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Received: 30 June 2019 | Accepted: 13 July 2020

Running head: TAYLOR ET AL.

ARTICLE

**Skin lesion prevalence of estuarine common bottlenose dolphins
(*Tursiops truncatus*) in North Carolina, with comparisons to
other east coast study sites**

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as doi: [10.1111/mms.12731](https://doi.org/10.1111/mms.12731)

Abstract

Common bottlenose dolphins (*Tursiops truncatus*) are sentinels of environmental health. Skin lesions may indicate disease and can be used to infer population health. We estimated the prevalence of skin lesions and identified major lesion types on coastal bottlenose dolphins in Roanoke Sound, North Carolina, over a 3-year period using photo-identification. Boat-based surveys were conducted from April 2012 through October 2014. High quality images of distinctive fins were examined for overall prevalence (P) of any skin lesion ($n = 169$, $P = 0.49$, 95% CI = 0.42–0.57). Lesion prevalence estimates varied little between years (2012 $P = 0.45$, 2013 $P = 0.56$, 2014 $P = 0.52$) and most lesions were observed in the spring ($P = 0.79$, 95% CI: 0.57–0.92). Of six lesion types examined, pale lesions were most common ($P = 0.41$, 95% CI: 0.30–0.52). Annual lesion prevalence estimates for dolphins in Roanoke Sound were comparable to published estimates for *T. truncatus* in Charleston, South Carolina, Brunswick, Georgia, and Sarasota, Florida ($p \geq .05$), although, seasonal differences in lesion occurrence and type were observed ($p < .05$). Future studies should examine relationships between

lesions and environmental variables and use stranded dolphins to investigate skin lesion etiology.

KEYWORDS

marine mammal, photo-identification, skin disease, wildlife epidemiology

1 | INTRODUCTION

As long-lived, apex predators, common bottlenose dolphins (*Tursiops truncatus*) are key indicators of marine environmental health (Wells et al., 2004). Long-term monitoring of local dolphin populations can provide insight into water quality and environmental contamination as bottlenose dolphins bioaccumulate contaminants from their prey in their blubber (Litz et al., 2007; Wells et al., 2004). Skin lesions have been linked with changes in environmental conditions such as water temperature and salinity (Hart et al., 2012; Wilson et al., 1999), as well as exposure to immunosuppressive environmental contaminants that could increase susceptibility to disease (Hansen et al. 2004; Schwacke et al. 2002; Van Bresseem et al. 2009a,b). Skin lesions can be observed on dorsal body surfaces visible during mark-recapture studies that rely on photo-identification methods. In fact, photo-identification has been a useful, noninvasive monitoring tool to detect, monitor, and categorize skin lesions on free-ranging bottlenose dolphins, thereby providing a mechanism to calculate and compare minimum prevalence estimates, and track lesion progression over time (Bearzi, Rapoport, Chau,

& Saylan, 2009; Bertulli, Checchetti, Van Bresseem, & Van Waerebeek, 2012; Hart et al., 2012; Hart, Wells, Adams, Rotstein, & Schwacke, 2010; Leone, Bonanno Ferraro, Boitani, & Blasi, 2019; Mullin et al., 2015; Thompson & Hammond, 1992; Toms, Stone, & Och-Adams, 2020; Van Bresseem et al., 2003a, 2009b; Wilson, Thompson, & Hammond, 1997).

Skin lesions have been observed among various species of cetaceans throughout the world (Bearzi et al., 2009; Bertulli et al., 2012; Hamilton & Marx, 2005; Hart et al., 2012; Hupman et al., 2017; Kiszka, Van Bresseem & Pusineri et al., 2009; Martinez-Levasseur et al., 2011; Thompson & Hammond, 1992; Van Bresseem et al., 2003b, 2015). Lesions may have a bacterial, viral, or fungal origin (Hart et al., 2012; Reif et al., 2008; Van Bresseem et al., 1999). In addition to local environmental factors or contaminants that may affect their severity and prevalence (Van Bresseem et al., 2003b, 2009a; Wilson et al., 1999), ultraviolet radiation is also known to influence cetacean skin lesions, particularly among species with lighter skin pigmentation such as blue whales (Martinez-Levasseur et al., 2011). Several studies have visually characterized lesions and

estimated prevalence at individual study sites (Hart et al., 2010, 2012; Hupman et al., 2017; Leone et al., 2019; Van Bressemer et al., 2007; Wilson et al., 1997), which have helped to standardize lesion characteristics and facilitate comparisons across geographically distinct study sites.

