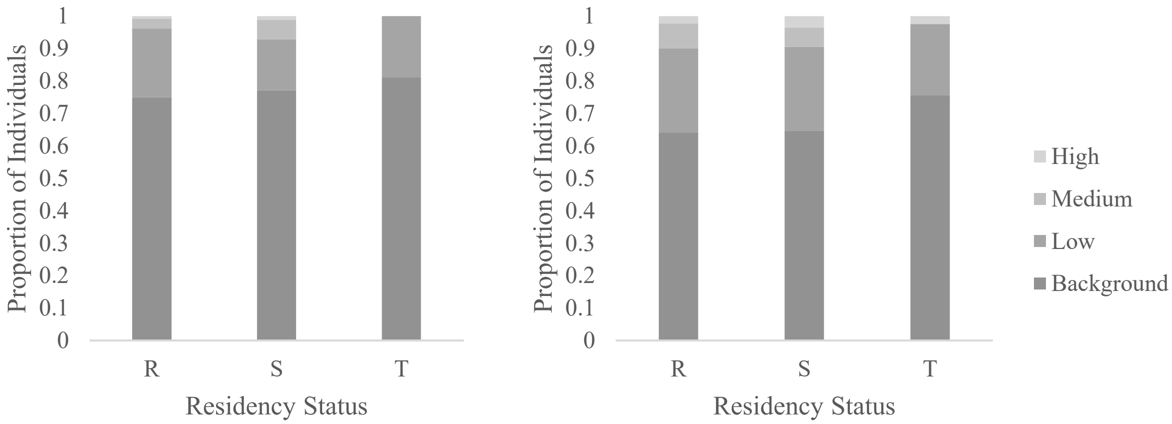


Fig. 5a-c: Lesion prevalence for residency categories in each period for a) the D data set b) the D+B data set and c) the mean of the three periods for both D and D+B data sets. Error bars represent the standard error of the mean.

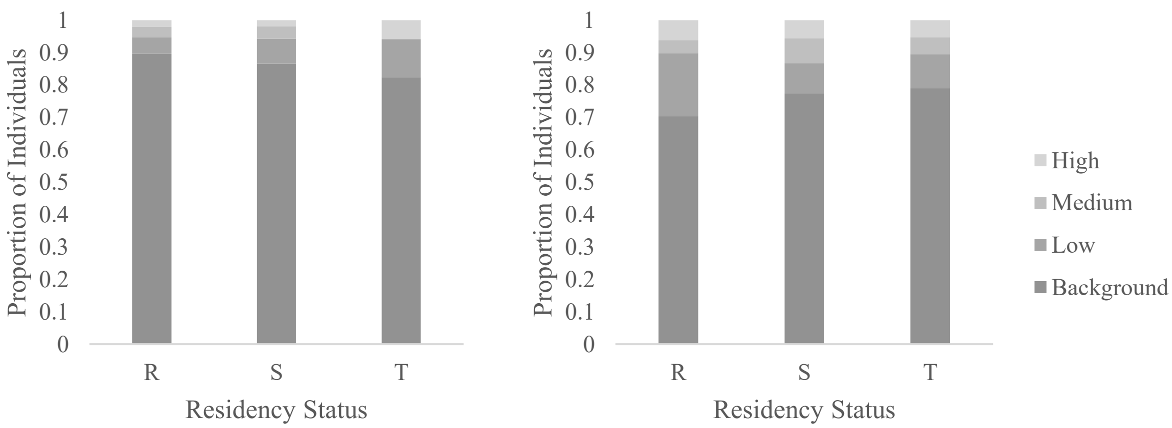
D D+B

(a)



1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Proportion of Individuals
R
S
T
Residency Status
Proportion of Individuals
R
S
T
Residency Status
High
Medium
Low
Background

(b)



