ABSTRACT

Title of Thesis:	AMBIENT SOUND AFFECTS MOVEMENT AND CALLS OF BOTTLENOSE DOLPHINS
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Abundant oceanic shipping and more frequent and intense storms are increasing sound levels in aquatic habitats. Understanding how changing soundscapes affect protected species, especially those that use sound to communicate and navigate, is critical. This study utilizes passive acoustic monitoring to investigate the effects of changing ambient sound levels on bottlenose dolphin (*Tursiops truncatus*) movements, spatial utilization, and social calls in the Mid-Atlantic Bight, USA. By localizing dolphin whistles, I determined that their habitat use changed under higher ambient sound levels and that these elevated sound levels caused dolphins to alter the acoustic characteristics of their calls. The acoustic characteristics of individually identifiable calls (signature whistles) also varied between the sites and regions in which they were recorded. As changes in the underwater soundscape continue in the future, these findings will help inform resource managers about how protected marine mammals may be affected by anthropogenic activities and sounds.

AMBIENT SOUND AFFECTS MOVEMENT AND CALLS OF BOTTLENOSE DOLPHINS

by

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Thesis submitted to the Faculty of the Graduate School of the University of Maryland, College Park, in partial fulfillment of the requirements for the degree of Master of Science 2022

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Chapter 1: Introduction

Background

Historically, knowledge of animals was gained through visual observation and little was known of aquatic organisms, particularly beyond nearshore environments, because of our inability to locate or track them. Now, technology allows us to remotely record aquatic environments and understand the organisms that inhabit them. One of these such approaches is passive acoustic monitoring, which offers opportunities for analysis of ambient sound levels, presence of specific sound sources (biotic, abiotic, or anthropogenic), acoustic behavioral information (e.g. foraging or different call types), and potentially sounds that can be localized (Sousa-Lima et al., 2013). With acoustic recordings spanning months, years, and decades, our understanding of the underwater soundscapes can include those questions that require broad temporal resolutions.

For vocalizing marine species such as marine mammals, changing soundscapes can be a threat to their physiology and communication. All soundscapes have abiotic and biotic components, but increasingly remote habitats are now being subjected to anthropogenic sounds (Lynch et al., 2011)). The ocean soundscape is becoming increasingly loud, primarily as a result of the rise in anthropogenic sound (Duarte et al., 2021). As the frequency and intensity of anthropogenic noise increases, its effects on species are also expected to increase. Exposure to anthropogenic sound has led to decreased life spans of organisms (Gurule-Small & Tinghitella, 2019), interruptions of foraging (Wisniewska et al., 2018), and individual fitness and populationlevel effects (Shannon et al., 2016). As development increases in coastal regions, acoustic disturbances will increase, and the cumulative effects of these sounds on marine species, both

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lethal and non-lethal, will be an important priority for natural resource managers and decision makers worldwide (Colbert, 2020; Kragh et al., 2019).

Study system

Since 2014, the coastal waters off Maryland in the southern Mid-Atlantic Bight, USA, have been monitored for the presence of marine mammals using passive acoustic monitoring. The Mid-Atlantic Bight extends from Cape Hatteras, North Carolina in the south to Martha's Vineyard, Massachusetts in the north. Despite being a migration corridor for several whale species and within the home range of several dolphin species (including multiple bottlenose dolphin populations; *Tursiops truncatus*), the Mid-Atlantic Bight is one of the least studied areas on the East Coast of the USA for marine mammals. This is especially true for the extensive and highly industrialized estuary, the Chesapeake Bay, whose mouth is at the Bight's southern extent. The southern Mid-Atlantic Bight region encompasses the busy Ports of Wilmington, Delaware and Philadelphia, Pennsylvania, and has abundant recreational fisheries and boating. This region, like much of the eastern coast of the USA, includes an area for offshore wind energy development over the next decade. It is critical to understand the effects of activities such as wind farm construction on marine mammals to help inform environmental impact assessments and management of these federally protected species.

The bottlenose dolphin, a highly vocal, mobile, and social species, inhabits the Mid-Atlantic Bight year-round. This species produces a plethora of echolocation clicks, whistles, and other vocalizations. Previous studies have localized dolphin clicks (Aubauer et al., 2000; Hastie et al., 2006), but localization of whistles has been limited to towed arrays (Quick & Janik, 2012) or captivity (Freitag & Tyack, 1993). Detecting and localizing whistles is advantageous because they can convey important information such as an individual dolphin's identity and because they occur at lower frequencies than echolocation clicks. Therefore, acoustic recorders can have a reduced sampling rate and increased deployment durations. Dolphin signature whistles are individually identifiable calls that can be identified by their repeated pattern of the same whistle. Signature whistles can be used to determine which and how many individuals are present in a group, and when and where individuals reoccur (Bailey et al., 2021; Erbe et al., 2020; Kriesell et al., 2014; Longden et al., 2020).

Goals and objectives

Our understanding of the effects of human activities and changing environments on animals should not be limited to the presence and absence of a species within visual range of an observer for daylight hours in good weather conditions. Passive acoustic monitoring offers unique insights into these species' lives and behavior. This study utilized passive acoustic data to understand the movements of bottlenose dolphin groups and signature whistles of individuals under different ambient sound conditions to understand the effect on their behavior and determine whether there is acoustic differentiation of individually identifiable dolphin calls throughout the region.

Marine mammals have been found to avoid loud environments by moving away from the source of the sound (Bain & Williams, 2006; Erbe et al., 2018; Reichmuth, 2007; Southall et al., 2016), but may suffer increased stress (Erbe et al., 2018; Romano et al., 2004) or physiological damage to their hearing when they are unable to avoid it (Erbe et al., 2018; National Research Council (U.S.), 2005; Weilgart, 2007). Most commonly, however, they exhibit behavioral responses such as a change in their movement orientation (Cox, 2006; Nowacek et al., 2004), altered respiration (Frankel & Clark, 2000), aggressive behavior, or cessation of reproduction

(Saplosky et al., 2000; Southall et al., 2007). In this study, I utilized bottlenose dolphin whistles to localize pods of dolphins and assess the effect of ambient sound levels on their movements.

Whistles of free-ranging dolphins in the Mid-Atlantic Bight were used to determine the location and movement paths of dolphin pods. I examined these measures in relation to the ambient sound conditions. I hypothesized that dolphins would increase their speed of travel, travel in more direct routes, and utilize a smaller area during higher sound conditions to minimize possible auditory damage or stress and to maintain group cohesion (Erbe et al., 2018; Janik, 2000; Reichmuth, 2007; Southall et al., 2016). I also expected a greater response to increased mid-frequency sound levels (2820 – 7080 Hz) compared to broadband levels (10 – 7080 Hz) due to bottlenose dolphins' hearing sensitivity and communication within that frequency range (Erbe et al., 2016, 2018; Ljungblad et al., 1982).

At sites across the study area, when dolphin signature whistles were detected, they were identified and reoccurrences within the study area were determined. The acoustic characteristics of these individually identifiable calls were analyzed across sites and I determined how they were affected by changing ambient sound levels.

Thesis contents

In Chapter 2, a passive acoustic monitoring array within the Maryland Wind Energy Lease Area approximately 26-30 km off Ocean City, MD, was used to detect and localize bottlenose dolphin whistles and assess the effect of ambient sound conditions on their movements. Hourly ambient sound measurements were calculated for broadband and midfrequencies, and hours were categorized as having low (sound level category 1 (SL1): 84.0 -92.4; 81.5 – 88.2 dB_{rms} re 1µPa, respectively), medium (SL2: 92.4 – 102.6; 88.2 – 95.7 dB_{rms} re 1μ Pa, respectively), or high (SL3: 102.6 – 126.1; 95.7 – 115.8 dB_{rms} re 1μ Pa, respectively) sound levels.

Under each sound level category, dolphin group locations were obtained by localizing dolphin whistles in the Raven 2.0 Correlation Sum Estimation (CSE) localization algorithm (Hawthorne & Salisbury, 2016). I then used a state-space movement model (Patterson et al., 2008) to account for location error and determine their speed and how directly they traveled. Finally, I calculated the kernel density and spatial distribution of a random selection of whistle locations for each sound level category. Bottlenose dolphins did not significantly alter their speed or how directly they traveled, nor did they alter the amount of space they inhabited under different ambient sound levels. However, dolphins did alter the region of the study area that they inhabited, occurring further east when broadband sound levels were highest and further south when mid-frequency sound levels were highest.

In Chapter 3, I tested the efficacy of the dolphin signature whistle identification methodology and then applied this approach in the field. Acoustic data were recorded from dolphins at the National Aquarium, and I evaluated the ability to detect reoccurrences of their signature whistles. In the Potomac River of the Chesapeake Bay, acoustic recordings occurred in proximity to visual surveys to determine how the number of unique signature whistles related to dolphin group size. Then, at five sites (two in the Chesapeake Bay, two coastal, and one offshore), the acoustic characteristics of signature whistles (start, end, minimum, maximum, and delta (maximum minus minimum) frequency, duration, and number of extrema) were measured and compared. When signature whistles reoccurred, I determined whether the characteristics of those signature whistles changed based on the ambient sound levels when they were emitted.

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A minimum of 347 individual bottlenose dolphins in the Chesapeake Bay were identified based on their signature whistles. The acoustic characteristics of signature whistles varied significantly between sites and regions. Signature whistles from offshore, where sound levels were consistently highest, were found to have higher minimum frequencies and fewer number of extrema compared to other locations. Increased ambient sound levels also caused shortening of the duration of signature whistles, likely to simplify these important calls and reduce information loss. Signature whistles most often reoccurred between the two coastal sites and between the most inshore coastal site and the Potomac River. None of the signature whistles that were detected offshore were detected within the Chesapeake Bay, indicating that these dolphins may belong to the Western North Atlantic Offshore bottlenose dolphin stock, about which very little is known.

Implications and future work

For the marine mammal species that inhabit the Mid-Atlantic Bight, wind energy development, increased shipping traffic, and intensifying storms as a result of climate change are on the horizon. As these abiotic events and anthropogenic actions increase ambient sound levels, management of protected species will require an understanding of the distribution and movements of individual animals and populations and their response to elevated sound levels. This thesis examined the effects of changing ambient sound levels on a variety of dolphin movement, communication, and distribution measures. It also investigated the efficacy and utility of identifiable dolphin calls to track individuals and determine how they adjust their calls to compensate for changing sound levels. This study established that dolphins altered their distribution and their identity communication calls under elevated ambient sound conditions, alerting managers to some of the ways in which increasing sound levels will affect this species. In addition to understanding the effects of changing ambient sound levels, this research illustrated that in an area separated by only tens of kilometers, the acoustic characteristics of dolphin's signature whistles varied significantly. Here, where the Western North Atlantic (WNA) Northern Migratory Coastal, WNA Southern Migratory Coastal, Northern North Carolina Estuarine System, and the WNA Offshore populations of bottlenose dolphins all occur, signature whistles may be used to distinguish between individuals and help delineate populations, which is an important management concern.