Since 2008 the Outer Banks Center for Dolphin Research (OBXCDR) has conducted a long-term photo-identification monitoring study of bottlenose dolphins (*T. truncatus*) in Roanoke Sound, North Carolina. Dolphins that occur regularly in the sound are part of the Northern North Carolina Estuarine System stock (NNCES; Hayes, Josephson, Maze-Foley, & Rosel, 2017). The NNCES stock comprises approximately 823 individual dolphins, ranging from southern Virginia south to central North Carolina and utilizing both coastal and estuarine waters (Hayes et al., 2017; Gorgone, Eguchi, Byrd, Altman, & Hohn, 2014). From 2013 to 2015, the NNCES stock was affected by an Unusual Mortality Event (UME) attributed to a morbillivirus epidemic (Hayes et al., 2017; Morris et al., 2015). Although the UME affected more than 1,600 bottlenose dolphins along the U.S. East Coast (Morris et al., 2015), the full extent of UME-related

impacts on the NNCES stock has not yet been assessed, and the health of the population occurring in Roanoke Sound is of particular interest. Opportunistic photo-identification efforts have identified the presence of skin lesions on dolphins sighted in Roanoke Sound since 2007. These anecdotal sightings warranted further investigation into prevalence and types of skin lesions that occur in this area.

The objectives of this study were to (1) use photo-identification to estimate the overall, annual, and seasonal minimum skin lesion prevalence among bottlenose dolphins in Roanoke Sound; (2) classify and quantify the types of skin lesions observed on bottlenose dolphins in Roanoke Sound using descriptions from previous studies; and (3) identify geographic differences in the occurrence of skin lesions by comparing minimum skin lesion prevalence estimates for dolphins in Roanoke Sound to estimates reported for coastal bottlenose dolphins at other southeastern U.S. sites. Lesion presence may provide insight into the condition of individual marine mammals (Pettis et al., 2004; Reif et al., 2008; Thompson & Hammond, 1992; Van Bresse et al., 2009b) and signal changes in local environmental

conditions (Hart et al., 2012; Harzen & Brunnick, 1997); thus, establishing baseline estimates for skin lesion prevalence may be useful for monitoring population health. In addition, comparisons of lesion prevalence estimates between sites can provide context for individual population assessments, especially among studies with similar methodology and consistent classification criteria.

2 | MATERIALS AND METHODS

2.1 | Study site

The study site encompassed the entirety of Roanoke Sound, approximately 140 km² from the northern tip of Roanoke Island south to Oregon Inlet (Figure 1). Located in northeastern North Carolina, Roanoke Sound is part of the Albemarle Pamlico Estuary Complex and consists of shallow areas containing submerged aquatic vegetation and sandbars, as well as a dredged channel (Giese, Wilder, & Parker, 1985). The sound receives freshwater inflow from the Albemarle Sound in the north as well as saltwater inflow from the Atlantic Ocean through Oregon Inlet in the south. Water circulation throughout the sound is primarily wind-driven (Carpenter & Dubbs, 2012). In addition to freshwater

inflow, Roanoke Sound receives agricultural runoff from the Albemarle Sound (Giese et al., 1985). Roanoke Sound is heavily used for recreational fishing, boating, and swimming during the summer season. Dolphins exhibit seasonal residency in the sound with individuals occurring in Roanoke Sound from May through October. Although many dolphins sighted in the area exhibit low site fidelity, high and moderate site fidelity have been documented for a small number of individuals (Taylor, Fearnbach, & Adams, 2017).

2.2 | Field data collection

Dedicated monthly transect surveys were conducted from a 5 m outboard vessel in April–October between 2012 and 2014. Surveys were not conducted in August or September 2014 due to poor weather conditions, nor during the coldest months of November–March due to lack of equipment needed to operate in cold water conditions. Standard photo-identification techniques were used for photographing dorsal fins (Würsig & Würsig, 1977). An attempt was made to photograph both sides of each dorsal fin. A survey was considered complete if the entire study area was covered within a single day. For this analysis, surveys were

aggregated into the following three seasons: spring (April and May), summer (June, July, and August), and fall (September and October).

2.3 | Dolphin identification

A customized Microsoft Access database, FinBase, was used for storage, management, and analysis of sightings data including dorsal fin images (Adams, Speakman, Zolman, & Schwacke, 2006). Dorsal fin images were sorted and graded for photo quality using the following criteria (focus, contrast, angle of fin, obstruction of fin, and distance of fin to observer; Adams et al., 2006; Urian, Hohn, & Hansen, 1999). Each dorsal fin was matched to an existing cataloged individual or was added as a new fin to the catalog and was verified by an independent researcher. Fins were assigned a distinctiveness rating (not distinct, low distinctiveness, average distinctiveness, or high distinctiveness) when added or matched to the catalog. Poor quality images in which focus was poor or the dorsal fin angle was oblique to the camera (photograph quality grade of >12 out of 33) and low distinctiveness/not distinct fins were excluded from the analyses. Limiting the analyses to images with

distinctive fins minimized the possibility of double-counting individuals with lesions.