1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
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Proportion of Individuals
R
S
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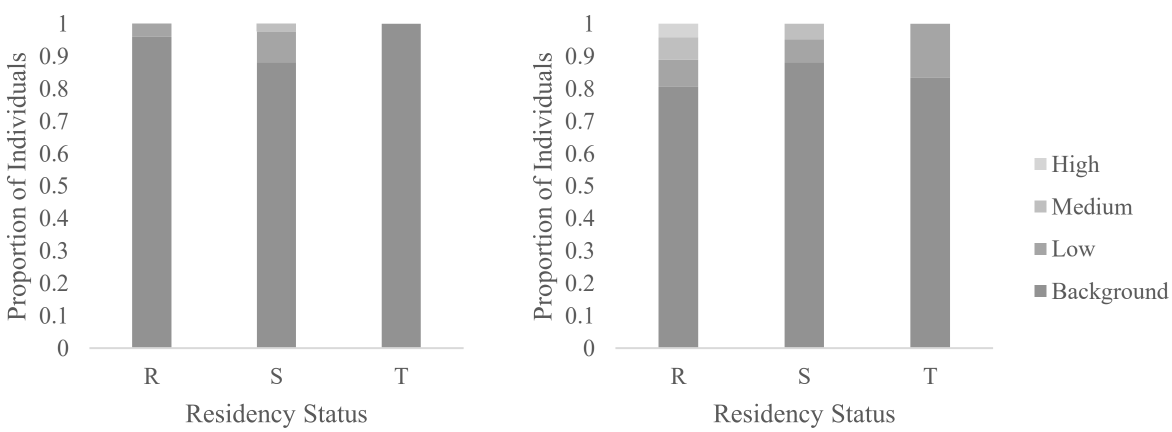
Residency Status
Proportion of Individuals
R
S
T
Residency Status
High
Medium
Low
Background

(c)



1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Proportion of Individuals
R
S
T
Residency Status
Proportion of Individuals
R
S
T
Residency Status
High
Medium
Low
Background

(d)

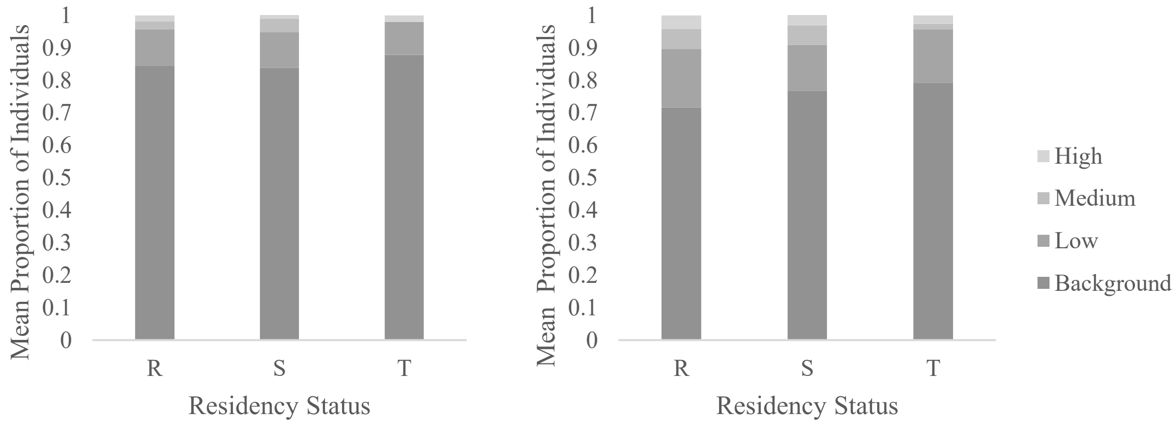


Figure 6 a-d: D extent of coverage (left) and D+B extent of coverage (right) by residency category for a) Pre-UME, b) During UME, and c) Post-UME time periods, and (d) the mean of the three time periods.