Future research should aim to improve localization algorithms for dolphin whistles and other sounds, which would allow finer spatiotemporal analysis. Additionally, comparing dolphin signature whistles in this region to those further north and south would allow larger scale movements of individuals and mixing of populations to be determined. This thesis also investigated the utility of signature whistles in providing a minimum estimate of group sizes. The development of an automated pipeline and machine learning to detect and identify dolphin signature whistles, localize those individuals, determine their stock or population identity, and match those signature whistles to a catalog of unique signature whistles to determine reoccurences would allow the increasing volumes of acoustic data to be processed in a more time and cost efficient way. Improvements in our understanding of individual identities, population structure, and abundance of individuals from passive acoustic monitoring could revolutionize monitoring of this species, particularly in offshore environments that are difficult to study.

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Chapter 2: Altered spatial distribution of a marine top predator under elevated ambient sound conditions

Introduction

Marine species are perpetually exposed to natural and anthropogenic sounds (Hildebrand, 2009). These sounds have been classified into the categories of abiotic (waves and rain; geophony), biological (biophony), and anthropogenic (produced by human activities such as construction and vessel traffic: Duarte et al., 2021; Pijanowski et al., 2011). Every soundscape is composed of varying levels of these sounds, and increasingly remote habitats, including the ocean (Duarte et al., 2021), are being subjected to anthrophony (Dziak et al., 2017; Lynch et al., 2011).

As the prevalence and intensity of anthropogenic sound increases, its effects on species are also expected to increase. Exposure to anthropogenic sound has led to decreased life spans (Gurule-Small & Tinghitella, 2019), interruptions of foraging (Wisniewska et al., 2018), and increased vulnerability to predation (Simpson et al., 2016) resulting in both individual fitness and population-level effects (Shannon et al., 2016). A wide variety of habitats and organisms have been shown to be affected by anthrophony (invertebrates: Gurule-Small & Tinghitella, 2019 bats: Bunkley et al., 2015, fish: Buehler et al., 2015, birds: Dooling & Popper, 2016, marine mammals: Erbe et al., 2016; Shannon et al., 2016, amphibians: Zhao et al., 2018). Understanding how organisms respond to varying sound levels is also critical for management, especially for protected species such as marine mammals that utilize sound for communication, navigation, and foraging.

Marine mammals are vocal, highly mobile species that have broad hearing ranges (less than 1 kHz to more than 150 kHz; Erbe et al., 2018; Wartzok & Ketten, 1999) and respond

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physically and behaviorally to the anthrophony (Nowacek et al., 2007). Marine mammal responses to the anthrophony include: altering their vocalizations (Fouda et al., 2018; Kragh et al., 2019; Parks et al., 2011) or hearing thresholds (Nachtigall et al., 2018); changes in orientation (Cox, 2006), speed (Nowacek et al., 2004), respiration, or group distribution (Frankel & Clark, 2000); avoidance vertically or horizontally (Bain & Williams, 2006; Erbe et al., 2018; Morton & Symonds, 2002; Reichmuth, 2007; Southall et al., 2016); aggressive behavior; and cessation of reproduction (Saplosky et al., 2000; Southall et al., 2007). These animals are unlikely to be able to avoid sounds when it occurs suddenly, for prolonged periods of time, over large areas, or within critical habitat. When exposed to loud sounds, marine mammals may suffer increased stress (Erbe et al., 2018; Romano et al., 2004) and auditory (Board & National Research Council, 2005; Erbe et al., 2018; Weilgart, 2007) or organ injury (Board & National Research Council, 2005; Erbe et al., 2018; Finneran et al., 2003). Due to the possible effects of increased sound levels, governments around the world are increasingly regulating the underwater anthrophony (Colbert, 2020; Francis & Barber, 2013).

To better understand individual organisms' responses to the anthrophony, controlled sound exposure experiments and behavioral monitoring with tagged whales have been utilized (Southall et al., 2016). However, these methods have limited sample sizes and may be challenging for smaller cetaceans, such as dolphins (Schneider et al., 1998). A non-invasive alternative for tracking animal movements and ambient sound levels is to use passive acoustic monitoring and localization (Blumstein et al., 2011; Hawthorne & Salisbury, 2016; Van Parijs et al., 2009). This can aid in our understanding of marine mammals' activities across specific landscapes and for long durations as they are exposed to different forms and levels of anthrophony.

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A key question in understanding its impacts on animals is how increasing sound levels influence spatial position and habitat use. Here, I investigated whether a common coastal marine top predator, the bottlenose dolphin (*Tursiops truncatus*), altered its spatial location and movements under different ambient sound conditions. Unlike previous passive acoustic monitoring studies that have localized dolphin clicks (Aubauer et al., 2000; Hastie et al., 2006) or whistles from a towed array (Quick & Janik, 2012) or in captivity (Freitag & Tyack, 1993), this study localized the whistles of free-ranging dolphins in the open ocean. Using these localized positions, I examined whether the distribution and movements of dolphin pods differed in relation to ambient sound conditions. I hypothesized that dolphins would increase their speed of travel, travel in more direct routes, and utilize a smaller area during higher sound conditions to minimize possible auditory damage or stress and maintain group cohesion (Erbe et al., 2018; Janik, 2000a; Reichmuth, 2007; Southall et al., 2016). I also expected a greater response to increased mid-frequency sound levels compared to broadband levels due to bottlenose dolphins' hearing sensitivity and communication within this range (Erbe et al., 2016, 2018; Ljungblad et al., 1982)

Methods

Study area

My study area was located in the southern Mid-Atlantic Bight, USA between 26 and 30 km east of Ocean City, Maryland, USA (Fig. 2.1). This study area contains the leased wind energy area (WEA) offshore of Maryland and is adjacent to the traffic separation scheme leading into the Ports of Wilmington, Delaware and Philadelphia, Pennsylvania (Breithaupt et al., 2017; Samoteskul et al., 2014). Water depths ranged from approximately 25 to 28 m (Fig. 2.1), and the

hydrophones were deployed approximately 3 m above the ocean floor using bottom-anchored moorings.

Data collection

Acoustic recordings were obtained using the Marine Autonomous Recording Units (MARUs; Cornell University, NY, USA). Instruments were positioned in an array with a mean of 2.76 km between recording units and deployed from July 13, 2018 to August 20, 2018. MARUs were equipped with an HTI-94-SSQ hydrophone (High Tech Inc., MS, USA; sensitivity: -162 dB re $1V/\mu$ Pa, frequency response: 2 Hz- 30 kHz) sampling at 16 kHz. High (10 Hz) and low-pass (7080 Hz) filters were applied to the acoustic data [effective frequency range 10 - 7080 Hz]. The four MARUs (A-1M, A-2M, A-3M, A-4M; Fig. 2.1) were time-synchronized using sounds played-back through an underwater speaker at the beginning and end of the deployment (Bailey et al., 2018).

Ambient sound analysis

Ambient sound levels at A-2M were calculated using the Raven-X toolbox (Dugan et al., 2016) in MATLAB (Mathworks, Inc., MA, U.S.A.) as representative sound levels of the region. Sound levels (dB re 1 μ Pa root-mean-square (rms)) were computed for each 1-s time bin and 1-Hz frequency band. Hourly average broadband (10 – 7080 Hz) and mid-frequency (2820 – 7080 Hz) sound levels were calculated. Hourly broadband and mid-frequency ambient sound level categories (SLs) were assigned as follows: SL1 was assigned to hours in the quartile representing the lower 25% of sound levels; SL2 was assigned to the second and third quartiles representing 50% of sound levels; and SL3 was assigned to the fourth quartile, the upper 25% of sound levels.

Dolphin whistle detection and localization

Bottlenose dolphins are the primary marine mammal species present during the summer months in this region (Bailey et al., 2021; Barco et al., 1999, 2015). Acoustic recordings from each MARU were first processed using the PAMGUARD Whistle and Moan Detector (Gillespie et al., 2011) to identify when dolphin whistles were present. Acoustic data were then manually reviewed in time-aligned concatenated multi-channel spectrograms in Raven Pro 2.0 (Cornell University, NY, USA). Spectrograms were reviewed in 15-second windows with a 256-point fast Fourier transform (FFT) and Hann window with 75% overlap with a time resolution of 4 ms and frequency resolution of 62.5 Hz. Identified whistles were then manually compared across MARUs to determine when whistles had been detected on at least three MARUs and were therefore potentially locatable.

The localization process was conducted using the Raven 2.0 Correlation Sum Estimation (CSE) localization algorithm (Hawthorne & Salisbury, 2016). The CSE algorithm provided the estimated latitude and longitude of the vocalizing animal. It also estimated the expected arrival time of the dolphin whistle at each MARU given the estimated location of the vocalizing dolphin. These predicted arrival times were displayed as boxes on concatenated spectrograms (Fig. 2.2).

Arrival boxes were reviewed in Raven for proper alignment in 15 s, 10 – 8000 Hz timealigned concatenated spectrograms, and location estimates were considered reliable if the arrival boxes aligned properly with the actual arrival of the dolphin whistle (Fig. 2.2). Dolphin whistle location accuracy was categorized based on the alignment of predicted arrival boxes. A location estimate was considered of good quality if the received signal's arrival time at the other two MARUs was entirely within the estimated arrival boxes. The location estimate was considered of fair or poor quality if 25-99% or 0-25%, respectively, of the received signal was within the estimated arrival boxes. Poor quality location estimates were considered unreliable and were removed from subsequent analyses.

The CSE algorithm searches for an energy maximum within the recording using a stochastic search algorithm. It then compares the waveform of the whistle from each recorder and calculates the time of arrival differences for the peak energy in the waveforms. In complex acoustic environments, a local maxima or "subpeak" may be selected as the peak to be used in the comparison (Frankel et al., 2014). This variation in peak selection can decrease the precision of the algorithm.

To determine the precision of the CSE algorithm, 100 randomly selected whistles in each sound level category (300 whistles total) were localized five times, yielding five location estimates for each. I determined the mean distance between all five location estimates (how close they were to one another) and the mean distance of each location from the center of the localization array were calculated and tested for a correlation between them. An ANOVA was used to determine whether the mean distances between the localized positions of each whistle varied depending on the broadband (10 – 7080 Hz) or mid-frequency (2820 – 7080 Hz) SLs (SL1: low, SL2: medium, SL3: high) in which they were detected. This allowed me to identify whether the precision of the CSE algorithm varied in relation to the ambient sound level.

Dolphin spatial distribution

Kernel density estimates of the 100 randomly selected dolphin localizations (using the mean location of all five localizations) in each broadband and mid-frequency sound level were calculated using the Home Range Tool (Rodgers et al., 2011) in ArcGIS 10.4 (www.esri.com). These randomly selected whistles provided an independent sample of dolphin locations. Because

the distributions of these kernel densities were non-parametric, a Wilcoxon rank-sum test was used to determine whether the areas of the 25, 50, 75, and 90th percentiles significantly differed in relation to the ambient sound level categories. ANOVAs and Tukey's post-hoc analyses (Tukey-HSD) were utilized to investigate whether and how the spatial distribution (latitude and longitude) of the dolphins' locations varied under different ambient broadband and midfrequency sound levels.