2.4 | Skin lesion detection and classification

Original images of distinctive individuals with good quality photos in the FinBase catalog were visually screened for lesions. The number of lesions per individual was classified as singular or multiple. Lesion type was categorized based on macroscopic features, including form, shape, and color and descriptions from previous studies (Hart et al., 2012; Hupman et al., 2017; Sanino et al., 2014; Van Bresseem et al., 2007, 2015; Wilson et al., 1997). These categories included: (1) black, (2) pale, (3) dark-fringed, (4) spotted, (5) vesicular, or (6) white-fringed. Figure 2 provides a description of each lesion type. "Major" lesion types were defined as those with an overall prevalence >0.15 (Hart et al., 2012).

2.5 | Statistical analysis

2.5.1 | Minimum overall, annual, and seasonal lesion prevalence

Overall, annual, and seasonal lesion prevalence (P) were calculated according to Equations 1-3, respectively (see below). If an individual was sighted with lesions on multiple occasions

during the study period or within a given year or season, only the first occurrence of the lesion was used to estimate skin lesion prevalence. Ninety-five percent confidence intervals were calculated for all skin lesion prevalence estimates. Overall lesion prevalence was calculated using the following equation:

$$P_{\text{overall}(2012-2014)} = \frac{\text{\# distinct individuals with at least one skin lesion (2012-2014)}}{\text{total \# distinct individuals screened (2012-2014)}} \quad (1)$$

Annual lesion prevalence was calculated using the following

equation:

$$P_{\text{annual}} = \frac{\text{\# distinct individuals with at least one skin lesion during a particular year}}{\text{total \# distinct individuals screened during a particular year}} \quad (2)$$

Seasonal lesion prevalence was calculated using the following

equation

$$P_{\text{seasonal}} = \frac{\text{\# distinct individuals with at least one skin lesion during a particular season}}{\text{total \# distinct individuals screened during a particular season}} \quad (3)$$

:

(3)

2.5.2 | Major lesion type minimum prevalence estimates

The prevalence of each major lesion type was calculated as:

(4)

(4)

Individuals were included in the numerator of Equation 4 if they were seen with a given lesion type at any point during the study period. If an individual was sighted with the same type of lesion multiple times during the study, only one occurrence of the individual was counted. If an individual was sighted with multiple types of lesions throughout the study period, each occurrence of the lesion type was counted. The proportion of individuals sighted with more than one type of lesion was also calculated to estimate the prevalence of individuals displaying multiple lesion types.

2.5.3 | Comparison to other southeast U.S. study sites

Annual, seasonal, and lesion type prevalence estimates for dolphins in Roanoke Sound were compared to published estimates for coastal bottlenose dolphins in Charleston, South Carolina

(approxim

$$P_{\text{lesion type}} = \frac{\text{\# distinct individuals with at least one skin lesion of a particular type}}{\text{total \# distinct individuals with skin lesions}}$$

ately 724

km south

of Roanoke Sound), Brunswick/Sapelo, Georgia (approximately 901

km south of Roanoke Sound), and Sarasota Bay, Florida (approximately 1,416 km southwest of Roanoke Sound; Hart et al., 2012). Mean water temperatures for these sites decreased with decreasing latitude, ranging from 21.1°C in Charleston, South Carolina, to 24.4°C in Sarasota, Florida (Hart et al., 2012). In contrast, salinity increased across sites with increasing latitude, ranging from 29.9‰ in Charleston, South Carolina to 33.1‰ in Sarasota, Florida (Hart et al., 2012). The Brunswick, Georgia, site exhibited the largest range in salinity (0.1‰–33‰) and water temperature (7.6°C–30.3°C) (Hart et al., 2012). In addition, dolphins in Brunswick, GA exhibited high exposure to polychlorinated biphenyls (PCBs) (Balmer et al., 2011). Lesion prevalence estimates for these southeastern sites also relied upon lesion detection in images taken during photo-identification surveys (Hart et al., 2012). Data collection for prevalence estimates calculated for these southeastern sites occurred in 2009 and are described in Hart et al. (2012). Annual, seasonal, and lesion type prevalence comparisons between dolphins in Roanoke Sound and each southeastern U.S. site relied upon pairwise chi-square or Fisher's exact tests ($\alpha = 0.05$) and

comparison of 95% confidence intervals. Statistical analyses were conducted using Statistica v. 13.3 (TIBCO Software Inc., Palo Alto, CA).