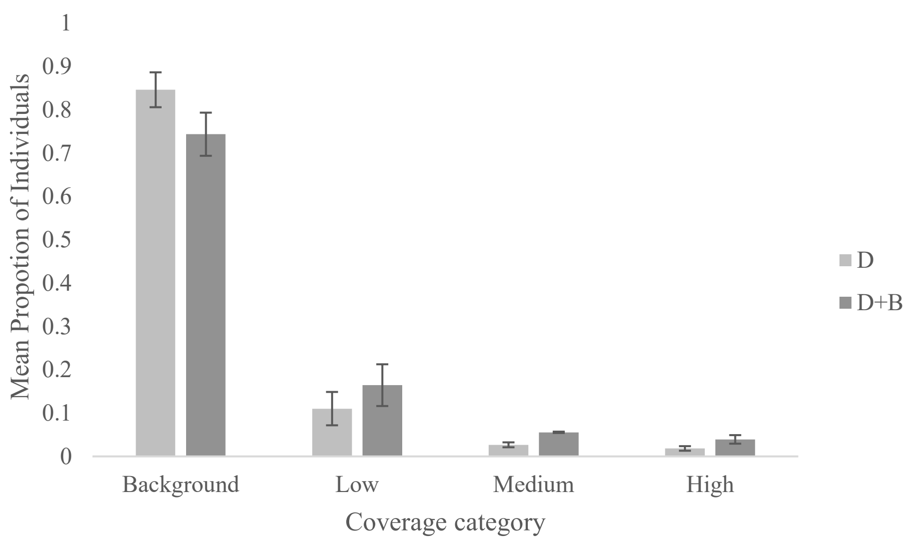
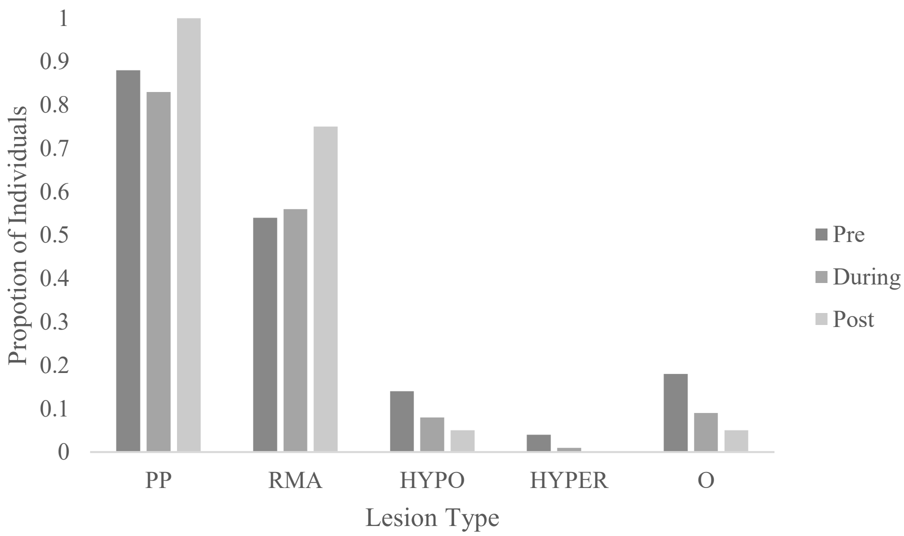


Figure 7: The mean proportion of lesioned individuals in each coverage category for all periods. Error bars represent the standard error.

(a)



(b)

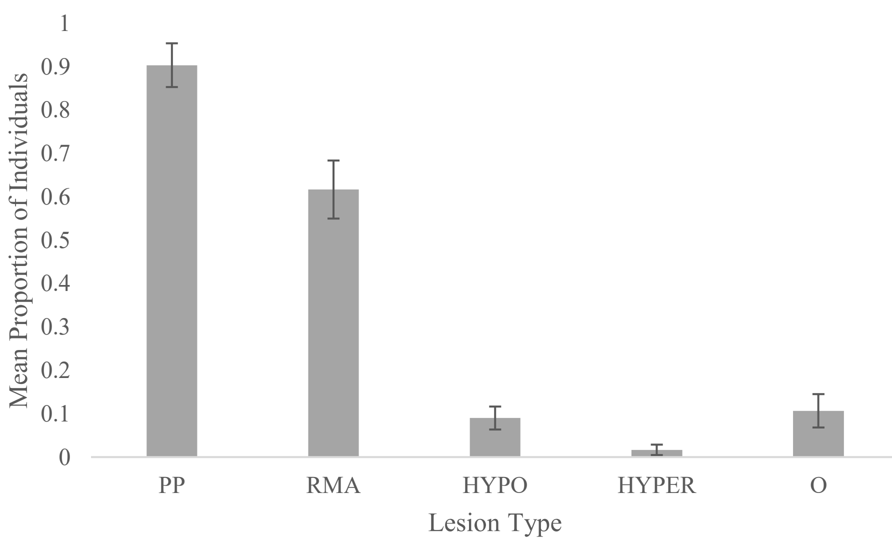


Figure 8: Proportion of lesioned individuals (D+B) with each lesion type by a) each period of study, and b) the mean of the three periods. Error bars represent the standard error. Multiple lesion types may occur in the same individual.

Appendix A

Table A1: Results of Fisher's Exact test for differences in prevalence between residency groups.

	Pre-UME		During UME		Post-UME	
	D	D+B	D	D+B	D	D+B
p	1.64E-15*	5.75E-12*	3.17E-10*	8.15E-10*	2.02E-11*	6.96E-09*

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