Dolphin movement patterns

The detection range of the localization array was estimated to be approximately 15 km from the center of the array based on the source level of dolphin whistles reported in the literature, accounting for background noise (following Frankel et al., 2014). Dolphin locations at distances greater than 15 km were not included in the analyses. Whistles were abundant in the recordings, so another subset of whistles from 14 random hours in each broadband sound level category were selected for localization. I did not specifically localize signature whistles (individually identifiable calls,(Janik & Sayigh, 2013) and thus I cannot attribute whistles to a particular dolphin. Therefore, the sequential locations of the dolphin pod over the selected time period constituted putative tracks and the dolphin pod's movements.

Because of the potential variability in the CSE localization algorithm and the subsequent potential error in the accuracy and precision of the estimated locations, I utilized a state-space movement model (SSM) to estimate the likely path of dolphin movement (Patterson et al., 2008). An SSM couples a measurement equation that accounts for the observation error and a transition equation that describes the movement process (Bailey et al., 2008; Jonsen, 2016; Jonsen et al., 2005; Patterson et al., 2008). These models have generally been applied to satellite telemetry data, although they can be adapted for other types of data (Bailey et al., 2014; Breed et al., 2012). As far as I am aware, SSMs have not previously been applied to positions derived from passive acoustic localization. The SSM was fit using the bsam package (Jonsen et al., 2013) in R (version 4.0.0). All five localized positions for each whistle were included as the input with a location quality class of 0. An SSM is typically used on movement data collected over periods of days, weeks, or months. With as many as 614 dolphin location estimates within an hour (mean = 84 whistles per hour, sd = 85), the SSM was set to produce location estimates at intervals of 4.5 mins. Using the regularized positions from the SSM, the locations for each putative track were plotted spatially. A maximum swim speed of 9.3 m/s (Hartel et al., 2020; Johannessen & Harder, 1960) between adjacent positions was used to identify biologically unreasonable position estimates that were removed and consequent speeds re-checked.

I calculated two metrics to analyze the temporal and spatial aspects of the dolphin pod's movement in relation to ambient sound levels. First, the speed of travel was calculated between the tracks output from the SSM. To determine if dolphin speeds varied in relation to differing broadband and mid-frequency sound level categories, a generalized linear mixed-effects model with a Poisson distribution was utilized in R with the track's identity included as a random effect.

Second, a straightness index (Benhamou, 2004) was calculated to determine whether dolphins travelled in a more direct, linear manner or a more circuitous path in relation to the ambient sound conditions. The straightness index was calculated by dividing the distance between the first and last locations of the track (derived from the SSM) by the total distance traveled, resulting in an index between 0 and 1. A straightness index of 1 indicated that the path was a straight line, or a direct path, and values closer to 0 indicated a more circuitous path. An ANOVA was used to determine whether there were any differences in the straightness index for each track in relation to the sound level category they occurred in.

Results

Ambient sound analysis

Hourly broadband sound levels at site A-2M ranged from $84.0 - 126.1 \text{ dB}_{rms}$ re 1µPa and mid-frequency sound levels ranged from $81.5 - 115.8 \text{ dB}_{rms}$ re 1µPa (Table 2.1). During hours that contained bottlenose dolphin whistles, mid-frequency sound levels ranged from $81.6 - 110.4 \text{ dB}_{rms}$ re 1µPa (Table 2.2). Broadband sound levels were significantly correlated with mid-frequency sound levels (Pearson correlation; r(933) = 0.15, p < 0.01).

Dolphin whistle detection and localization

There were 9,267 whistles detected during the recording period. Whistles were detected less frequently during the afternoon and early evening with only 121 of the whistles detected between 1300 and 2100 EST. During those hours, an average of only 15 whistles were detected per hour, but at other times of day averaged 572 whistles per hour. As a result, the whistles we localized occurred primarily from 2100 to 1200 in the late evening to morning. The random selection of 100 whistles in each broadband sound level category (300 whistles total) occurred over 99 hours (mean = 3.03 whistles per hour, sd = 4.16 whistles) on 29 days (mean = 3.41 hours per day, sd = 2.50 hours; Table 2.3). Because A-3M was prematurely trawled from its location, I was unable to precisely time-align that MARU. As a result, many whistles were not localizable because they were only detected on two of the three other MARUs.

The median distance between the five locations estimated by the CSE algorithm for each whistle (Fig. 2.3) was not significantly related to either the broadband or mid-frequency ambient

sound level ($F_{2,29} = 1.23 \text{ p} = 0.29$, and $F_{2,297} = 0.59$, p = 0.56, respectively; Fig. 2.3a, b). However, the variability in the estimated locations did significantly increase with increasing distance from the center of the array (r(142) = 0.19, p = 0.02; Fig. 2.3c). The median distance between estimated locations for a single localized whistle was 3.28 km (sd = 5.34 km).

Dolphin spatial distribution

Dolphins were located throughout and around the WEA and were not clustered within the localization array. There was no significant difference in the size of the kernel densities under different ambient sound conditions (Fig. 2.4, Table 2.4). There was a difference, however, in the spatial distributions of the dolphins in relation to ambient sound levels. Under elevated broadband sound levels (SL3), dolphins were found significantly further east than under SL1 or SL2 (ANOVA: $F_{2, 287} = 13.31$, p < 0.01; Fig. 2.4). Under elevated mid-frequency sound levels (SL3), dolphins were found further south than in mid-frequency SL1 or 2 (ANOVA: $F_{2,287} = 13.67$, p < 0.01; Fig. 2.5). The Tukey-HSD test indicated that only the differences in spatial distribution between broadband SL1 and SL3 (3.50 km) and mid-frequency SL2 and 3 (3.36 km) were larger than the mean distances between localization results (3.28 km).

Dolphin movement patterns

Movement tracks contained 643 dolphin whistles and spanned 18 days (Table 2.3). Broadband sound levels for these hours ranged from $84.5 - 20.7 \, dB_{rms}$ re 1µPa and midfrequency sound levels ranged from $83.1 - 104.6 \, dB_{rms}$ re 1µPa. Given the variability in location estimates for each whistle (approximately 3 km), the raw locations were all assigned an Argos location class of 0 in the SSM, which had an equivalent mean error (Costa et al., 2010). Output locations from the SSM that had unrealistic dolphin travel speeds were removed (n = 42 locations). The final dataset had 35 tracks comprised of 133 locations at 4.5 min intervals (Fig. 2.6).

A linear mixed-effects model indicated that dolphin pod speeds were not significantly related to either broadband or mid-frequency ambient sound levels (Fig. 2.7, Table 2.5, Table 2.6). The straightness index also did not vary significantly in relation to broadband or mid-frequency sound levels (ANOVA: $F_{2,22} = 0.53$, p = 0.60, $F_{2,22} = 0.01$, p = 0.99, respectively; Fig. 2.8, Table 2.6).

Discussion

Bottlenose dolphins utilized different regions of my study area under elevated broadband and mid-frequency sound levels (Fig. 2.4, 2.5). My analysis indicated that dolphins did not, however, move at different speeds (Fig. 2.7, Table 2.6), utilize more or less space (Table 2.4), or alter the directness of their travel (Fig. 2.8) under different ambient sound conditions at these spatiotemporal scales. This study provides important information on the impacts of varying ambient sound conditions on bottlenose dolphins and can help to interpret future changes in movement that occur during and after the construction of an offshore wind farm in the Maryland WEA. I also demonstrated the utility of a non-invasive approach (passive acoustic localization) to concurrently monitor sound levels and the movements of a vocalizing marine species over a scale of several kilometers.

Though coastal bottlenose dolphins are often subjected to numerous natural and anthropogenic disturbances and may become resilient (New et al., 2013; Smith & Reeves, 2012) or habituated (Fandel et al., 2015; Papale et al., 2012) to those disturbances, a change in dolphins' habitat utilization was documented in this region during elevated ambient sound conditions. The lack of change in dolphins' speed and straightness of travel under different sound conditions may, however, be the result of some habituation to elevated ambient sound levels. While much attention has been paid to physiological injury and deaths caused by sounds (Board & National Research Council, 2005; Erbe et al., 2018; Finneran et al., 2003; Romano et al., 2004; Weilgart, 2007), non-lethal effects are more common and more challenging to understand. My study area is located southwest of the shipping lanes for the busy Ports of Wilmington, Delaware and Philadelphia, Pennsylvania (Fig. 5d) and can be subjected to intense storms and hurricanes (particularly in the late summer and autumn). Bottlenose dolphins in this region are therefore regularly exposed to elevated sound levels from anthropogenic and physical environmental sources (Fandel et al., 2020).

The maximum hourly broadband sound level in this study was 126.1 dB_{rms} re 1µPa, but the accuracy of this localization algorithm was not significantly affected by the ambient sound level. Fourteen hours overall (1.50%, n = 935) and four hours that contained dolphin whistles (1.25%, n = 321) had average sound levels over 120 dB_{rms} re 1µPa, the threshold for continuous sound to be classified as a disturbance to marine mammals (National Research Council, 2005). I found that dolphins altered their broad-scale spatial distributions under broadband sound levels starting at 102.6 dB_{rms} re 1µPa and mid-frequency sound levels from 95.7 dB_{rms} re 1µPa. These values were based on the designated sound level categories and in the future these values may be further refined to determine precise thresholds.

The altered distribution of free-ranging bottlenose dolphins under elevated sound levels in this study could be classified with a severity response rank of 6 (Southall et al., 2007, 2021). More severe responses could include individual and/or group avoidance behaviors (Donovan et al., 2017) and increased vigilance (Colbert, 2020) resulting in reduced foraging efficiency (Luo et al., 2015) or abandonment of critical habitat (Colbert, 2020). Avoidance behaviors and displacement in this study area, which has been established as a dolphin foraging habitat (Bailey et al., 2019; Fandel et al., 2020), could have serious consequences. The population consequences of disturbance model could be developed to indicate whether these altered habitat preferences (behavioral changes) affect the population dynamics of the animals in this region through changes to their health and vital rates (Erbe et al., 2018; Harwood et al., 2014; New et al., 2014).