3 | RESULTS

3.1 | Skin lesion prevalence estimates in Roanoke Sound

Eighteen surveys were conducted over the course of the study period (Figure 3). Between 2012 and 2014, good and average quality digital images of 169 different dolphins were screened for the presence of skin lesions. The amount of visible body surface varied by photograph; thus, reported lesion prevalence estimates should be considered minimum estimates. Prevalence (P) estimates indicated the proportion of individuals screened with visible lesions. Of the total individuals screened, 83 dolphins (P = 0.49, 95% CI: 0.42-0.57) had visible lesions at some time during the study period based upon prevalence estimate calculation using Equation 1. The highest annual prevalence (P; Equation 2) was observed in 2013 (P = 0.56, 95% CI: 0.36-0.74), with no observable annual trend during the study period (Table 1). Of the 169 individual dolphins observed throughout the study, six were sighted in all 3 years. Three of these

individuals were observed with lesions once during the study, and one was observed with lesions in two consecutive years. Skin lesions were most commonly observed during spring ($P = 0.79$, 95% CI: 0.57–0.92; Equation 3; Table 2) and least commonly observed during summer ($P = 0.36$, 95% CI: 0.26–0.47; Table 3).

3.2 | Classification of skin lesion types

Six different types of lesions were observed (Figure 2), with three representing major lesion types ($P > 0.15$; Hart et al., 2012; Equation 4). These major lesion types included pale lesions ($P = 0.41$; 95% CI: 0.30–0.52), black lesions ($P = 0.37$, 95% CI: 0.27–0.49), and dark-fringed lesions ($P = 0.27$, 95% CI: 0.18–0.38; Table 4; Hart et al., 2012; Hupman et al., 2017; Sanino et al., 2014; Van Bresseem et al., 2015; Wilson et al., 1997). While pale and black lesions were observed in each of the three seasons evaluated, dark-fringed lesions were only observed during spring and summer. Approximately one third ($n = 28$) of the dolphins with lesions presented with more than one lesion type; dolphins with multiple lesion types were primarily observed in the summer.

3.3 | Comparisons to other southeast U.S. study sites

Annual skin lesion prevalence estimates for dolphins in Roanoke Sound were comparable to estimates from other southeastern sites, with the exception of 2012, when lesion prevalence in Roanoke Sound ($P = 0.45$, 95% CI: 0.34–0.56) was significantly lower than Brunswick ($P = 0.59$, 95% CI: 0.53–0.64, $p = .02$; Table 1). Seasonally, the prevalence of skin lesions in the fall on dolphins in Roanoke Sound ($P = 0.44$, 95% CI: 0.34–0.56) was significantly higher than on dolphins in Charleston ($P = 0.26$, 95% CI: 0.19–0.35, $p = .006$) and Brunswick ($P = 0.30$, 95% CI: 0.21–0.40, $p = .04$; Table 5). Compared to estimates among dolphins observed in Sarasota Bay, lesion prevalence for dolphins in Roanoke Sound were significantly higher for all seasons included in this study (spring $p = .0001$, summer $p = .0003$, fall $p < .0001$; Tables 2,3,5). Of the three major lesion types, pale and black lesions were more commonly observed in Roanoke Sound than in the other southeastern study sites (Table 4). Dark-fringed lesions, however, were observed more among dolphins in Charleston ($P = 0.55$, 95% CI: 0.47–0.63) and Brunswick ($P = 0.58$, 95% CI: 0.50–0.65) than in Roanoke Sound ($P = 0.27$, 95% CI: 0.18–0.38, $p < .0001$ and $p < .0001$,

respectively; Table 4).

4 | DISCUSSION

4.1 | Overall lesion prevalence

Across all sampling years (2012–2014), approximately half of distinct dolphins sighted in Roanoke Sound were observed with at least one skin lesion. This estimate is comparable to observations of bottlenose dolphins from other southeastern U.S. study sites ($P = 0.38\text{--}0.59$; Hart et al., 2012), which may be attributed to similar stressor exposure (i.e., water temperatures, salinities, prey availability, disease outbreaks) among dolphins inhabiting the southeastern U.S. coast.

Contrarily, skin lesion estimates for bottlenose dolphins inhabiting other regions of the globe were higher (Bearzi et al., 2009; Harzen & Brunnick, 1997; Wilson et al., 1999). For example, Bearzi et al. (2009) estimated an overall skin lesion prevalence of 0.73 among coastal bottlenose dolphins in and around Santa Monica Bay, California, and skin lesion prevalence for inshore bottlenose dolphins in the Sado estuary of Portugal was estimated to be 0.85 (Harzen & Brunnick, 1997). Furthermore, when Wilson et al. (1999) compared skin lesion prevalence

estimates of coastal bottlenose dolphins between ten sites throughout the world, the overall prevalence estimate exceeded 0.60 for each site.

These larger-scale geographic differences in lesion prevalence estimates may be due to differences in sampling methodology, variability in exposure to natural and anthropogenic pollutants (Harzen & Brunnick, 1997; Van Bresse et al., 2003b, 2009a), or global variation in climate factors (Hart et al., 2012; Wilson et al., 1999). For example, Wilson et al. (1999) and Hart et al. (2012) demonstrated inverse relationships between water temperature and skin lesion prevalence on coastal bottlenose dolphins. More specifically, Wilson et al. (1999) found the greatest prevalence of lesions ($P = 0.99$) on bottlenose dolphins that inhabited the more (boreal) northern site, the Moray Firth, where dolphins would be exposed to the coldest water temperatures. Hart et al. (2012) also found the greatest prevalence of lesions ($P = 0.59$) to occur in Brunswick, Georgia, the site with the lowest mean water temperature. Studies also suggest a relationship between salinity and skin lesions (Gulland et al., 2008; Mullin et al.,

2015), whereby low salinity may act as an immune system stressor, increasing vulnerability to viral and bacterial diseases, or alter the integrity of the skin thereby leading to skin lesions (Ewing et al., 2017; Fazioli & Mintzer, 2020; Mullin et al., 2015; Wilson et al., 1999).

Skin lesions may also be indicative of climate change impacts, such as the emergence of new diseases (Burek, Gulland, & O'Hara, 2008; Rotstein et al., 2009). For example, Paracoccidioidomycosis ceti (*Paracoccidioides brasiliensis* var. *ceti*; Vilela & Mendoza, 2018; Vilela et al., 2016) is typically known to occur in tropical climates yet was identified on two offshore bottlenose dolphins stranded off North Carolina in 2005 and 2008 (Rotstein et al., 2009). Given the long-term study of dolphins in Roanoke Sound, future research on skin lesions in this area should include longitudinal analyses of lesion presence and corresponding changes in water temperature and salinity. Continued monitoring efforts will also aid in the detection of new diseases in Roanoke Sound and enhance our understanding of environmental factors that may promote their occurrence.

4.2 | Annual and seasonal lesion prevalence

Annually, lesion prevalence was consistent across years; approximately 45%-56% of individuals sighted during photo-identification surveys were observed with at least one skin lesion each year. The persistence of lesions over time on dolphins in Roanoke Sound may reflect new individuals acquiring lesions or affected individuals retaining their lesions each year. For example, ID 52, a female, was observed in all three years of the study, and presented with skin lesions in summer 2013 and spring 2014. Temporal differences in lesion presence have been observed on various species of cetaceans in other sites (Hamilton & Marx, 2005; Van Bresseem & Van Waerebeek, 1996; Wilson, Grellier, Hammond, Brown, & Thompson, 2000). For example, Wilson et al. (2000) found that the overall extent of lesions increased on individual coastal bottlenose dolphins throughout an 8-year study. In addition, Hamilton & Marx (2005) found the occurrence of white lesions on North Atlantic right whales (*Eubalaena glacialis*) in the Bay of Fundy to steadily increase over a span of 7 years. As the current study comprised three years, continued longitudinal monitoring of lesions on

seasonally resident dolphins in Roanoke Sound would provide insight into whether temporal trends in skin lesions exist in this study population.

Seasonally, the greatest prevalence of skin lesions on dolphins in Roanoke Sound occurred in the spring. Our findings of a predominance of skin lesions in the spring are consistent with other studies in the southeastern U.S. (Hart et al., 2012). Seasonal variation in lesion occurrence may be influenced by water temperature or salinity. Measured water temperature was lowest in the spring (mean = 22.7°C) and highest in the summer (mean = 27.1°C), and previous studies within this region have demonstrated that salinity is lowest during spring due to increased freshwater inflow from precipitation (Carpenter & Dubbs, 2002; Giese et al., 1985). Future studies should focus on exploring the relationship between skin lesion presence and environmental factors to gain insight into how these factors influence skin lesion occurrence in Roanoke Sound.