The hour with the highest ambient (broadband) sound level (August 11, 2018 18:00; 126 dB_{rms} re 1µPa) and the loudest hour with whistles (third loudest hour overall; July 27, 2018) 23:00; 123 dB_{rms} re 1µPa) occurred during storms (0.21% of the recording period), but the remaining twelve hours with sound levels over 120 dB did not. Intense storms have been shown to affect dolphin foraging and how long animals spend in a region (Fandel et al., 2020; Smith et al., 2013). Elevated broadband ambient sound levels in this study not resulting from storms were largely due to the presence of vessels. Shipping traffic has been repeatedly shown to interrupt dolphin behaviors (Papale et al., 2012; Pirotta et al., 2015; Wisniewska et al., 2018). In my study, dolphins moved further east (closer to the shipping lanes) under elevated sound conditions. While this could suggest that this habitat is particularly valuable (perhaps because of altered prey distribution in the presence of large vessels; (Ivanova et al., 2020), it is more likely that the dolphins are being displaced to a more marginal habitat by an acoustic disturbance. In this area, where over a thousand bottlenose dolphins have been identified (Bailey et al., 2021), increased vessel traffic and therefore elevated sound levels (Tougaard et al., 2008) during the construction and maintenance of the offshore wind farm will contribute further anthropogenic sound to a region that already has high ambient sound levels (Grade & Sieving, 2016).

Localization of dolphin whistles to investigate movement tracks at the spatiotemporal scales of this study did not indicate any changes in speed or directness of travel under varied

ambient sound conditions. The premature surfacing of a hydrophone rendered the acoustic recordings from that site unusable in the localization process, decreasing the precision of my array. Like other localisation studies, the precision of this localization significantly decreased as the dolphins' distance from the array increased (Watkins & Schevill, 1972; Zhang et al., 2016). I utilized a several kilometer array and a localization algorithm built for the stereotyped vocalizations of whales to localize dolphin groups. The CSE algorithm's precision error of approximately 3 km may be less of an issue for large, slowly traveling whale species with body lengths of tens of meters, but further refining is recommended to provide precision appropriate for relatively fast-moving, smaller (2-3 m) cetacean species such as bottlenose dolphins. For this species, with whistles of 12 kHz in a high sea state, the communication range is as low as 1.5 km (Janik, 2000). Despite these challenges, I localized dolphins at greater distances than other whistle localisation studies which have ranged from tens of meters (Gillespie et al., 2020) to 3.7 (Brunoldi et al., 2016) or 5 km (Kim et al., 2006) and up to 8 km (Wiggins et al., 2013).

The SSM was utilized to account for both the challenges of localizing a pod of dolphins and to account for the level of imprecision of the CSE algorithm. However, the SSM was designed for tracking the movement of a single animal. As a result, the SSM produced some locations that were deemed unreasonable given the known maximum speed of dolphin travel. This may have been due to the presence of more than one dolphin pod within the localization range, which would have produced what seemed to be spurious locations for the track of a single pod. Furthermore, my analysis was aimed at understanding the movement of the dolphin pod under hourly ambient sound conditions. I did not determine the instantaneous behavioral response of an individual to sound events, which may be more appropriate for acute or pulsed activities, such as pile driving. Localizing individually-identifiable calls such as signature whistles (Janik et al., 2006) could also allow tracking of individuals, although the repetition rate of identity calls may limit the number of derived locations.

This study illustrates the altered habitat utilization of dolphins under elevated broadband and mid-frequency sound levels and provides important information contributing to the understanding of non-lethal effects of acoustic disturbances on protected marine mammals. These altered spatial distributions, especially when exacerbated by increased vessel traffic and wind farm construction, could lead to population-level disturbances (Erbe et al., 2018; Harwood et al., 2014; New et al., 2014). As WEAs are developed along the east coast of the United States and throughout this population's range, acoustic disturbances will increase, and assessing the cumulative effects will become a critical priority for decision makers (Kragh et al., 2019).
Tables

Table 2.1. Broadband (10 – 7080 Hz) and mid-frequency (2820 – 7080 Hz) sound levels (dB_{rms} re 1 μ Pa) in each sound level category at survey site A-2M from July 13 to August 29, 2018.

Broadband sound level category	Broadband sound level (dB _{rms} re 1μPa)	Mean ± SE mid- frequency sound level	Mid- frequency range (dB _{rms} re 1µPa)	Mid- frequency sound level category	Mid- frequency sound level (dB _{rms} re 1µPa)
				0	
1	84.0 - 92.4	89.7 ± 0.6	81.6 - 107.5	<u>1</u>	81.5 - 88.2
1 2	84.0 - 92.4 92.4 - 102.6	$\begin{array}{c} 89.7 \pm 0.6 \\ 92.2 \pm 0.5 \end{array}$	$\frac{81.6 - 107.5}{82.2 - 110.4}$	$\frac{1}{2}$	81.5 - 88.2 88.2 - 95.7

Table 2.2. The number of hours with whistles, number of whistles detected, and the number successfully localized in each broadband (10 - 7080 Hz) and mid-frequency (2820 - 7080 Hz) sound level (dB_{rms} re 1µPa) categories at survey site A-2M from July 13 to August 29, 2018.

Broadband sound level category	Total hours with whistles	Total number of whistles	Number of whistles successfully localized	Mid- frequency sound level category	Total hours with whistles
1	76	2,678	189	1	76
2	152	4,583	261	2	186
3	93	2,006	204	3	59

Table 2.3. Number of locations in each broadband (10 - 7080 Hz) and mid-frequency (2820 - 7080 Hz) sound level (SL) category used in the analysis of dolphin pod tracks and the number of hours and days from which the whistles in each broadband and mid-frequency sound level category originated.

Tracks							
	Broadband SL1	Broadband SL2	Broadband SL3	Mid- frequency SL1	Mid- frequency SL2	Mid- frequency SL3	
Number of locations	51	42	40	49	62	22	
Randomly selected 100 whistles per broadband sound level							
Number of hours	30	37	33	21	61	16	
Number of days	11	18	22	9	24	10	

	Cumulative Area (km ²)			
SL	25 th	50 th	75 th	90 th
Broadband				
1	45.58	113.05	228.23	370.80
2	54.52	128.80	251.51	412.17
3	43.10	112.45	251.13	466.06
Mid-frequency				
1	51.33	123.75	247.52	404.23
2	46.20	115.37	243.42	428.79
3	47.36	113.71	238.27	411.91

Table 2.4. Cumulative area (km²) for each percentile of the kernel density for the randomly selected whistles in each broadband (10 - 7080 Hz) and mid-frequency (2820 - 7080 Hz) sound level (SL) category.

Table 2.5. Results of the linear mixed-effects models for dolphin pod speeds in relation to the broadband (10 - 7080 Hz) or mid-frequency (2820 - 7080 Hz) ambient sound levels (SLs). The SL1 (lowest sound level) category was used as the reference level in each model.

Broadband	Coefficient value	Std. Error	DF	t-value	p-value	
Intercept	3.71	0.70	59	5.32	0.00	
SL 2	-0.66	1.02	32	-0.65	0.52	
SL 3	0.28	1.03	32	0.27	0.79	
Mid-Frequency						
Intercept	3.10	0.74	59	4.18	0.00	
SL 2	0.71	0.95	32	0.74	0.46	
SL 3	0.74	1.23	32	0.60	0.55	

Table 2.6. Mean and standard deviation (SD) of speed and straightness index (between 0 and 1) for each sound level category.

Sound level category	Broadband mean speed [mid- frequency mean speed] (m/s)	Broadband SD of mean speed [mid- frequency SD]	Broadband mean straightness index [mid- frequency straightness]	Broadband SD of straightness index [mid- frequency SD]
1	4.00 [3.23]	2.85 [2.42]	0.70 [0.74]	0.35 [0.28]
2	3.34 [4.03]	2.84 [2.98]	0.72 [0.71]	0.29 [0.36]
3	3 53 [3 57]	2 79 [3 20]	0 75 [0 72]	0 35 [0 30]



Figures

Figure 2.1. Map of study area including locations for the Marine Autonomous Recording Units (MARUs; green circles), leased Wind Energy Area (WEA; yellow), and the MARU's 15 km localization radius (dashed line).



Figure 2.2. Example of a reliable location estimate for dolphin whistles recorded on August 14, 2018. The red box indicates the first arrival (or reference selection) of the dolphin whistle, and the purple boxes (placed by the Correlation Sum Estimation (CSE) localization algorithm) indicate the predicted arrival times of the reference dolphin whistle in the synchronized localization array. Spectrogram parameters: 512-point fast Fourier transform (FFT), Hann window, 90% overlap, 15-second duration.





Figure 2.3. Mean and standard error of the distances between the five locations for each of the 100 randomly selected whistles in each (a) broadband (b) and mid-frequency sound level (SL) category, and (c) median distance between location results in relation to the median distance from the center of the localization array with a linear line of best fit (black line, confidence interval (95%): shaded grey).





Figure 2.4. Plot of kernel densities for localizations of 100 randomly selected dolphin whistles (black circles) during low (a; 84.0 - 92.4 dB_{rms} re 1 μ Pa; SL1), medium (b; 92.4 – 102.6 dB_{rms} re 1 μ Pa; SL2), and high (c; 102.6 – 126.1 dB_{rms} re 1 μ Pa; SL3) hourly broadband (10 – 7080 Hz) ambient sound levels.

c)





Figure 2.5. Plot of kernel densities for localizations of randomly selected dolphin whistles (black circles) during a) low ($\leq 88.2 \text{ dB}_{rms}$ re 1µPa: SL1), b) median ($88.2 - 95.7 \text{ dB}_{rms}$ re 1µPa; SL2), and c) high ($\geq 95.7 \text{ dB}_{rms}$ re 1µPa; SL3) hourly mid-frequency (2820 - 7080 Hz) sound levels, and d) the Maryland Wind Energy Area (WEA; white), 100 randomly selected whistle localizations under higher ($102.6 - 126.1 \text{ dB}_{rms}$ re 1µPa) broadband (10 - 7080 Hz) sound conditions, and tracks of all vessels utilizing AIS in 2018 (MarineCadastre.gov, coast.noaa.gov/digitalcoast/data/).



Figure 2.6. All dolphin movement tracks used in analysis (broadband sound level (SL)1: red circle, SL2: blue diamond, SL3: green square, beginning of the track: open black circle) and Maryland Wind Energy Area (WEA; yellow).



Figure 2.7. Box plots of the dolphin pod speeds (m/s) in relation to the a) broadband (10 - 7080 Hz) and b) mid-frequency (2820 - 7080 Hz) ambient sound levels (SLs).



b)

Figure 2.8. Straightness Index (± standard error) for each dolphin track in relation to a) broadband and b) mid-frequency ambient sound levels (SLs) with values nearer to 1.0 indicating a more direct path of travel.

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Chapter 3: Bottlenose dolphin signature whistle identification, use as a measure of abundance, and determination of site and regionally specific acoustic characteristics.

Introduction

Advances in our ability to record and process large volumes of acoustic data (Blumstein et al., 2011; Luczkovich et al., 2008; Stowell et al., 2019) have made passive acoustic monitoring an increasingly important method of environmental monitoring. In habitats where visual monitoring is challenging and animals vocalize frequently, passive acoustic monitoring is particularly beneficial. However, we still know relatively little about how organisms' vocalizations may vary in different environments and under changing acoustic conditions (Fouda et al., 2018; Slabbekoorn & Peet, 2003).

A highly vocal group of species in the marine environment are marine mammals. In addition to detecting their presence from their calls, some species have individual- (Janik & Sayigh, 2013) and pod-specific calls, allowing detection of individuals (Filatova et al., 2015; Ford & Fisher, 1982; Scheer et al., 1998). The vocal and social bottlenose dolphin (*Tursiops truncatus*) produces individually-distinct calls called signature whistles (Sayigh et al., 1990). These whistles comprise more than half of all the whistles they produce in the wild (Cook et al., 2004; Smolker et al., 1993). Signature whistles are formed in the first year of the dolphin's life (Caldwell et al., 1990) and remain relatively stable throughout their lifetime (Caldwell & Caldwell, 1968; Sayigh et al., 1990). Use of signature whistles in captive (Gridley et al., 2014; Janik et al., 2013; Lopez-Marulanda et al., 2020; Rachinas-Lopes et al., 2017) and free-ranging animals (Bailey et al., 2021; Erbe et al., 2020; Gridley et al., 2014; Janik et al., 2013; Kriesell et al., 2014; Longden et al., 2020; Quick & Janik, 2012) has been documented. These unique whistles have been used to both identify (Janik & Sayigh, 2013; Quick & Janik, 2012) and to estimate the number of individuals within a population (Longden et al., 2020).

Signature whistles can be identified by the repetition (called a bout) of the same whistle (at least 75% similarity), at least one second apart and typically within 5 to 10 s (Janik et al., 2013). Some studies have used stricter criteria for classifying signature whistles such as requiring 3 to 5 whistles in a bout (Longden et al., 2020; Lopez-Marulanda et al., 2021; Rachinas-Lopes et al., 2017). The method of determining reoccurrences of signature whistles varies from fully manual to a combination of manual and automated matching. The ability of these criteria to identify the signature whistles of captive dolphins without isolation of individuals has not yet been tested (Janik et al., 2013; Rachinas-Lopes et al., 2017).

Signature whistles have been used to verify population sizes for small (< 100 individuals), resident (Erbe et al., 2020; Kriesell et al., 2014; Longden et al., 2020) populations by comparing simultaneous photo-identification and acoustic recordings containing signature whistles (Erbe et al., 2020; Kriesell et al., 2014). This method has not yet been tested on larger, free-ranging populations. In orcas (*Orcinus orca*), family pods are differentiated based on the characteristics of their vocalizations (Ford & Fisher, 1982). While some examination of dolphin vocalization differentiation has been examined (Campbell, 2004; Morisaka et al., 2005), it is not yet known whether population-specific differences occur in bottlenose dolphin's signature whistles.

The Atlantic coast of the USA is home to 19 different populations of bottlenose dolphins (Hayes et al., 2021). The region along the coasts of Delaware, Maryland, and Virginia, or the southern Mid-Atlantic Bight, is one of the least studied areas for bottlenose dolphins on the east coast of the USA. This study aims to determine 1) the efficacy of the current methodology for detecting and matching bottlenose dolphin signature whistles, 2) whether detection of individual

signature whistles can be used as a proxy for the group size of free-ranging animals, 3) a minimum estimate of the number of bottlenose dolphins in the Chesapeake Bay and waters of coastal Maryland, 4) whether there are site- or regionally-specific differences in signature whistle characteristics and how they relate to existing population boundaries, and 5) whether individual dolphins alter their signature whistles in response to ambient sound conditions. The findings from this study will help to determine bottlenose dolphin identities and populations from signature whistles and has implications for population management in this urbanized waterway and in an area proposed for offshore wind development.

Methods

Signature whistle detection and matching efficacy

To determine how effectively I could detect and match signature whistles when a known number of dolphins were present, I recorded the vocalizations of six bottlenose dolphins at the National Aquarium in Baltimore, Maryland, USA. Two dolphins (one female; Bayley and one male; Beau) were suspected of having signature whistles. It was not known whether the other four dolphins (three females and one male) produced signature whistles. All dolphins were captive-born but have lived with dolphins who were wild-born. Their ages ranged from to 13 to 29 years old. The youngest female (13 years old, Bayley) and male (14 years old, Foster) were the offspring of the two eldest females (Chesapeake, 29 and Jade, 22 years old, respectively). Beau (16 years old) and Spirit (20 years old) share a mother but have different fathers.

To obtain recordings of the dolphins' vocalizations, a cabled hydrophone (Aquarian H2a-XLR, Aquarian Hydrophones, WA, USA) was positioned behind a metal gate in a training pool adjacent to the dolphins (1 m below surface in 2 m of water). The hydrophone sampled at 48 kHz with a sensitivity of 180 dB re $1V/\mu$ Pa. The hydrophone was attached to a Zoom U-22 audio interface (Zoom North America, NY, USA), which allowed a variable gain (between 7 and 10 dB re 1μ Pa, manually adjusted within and between recordings as needed), and the recording was saved using Adobe Audition (Version 11.0.2.2, CA, USA).

Recordings were taken during the dolphins' sessions with their trainers (training sessions) as well as during free swim periods when all dolphins socialized normally in their groups (females together and males together in separate tanks). During training sessions, a trainer positioned each dolphin as close to the hydrophone as possible and signaled the dolphin to produce their whistle or a vocalization. The time of the vocalization and type (if known) were recorded. Vocalizations from other individuals were noted whenever possible to assist with the matching of vocalizations to individual dolphins. All individuals of the same sex were in the same pools during training periods and during free swims.

All audio recordings were manually inspected in Raven 2.0 Interactive Sound Software (Cornell University, NY, USA) for the presence of signature whistles meeting the SIGnature IDentification (SIGID) criteria (Janik et al., 2013). The SIGID criteria for identifying signature whistles requires that whistles with 75% similarity appear in a repeated pattern of two or more whistles (bout; (Cook et al., 2004) within 1 -10 seconds of the same whistle and with a duration of at least 0.2 seconds (Gridley et al., 2014; Janik & Sayigh, 2013; Fig. 3.1). Whistles that appeared during the free swim recordings and met the SIGID criteria were considered signature whistles, regardless of whether they were produced when trainers signaled them to do so.

Using the methodology of (Bailey et al., 2021), signature whistles were contoured using Beluga software (https://synergy.st-andrews.ac.uk/soundanalysis) and analyzed with ARTwarp (Deecke & Janik, 2006) to determine reoccurrences. All signature whistles were contoured and processed in ARTwarp at vigilance (minimum matching criteria) thresholds of 75, 80, 90, and 94%. The ARTwarp results were compared to the manual comparisons of signature whistles. The number of true positive reoccurrences was calculated as the percentage of ARTwarp whistle reoccurrences that matched the manual categorizations.

Signature whistles as an indicator of group size

To determine whether the number of bottlenose dolphin signature whistles could be used as a proxy for the group size, visual sightings of dolphins were compared to the number of unique signature whistles during corresponding times. Visual sighting data from the Potomac-Chesapeake Dolphin Project (pcdolphinproject.org) included the start location and time as well as the number of dolphins present (group size) as indicated by individually identifiable dorsal fin photographs. The number of unique dorsal fin photographs was treated as the true number of dolphins present whenever available. When dorsal fin photographs were not available (if a group was sighted from shore, for example), a best estimate of group size taken by the survey team was used. Pseudo-replication of individuals in photographs was possible when sightings occurred in close spatial or temporal proximity and individuals possibly moved between subgroups. Signature whistles were recorded in the Potomac River (Site 4; Fig. 3.2) on a Snap acoustic recorder (Loggerhead Instruments, FL, USA) and identified using the SIGID methodology.

The dolphin group size was compared to the number of unique signature whistles during the time of the sighting and in adjacent hours if they contained signature whistles. For example, if a sighting occurred between 0900-1000 and signature whistles were detected between 0800 and 1100, the signature whistles in all four hours were included in the comparison. Sightings that occurred less than an hour apart were combined into a single encounter. The distance from the start location of the visual sighting to the acoustic recorder at Site 4 was also calculated for each encounter.

Linear regression models (lms) and generalized additive models (GAMs) were applied in R (version 4.1.0) to ascertain the relationships between a) the number of unique signature whistles and the group size, and b) the distance between the sighting and the acoustic recorder and the difference between the number of unique signature whistles and the group size. I also included in the GAM the presence of foraging (as a percentage of hours containing either presence (1) or absence (0) of foraging) and the number of hours with signature whistles. Foraging was determined by the presence of feeding buzzes, or when dolphin clicks occurred with an inter-click interval of \leq 9.9 ms (Bailey et al., 2019).

Signature whistles as a measure of minimum abundance

Passive acoustic monitoring occurred at five sites: two in the southern coastal Mid-Atlantic Bight (blue triangles; Sites 1, 2, Coastal region), two in the Chesapeake Bay (black circles; Site 3, 4, Bay region), and one offshore in the southern Mid-Atlantic Bight (green square; Site 5, Offshore region; Fig. 3.2). Sites 1, 2, and 5 were located 12, 31, and 64 km east of Ocean City, Maryland, USA, respectively (Fig. 3.2). Water depths at these sites ranged from approximately 20 - 42 m, and the acoustic recording instruments were deployed approximately 1 m above the ocean floor using bottom-anchored moorings (Garrod et al., 2018).

Details on acoustic data recording and identified signature whistles from 2016 through 2018 at Sites 1, 2, and 3 were described in (Bailey et al., 2021). Snap acoustic recorders (Loggerhead Instruments, FL, USA) were deployed at Sites 4 and 5. At Site 4 in the Potomac River, the Snap was deployed in approximately 3 m of water from June to September 2019. At Site 5, 64 km from the Maryland coastline, the Snap was deployed from July to September 2018 in 42 m of water. At both sites, the Snap was approximately 1 m above the seabed sampling at 48 kHz with a sensitivity of 180.2 dB re $1V/\mu$ Pa and a gain of 2.05 dB re 1μ Pa. To increase the time span of the recordings, the Snap was duty-cycled for two minutes on and eight minutes off at Site 4 and one minute on and nine minutes off at Site 5. At Site 4, recordings were investigated for signature whistles only on days during which a visual sighting occurred. At Site 5, the PAMGUARD Whistle and Moan Detector (Gillespie et al., 2011) was utilized to determine hours with possible dolphin whistle presence and these hours were manually searched for signature whistles.

The SIGID criteria and Bailey et al. (2021) methodology were utilized to identify signature whistles and determine when they reoccurred. Signature whistles were considered reoccurrences when they occurred on different days or in different locations. When signature whistles reoccurred within a site, the whistle was only counted as one unique whistle for that location. Only the best whistle, that which had the highest signal-to-noise ratio and clearest shape, was used in further analyses. Signature whistle catalogs (containing only the best signature whistles) from each site were matched against all other sites to determine reoccurrences.