4.3 | Prevalent skin lesion types

Among the six lesion categories determined by macroscopic characteristics, pale lesions were most commonly observed.

Sanino et al. (2014) and Van Bresseem et al. (2015) described pale lesions as infectious and possibly linked to low water temperature or habitat degradation. Hart et al. (2012) also described pale lesions as potential indications of healing due to trauma, inflammation, or prior viral infection. Black and dark-fringed lesions were also commonly observed on dolphins in Roanoke Sound. These lesion types have been described in other studies (black: Wilson et al., 1997; Toms et al., 2020; dark-fringed: Thompson & Hammond, 1992), although etiology is not well understood. Hart et al. (2012) hypothesized a potential association between dark-fringed lesions and pox virus, although this association is not confirmed.

The prevalence of multiple types of skin lesions on bottlenose dolphins in Roanoke Sound was comparable to observations among dolphins in Charleston, Brunswick, and Sarasota Bay ($P = 0.38, 0.36, 0.30$, respectively; Hart et al., 2012). Wilson et al. (1997) also found a high occurrence of coastal bottlenose dolphins presenting with multiple types of lesions ($P = 0.61$), but the implications of this are not well understood. While different lesion types may indicate different

disease or pathology, some studies suggest that different lesion types may have the same etiology. For example, poxvirus has been associated with both tattoo and ring lesions; thus, despite different macroscopic features, these types of lesions may result from the same pathogen (Van Bresse et al. 1999, 2006). Given the high prevalence of skin lesions on dolphins in Roanoke Sound, the common co-occurrence of multiple lesion types, and overlap with the Outer Banks Marine Mammal Stranding Network coverage area, future studies should sample lesions on stranded dolphins to better define the etiology of skin lesions with different macroscopic features.

4.4 | Limitations

Skin lesion prevalence estimates were derived by examining the dorsal regions of the body for the presence of lesions at a single point in time. It is possible that the true prevalence of skin lesions among dolphins in Roanoke Sound may be greater if other parts of the body were visible during photo-identification surveys. For example, Hupman et al. (2017) examined skin lesion prevalence among common dolphins (*Delphinus* sp.) in Hauraki Gulf, New Zealand, where anterior, ventral, and dorsal regions

of the dolphins were photographed. Prevalence estimates were calculated based upon lesion presence in all body regions, and estimates were relatively high compared to other study sites (Hupman et al. (2017)). In our study, it was generally not possible to photograph anterior or ventral regions of the dolphins, however, including these regions in our examination may yield higher lesion prevalence estimates.

Due to the inability to distinguish between clean and minimally distinctive fins, these individuals were excluded from the analysis. Many clean/low distinct fins are juveniles and calves. Several studies (Powell, Wallen, Bansal, & Mann, 2018; Van Bresseem & Van Waerebeek, 1996; Wilson et al., 2000) have found that the prevalence of lesions varies by demography, and that juveniles/calves may be more susceptible to lesions. As such, lesion prevalence estimates reported in this study should be considered minimum estimates.

4.5 | Conclusions

This study represents the first formal examination of skin lesion occurrence on bottlenose dolphins in Roanoke Sound. Overall lesion prevalence for dolphins in Roanoke Sound is

within the range of estimates for other southeastern U.S. coastal sites, suggesting chronic persistence of skin lesions for southeastern dolphin populations, albeit at lower levels than coastal bottlenose dolphins studied in other regions of the globe. However, comparisons across geographically distinct sites can be challenging due to differences in environmental factors (e.g., water temperature and salinity), anthropogenic stressors (e.g., environmental contaminants), uncertainties regarding etiology, and inconsistencies in lesion classification. For example, spotted lesions observed in this study (Figure 2) resemble "focal skin disease" observed by Sanino et al. (2014) on Peale's dolphins (*Lagenorhynchus australis*) and Chilean dolphins (*Cephalorhynchus eutropia*) in the Añihué Reserve of Chilean Patagonia. Similarly, the pale lesions described in our study are similar in appearance to the "pale dermatitis" (PAD) described by Van Bresse et al. (2015) on inshore common bottlenose dolphins in Paracas Bay, Peru, white and cream lesions described by Wilson et al. (1997) on coastal common bottlenose dolphins in the Moray Firth, and "pale skin patches" described by Sanino et al. (2014) on Peale's dolphins and

Chilean dolphins in the Añihué Reserve. While evidence suggests lesion occurrence can be influenced by environmental factors that would vary by geography, differences in the subjective categorization of lesion types cannot be discounted as a possible explanation of differences in the types observed across sites. Despite these limitations, this study is the first step in understanding baseline levels of skin lesions among dolphins in Roanoke Sound, and continued lesion monitoring during photo-identification surveys will help detect future perturbations in this marker of population health (Pettis et al., 2004).

Finally, the relationship between skin lesions and environmental contaminants was not explored in this study, although prior studies have suggested a link between skin lesions and contaminants such as PCBs (Harzen & Brunnick, 1997; Van Bresse et al., 2003b, 2009a). Very little toxicological data exist for dolphins inhabiting waters near Roanoke Sound, however, PCBs have been detected in dolphins in Beaufort, North Carolina (Hansen et al., 2004), a potential overwintering area for this population (Urian, 2016). Roanoke Sound is also susceptible to agricultural runoff through the Albemarle Sound

(Giese et al., 1985), so additional research to explore the relationship between contaminants and skin lesions is warranted. Remote biopsy sampling serves as a cost-efficient mechanism to study contaminant concentrations in bottlenose dolphins (Balmer et al., 2011), and could provide further insight into potential anthropogenic effects on dolphin lesions in Roanoke Sound.

ACKNOWLEDGMENTS

We would like to thank OBXCDR volunteers and interns for assistance with field surveys and FinBase processing, Todd Speakman for aid in creating transect survey routes, Jay Taylor and John Kerner for assistance in dorsal fin matching and boat maintenance, and Kim Urian for advice on sampling protocols. Shauna McBride-Kebert provided a helpful review of an earlier draft of this manuscript. This manuscript also benefited from the comments of two anonymous reviewers. Funding was provided by fundraisers and donations to the Outer Banks Center for Dolphin Research. Field research was conducted under General Authorization permits awarded to J. Taylor (LOC-13416, LOC-17988).

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TABLE 1 Annual prevalence estimates in Roanoke Sound with comparisons to Charleston, South Carolina, Brunswick, Georgia, and Sarasota, Florida (Hart et al., 2012).

	RS 2012	RS 2013	RS 2014	CHS	BSG	SSB
<i>n</i>	85	27	60	351	322	266
<i>L</i>	38	15	31	171	189	101
<i>P</i>	0.45	0.56	0.52	0.49	0.59	0.38
95% CI	0.34-0.56	0.36-0.74	0.39-0.65	0.43-0.54	0.53-0.64	0.32-0.44
<i>p</i> RS 2012	–	–	–	.51	.02*	.27
<i>p</i> RS 2013	–	–	–	.49	.75	.08
<i>p</i> RS 2014	–	–	–	.67	.31	.05

Note. *n* = number of distinctive dolphins evaluated for lesions with good quality photographs; *L* = number of distinctive dolphins photographed with visible skin lesions; *P* = minimum prevalence estimate; *indicates a significant difference ($\alpha = 0.05$).

TABLE 2 Spring prevalence estimates in Roanoke Sound with comparisons to Charleston, South Carolina, Brunswick, Georgia, and Sarasota, Florida (Hart et al., 2012).

	RS	CHS	BSG	SSB
<i>n</i>	24	108	139	127
L	19	74	96	45
P	0.79	0.69	0.69	0.35
95% CI	0.57-0.92	0.59-0.77	0.61-0.77	0.27-0.44
<i>p</i> to RS	–	.30	.32	.0001*

Note. *n* = number of distinctive RS dolphins evaluated for lesions with good quality photographs; L = number of distinctive RS dolphins photographed with visible skin lesions; P = minimum prevalence estimate; *indicates a significant difference ($\alpha = 0.05$).

TABLE 3 Summer prevalence estimates in Roanoke Sound with comparisons to Charleston, South Carolina, Brunswick, Georgia, and Sarasota, Florida (Hart et al., 2012).

	RS	CHS	BSG	SSB
<i>n</i>	89	163	195	124
L	32	35	50	18
P	0.36	0.22	0.26	0.15
95% CI	0.26-0.47	0.16-0.29	0.20-0.032	0.09-0.22
<i>p</i> to RS	–	.01*	.08	.0003*

Note. *n* = number of distinctive RS dolphins evaluated for lesions with good quality photographs; L = number of distinctive RS dolphins photographed with visible skin lesions; P = minimum prevalence estimate; *indicates a significant difference ($\alpha = 0.05$).

TABLE 4 Major lesion type prevalence estimates in Roanoke Sound with comparisons to Charleston, South Carolina, Brunswick, Georgia, and Sarasota, Florida.