Acoustic characteristics of signature whistles

To determine the acoustic characteristics of signature whistles from each site, I selected a subset of 100 signature whistles from each site (or the number of unique signature whistles identified if <100) whose full shape was clear. Manual measurements were taken of the duration, start, end, maximum, minimum, and delta frequencies (maximum minus minimum frequency), and number of local extrema (the start and end of the whistle were considered local extrema) for each whistle (similar to Fouda et al., 2018; Kriesell et al., 2014; Fig 3.3). The distribution of the signature whistles' characteristics were analyzed for normality using Shapiro-Wilk tests, and

Box-Cox analyses (MASS package in R; Venables & Ripley, 2002) were used to determine whether transformations of the characteristics were necessary for parametric analyses.

A multivariate analysis of variance (MANOVA) in R was utilized to determine whether the whistle characteristics (response variables) were related to the site (Sites 1-5) or region (Bay, Coastal, Offshore) at which the signature whistle was detected (explanatory variables). Post-hoc pairwise t-tests with Bonferroni adjusted p-values were used to investigate which and how signature whistle characteristics varied between sites and regions. The Boruta package in R (Kursa & Rudnicki, 2010) utilizes random forest models to assess factor importance in determining an identity (e.g. site or region) and was employed to determine the relative importance of each signature whistle characteristic in that process. The maximum number of runs for the Boruta model was 10,000.

Signature whistle variation in relation to ambient sound levels

To understand whether bottlenose dolphins alter their signature whistles in response to ambient sound levels, a subset of 100 clear signature whistles that reoccurred at least once, either at the same or at a different site, were selected for acoustic characteristic measurement (duration, start, end, maximum, minimum, and delta frequencies, number of local extrema). Generalized estimating equations-generalized linear models (GEEs) in R (geepack package in R; Højsgaard & Halekoh, 2006) were used to determine which signature whistles characteristics were affected by changes in ambient sound levels. The identity of the signature whistle was treated as a cluster and an exchangeable correlation structure was used in the GEEs. Ambient sound levels were calculated as the broadband (up to 24 kHz) sound pressure level (SPL; dB re 1µPa root-meansquare (rms)) during the recording in which the signature whistle occurred.

Results

Signature whistle detection and matching efficacy

Dolphins at the National Aquarium were acoustically recorded for 384 minutes during six free swim sessions over three days (mean recording length per session = 64.11 min, sd = 25.35 min). Male and female dolphin training sessions occurred separately for a total of 181 minutes and 17 training sessions (86.33 min, 7 sessions for females, mean = 12.33 min per training session, sd = 3.96 min; 94.42 min, 10 sessions for males, mean = 9.44 min per training session, sd = 3.77 min). Six unique signature whistles were manually identified during free swim recordings (Table 3.1), which matched the number of dolphins at the National Aquarium. Because of the placement of the hydrophone in an adjacent tank, many recordings of signature whistles (36%) had low signal-to-noise ratios (SNRs) and were therefore unable to be contoured and matched with other whistles using ARTwarp (Table 3.1).

Whistles produced during training sessions by the two captive dolphins suspected of having signature whistles (Bayley and Beau) were present in the recordings when dolphins were freely socializing (Table 3.1). Beau's signature whistle appeared in the training session as a repeated pattern (whistle b) of the single-looped signature whistle (whistle a) produced during the free swim recordings. Foster repeated the other male dolphin's (Beau's) looped signature whistle (Beau whistle b) when signaled by the trainer to whistle. When Beau's signature whistle was recorded in training sessions, the number of repetitions varied between two and ten looped whistles (e.g. whistle b). The signature whistles from other dolphins were identified during free swim sessions and I attempted to assign them to an individual dolphin based on noted vocalizations during the training sessions. The signature whistle suspected of belonging to Foster was found most often, appearing in five of the six free swim recording sessions (83%; Table 3.1). Bayley's signature whistle was recorded in a signature whistle pattern in three recordings and singly (i.e. not in a bout) in a fourth recording (67%). The signature whistle suspected of belonging to Jade was found in three of six free swim recordings (50%) and ARTwarp was most successful in classifying this whistle (100% true positive classifications; Table 3.1). Beau's signature whistle was recorded in only one free swim recording in the signature whistle pattern and in one session singly (33%). The other two signature whistles (suspected of belonging to Chesapeake and Spirit) were recorded in only one free swim session each. Only one recording contained all six signature whistles (though one whistle was not in a bout). There was also one whistle that appeared in a signature whistle may have been produced by Chesapeake or Spirit, but there were no noted vocalizations for any particular dolphin at the times when the whistles occurred.

An ARTwarp vigilance threshold of 90% yielded the highest true positive detection rate (ARTwarp matched the manual analysis), correctly matching 100% of Jade's signature whistle (n = 5), 57% of Foster's signature whistle (n = 7; Table 3.1), and 50% of all signature whistles. At a 94% vigilance threshold, ARTwarp correctly matched 38% of all signature whistles contoured, and at 75% and 80% vigilance, ARTwarp correctly matched 42% of signature whistles. Because Chesapeake and Spirit's signature whistles appeared only once in the recordings, I could not evaluate ARTwarp's ability to correctly match these whistles.

Signature whistles as indicators of group size

A total of 81 visual sightings of bottlenose dolphins were paired with 25 hours that contained signature whistles and 55 hours that did not. When the sightings and signature whistles were combined into encounters, 72 comparisons were made between visual group sizes (1-4 sightings per encounter) and the number of unique signature whistles (0-2 hours containing signature whistles per encounter). Within these comparisons, 55 encounters contained no signature whistles (76.4%) and 45 contained no dolphin echolocation clicks (62.5%). When dolphin clicks were detected during the encounter, foraging occurred 44.4% of the time (n = 20).

The group size of dolphin pods ranged from 1 to 90 dolphins (mean = 18, sd = 20) and the number of unique signature whistles ranged from 0 to 14 (mean = 2, sd = 3). There were three occasions on which more signature whistles were recorded than dolphins visually observed. One sighting that occurred nearly 20 km from the hydrophone was not included in analyses. When group size was larger than the number of signature whistles, the difference between these two ranged from 1 to 86 (mean = 17, sd = 20).

The number of unique signature whistles detected had a significant, positive, linear relationship with the dolphin group size ($R^2 = 0.064$, $F_{1,70} = 4.75$, p = 0.03), but this relationship only explained 6.4% of the variance between these two variables (Fig. 3.4a, Table 3.2 model a). The difference between the group size and the number of signature whistles did not increase as the distance between the sighting and the acoustic recorder increased (Table 3.2 model b). A GAM including the number of signature whistles detected, hours with signature whistles, percentage of foraging during the encounter, and distance between the sighting and the recorder as the explanatory variables did not have any significant relationship with group size as the response variable (Table 3.2 model c). The number of dolphins observed (group size) increased as the duration of the encounter increased (Table 3.2 model d, Fig. 3.4c).

Signature whistles as an indicator of minimum abundance

Previous research at Sites 1, 2, and 3 detected a total of 1,172, 327, and 19 unique signature whistles, respectively (Bailey et al., 2021). A total of 333 unique signature whistles were detected at Site 4 in the Potomac River in the Chesapeake Bay, and 38 at the offshore site, Site 5 (Table 3.3). These counts of signature whistles represent a minimum abundance of dolphins at these sites.

Acoustic characteristics of signature whistles

The distribution of the signature whistle characteristics (start, end, maximum, minimum, and delta frequencies, duration, number of local extrema) were non-parametric (Shapiro-Wilk test; p < 0.05). Box-Cox analyses indicated that square root (sqrt) transformations were appropriate for the start, end, minimum, and maximum frequencies, and log transformations were appropriate for the end frequencies, duration, and number of local extrema. The data were tested again for normality after transformation, but only the start, minimum, and delta frequencies became normally distributed. Because the distribution of end frequencies remained non-parametric after transformation, a sqrt transformation was used instead to keep the scale of this characteristic consistent with the other frequency measures. Only transformed values of signature whistle characteristics were used in the subsequent analyses of these whistles.

A MANOVA indicated that start ($F_{4,352} = 2.71$, p = 0.03), end ($F_{4,352} = 4.52$, p = 0.01), and minimum frequencies ($F_{4,352} = 10.3$, p < 0.01) as well as duration ($F_{4,352} = 9.36$, p < 0.01) and number of extrema ($F_{4,352} = 7.28$, p < 0.01) of signature whistles varied significantly by site. Pairwise t-tests indicated that the start and end frequencies of signature whistles were higher (Fig. 3.5a, b) and delta frequencies were lower (Fig. 3.5d) at Site 5 compared to Site 4. Minimum frequencies of signature whistles at Site 5 were higher than all other sites (Fig. 3.5c), and
signature whistles from Site 2 had significantly longer durations than whistles from Site 1, 3, and 4 (Fig. 3.5e). The number of extrema were significantly fewer in signature whistles from Site 5 compared to any site other than Site 3, and there were significantly more local extrema at Site 1 compared to Site 2 (Fig. 3.5f).

A MANOVA also indicated that start, end, and minimum frequencies as well as the duration and number of extrema varied significantly by region. Offshore signature whistles had significantly higher start, end, and minimum frequencies compared to those from the Coastal and Bay regions (Fig. 3.6a, b, c). The duration of signature whistles from the Bay were shorter than those from the Coastal or Offshore regions (Fig. 3.6d), and Offshore signature whistles had significantly fewer extrema than the Bay or Coastal whistles (Fig. 3.6e).

All signature whistle characteristics were deemed important for determining site identity, but minimum frequency and duration were the most important factors (Fig. 3.7a). The duration, minimum, maximum, end, and delta frequencies were the important characteristics in determining the region identity of signature whistles (Fig. 3.7b). Consideration of the start frequency and number of extrema decreased the performance of the model when determining the region to which a signature whistle belonged (Fig 3.7b).

Median daily sound levels in all sites and regions were significantly different from one another (p < 0.01). Site 1 was loudest (median daily sound level of 131 dB re 1µPa, range (lowest minimum to highest maximum) = 123-141 dB) followed by Site 5 (median daily sound level = 127 dB re 1µPa, full range = 126-140 dB), Site 2 (median = 114 dB re 1µPa, full range = 109-141 dB), Site 3 (median = 114 dB re 1µPa, full range = 88-145 dB), and Site 4 (median = 109 dB re 1µPa, full range = 88-161 dB; Fig. 3.8). The Offshore region had the second highest daily median ambient sound levels (Fig. 3.8) and the highest median daily minimum sound levels (127 dB re 1 μ Pa, sd= 0.2 dB). Site 4 had the largest range in sound levels (Fig. 3.8).

Signature whistle variation in relation to ambient sound levels

In total, 201 unique signature whistles reoccurred a total of 252 times at the five different sites (Fig. 3.9). The largest number of reoccurrences occurred between Sites 1 and 2 (n = 49; 15% of all unique signature whistles from Site 2) and Sites 1 and 4 (n = 30, 9% of unique signature whistles from Site 4; Fig. 3.9). Of the 19 unique signature whistles at Site 3, 26% (n = 5) reoccurred at Site 4 (Fig. 3.9). Of the 333 unique signature whistles identified at Site 4, 24 unique signature whistles reoccurred 27 times within the site. Six signature whistles from Site 4 reoccurred at Site 2 (2% of all Site 4 unique signature whistles; Fig. 3.9). Of the 38 unique signature whistles detected at Site 5, three signature whistles reoccurred within the site. There were three reoccurrences between Site 5 and Site 2 (two of the same whistle; 5% of all unique signature whistles from Site 5) and one at Site 1 (3%; Fig. 3.9).

The random selection of 100 unique signature whistles included 230 total whistle occurrences: 132 whistles at Site 1, 49 at Site 2, 9 at Site 3, 36 at Site 4, and 4 at Site 5. The duration of signature whistles significantly varied in relation to changing ambient sound levels, decreasing as sound levels increased (Estimate = -0.005, S.E. = 0.003, Wald = 4.08, p = 0.04).

Discussion

I found that bottlenose dolphin signature whistles can be effectively used to provide a minimum estimate of the number of individual dolphins present in a region. This use of signature whistles give larger underestimations of the number of animals as the group size increases, likely due to the same number of individuals calling when many animals are present. I also determined

that the characteristics of signature whistles significantly varied by site and region, which may represent acoustic differences amongst the signature whistles of estuarine, coastal, and offshore bottlenose dolphin populations. Notably, the same individual adjusted the duration of its signature whistle in relation to the ambient sound level when it was emitted. This is the first quantitative investigation into the effects of location and ambient sound levels on individually identifiable dolphin calls.

The identification of signature whistles provides a non-invasive approach for the continuous monitoring and detection of individual bottlenose dolphins (and some other delphinids), particularly in locations where visual surveys and photo-identification methods may be difficult, such as offshore. The SIGID method used to detect signature whistles gives guidelines for identifying and matching signature whistles to one another but acknowledges that these criteria may identify only about half of signature whistles (Janik et al., 2013). While signature whistles are typically emitted between 1 and 10 seconds apart, they can be separated by more than 89 minutes, and 100 renditions of a signature whistle may not result in successful classification based on the SIGID detection methods (Janik et al., 2013). This finding was verified by my recordings from the National Aquarium, during which signature whistles were sometimes emitted in patterns that did not fit the standard SIGID criteria. These animals' propensity for location and context-specific vocalization variation may further confound detection of signature whistles. At the National Aquarium and in the Potomac River, I found that the emission rates of signature whistles varied depending on the individual dolphin and on the group size.

This study tested the SIGID methodology's ability to identify and match the signature whistles of six dolphins in captivity. Dolphins in captivity may have less complex whistles

compared to free-ranging individuals (Miksis et al., 2002), and some dolphins in captivity do not utilize their signature whistles (Rachinas-Lopes et al., 2017) or may not have them (Quick & Janik, 2008). Signature whistles from all captive dolphins at the National Aquarium were not present in every recording, indicating that they do not use these whistles as often as free-ranging populations (Cook et al., 2004; Smolker et al., 1993) and may whistle less overall (Quick & Janik, 2008). There was high inter-dolphin variability in whistle emission rates and no dolphins emitted their whistle in every recording. Signature whistles from the youngest male (Foster) and youngest female (Bayley) were recorded most often.

For use of signature whistles for identification of individuals, as in the use of dorsal fins photographs, the probability of obtaining an identity for every individual during a survey is not equal; some individuals will be less averse to boats and have more distinctive fin features than others. Similarly, dolphins that have more distinct signature whistles and emit them frequently are more likely to be identified. Additionally, as observed when Foster emitted the looped pattern of the Beau's signature whistle at the National Aquarium, some paired male dolphins may share or have highly similar signature whistles (Smolker & Pepper, 1999; Watwood et al., 2004) or imitate the whistle of a con-specific (Janik, 2000). In free-ranging individuals, instances of imitation in which the true owner of the signature whistle is not known can confound attribution of identities based solely on signature whistles. Similarly, I was unable to record dolphins in isolation at the National Aquarium, so whistles could have been emitted by any dolphin, making attribution challenging.

Whether the process is manual or automated with manual validation, signature whistle identification and matching is currently a subjective practice. While agreement on matches of signature whistles between analysts is typically high (Erbe et al., 2020; Kriesell et al., 2014),

manual matching is time intensive. Automated matching of signature whistles with ARTwarp provides possible matches for manual validation and is best suited for large catalogs of whistles when manual matching of all whistles is not feasible. While other studies have suggested whistle matching thresholds of 94% (Bailey et al., 2021) or 75% (Janik et al., 2013), the appropriate value depends on the recording conditions, especially the ability to obtain recordings of whistles with high SNRs making the whistle's shape clearly discernable. Low SNR whistles from the National Aquarium resulted in difficulties both in identifying the contour of the whistle and in manual matching (Morrison et al., 2020).

Less than two-thirds of signature whistles from captive dolphins at the National Aquarium could be contoured, primarily due to low SNR whistles. I was unable to place the hydrophone in the tank with the dolphins out of concern for them damaging it or injuring themselves. When dolphins were positioned close to the hydrophone, the full whistle was clear, despite the hydrophone being behind a metal gate. However, when dolphins were socializing in another pool during the free swim sessions, the full whistle was often not clearly recorded, making analysis challenging. Thus, the strict methodology of SIGID may need refining for challenging recording environments that prohibit clear visualizations of whistles. It may be beneficial to contour all whistles in a bout and employ an automated analysis software such as ARTwarp to match these whistles and determine the vigilance threshold appropriate for that environment. Advances in machine learning may allow the development of pipelines to perform signature whistle identification and matching from acoustic files, helping to reduce the currently time-intensive effort.

In the Chesapeake Bay's Potomac River, the number of unique signature whistles generally represented the minimum number of dolphins present and underestimated the group

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size by a larger amount as the number increased. A previous study indicated that the number of unique signature whistles is significantly related to the total number of dolphins in the group, although it consistently underestimates the number of dolphins present (Kriesell et al., 2014). As expected, individual call rates increase to a certain group size then decrease in larger groups (Quick & Janik, 2008). Bottlenose dolphins are rarely found singly and have potential group sizes of as many as 10,000 individuals in other regions (Scott & Chivers, 1990), so I would not expect individuals to vocalize concurrently, as this would result in the masking of calls.

Call rates can also vary depending on behavioral states (Cook et al., 2004; Janik & Slater, 1998; Marley et al., 2017; Quick & Janik, 2008). Dolphins engaged in surface travel can have either lower (Quick & Janik, 2008) or higher (Cook et al., 2004) calls rates, and when calves are present, mother-calf pairs frequently emit signature whistles to maintain contact but may emit fewer whistles overall (Kriesell et al., 2014; Marley et al., 2017; Van Parijs & Corkeron, 2001). While other studies have suggested that bottlenose dolphins call more frequently when foraging (Acevedo-Gutiérrez & Stienessen, 2005), this behavior was not found to be a significant factor in determining dolphin group size. Assessing the total number of whistles present or the sound levels in the frequency band containing whistles may also aid in determining the true abundance of dolphins present. A conversion factor to calculate the group size based on the number of unique signature whistles detected was not established in my study, but signature whistles may be used to obtain a minimum group size.

Measurements of the acoustic characteristics of signature whistles in this region adds to a growing understanding of dolphin communication for both signature (Campbell, 2004; Esch et al., 2009; Kriesell et al., 2014) and non-signature whistle types (Fouda et al., 2018; Tellechea et al., 2014) worldwide. Signature whistles from bottlenose dolphins in this study area were longer

(0.16-4.42 s) and had more varied minimum (1656-13254 Hz) and maximum frequencies (4026-23109 Hz) compared to signature whistles from Walvis Bay, Namibia (0.46-2.35 s, 2590-8230 Hz, 9880-20060 Hz (Esch et al., 2009) and Sarasota Bay, Florida, USA (0.5-2.3 s, 3000-13300 Hz, 9300-27300 Hz; Kriesell et al., 2014). Signature whistles from this study area also had mean minimum (5757 Hz), maximum (12997 Hz), and delta frequencies (7240 Hz) similar to signature whistles from Turneffe Atoll, Belize (5320 Hz, 12850 Hz, 7530 Hz, respectively) and similar durations (0.91 s) to whistles from San Diego, California, USA (1.26 s; Campbell, 2004).

The importance of signature whistles in communicating an individual's identity may necessitate its acoustic differentiation from other whistle types. A random selection of whistles from Site 2 in this study area (Fouda et al., 2018) were similar in duration (0.40 s) and maximum frequency (10075 Hz) to a random selection of whistles from Uruguay (0.628 s, 9720 Hz; Tellechea et al., 2014), but different from the signature whistles at Site 2. Signature whistles at Site 2 were generally longer (1.13 s), had lower minimum (5966 Hz), higher maximum (12750 Hz), and larger delta frequencies (6784 Hz) than that random selection of whistles from the same site (0.40 s, 6792 Hz, 100075 Hz, 3282 Hz; Fouda et al., 2018). This dissimilarity of characteristics between a random selection of whistles and the signature whistles from Site 2 suggests that there are some acoustic properties of signature whistles. This is further supported by the emission of a signature whistle singly (i.e. not in a bout) by dolphins at the National Aquarium and elsewhere (Janik et al., 2013; Kriesell et al., 2014).

Characterizing signature whistles may allow for the acoustic differentiation of populations or stocks. This is especially valuable in areas of population overlap such as the Mid-Atlantic Bight and the Chesapeake Bay. My study area is within the home range of up to four different populations of bottlenose dolphins; the Western North Atlantic (WNA) Northern Migratory Coastal, WNA Southern Migratory Coastal, Northern North Carolina Estuarine System, and the WNA Offshore stocks. The numerous differences in the acoustic characteristics of signature whistles from bottlenose dolphins in the Offshore region (Site 5, 42 m depth) and lack of reoccurrences of these whistles in the Chesapeake Bay suggests that these dolphins may be from the WNA Offshore stock. I would expect the WNA Offshore population to be found only occasionally inshore, consistent with the finding that offshore populations are morphologically different and bathymetrically isolated (at depths greater than 25 or 34 m depending on the region) from the coastal populations (CETAP, 1982; Garrison et al., 2003; Kenney, 1990; Torres et al., 2003). The movement patterns and number of individuals in this offshore population are poorly understood. Signature whistle analysis may allow us to track individuals over time and space, which is especially valuable in this area where visual methods of tracking are challenging and offshore renewable energy is being developed.

Large numbers of bottlenose dolphins seasonally inhabit the Chesapeake Bay (Barco et al., 1999; Rodriguez et al., 2021), and acoustic niche partitioning (Hart et al., 2020; Krause & Farina, 2016) in signature whistles may be occurring (Campbell, 2004; Luís et al., 2021) due to population overlap. However, it is still unclear to which population(s) these bottlenose dolphins in the Bay belong. Population-level variation in vocal calls (i.e. vocal dialects) have been detected in multiple species (Astrom & Stolt, 1993; Barker et al., 2021; Ford & Fisher, 1982; Marler & Tamura, 1962) and are likely utilized in this highly vocal and mobile species. Some signature whistles, but not all, detected in the Chesapeake Bay (Sites 3 and 4) also occurred in the Coastal region (Sites 1 and 2), suggesting that the Chesapeake Bay may be a mixing ground for multiple populations including the WNA Northern Migratory Coastal, WNA Southern Migratory Coastal, and Northern North Carolina Estuarine System stocks. It also indicates that the ranges of these populations may be more extensive than previously thought and reconsideration of stock delineations may be necessary.