Site	n	Pale			Black			Dark-fringed		
		L	P	95%CI	L	P	95%CI	L	P	95%CI
RS	83	34	0.41	0.30-0.52	31	0.37	0.27-0.49	22	0.27	(0.18-0.38)
CHS	171	21	0.12	(0.08-0.18)	44	0.26	(0.20-0.33)	94	0.55	(0.47-0.63)
BSG	189	40	0.21	(0.16-0.28)	38	0.20	(0.15-0.27)	109	0.58	(0.50-0.65)
SSB	101	16	0.16	(0.10-0.25)	29	0.29	(0.20-0.39)	24	0.24	(0.16-0.33)
Comparisons to RS										
		Pale			Black			Dark-fringed		
		CHS	BSG	SSB	CHS	BSG	SSB	CHS	BSG	SSB
<i>p</i>		<.0001*	.0007*	.0001*	.06	.003*	.21	<.0001	<.0001	.67

Note. *n* = number of distinctive RS dolphins identified with lesions from good quality photographs; L = number of distinctive RS dolphins photographed with visible skin lesion type; P = minimum prevalence estimate; *indicates a significant difference ($\alpha = 0.05$).

TABLE 5 Fall prevalence estimates in Roanoke Sound with comparisons to Charleston, South Carolina , Brunswick, Georgia, and Sarasota, Florida (Hart et al., 2012).

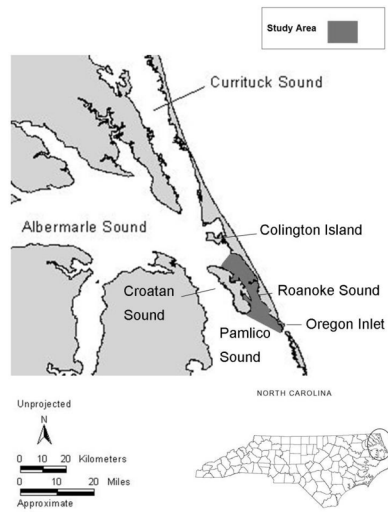
	RS	CHS	BSG	SSB
<i>n</i>	81	123	98	132
L	36	32	29	20
P	0.44	0.26	0.30	0.15
95% CI	0.34-0.56	0.19-0.35	0.21-0.40	0.10-0.23
<i>p</i> to RS	–	.006*	.04*	<.0001*

Note. *n* = number of distinctive RS dolphins evaluated for lesions with good quality photographs; L = number of distinctive RS dolphins photographed with visible skin lesions; P = minimum prevalence estimate; *indicates a significant difference ($\alpha = 0.05$).







FIGURE 1 Roanoke Sound study area.

FIGURE 2 Descriptions and examples of skin lesion types identified on common bottlenose dolphins (*Tursiops truncatus*) in Roanoke Sound.

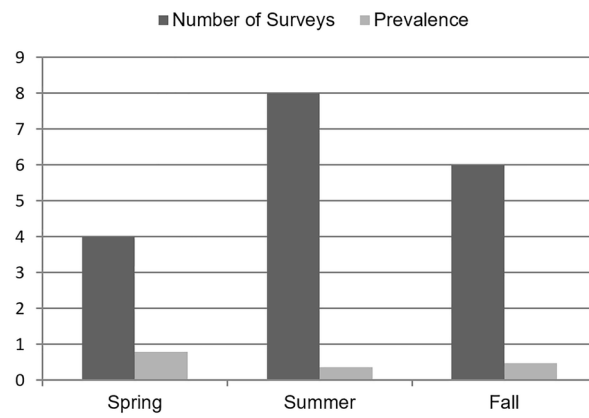
FIGURE 3 Number of surveys conducted per season and seasonal skin lesion prevalence estimates among common bottlenose dolphins (*T. truncatus*) sampled in Roanoke Sound, 2012–2014.



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Black beavers	Uniform in color and circular or elongate in shape. Lightly depressed.	Wilson et al., 1987 Tynes et al., 2020	
Pink beavers	Pink in color and angular in shape with a localized distribution.	Wilson et al., 1987 Carroll et al., 2014 Van Dyke et al., 2015	
Dark ringed beavers	Circular pink areas of dark ring, and hence a ringed or ringed distribution.	Wilson et al., 1987 Happan et al., 2017	
Spotted beavers	Lesser area under in color than surrounding area, circular in shape, and having a localized distribution.	Hart et al., 2012 Happan et al., 2017	
Vertical beavers	Flattened, ribbon-like sites, notches.	Van Dyke et al., 2007, 2015	
White ringed beavers	White holes, surrounding normally colored or dark areas.	Wilson et al., 1987 Happan et al., 2017	

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MMS_12731_4755_Fig3.tif