The importance of identifying the stock identity of dolphins in the Chesapeake Bay is further necessitated by the evidence of calves (Barco et al., 1999). Expanding the study area to the north and to the south would extend into the known ranges of other populations of this species and offer the opportunity to record signature whistles from individuals who have been photo-identified. The feasibility of using signature whistles and their characteristics for stock assignment could then be determined, allowing a better understanding of stock structure, which is vital for management and consideration during environmental impact assessments.

High ambient sound levels (> 120 dB re 1 μ Pa) were recorded in my study area, and the duration of dolphin signature whistles decreased as ambient sound levels increased. Other investigations of the effects of sound levels on dolphin whistle characteristics (not specific to signature whistles) have found that as sound levels increased, delta frequencies increased and the number of extrema decreased (Fouda et al., 2018; Marley et al., 2017). While an increase in maximum frequency in response to higher sound levels was found in some studies (Fouda et al., 2018; Marley et al., 2017; van Ginkel et al., 2018), the maximum frequency of some signature whistles in this region were near the highest recorded values for a whistle's frequency (Caldwell et al., 1990) and the upper limit of our sampling frequency. Thus, alternative acoustic compensation strategies such as shortening the duration of signature whistles may have been necessary (Fournet et al., 2021; Hotchkin et al., 2013; Krahforst et al., 2016; Miksis-Olds & Tyack, 2009; Picciulin et al., 2012). These compensation strategies, however, may not be adequate to avoid signal masking (Templeton et al., 2016).

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Offshore, where median daily ambient sound levels were highest, signature whistles were higher frequency (start, end, minimum) and simpler (with fewer extrema). Whistles with higher frequencies (Fouda et al., 2018; van Ginkel et al., 2018) and fewer extrema (Fouda et al., 2018; Marley et al., 2017) are common where low frequency ambient sound levels are higher. Large vessels and high sea states are the primary contributors to consistently high ambient sound levels at this location near the vessel separation schema leading into the busy Port of Wilmington, Delaware. A location offshore New Jersey, USA, only 130 km north of my study region, had the highest ambient sound levels and sound levels more consistently above 120 dB compared to nine other sites along the Atlantic Coast of the USA (Rice et al., 2014). New Jersey's soundscape, whose elevated sound levels were attributed to an abundance of vessel traffic (Rice et al., 2014), is similar to that of this study's Offshore region. Dolphins of this region are likely utilizing higher frequency whistles to avoid acoustic masking from vessel traffic (Ansmann et al., 2007; May-Collado & Wartzok, 2008).

Sound levels in the Chesapeake Bay were highly variable, but had lower median sound levels compared to the Coastal and Offshore regions. As in other coastal and shallow water regions (Haviland-Howell et al., 2007), higher sound levels in the Bay and at Site 1 are likely due to recreational vessel presence. Increased sound levels from vessels in the Bay, however, likely dissipate more quickly in the shallow, muddy bottom environment (Bedford & Stern, 1983; Forrest et al., 1993). At Site 1, only 12 km from the busy recreational area of Ocean City, Maryland, sound levels were highest, likely due to an abundance of recreational vessels increasing their speeds as they exit the channel during summer excursions (Jensen et al., 2009). Both the acoustic environment's characteristics (i.e. bottom substrate and depth) and anthropogenic activity (i.e. recreational and shipping vessels) are important influences on sound levels experienced by dolphins in this region.

This study found that the characteristics of bottlenose dolphin signature whistles differed significantly between sites and regions. In addition, bottlenose dolphins adjusted the duration of their signature whistles in relation to the ambient sound environment in which they were emitted, possibly to reduce the possibility of interruption of their call. Increasing sound levels in the ocean may continue to cause changes in dolphin calls leading to information loss and reducing cohesion amongst dolphin groups, including mother-calf pairs. These findings help us to understand how the changing ocean is affecting this critical communication and will be vital for management of this region, which is subject to rising sound levels due to offshore wind development, coastal development, bridge construction, and increasing vessel traffic.

Tables

Table 3.1. Dolphin sex (F: female, M: male), name, age in years, and spectrogram of their signature whistle as well as what percentage of their signature whistles were contoured and how successful ARTwarp was at classifying these signature whistles at the 90% vigilance threshold (i.e. true classifications).

Sex	Dolphin, age in years	Signature whistle	Percentage of free swim recordings containing signature whistle	Number, and percentage of whistles contoured (Total number of whistles)	True classification at 90% vigilance %
F	Bayley, 13	22 - 20 - 20 - 20 - 20 - 20 - 20 - 20 -	67%	7, 54 % (13)	29%
М	Beau, 16	a) white most best who	33%	3, 50% (6)	33%

F	Jade, 22	22- 20- 18- 16- 14- 12- 10- 8- 6- 4- 2- 10- 8- 6- 4- 2- 10- 10- 8- 8- 6- 4- 2- 10- 10- 8- 8- 10- 10- 10- 10- 10- 10- 10- 10- 10- 10	50%	5, 83% (6)	100%
М	Foster, 14	22 - 20 - 20 - 20 - 20 - 20 - 20 - 20 -	83%	7, 70% (10)	57%
F	Chesapeake, 29	22- 20- 18- 14- 12- 10- 8- 6- 4- 2- 5- 6- 4- 2- 5- 6- 5- 6- 5- 6- 14- 12- 10- 8- 6- 14- 12- 10- 8- 12- 10- 8- 10- 10- 10- 10- 10- 10- 10- 10- 10- 10	17%	1, 100% (1)	N/A
F	Spirit, 20	22- 20- 18- 16- 14- 12- 10- 8- 6- 4- 2- 8- 6- 4- 2- 8- 6- 4- 2- 15-1653.5	17%	1, 100% (1)	N/A

Table 3.2. Results of linear models (lm) and generalized additive models (GAM) relating visual group size (GroupSize), number of unique signature whistles (SW), the difference between the group size and number of unique signature whistles (GroupSize-SW), distance from the start of the sighting from the hydrophone (DistFromSite; km), percentage of hours during the visual sighting that contained foraging (Foraging), number of hours of containing signature whistles (SW) in a sighting, and duration of the visual sighting (Dur, min). Smoothers for the GAM (indicted by "s()") were restricted to 4 knots.

Model	Estimate	Std. Error	P- value	R ²
a: lm(GroupSize ~ SW)	1.34	1.24	0.30	0.08
b: lm((GroupSize-SW) ~ DistFromSite)	5.66	3.46	0.12	0.16
c: GAM(GroupSize ~ SW + (Number				
of hours with SW) + $s(Foraging, 4)$ +				
s(DistFromSite, 4))				0.35
SW	1.77	1.18	0.17	
Number of hours with SW	-10.94	10.26	0.31	
Foraging	edf=1.00	F=1.09	0.32	
DistFromSite	edf=1.99	F=2.61	0.12	
d: GAM(GroupSize \sim s(Dur,4))	edf=2.67	F=3.99	< 0.03*	0.43

Table 3.3. For each site and region, the recording period, total number of signature whistles
detected, and total number and percentage of signature whistles selected for acoustic
characteristic measurement.

Site	Region	Recording period	Hours analyzed	Total number of unique SW	Number of SWs with measured characteristics	Percentage of total SW with measured characteristics (%)
1	Coastal	June-Aug 2017, July-Oct 2018	268	1172	100	9
2	Coastal	July-Sept 2016, Jan-April, June- Oct 2017, June-Dec 2018	521	327	100	31
3	Bay	May-Sept 2018	2488	19	19	100
4	Bay	June-Sep 2019	311	333	100	33
5	Offshore	July- Sep 2018	415	38	38	100

Figures



Figure 3.1. A spectrogram of a whistle (box selections 1 and 2) that meets the signature whistle criteria of a duration of greater than 0.2 seconds with 75% contour similarity (red dashed line), repeated within 1-10 seconds of one another.



Figure 3.2. Map of acoustic recording sites in the southern coastal Mid-Atlantic Bight, USA (blue triangles; Sites 1, 2), Chesapeake Bay (black circles; Site 3, 4), and offshore in the Mid-Atlantic Bight (red square; Site 5; following site nomenclature in Bailey et al. 2021 with additional sites).

Figure 3.3. Diagram of whistle showing characteristics measured including start, end, minimum, maximum, and delta frequencies, number of extrema, and duration.





Figure 3.4. Plots and results of linear regression models for a) the number of unique signature whistles detected and the number of dolphins sighted during an encounter and (Table 3.2 model a), b) plot of the generalized additive model's (GAM) smoother (i.e. partial residual) for the difference between the group size and number of signature whistles and distance between the sighting and the acoustic recorder (in km; Table 3.2 model c), and c) plot of the GAM smoother for the group size and duration (Dur) of the encounter (Table 3.2 model d).



Figure 3.5. Boxplot of the a) square root (sqrt) of the start frequency, b) sqrt of the end frequency, c) sqrt of the minimum frequency, d) sqrt of the delta frequency, e) log of the duration, and f) log of the number of extrema for signature whistles at each site. Asterisks indicate an adjusted p-value (Bonferonni method) of less than 0.05 in pairwise t-tests. Blue boxes

indicate sites in the Coastal region, black are sites in the Chesapeake Bay, and red is the site in the Offshore region.



Figure 3.6. Boxplot of the a) square root (sqrt) of the start frequency, b) sqrt of the end frequency c) sqrt of the minimum frequency, d) log of the duration, and e) log of the number of

extrema for signature whistles in each region. Asterisks indicate an adjusted p-value of less than 0.05 (Bonferonni method) in pairwise t-tests.



Figure 3.7. Results of the Boruta random forest model indicating the ranked importance of signature whistle characteristics in determining a whistle's (a) site and (b) regional identities. Green boxes indicate important characteristics, red indicates characteristics that decreased the performance of the model, and the blue are shadow factors used to test the model. Shadow factors are shuffled duplicate copies of factors added to remove correlations between variables.

Region 🛱 Bay 🛱 Coastal 🛱 Offshore



Figure 3.8. Median daily sound levels (SPL rms: dB re 1μ Pa) at each site (a) and region (b). Asterisks indicate an adjusted p-value (Bonferonni method) of less than 0.05 in the pairwise t-tests.



Figure 3.9. Network diagram showing signature whistle reoccurrences (gray lines) between sites (circles labeled with site number) within the study area. Blue nodes indicate sites in the Coastal region, black indicate sites in the Chesapeake Bay, and red indicates a site in the Offshore region.

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